Combination of an anaerobic membrane bioreactor (AnMBR) with microalgae production for decentralized wastewater treatment

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Abstract

Most of the world's inhabitants still do not have access to sanitation facilities, particularly in remote or poor regions. Consequently, efficient solutions for decentralized wastewater treatment are urgently needed. This study investigates the technical feasibility of the coupling of an anaerobic membrane bioreactor (AnMBR) process with microalgae cultivation for decentralized domestic wastewater treatment. The results presented in this work are based on the 327-days operation of an 850-L AnMBR pilot-plant installed in a residential building in Hamburg, Germany, as well as on lab-scale and full-scale experiments conducted with the effluent of the AnMBR process (permeate) and with three microalgae species (*Acutodesmus obliquus, Chlorella vulgaris* and *Chlorella sorokiniana*).

Over the eight main experimental phases of the AnMBR operation, chemical oxygen demand (COD) concentration in wastewater amounted to between 0.97 and 2.49 $g \cdot L^{-1}$. Since between 86 % and 94 % of the COD was removed during the process, this showed a higher performance than other small decentralized wastewater treatment plants (WWTPs) and even competed with conventional German WWTPs. However, the organic loading rate, methane production and the hydraulic retention time were in the lower range of reported AnMBR-related values. This was due to a lower microorganism concentration in the reactor.

Overall, 95 - 99 % of total nitrogen (TN) and 79 - 87 % of total phosphorus (TP) present in the wastewater were recovered in the permeate. During the lab-scale and full-scale experiments, the three microalgae species were able to assimilate the nutrients contained in the permeate and grow in this culture medium. Altogether, a similar performance to synthetic fertilizers was achieved. During the lab-scale experiments with *Acutodesmus obliquus* and *Chlorella vulgaris*, initial TN and TP concentrations were 64 - 115 $mg \cdot L^{-1}$ and 7.1 - 14 $mg \cdot L^{-1}$ respectively. During these experiments, a decrease of TN removal and biomass growth was punctually observed. This was due to a lack of iron in the permeate and could be easily counterbalanced by the supply of an iron salt and a chelating agent into the culture. Initial TN and TP concentrations were comparatively higher during the experiments conducted with *Chlorella sorokiniana* and ranged from 98 to 160 $mg \cdot L^{-1}$ and 16 to 37 $mg \cdot L^{-1}$ respectively. The reduced TN/TP ratio in the permeate led to an incomplete TP removal. For unfavorable TN/TP ratios, a solution leading to a complete TP removal must be implemented, such as the use of another microalgae species capable of adapting to low TN/TP ratios, pH increase or the use of iron or aluminum salts leading to TP precipitation.

During the two full-scale experiments conducted with two flat panel photobioreactors in the fall and summer, ammonium and TP were completely removed. However, TN was totally assimilated only during the summer. The incomplete TN removal during the fall season was caused by the supply of flue gas containing NO_x combined with a low irradiance making the assimilation of NO_x by the microalgae impossible. Nutrient uptake was usually higher than and biomass production similar to the values for similar light conditions reported in the literature. Overall, the results related to nutrient removal during microalgae cultivation indicate that this new process can compete with conventional WWTPs.

In this study, the technical feasibility of the combination of an AnMBR process and microalgae cultivation was proved. If the different processes, especially the cultivation and harvesting of microalgal biomass, are energetically optimized, this new technology will be an adequate solution to the need for efficient decentralized wastewater treatment technologies.

Zusammenfassung

Ein Großteil der Weltbevölkerung hat keinen Zugang zu sanitären Einrichtungen, insbesondere in abgelegenen oder armen Regionen. Daher sind effiziente Lösungen für dezentrale Abwasserbehandlung dringend erforderlich. Diese Studie untersucht die Machbarkeit der Kopplung eines anaeroben Membranbioreaktors (AnMBR) mit Mikroalgenkultivierung zur dezentralen häuslichen Abwasserbehandlung. Die vorgestellten Ergebnisse basieren auf einem 327-Tage langen Betrieb einer 850-L AnMBR Pilotanlage in einem Hamburger Wohnhaus, sowie auf Labor- und Full-Scale-Versuchen mit dem Ablauf des AnMBR Prozesses (Permeat) und drei Mikroalgenarten (*Acutodesmus obliquus, Chlorella vulgaris* und *Chlorella sorokiniana*).

Während der acht Hauptversuchsphasen des AnMBR-Betriebs betrug der chemische Sauerstoffbedarf (COD) im Abwasser zwischen 0,97 und 2,49 $g \cdot L^{-1}$. Mit einer Entfernung des COD zwischen 86 % und 94 % zeigte der Prozess eine höhere Leistung als dezentrale Kleinkläranlagen und kann diesbezüglich auch mit konventionellen deutschen Kläranlagen konkurrieren. Die Leistung bezüglich Raumbelastung, Methanproduktion und hydraulischer Verweilzeit war jedoch im unteren Bereich der Literaturwerte. Dies ist auf eine geringere Konzentration von Mikroorganismen im Reaktor zurückzuführen.

Insgesamt wurden 95 - 99 % des gesamten Stickstoffs (TN) und 79 - 87 % des gesamten Phosphors (TP) im Permeat zurückgewonnen. Während der Labor- und Full-Scale-Versuche konnten die drei Mikroalgenarten die Nährstoffe erfolgreich aufnehmen und wachsen. Insgesamt wurde eine ähnliche Leistung wie bei synthetischen Düngemitteln erzielt. Während der Laborversuche mit *Acutodesmus obliquus* und *Chlorella vulgaris* lagen die TN- und TP-Konzentrationen zunächst bei 64 - 115 $mg \cdot L^{-1}$ bzw. 7.1 - 14 $mg \cdot L^{-1}$. Während dieser Experimente wurde zum Teil eine Senkung der TN-Entfernung und des Biomassewachstums durch Eisenmangel im Permeat beobachtet, was durch die Zufuhr eines Eisensalzes und eines Chelatbildners problemlos ausgeglichen werden konnte. Die anfänglichen TN- und TP-Konzentrationen während der Laborversuche mit *Chlorella sorokiniana* waren im Vergleich höher und betrugen 98 - 160 bzw. 16 - 37 $mg \cdot L^{-1}$. Das reduzierte TN/TP-Verhältnis führte zu einer unvollständigen TP-Entfernung. Für ungünstige TN/TP-Verhältnisse sind daher entsprechende Konzepte nötig, wie z.B. die Verwendung einer anderen Mikroalgenart, die sich an niedrige TN/TP Verhältnisse anpassen kann, eine pH-Erhöhung oder die Verwendung von Eisen- oder Aluminiumsalzen um TP-Ausfällungen herbeizuführen.

Bei zwei Full-Scale-Versuchen im Herbst und im Sommer mit zwei Flach-Photobioreaktoren wurden Ammonium und TP vollständig entfernt. Allerdings wurde TN nur während des Sommers vollständig aufgenommen. Eine unvollständige TN-Entfernung im Herbst wurde durch die Zufuhr von NO_x-haltigem Rauchgas in Verbindung mit schlechten Lichtverhältnissen verursacht. Im Vergleich zu Literaturwerten für ähnliche Lichtverhältnisse war die Nährstoffaufnahme höher und die Biomasseproduktion ähnlich. Bezüglich der Nährstoffentfernung konnte der Prozess mit konventionellen Kläranlagen konkurrieren.

In dieser Studie wurde die technische Machbarkeit der Kombination eines AnMBR-Verfahrens mit Mikroalgenkultivierung nachgewiesen. Wenn die verschiedenen Prozesse, insbesondere die Kultivierung und Ernte von Mikroalgenbiomasse, energetisch optimiert werden, wird diese neue Technologie eine adäquate Lösung für den Bedarf an effizienten dezentralen Abwasserbehandlungstechnologien werden.

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Nomenclature

Abbreviations

ADP	-	adenosine diphosphate
AnMBR	-	anaerobic membrane bioreactor
ASA	-	aerobic sludge stabilization
ASAN	-	anaerobic sludge stabilization
ATP	-	adenosine triphosphate
BIQ - The Algae House	-	the “building with bio-intelligent quotient” - innovative residential building in Hamburg-Wilhelmsburg, Germany
BOD_5	$g \cdot L^{-1}$	biochemical oxygen demand after 5 days
BPR	$g \cdot L^{-1} \cdot d^{-1}$	biomass production rate
BPR_α	$g \cdot m^{-2} \cdot d^{-1}$	biomass production rate referring to the illuminated area of a photobioreactor
BSP	kg $VS \cdot kg^{-1}$ COD_{rem}	biosolids production
CH_4	-	methane
CHP	-	combined heat and power plant
CO_2	-	carbon dioxide
COD	$g \cdot L^{-1}$	chemical oxygen demand
cRIO	-	Compact RIO system (National Instruments)
CSTR	-	continuous stirred tank reactor
DIN	-	from the German “Deutsches Institut für Normung” - German Institute for Standardization
DNA	-	deoxyribonucleic acid
DOM	-	dissolved organic matter
DTPA	-	diethylenetriaminepentaacetic acid
DWA	-	from the German „Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall“ - German Association for Water, Wastewater and Waste
EDDHA	-	ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid)
EDTA	-	ethylenediaminetetraacetic acid
EEC	-	European Economic Community
EN	-	European norm
EU	-	European Union
F/M	kg $COD \cdot kg^{-1}$ $VS \cdot d^{-1}$	food-to-microorganisms ratio
Fe	-	iron
Fe_2S_3	-	iron(III) sulfide
$Fe(OH)_3$	-	iron(III) hydroxide
$FeSO_4$	-	iron(II) sulfate
FPGA	-	field programmable gate arrays
GC		gas chromatography
H_2	-	hydrogen
H_2O	-	water
H_2S	-	hydrogen sulfide

HAWANA	-	from the German „Nutzung von Haushaltsabwässern zur Versorgung einer Bioreaktorfassade mit Wasser und Nährstoffen“ - Use of wastewater to supply a bioreactor facade with water and nutrients
HRAP	-	high rate algal pond
HRT	d	hydraulic retention time
ISO	-	international organization for standardization
KNO_3	-	potassium nitrate
LED	-	light-emitting diode
LHV	$MJ \cdot kg^{-1}$	low heating value
MPBR	-	membrane photobioreactor
Mg	-	magnesium
$MgSO_4$	-	magnesium sulfate
Mn	-	manganese
$MnCl_2$	-	manganese(II) chloride
$MnSO_4$	-	manganese(II) sulfate
N_2	-	nitrogen
N_2O	-	nitrous oxide
NAD	-	nicotinamide adenine dinucleotide
NADH		Reduced form of NAD
$NADP^+$		Oxidized form of NAD
NaOH	-	sodium hydroxide
NH_4-N	-	ammonium
NH_4NO_3	-	ammonium nitrate
$(NH_4)_2SO_4$	-	ammonium sulfate
NO_x	-	nitrogen oxide
NO_2-N	-	nitrite
NO_3-N	-	nitrate
NTA	-	nitrilotriacetic acid
O_2		dioxygen
OLR	$kg\ COD \cdot m^{-3} \cdot d^{-1}$	organic loading rate
PBR		photobioreactor
PE	-	population equivalent
PE	%	photosynthetic efficiency
PO_4-P	-	phosphate
PPFD	$\mu mol \cdot s^{-1} \cdot m^{-2}$	photosynthetic photon flux density
PVDF	-	polyvinylidene fluoride
RC	$g \cdot L^{-1} \cdot d^{-1}$	removal capacity
RC_α	$g \cdot m^{-2} \cdot d^{-1}$	removal capacity referring to the illuminated area of a photobioreactor
RE	%	removal efficiency
RT	-	real-time system
S	-	sulfur
SBP	$m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}$	specific biomethane production
SBR	-	sequencing batch reactor

SO_4-S	$g \cdot L^{-1}$	sulfate
SRT	d	solid retention time
TN	$g \cdot L^{-1}$	total nitrogen
TP	$g \cdot L^{-1}$	total phosphorus
TS	$g \cdot kg^{-1}$	total solids
TU	-	technical University
UASB	-	upflow anaerobic sludge blanket
UV		ultraviolet
VDI	-	from the German „Verein Deutscher Ingenieure“ - association of German engineers
VFA	-	volatile fatty acids
VS	$g \cdot kg^{-1}$	volatile solids
WWTP		wastewater treatment plant

Greek and Latin letters

α	-	proportion
Δ	-	fluctuation
η	Pa·s	dynamic viscosity
μ	d^{-1}	microalgae specific growth rate
A	m^2	area
C	$g \cdot L^{-1}$	concentration
E_e	$W \cdot m^{-2}$	solar irradiance
m	kg	mass
n	mol	amount of substance
N	-	number of days of an experimental period
p	bar	pressure
Q	$m^3 \cdot s^{-1}$	flow rate
r	$g \cdot d^{-1}$	daily biomass production
R	$J \cdot mol^{-1} \cdot K^{-1}$	gas constant
t	d	time
T	K	temperature
V	m^3	volume
W	kg	weight

Indices

0	-	under standard conditions
b	-	biologically
B	-	beginning
E	-	end
f	-	final
i	-	initial
max	-	maximum
M	-	membrane

n	-	normalized
p	-	permeate
pers	-	person
rem	-	removed
ret	-	retentate
R	-	reactor
w	-	wastewater

1 Introduction

The access to drinking water and sanitation facilities for the 7.6 billion people living on Earth remains one of the greatest challenges of the 21st century. The world population suffering from water shortages has risen from 14 % to 58 % in the last century (Kummu *et al.* 2016). This threat is not limited to countries of the southern regions, but also on several industrialized countries (Stiefel 2013)**.** Overall, four billion people face severe water scarcity (Mekonnen and Hoekstra 2016 - Figure 1). Meanwhile, the sanitation problem concerned in 2015 2.4 billion people (UNICEF and World Health Organization 2015). Especially in segregated or poor regions without sewer systems, household wastewater is often not efficiently cleaned, leading to many environmental and health problems. Overall, 80 % of the global population has no form of treatment before wastewater discharge (WWAP 2017).

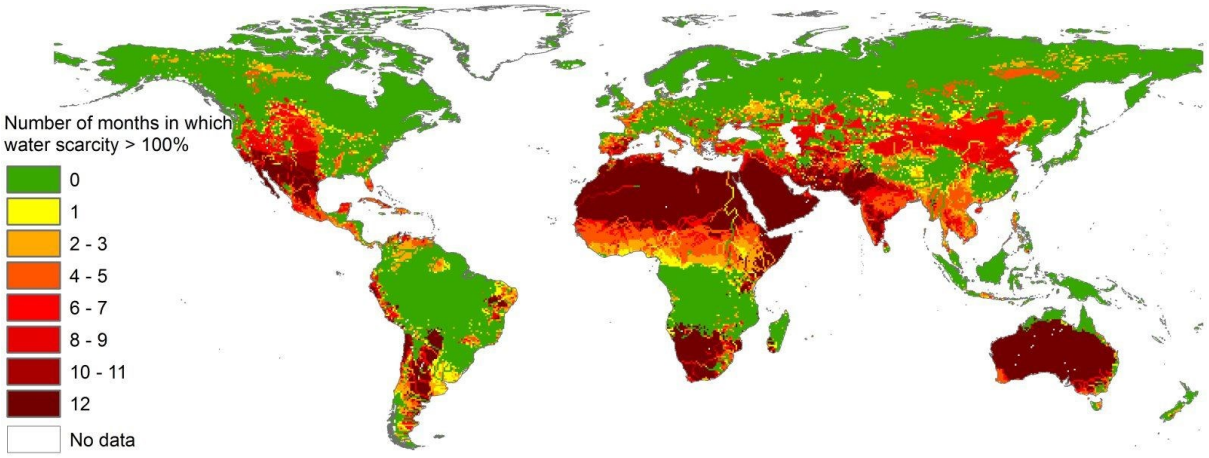

Figure 1: Global water scarcity (Mekonnen and Hoekstra 2016)

The application of small size decentralized wastewater treatment plants (WWTPs) seems there the most appropriate solution. In these regions, the treatment of the wastewater onsite is cheaper than a complex construction of pipe networks and the use of pumps. This low-cost solution allows also more autonomy to the communities and helps to combat water shortages, as the wastewater can be directly reused onsite (Chirisa *et al.* 2017). Also in urban areas, the use of centralized WWTPs already showed some limits. High investments, long planning as well as long construction times are needed and difficulties are encountered to expand the plants with a growing population. On the contrary, onsite treatment is seen as a viable, financially sustainable and ecological alternative, presenting more flexibility and reliability, reducing the amount of waste at the source and facilitating resource recovery (Bernal and Restrepo 2012; Kohler *et al.* 2016). However, the wastewater processes used worldwide for decentralized applications, such as septic systems, man-made wetlands and sand filters are usually not suitable as a long-term solution (Pang *et al.* 2017).

The use of anaerobic membrane bioreactors (AnMBR) for wastewater treatment has gained increasing interest over the last decades. An AnMBR consists of a reactor where wastewater is biologically degraded in absence of oxygen coupled with solids separation by the means of a membrane filtration unit. This technology cumulates the advantages of anaerobic processes, which are low sludge production, low energy consumption, high organic removal, stable operation and pathogens and parasites deactivation (Dvořák *et al.* 2015). Because of the low space requirement combined with the low maintenance (Pretel *et al.* 2016), the use of an AnMBR is especially adapted for decentralized applications.

As an anaerobic process does not degrade nitrogen and phosphorus compounds, efficient strategies for both the removal and recovery of these macronutrients from the permeate need to be investigated *(*Ozgun *et al.* 2013). First, nitrogen and phosphorus removal is essential, since an incomplete nutrient removal from wastewater streams leads to eutrophication of the water bodies. The consequences for the aquatic systems are as various as tragic. Nuisance algae proliferation, low dissolved oxygen concentration and cyanotoxin production lead to fauna suffocating and poisoning (Christenson and Sims 2011). Nevertheless, not only the removal but also the recovery of these nutrients is crucial. Indeed, phosphorus is an essential but finite resource becoming scarce and expensive. While the depletion of this resource is expected within the next 100 years (Withers *et al.* 2015), phosphorus from human feces and urine represents 20 % of the phosphorus global demand for fertilizers (Mihelcic *et al.* 2011, Kok *et al.* 2018). Hence, its efficient recovery would represent a partial solution against the depletion of this resource. Nitrogen, on the other hand, can be synthesized, but this is energetically and environmentally costly. For instance, the production and use of 1 kg of ammonium nitrate-based fertilizer cause the emission of 3 kg of carbon dioxide (Fertilizers Europe 2011). Based on DWA (2008), 20 % of the global nitrogen demand would be guaranteed if all the nitrogen contained in human feces and urine was recovered.

The most common way to recover the water and the nutrients contained in the effluent of an AnMBR is fertigation, a process that combines irrigation and fertilization by injecting fertilizers into an irrigation system. This onsite recycling avoids the use of synthetic fertilizers and assures economic and environmental sustainability to the communities living in the area. For this purpose, organic and pathogen removal throughout the AnMBR process needs to be total and the treatment facility needs to be closed from agricultural areas. Furthermore, the local legislation must allow wastewater streams recycling for irrigation purposes and the effluent quality must respect the local standards for reuse with the purpose of crop irrigation.

Using the nutrient-rich permeate for microalgal biomass production also represents a consistent strategy. For their growth, microalgae need three macronutrients, total nitrogen (TN), total phosphorus (TP) and carbon, with TN and TP in the preferred form of ammonium (NH_4-N) and phosphate (PO_4-P). In the permeate, TN and TP are as well mostly in the form of NH_4-N and (PO_4-P). Consequently, the combination of the AnMBR process with microalgae culture permits to close the nutrient cycle, as these main macronutrients are recycled into biomass. Furthermore, compared to other existing processes, both TN and TP removal occur in only one step and without the addition of any chemical product. Microalgae also permit the reduction of heavy metals and pathogen concentration in the effluent as well as the removal of organic micropollutants (Whitton *et al.* 2015).

As microalgae are photosynthetic organisms needing carbon dioxide to grow, this also enables the recycling of CO_2 rich flue gas. As 1.83 kg $CO_2 \cdot kg^{-1}_{biomass}$ are sequestered by the microalgae (Rosenberg *et al.* 2011), these organisms could play a great role to reduce the greenhouse gas emissions. Microalgal biomass can then be used in several applications like biofuel production, anaerobic digestion and pigments, lipids, proteins and fatty acids production. Consequently, microalgae production transforms waste streams into high-value products with an ecological gain.

In the present study, the combination of an 850-L decentralized AnMBR pilot-plant with microalgae cultivation at both lab-scale and full-scale is intensively investigated. The aims of the thesis, the backgrounds of the project as well as the structure of the thesis are described precisely in the following sections.

2 Background and outlines of this study

2.1 Aims of the study

The present work aims at decentralized domestic wastewater treatment and biogas production using an AnMBR combined with water and nutrient recycling from the permeate through microalgal biomass production. While industrial wastewater treatment using AnMBRs has been intensively studied, only limited full-scale applications can be found for municipal wastewater (Dvořák *et al.* 2015). Furthermore, these studies focused on low or medium strength municipal wastewater, which consists of domestic wastewater diluted with rainwater, street cleaning water and low strength industrial wastewater. On the contrary, the present work evaluates the performance of a decentralized AnMBR at a house scale characterized by high strength domestic wastewater. As the specificity of wastewater composition of such plants has to be taken more precisely into account, this work is essential to further expand this technology in small size onsite applications.

Moreover, microalgae cultivation using the permeate of an AnMBR has been scarcely investigated and only one author reported the use of permeate for microalgae cultivation in an outdoor pilot plant facing weather dependence in terms of temperature and light (Viruela *et al.* 2016; Viruela *et al.* 2018). To the knowledge of the author, the use of permeate from a decentralized AnMBR treating domestic wastewater and characterized by such high nutrient concentrations for microalgae cultivation has never been investigated.

Figure 2 represents the global aim of the study. This study was part of the project HAWANA that is described in the following section.

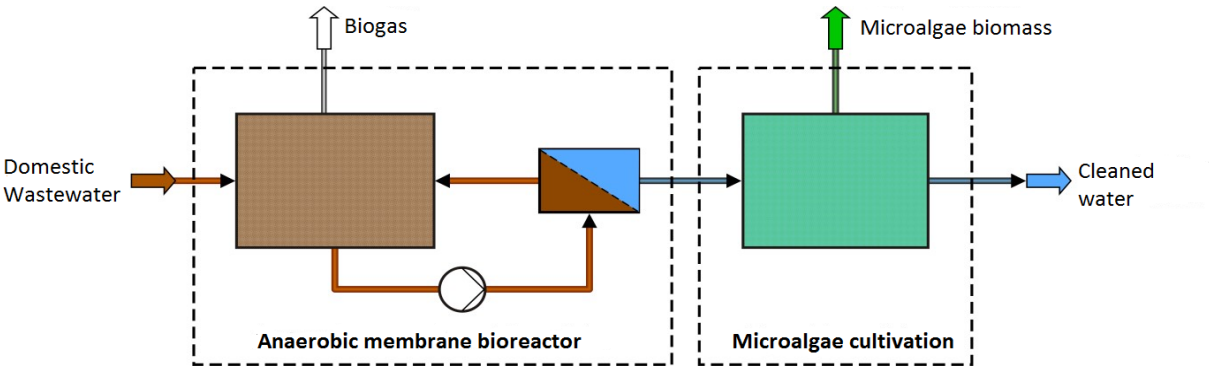

Figure 2: Aim of the study: combination of an AnMBR process and microalgae cultivation

2.2 Project HAWANA

The present study was conducted as part of the three-year research project HAWANA, which was funded by the Federal Ministry of Education and Research. The project took partially place in the residential building “BIQ - The Algae House”, which was constructed during the International Building Exhibition 2013 in Hamburg-Wilhelmsburg, Germany (Figure 3). This 15-residential unit building has the specificity to possess two photobioreactor facades with a total area and volume of 200 m² and 4,000 L respectively. These bioreactor facades are the key elements of the BIQ passive energy concept.

They consist of a closed circuit of several glass panels in which microalgae are cultivated in an aqueous culture medium containing essential nutrients. The CO_2 required for the photosynthesis of the microalgae is supplied at regular time intervals via compressed air to the bottom of each panel. The large air bubbles formed in the culture enable upstream water flow. Simultaneously, the turbulences ensure a constant mixing of the culture and stimulate CO_2 and light energy uptake. The CO_2 is flue gas from a combined heat and power plant (CHP) belonging to a gas-fired central heating system. Hence, this process leads to CO_2 recycling and the decrease of its emissions in the atmosphere.

The southwestern and southeast orientation of the facades ensures maximum exposure to the sunlight. Since microalgae usually accumulate on the glass surface, small plastic balls are also added to the culture medium. Through the upstream flow, they clean the glass panels from the inside. Hence, the facade orientation and the plastic balls enable efficient photosynthesis and biomass growth, and, at the same time, the culture medium is heated. Throughout the utilization of heat exchangers, the heat is used to produce hot water and to heat the flats. The excess of thermal energy is geothermally stored by probes and used to heat the photobioreactors in the cold seasons. The heat gain generated by the facade is around 150 kWh per square meter of facade per year. Furthermore, around 2.5 tons of CO_2 are annually absorbed throughout photosynthesis (COLT, ARUP and SSC GmbH 2013).

Figure 3: "BIQ - The Algae House" in Hamburg-Wilhelmsburg, Germany

The project HAWANA adds environmental and energy components to the BIQ model of sustainability. For this purpose, an AnMBR was installed in the technical room of the BIQ. A part of the wastewater produced by the inhabitants was anaerobically treated in the AnMBR. The biogas produced can be burned in a CHP or a boiler for onsite utilization, reinforcing the energy autonomy of the building. Furthermore, the effluent of the AnMBR is nutrient-rich and solid-free and may replace drinking water and fertilizers used for the preparation of the microalgae culture medium. Consequently, drinking water consumption would significantly decrease, which would lead to the protection of the water resource. To strengthen even more the autonomy of the BIQ concerning energy and water resources, utilization of the microalgae biomass as a substrate for the AnMBR or the burning of the biomass to produce heat and electricity can be imagined. Likewise, the effluent of the AnMBR could be used for non-drinking water usages (for example flush water) after microalgae cultivation.

The present work aims to investigate if the effluent of the AnMBR installed in the BIQ is an adequate culture medium for nutrient recovery throughout microalgal biomass production. For this purpose, several experimental works were conducted. These are described in detail in the following section.

2.3 General structure of the thesis

This thesis is divided into six chapters, which all have specific goals and bring supplementary information to the transdisciplinary problematic of the present study.

Chapter 1 introduces the global problematic related to both water and sanitation access as well as nutrient recovery from wastewater streams. In this chapter, the advantages and perspectives of decentralized wastewater treatment, the AnMBR technology and its combination with microalgae production are presented, too.

Chapter 2 shows the background of the study and precisely defines the aims and the structure of the thesis.

Chapter 3 is the main theoretical chapter and summarizes the state of the art in the transdisciplinary fields of wastewater treatment, nutrient removal and recovery, anaerobic digestion, membrane technique and microalgae cultivation. The theoretical knowledge, advantages, challenges and full-scale applications of the related processes and technologies are introduced.

Chapter 4 presents the operation of the AnMBR pilot-plant installed in the BIQ and the material used for microalgae cultivation in the laboratories of the TU Berlin and the University of Hamburg as well as in the pilot-plant Hamburg-Reitbrook. The specific parameters and equations used in this work are detailed, too. Finally, the temporal development of experimental work is represented.

Chapter 5 analyzes and discusses the main results showed in Chapter 4. Comparisons with existing and similar literature are made, too. Finally, an energy balance of the combination of the AnMBR technology and microalgae production is conducted. The pertinence and perspective of the coupling of both technologies are then discussed.

Chapter 6 provides a synthesis of the findings from the entire work as well as concluding remarks about the new perspectives given by this work and the further research needed.

3 State of the Art

3.1 Domestic wastewater treatment

3.1.1 Definition and composition of domestic wastewater

3.1.1.1 Definition of wastewater

Water is the basis of life and is daily used in industry, agriculture and private households. Consequently, the production of polluted water streams, also named wastewater streams, is unavoidable and represents a challenge for the natural water balance and the environment. Throughout adequate wastewater treatment, the environmental consequences of polluted water streams should be limited.

Wastewater can be classified according to its origin. According to Pöppinghaus *et al.* (1994), it is distinguished between:

- domestic wastewater from households or public buildings, including wastewater streams from street cleaning
- wastewater from small industries
- industrial effluents from the larger industries
- wastewater from agricultural use
- percolate water from groundwater
- rainwater from precipitation

Domestic wastewater consists of blackwater, which is water from the toilets (flushing water, toilet paper, faeces and urine), and greywater, which is the wastewater streams obtained when showering, bathing, with dishwashers or other domestic appliances. According to Tchobanoglous *et al.* (2014), wastewater can be classified in the following groups:

- biologically degradable compounds, such as organic compounds (proteins, carbohydrates and fats), organic nitrogen or sulfur compounds
- compounds that are non-biologically degradable or slowly biodegradable such as salts, acids, bases, mineral sludge, humic acid and persistent compounds (hormones, pharmaceuticals)
- nutrients like nitrogen or phosphorus compounds, which lead to eutrophication of the water bodies if they are not correctly treated
- toxic substances or organisms leading to diseases or poisoning such as heavy metals, pesticides, carcinogenic compounds, bacteria, viruses or mushrooms
- impurities such as gravel, sand, oil and grease.

In order to quantify the degree of contamination of wastewater and the efficiency of wastewater treatment, several parameters are used. These are defined in the following section.

3.1.1.2 Parameters for water and wastewater quantification

3.1.1.2.1 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is a sum parameter, which is representative for the mass of oxygen that is needed to oxidize all the substances and particularly the organic substances of a sample. This parameter refers to both dissolved as well as particulate compounds. COD is often used as the main parameter for the assessment of water quality and wastewater pollution levels.

3.1.1.2.2 Biochemical oxygen demand after 5 days (BOD_5)

Biochemical oxygen demand after 5 days is defined by the amount of oxygen that is used by bacteria within 5 days for the biodegradation of the organic substances contained in a sample. The BOD_5 value is always lower than the related COD value, as the COD measures all oxidizable organic substances and BOD_5 exclusively the biodegradable substances. According to Rosenwinkel *et al.* (2015), the COD/BOD_5 ratios permit a statement of the degradability of a wastewater sample. By values less than 2, the wastewater is easily biodegradable.

3.1.1.2.3 Total solids (TS) and volatile solids (VS)

Total solids and volatile solids are essential parameters that indicate the biomass concentration of a wastewater sample. TS is the part of the solid mass that remains after drying a sample at 105 °C for 24 hours. TS is divided into volatile and inert solids. Inert solids, such as sand or clay, consist of the inorganic ash residue obtained after heating the dried sample at 550 °C in a muffle furnace. VS is obtained by subtracting TS and the inert solids. This parameter includes the organic solid compounds that are biodegradable or non-biodegradable.

3.1.1.2.4 Nutrients

Discharge of wastewater without removal of the main nutrients TN and TP can lead to major environmental damages such as eutrophication. The accumulation of nutrients in the water bodies results in a reinforced growth of algae. These algae can displace other plants, thereby reducing biodiversity. The bacterial degradation of dead algae cells results in oxygen deficiency at the bottom of the water, which damages soil-living animals and plants. If the oxygen concentration in the water falls so far that it leads to anaerobic decomposition processes, toxic products are produced, as methane, ammonia and hydrogen sulfide, and kill the fauna present in the water (Umweltbundesamt 2017). The main two macronutrients TN and TP are defined as follows.

Total nitrogen can be bound in organic compounds, such as proteins, urea, nucleic acids and amino-acids or be present in the inorganic form of nitrates, nitrites and ammonium. According to Rosenwinkel *et al.* (2015), it can be assumed that TN forms 8 - 9 % of the COD. The nitrogen present in the wastewater comes mainly from urea.

TP is found in both organic and inorganic forms. The inorganic forms are mostly orthophosphate and polyphosphate. Compared to nitrogen compounds, phosphorus compounds have a lower proportion in wastewater streams.

3.1.1.3 Municipal and domestic wastewater composition

The quantity and quality of wastewater is determined by many factors such as the behavior, lifestyle and standard of living of the inhabitants of a region. In the literature, the values reported are usually

given for municipal wastewater, which is mostly composed of domestic wastewater diluted with low-strength industrial wastewater, rainwater and street cleaning water. Table 1 presents the average composition of wastewater at the input of a municipal WWTP and the median values for wastewater characterization in decentralized WWTPs. In the municipal wastewater composition, the streams are divided into three categories: low-strength, medium and high-strength wastewater. Domestic wastewater in decentralized WWTPs is logically much more concentrated than in centralized municipal WWTPs.

Table 1: Composition of wastewater at the input of a municipal WWTP and in a decentralized WWTP

Parameter	Municipal wastewater composition (Rawat *et al.* 2011)			Domestic wastewater composition in decentralized WWTPs (DWA 2008)
	Low-strength	Medium	High-strength	
TS ($g \cdot kg^{-1}$)	0.350	0.720	1.200	1.52
VS ($g \cdot kg^{-1}$)	0.185	0.365	0.600	1.10
COD ($g \cdot L^{-1}$)	0.250	0.500	1.000	1.07
BOD_5 ($mg \cdot L^{-1}$)	110	220	400	393
TN ($mg \cdot L^{-1}$)	20	40	85	118
TP ($mg \cdot L^{-1}$)	4	8	15	18.3

Table 2: Median daily wastewater discharge for each person in Central and Western Europe according to the different streams (urine, feces and greywater) (DWA 2008)

Parameter	Urine		Feces		Greywater		Sum
Unit	**$g \cdot pers^{-1} \cdot d^{-1}$**	**%**	**$g \cdot pers^{-1} \cdot d^{-1}$**	**%**	**$g \cdot pers^{-1} \cdot d^{-1}$**	**%**	**$g \cdot pers^{-1} \cdot d^{-1}$**
Daily discharge	1,370	1.3	140	0.1	108,000	98.6	109,510
TS	57	34	38	23	71	43	166
VS	41	34	35	29	44	37	120
COD	10	8.5	60	51	47	40	117
BOD_5	5	12	20	47	18	41	43
TN	10.4	81	1.5	12	1.0	7.0	12.9
TP	1.0	50	0.5	25	0.5	25	2.0

Table 2 presents for urine, feces and greywater the median daily discharge for each person in Central and Western Europe relating to the parameters TS, VS, COD, BOD_5, TN and TP. Although urine represents only 1.3 % of the daily volume of wastewater produced, it represents 81 % and 50 % respectively of the total TN and TP discharge. Relating to the feces that account for 0.1 % of the daily volume of wastewater, 47 % and 51 % of the COD and the BOD_5 are discharged via this stream. Finally, greywater represents almost 99 % of the daily wastewater discharge and the main pollutants contained in this stream are TS, VS, COD and BOD_5. Overall, each person produces an average of 110 L of wastewater each day.

3.1.2 Wastewater treatment in conventional wastewater treatment plants (WWTPs)

In centralized WWTPs, the process consists of three steps: wastewater collection system, wastewater treatment and disposal of the purified water. In Germany, over 95 % of the population is connected to the public sewerage system, which represents more than 10,000 existing WWTPs (Bundesministerium für Umwelt, Naturschutz, Bau und Reaktorsicherheit 2014). Conventional wastewater treatment generally consists of three stages: the mechanical stage, the biological stage and the chemical stage.

3.1.2.1 Mechanical stage

Wastewater, as the sum of domestic wastewater, low-strength industrial wastewater, rainwater, percolate water and street cleaning water, is separated mechanically at the inlet of the plant (Figure 4). Larger solids and floating materials in the size range of 3-100 mm are removed using sieves and rakes. This is followed by the grit chamber, where sand and gravels up to a particle size of 0.1 mm are removed. Finally, the wastewater usually stays two hours in the primary clarifier, where the finer particles are also separated by sedimentation (Barjenbruch 2015).

3.1.2.2 Biological stage

This process aims the oxidation of organic compounds with the help of microorganisms. The most widespread process is the activated sludge process. First, in the aeration tank, a sludge with suspended microorganisms aerobically degrades the organic components contained in the effluent. The mixture is then supplied into a secondary clarifier, where sludge and purified wastewater are separated. A part of the sludge is then returned to the aeration tank in order to reseed new wastewater and ensure the desired sludge concentration in the tank. The removal of nitrogen occurs throughout nitrification and denitrification simultaneously to aerobic organic matter degradation. During the nitrification process, under aerobic conditions, ammonium is first oxidized to nitrite and then, the nitrite is oxidized to nitrate. This compound is removed from the liquid phase by denitrification. In this step that occurs under anaerobic conditions, nitrate is reduced to nitrogen gas with the help of microorganisms. In the case of these nitrification/denitrification steps, the aeration tank represented in Figure 4 is replaced by a basin with both aerobic and anoxic zones.

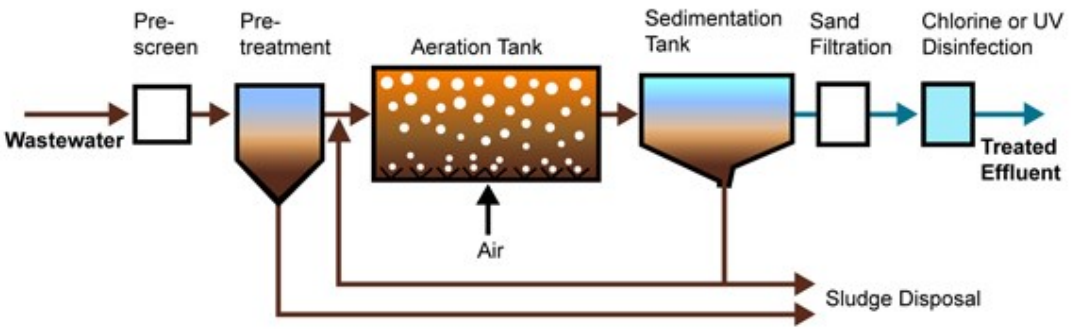

Figure 4: Conventional wastewater treatment in a municipal WWTP (Ionics Freshwater Ltd. 2010)

3.1.2.3 Chemical stage

The chemical stage is divided into neutralization (pH correction), precipitation and flocculation. Phosphorus elimination is particularly relevant since this compound is a eutrophication factor in water bodies. Precipitants are dosed and mixed into the wastewater stream. The precipitation reactions of the precipitant cations (Fe^{3+}, Al^{3+}, Ca^{2+}) with the phosphate anions produce micro- and then macroflocs, which are mechanically removed (Barjenbruch 2015).

The sludge produced in the different stages is transferred to an anaerobic reactor, where energy needed by the WWTP can be produced throughout methane production. In addition, different measures may be taken to disinfect the purified water. Treatments with chlorine dioxide, ultraviolet light or ozone are the most common. The stages presented here are not always systematic. Despite the danger of eutrophication and irreversible damage to the environment, efficient pollutant elimination is not always aimed. For example, in Germany, no requirement is set for small WWTPs (< 300 kg $BOD_5 \cdot d^{-1}$) with regard to phosphorus and nitrogen removal.

3.1.3 Decentralized domestic wastewater treatment

Decentralized wastewater treatment is defined by the handling of wastewater streams next to the source. These systems can be divided into three categories:

- simple sanitation systems aiming at a minimum of hygiene standards. In these systems, the discharge of a purified water with low pollutants levels is not the main aim.
- advanced systems that can be considered as small WWTP. These systems aim to limit water bodies pollution and have higher hygiene standards compared to the previous systems.
- recycling systems, where the main goal is to treat the pollution but also to transform the waste into a resource and reuse it. The present study is related to this kind of decentralized WWTPs, as it aims to use the organic matter and the nutrients contained in wastewater for methane production and microalgal biomass production respectively.

Table 3: Main differences between centralized and decentralized WWTP

Parameter	Centralized WWTP	Decentralized WWTP
Collecting system	Large diameters, long distances	Small diameters, short distances
Requirements space	Large area in one place	Small areas in many places
Operation and maintenance	Full time technical staff requirements	Less demanding, can be monitored remotely
Uniformity of water	Many types of water	More uniform water
Dilution grade	Less control over the storm water, more dilution	More control over the storm water, more concentrate
Risk	Risk on a larger scale	Risk distributed
Water transfer	Need for water transfer	Water is used and reused in the same area
Ease of expansion	High cost, more complexity of implementation	Low cost, less complexity of implementation
Potential to reuse	All water is concentrated in one point	Water can be reused locally

Although conventional WWTPs show a very high efficiency in pollutants removal, some disadvantages remain, as the high requirement of energy for aeration or the absence of pollutant reuse. At the contrary, decentralized treatments systems are appealing technologies due to several reasons. These are described in Table 3, which summarizes the main differences between a decentralized and a conventional centralized WWTP (Chirisa *et al.* 2017).

Overall, decentralized WWTPs make a major contribution to the autonomy of the municipalities, as wastewater is collected locally and the treated water can be reused locally. The recycling of wastewater is crucial in the remote or arm areas, especially when there is no access to a water supply network or when the region knows water scarcity. The operation of these decentralized systems is also less complex, so that little technical understanding and monitoring is necessary. Moreover, the absence of expensive pumping systems and pipe networks can make the decentralized solution a more cost-effective alternative. Because only one type of wastewater is treated and no dilution with other wastewater streams occurs, treatment processes are optimized. Hence, decentralized WWTPs are most of the time financially viable, environmentally friendly, sustainable and enables flexibility. According to Bernal and Restrepo (2012), in communities with low population densities, the costs for wastewater treatment can be reduced up to 60 % throughout the use of decentralized systems.

Nowadays, the most widespread decentralized systems are septic tanks, constructed wetlands, sequencing batch reactors (SBR), and membrane bioreactors (MBR). Although these systems have several advantages, some challenges remain. Septic tanks are very easy to implement and cost-efficient but removal of organic matter is low, the HRT is comparatively high and no water reuse is possible. Constructed wetlands have similar advantages to septic tanks. However, plant operation is complex and the system is surface-intensive (2 $m^2 \cdot PE^{-1}$). SBR systems achieve a high removal of organic and inorganic pollutants but the monitoring and maintenance requirements are high. Finally, MBR processes lead to extremely high pollutants elimination, low HRTs and an entire biomass and bacteria retention. However, the energy and costs requirements for this technology are comparatively high (Capodaglio 2017).

AnMBR processes have similar advantages to MBR systems but have lower energy requirements because of the absence of aeration in the reactor. They are characterized by a lower sludge production and usually require low space and maintenance. Compared to MBR systems, AnMBR processes possess the advantages of anaerobic processes. These advantages are the production of biogas for energy recovery, lower odor emissions and the possible degradation of compounds that are hardly biodegraded under aerobic conditions. Furthermore, after cessation of the AnMBR operation for several months, the anaerobic microorganisms can be reactivated within a few days. Hence, the AnMBR processes are a very appealing technology, which is precisely described in the following section.

3.2 AnMBR technology

An AnMBR process is a process defined by the coupling of an anaerobic reactor with a membrane unit. While the organic matter is biodegraded and the inorganic matter is mineralized in the anaerobic reactor, the use of a membrane guarantees the perfect retention of biomass, bacteria and germs and a high-quality solid-free effluent. As above-mentioned, this technology is very appealing for decentralized applications and is presented in the following section.

3.2.1 Definition of a membrane

A membrane is defined as a thin, partially permeable layer of a material, which, in contact with a fluid, a liquid or a gas, is permeable to at least one component, but impermeable to the other components (Melin and Rautenbach 2007). Compared to other separation processes, the separating material does not need to be chemically, biologically or thermally modified (Pinnekamp and Friedrich 2003). The driving force behind the separation passage is the difference in pressure between the side of the feed and the side of the permeate, called transmembrane pressure. This is obtained by overpressure on the feed side or low pressure on the effluent side. The initial stream, called feed, flows along (crossflow filtration) or through the membrane (dead-end filtration). The effluent that can pass through the membrane is called permeate. The remaining stream retained by the membrane is called concentrate or retentate (Melin and Rautenbach 2007). In wastewater treatment, membranes are primarily used for the retention of solids and dissolved substances as well as for disinfection.

3.2.2 Membrane operation

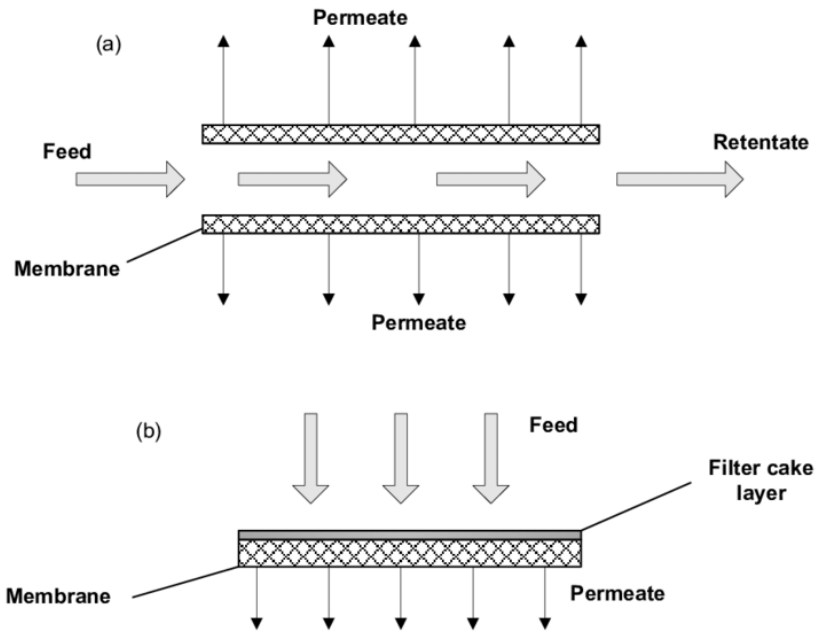

Figure 5: (a) Crossflow filtration, (b) dead-end filtration (Ishola 2014)

In dead-end operation, the feed stream flows perpendicularly to the membrane. Therefore, particles accumulate on the membrane and form a filter cake, which decreases the filter capacity of the membrane. As a result, the permeate flux decreases with increasing time. Consequently, the membrane must be regularly backwashed or cleaned in order to remove the filter cake (Figure 5).

In cross-flow filtration, the feed is guided parallel to the membrane surface and the permeate is extracted diagonally to it. The formation of a covering layer on the feed side, called membrane fouling, also occurs with crossflow operation. However, this problem is reduced. Indeed, because of the crossflow velocity of the stream and the shear forces acting on the surface, an equilibrium is established between fouling formation and fouling removal (Pinnekamp and Friedrich 2003).

3.2.3 Membrane material

Membranes can be made of organic or inorganic material. Organic membranes commonly consist of polymers and can be thermally, chemically and mechanically designed for specific separation problems. Besides, they are comparatively cost-efficient. Inorganic membranes are more regenerable and are generally used when the membrane must be often cleaned. They mostly consist of ceramics, aluminum, steel or glass materials, ceramic membranes being the most widespread for wastewater treatment. While inorganic membranes are extremely resistant to heat and chemicals and very durable, the main disadvantage remains the high acquisition costs (Pinnekamp and Friedrich 2003).

3.2.4 Membrane structure

Membranes can be symmetric or asymmetric. While symmetric membranes possess a nearly homogeneous structure, asymmetric membranes are composed of two layers. The so-called active layer on the feed side determines the separation behavior of the membrane, while the second layer ensures mechanical stability. Asymmetric membranes aim to keep the active layer as thin as possible and consequently keep the resistance of the membrane low. Compared to symmetric membranes, the flux is up 100 times higher in this configuration (Pinnekamp and Friedrich 2003).

3.2.5 Membrane classification

The pore size of a membrane has a decisive influence on which substances are separated and which not. In addition to the pore size, the molecular weight cut off can also be used. In this case, the separation limit is defined by the molecular mass of a compound (Melin and Rautenbach 2007). Figure 6 shows the different membrane categories, their pore size and the related compounds that are retained by the membrane or filtration processes.

3.2.5.1 Micro- and ultrafiltration membranes

Micro- and ultrafiltration membranes are pressure-operated membrane processes. While the microfiltration membranes have a pore size of about 0.1 - 5 µm, this ranges 0.01 - 0.1 µm for ultrafiltration membranes. The related transmembrane pressure amounts to between 0.1 and 10 bar. Microfiltration membranes mostly consist of symmetric polymers or ceramics membranes, while membranes made of asymmetric polymers, ceramics, or composite are usually used for ultrafiltration (Pinnekamp and Friedrich 2003). Both processes can be operated in dead-end or cross-flow configuration. All particles larger than the pore diameters of the membrane are retained. This creates a covering layer on the filter, which can also retain smaller particles. In municipal wastewater treatment, micro- and ultrafiltration are commonly used for the separation of the activated sludge, the separation of precipitated phosphorus and disinfection.

3.2.5.2 Nanofiltration

Nanofiltration is also a pressure-operated membrane process and is generally used to retain ions and organic compounds and to separate low and high molecular compounds. While nanofiltration is scarcely used in municipal wastewater treatment, this process is widespread for industrial wastewater treatment (Pinnekamp and Friedrich 2003).

3.2.5.3 Reverse osmosis

Reverse osmosis is carried out diffusively and is commonly used for seawater desalination or the production of ultrapure water. Only the compounds with a molecular weight of less than 200 $g \cdot mol^{-1}$ can pass through the membrane. In an osmosis process, the difference in concentration between the two phases is the driving force. A pure solvent, mostly water, diffuses through a dense, semi-permeable membrane into a concentrated solution. The process ends as soon as the same chemical potential has been set in both phases. In reverse osmosis, a pressure difference is applied to the concentrated solution and reverses the process. This results in a mass transfer contrary to the concentration difference.

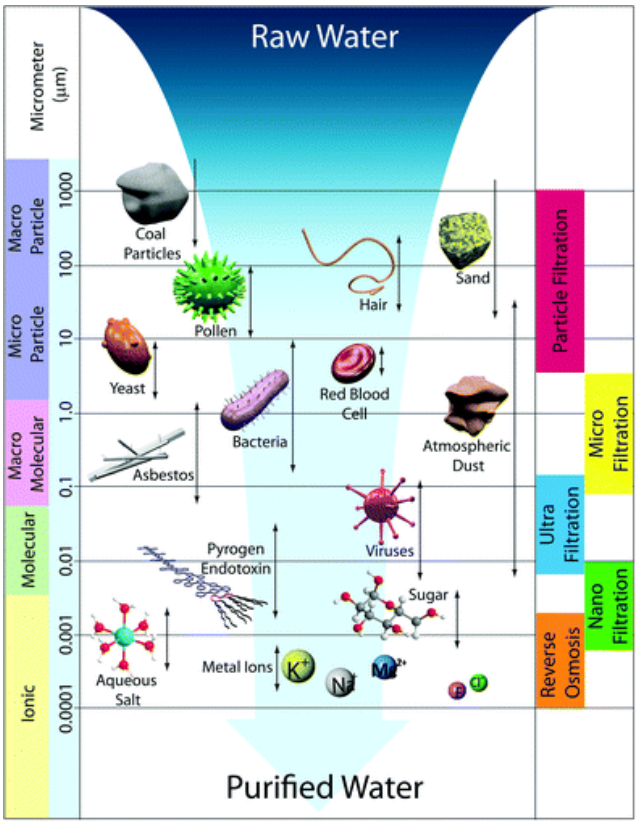

Figure 6: Presentation of the different filtration categories and the related components retained by the membrane (Lee et al. 2016)

3.2.6 AnMBR configurations

Three possibilities for the implementation of an AnMBR process are commonly used (Figure 7). The membrane is either immersed in the anaerobic reactor (one-stage submerged AnMBR or internal submerged AnMBR), immersed in a separate reactor (two-stage submerged AnMBR or external submerged AnMBR) or installed externally in a crossflow configuration. In the last case, the membrane is separate from the reactor and filtration requires a high transmembrane pressure and crossflow velocity to produce permeate and avoid fouling. Systems with submerged modules usually consume less energy as no pump is needed. Indeed, the membrane is directly immersed into the sludge in the anaerobic reactor or the separate tank and permeate is obtained by exerting vacuum on the membrane (Smith *et al.* 2012).

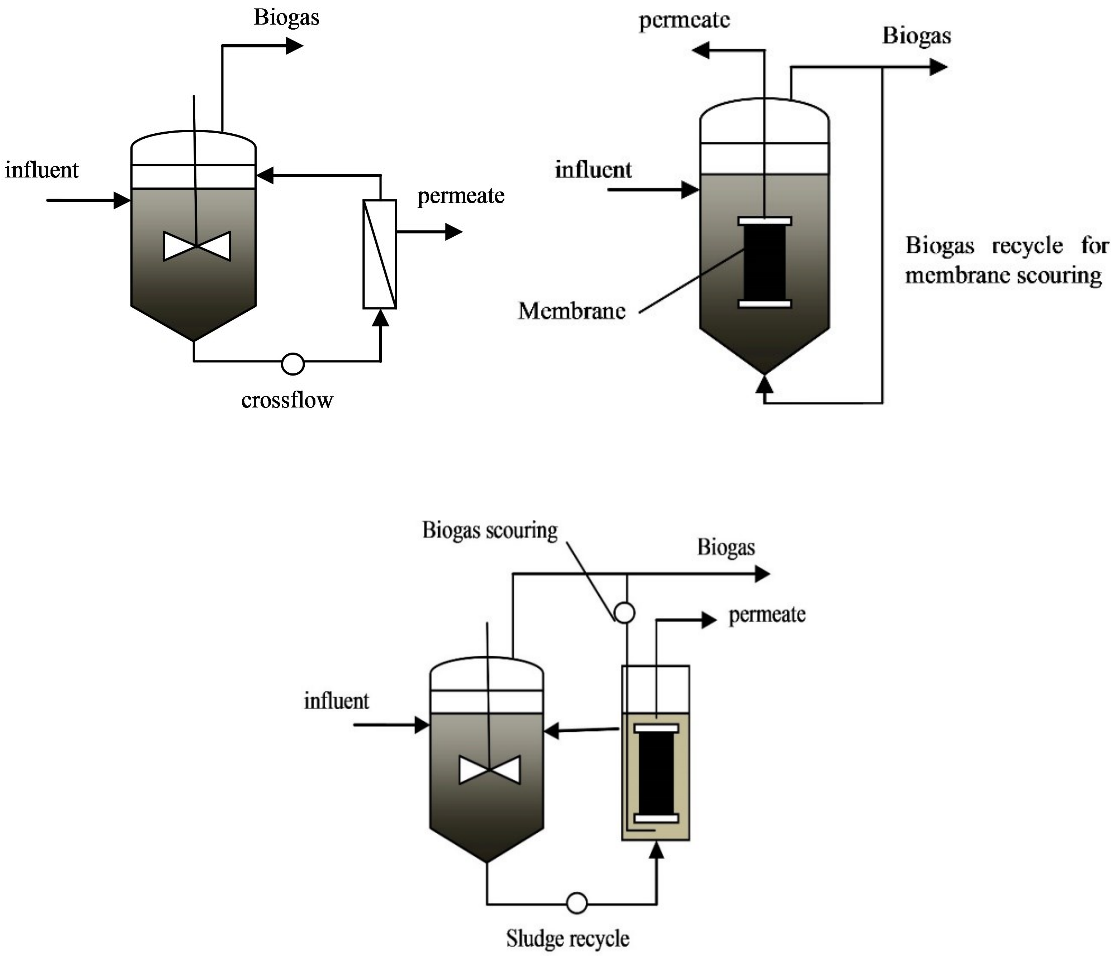

Figure 7: Different configurations of an AnMBR process - at the top left: crossflow configuration; at the top right: internal submerged AnMBR; at the bottom: external submerged AnMBR (Chang 2014)

For the submerged AnMBR processes, internal configurations have the significant advantage of a lower construction and maintenance cost but external submerged AnMBRs facilitate membrane maintenance. The most commonly used anaerobic reactors are continuously stirred reactors (CSTRs). Alternately, upflow anaerobic sludge blanket (UASB) can be considered, as an important proportion of the biomass is retained inside the reactor and could positively influence membrane fouling. Finally, micro- and ultrafiltration membranes are commonly used in AnMBR processes (Smith *et al.* 2012).

3.2.7 Definition of an anaerobic process

Substrates for anaerobic processes are mostly biopolymers as carbohydrates, proteins or fats (Figure 8). While, in aerobic processes, organic matter is converted to carbon dioxide and water, during an anaerobic process, the substrates are decomposed to methane and carbon dioxide. This transformation is divided into four steps, which occur in parallel: the hydrolysis, the acidogenesis, the acetogenesis and the methanogenesis.

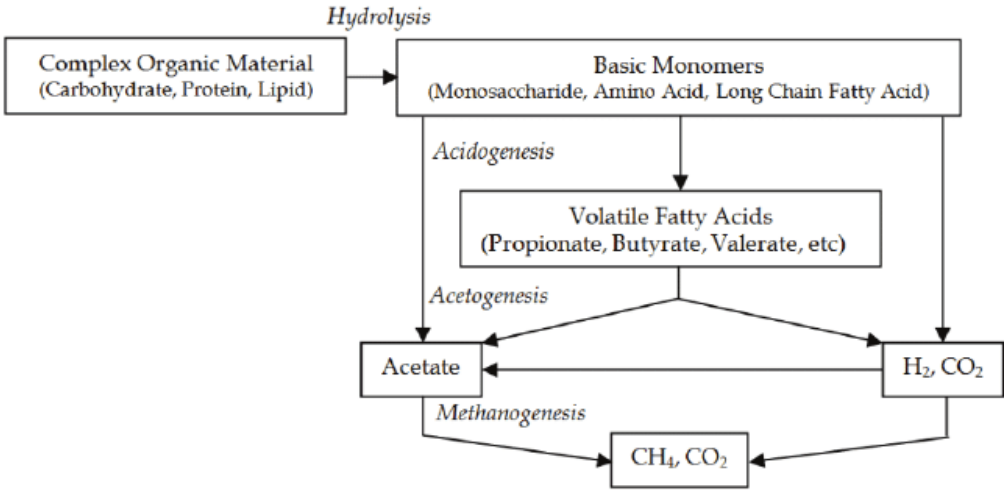

Figure 8: Presentation of the four steps of an anaerobic process (Ersahin et al. 2011)

During the hydrolysis, the biopolymers are transformed by extracellular enzymes of microorganisms into monomers and oligomers (sugar, amino acids and fatty acids). After this step, they are transported into the cytoplasm of the microorganisms (Rosenwinkel *et al.* 2015). During the acidogenesis, hydrogen partial pressure plays an important role. The fermentative bacteria can only further convert the monomers and oligomers into acetate, carbon dioxide and hydrogen if the hydrogen partial pressure is less than 0.1 Pa (Sahm *et al.* 2013). If the hydrogen partial pressure is higher, propionic acid, butyric acid, lactic acid and alcohols are produced more frequently.

During the acetogenesis, organic acids and alcohols that were produced during the acidogenesis are transformed via decarboxylation by acetogenic bacteria to acetate, hydrogen and carbon dioxide. This step also depends on the hydrogen partial pressure and can only take place if this is less than 0.1 Pa (Sahm *et al.* 2013). Methanogenesis is the process by which hydrogen, acetate and carbon dioxide are converted by the methanogenic archaea to methane. There are two possible ways. Hydrogenotrophic methanogens produce methane from hydrogen and carbon dioxide, while the acetoclastic methanogens produce methane from acetate and hydrogen. At high biomass concentrations, methane formation occurs mainly via hydrogen and carbon dioxide and vice-versa (FNR 2014).

3.2.8 Operation parameters of an anaerobic process

The organic loading rate (OLR) is one of the most important parameters for AnMBR processes. It indicates the mass of organic matter daily supplied into the reactor. Increases of this parameter lead to increases in microorganisms activity.

The hydraulic retention time (HRT) gives the theoretical residence time of the substrate in the reactor. The real HRT can deviate from the calculated value because of short circuits or dead zones in the reactor.

The solid retention time (SRT) describes the sludge age in the reactor. In contrast to other reactors, the SRT of an AnMBR is independent of the HRT because the membrane retains the biomass. The SRT ranges from 20 days to an infinite duration.

The food to microorganism ratio (F/M ratio) describes the ratio of the organic matter supplied into the reactor to the mass of microorganisms already present in the reactor. A high F/M ratio leads to an increase of the activity of microorganisms and their multiplication rate (Fernandes *et al.* 2013).

Methane production is also an essential factor, as it directly enables energy recovery. For instance, the specific biomethane production (SBP) indicates the daily methane volume produced related to the reactor volume. The methane yield represents the volume of methane produced related to the amount of organic matter supplied into the reactor.

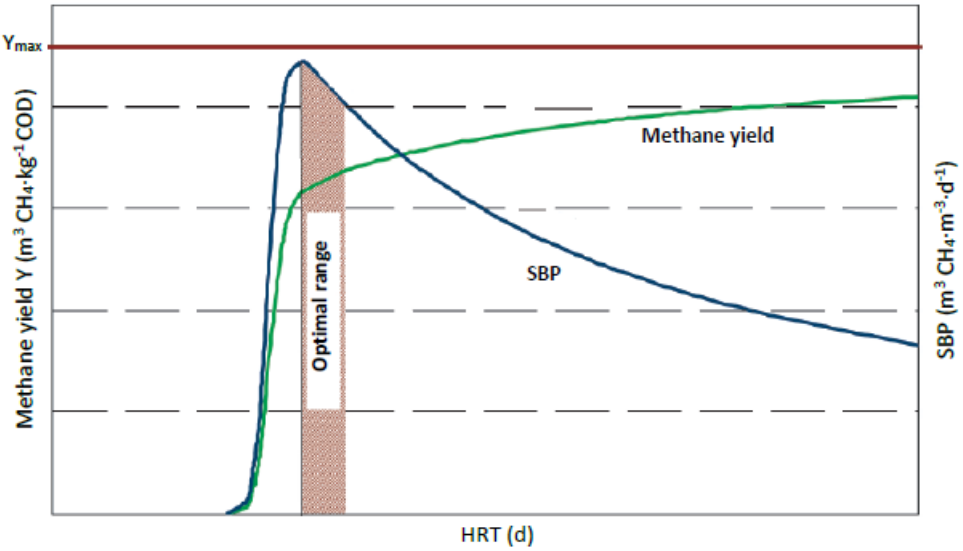

Figure 9: SBP and methane yield according to the average HRT (Linke et al. 2006)

As can be seen in Figure 9, the HRT has theoretically a direct influence on the methane yield and the SBP. When the HRT increases, the methane yield increases because more time is available for organic matter biodegradation by the microorganisms. Simultaneously, biogas production decreases because less biomass is introduced into the reactor. Hence, the optimum range of an AnMBR process needs to be experimentally determined. This is a key parameter, as a short HRT results in a higher OLR and consequently allows smaller reactor volumes.

3.2.9 Influencing factors

Several factors influence the performance of an AnMBR process. These are described in the following section.

3.2.9.1 pH

The pH value of the process depends both on the composition of the wastewater supplied into the process and on the acid-forming (acidogenesis) or acid consuming (acetogenesis, methanogenesis) processes in the reactor itself. According to FNR (2016), the optimal pH amounts to between 5.2 and 6.3 for the hydrolysis and the acidogenesis steps. For the acetogenesis of volatile fatty acids as well as for the methanogenesis, neutral conditions are optimal (6.5 to 8). As the microorganisms responsible for the methanogenesis are extremely sensitive to the pH values, neutral conditions in the reactor are to privilege. If the OLR parameter is too high, it can lead to an acidification of the process. In this case,

too many organic acids are produced in the reactor because the methanogenesis is a slower process compared to the acidogenesis. Consequently, the pH decreases and inhibits the proper operation of the methanogenic microorganisms.

3.2.9.2 Temperature

Temperature conditions for anaerobic processes can be divided into three categories: psychrophilic conditions with an optimum temperature of 25 °C, mesophilic conditions with a temperature range of 37 - 42 °C and thermophilic conditions with a temperature range of 50 - 60 °C. Under psychrophilic conditions, heating of the reactor is not necessary. However, substrate biodegradation and biogas production are low. In addition, a higher amount of methane is lost throughout dissolution in the sludge and in the permeate.

In the mesophilic temperature range, very high methane yields can be achieved, as most methane-forming bacteria have their optimum temperature in this range (FNR 2014). Furthermore, the process is very stable and can cope with temperature fluctuations up to 3 °C. Nevertheless, energy is needed to heat the reactor.

Under thermophilic conditions, the substrate biodegradation is comparatively very high but the energy needed to heat the reactor is much higher. Because of the very high temperature, pathogenic germs can be killed. Nevertheless, the process is much more sensitive than in the psychrophilic and mesophilic ranges and, therefore, an acidification of the process can occur extremely quickly if the OLR is set too high (Rosenwinkel *et al.* 2015).

3.2.9.3 Substrate composition

Like all other living creatures, microorganisms also need suitable compounds to maintain their vital functions and build new cells. In addition to the organic substances such as carbohydrates, fats and proteins, the microorganisms also require inorganic nutrients such as nitrogen, phosphorus, calcium, sodium and potassium. Trace elements such as chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum, iodine, nickel, arsenic and fluorine are also required (Rosenwinkel *et al.* 2015). In case of domestic or municipal wastewater, these nutrients and micronutrients are usually already contained in the substrate. Compared to aerobic processes, considerably less biomass is formed during anaerobic degradation. Consequently, the nutrient requirement is also significantly lower than for aerobic microorganisms. In addition, the nutrient ratio of COD/TN/TP should amount to approximately 800/5/1 (Rosenwinkel *et al.* 2015).

3.2.10 Challenges of AnMBR processes

AnMBR processes show high potential because of the high COD removal, the energy recovery of organic matter, the comparatively low energy-demand, the compactness and easy to implement processes as well as the obtaining of high-quality solids-free effluent. However, important challenges remain to further expand this technology. The two main challenges are the fouling of the membrane that requires regular membrane cleaning and the recovery of biomethane dissolved in the permeate. These two challenges are precisely described in the following section.

3.2.10.1 Membrane fouling

Membrane fouling is defined by the accumulation of organic and inorganic foulants in the membrane pores and on the membrane surface. As membrane fouling leads to an increase of energy demand for filtration, this is one of the main concerns of AnMBR processes. The foulants are among others suspended biomass, colloids, inorganic precipitates, soluble microbial products or extracellular polymeric substances (Smith *et al.* 2012). Fouling can occur throughout clogging of the membrane pores, adsorption of soluble compounds or deposition of solids forming a cake layer on the membrane surface. Membrane fouling is the main cause of the comparatively low expansion of the technology. This phenomenon is even not fully understood because of the variety of foulants, membrane characteristics and feed characteristics (Ozgun *et al.* 2013).

In crossflow operations, the crossflow velocity must avoid membrane fouling, while biogas sparging, backflushing or membrane relaxation are used in submerged AnMBR. Nowadays, the main researches related to this topic are the pretreatment of the feed, the modification of membrane surface properties and feed properties, as well as the optimization of operational parameters such as HRT, SRT, biomass concentration, pH, T, gas sparging intensity and crossflow velocity. This aims to operate the membrane with no flux decrease or transmembrane pressure increase for a duration as long as possible and to avoid regular membrane cleaning.

3.2.10.2 Recovery from methane dissolved in permeate

The release of methane in the water bodies throughout dissolution in the permeate leads to environmental concerns as well as economic losses. While Rongwong *et al.* (2018) outline that dissolved methane in permeate can reach up to 45 % of the total methane production, Smith *et al.* (2012) report values of 30 % at 35 °C and 50 % at 15 °C. The comparison with theoretical values obtained with Henry's law outlines the extremely important phenomenon of oversaturation in the reactor caused by mass transfer limitations. However, this topic has not been importantly investigated and remains challenging. Nowadays, two main technologies exist to recover the methane dissolved in the permeate. The first one is the stripping of the AnMBR effluent using post-treatment aeration. However, the efficiency of this process is still to increase, as well as the explosion danger caused by the contact of methane with air must be better controlled. The advantage of this process is the low energy demand of 0.05 $kWh \cdot m^{-3}$. The second recovery process is the use of a degassing membrane, which is a much more controlled process, as it does not need aeration. However, nowadays, the energy demand is 300 times the energy embedded in methane, so that the cost must be reduced before the expansion of this process. Hence, the topic of methane recovery of the methane dissolved in permeate remains a great challenge for the next decades (Smith *et al.* 2012).

3.3 Microalgae production

3.3.1 Definition and fundamentals of microalgae

Algae are defined as plants that have no roots, leaves or stems and that contain chlorophyll a as an elementary photosynthetic pigment (Lee and Shen 2013). A distinction is made between macroalgae and microalgae. Macroalgae are known to be multicellular visible algae, for example, seaweed. Microalgae, on the other hand, are single-cell algae, whose size mostly ranges between 1 µm and

1 mm. The exact number of species is still unknown but is estimated to be between 200,000 and 1,000,000. These unicellular species are mostly found in freshwater and marine systems and are considered healthy for any natural waterway. Although they can live independently, they are often found in symbiosis with other organisms. They usually use photosynthesis to grow, which means that they synthesize their biomass from CO_2 and H_2O with the help of the sunlight. This process is accompanied by a release of O_2, which makes the microalgae responsible for approximately 50 % of the O_2 production of the planet (Andersen 2005). Hence, they are the main suppliers of oxygen on the earth, and, consequently, an essential part of the ecosystem.

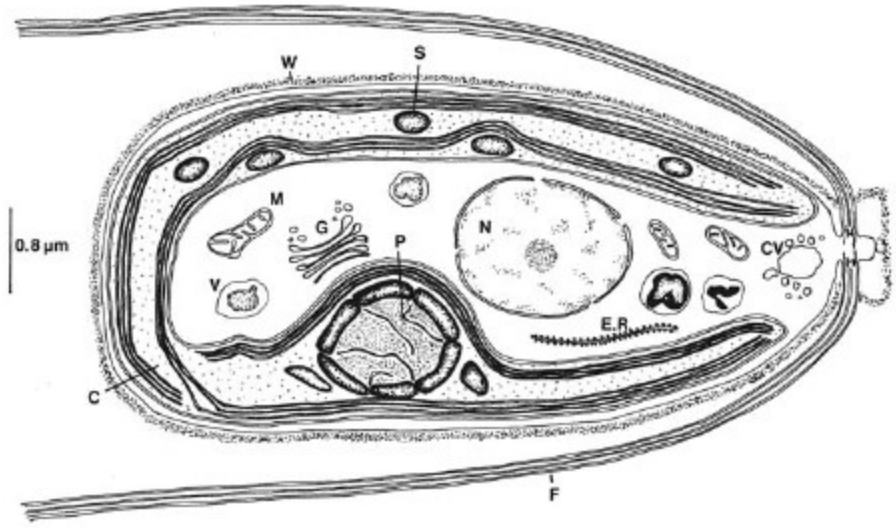

Figure 10: Structure of a microalgae cell - (C) Chloroplast; (CV) contractile vacuole; (E.R.) endoplasmic reticulum; (F) flagella; (G) Golgi apparatus; (M) mitochondrion; (N) nucleus; (P) pyrenoid; (S) starch; (V) vacuole; (W) cell wall (Lee 2008)

Microalgae cells are surrounded by a cell wall composed of polysaccharides and a thin plasma membrane that encloses the cell plasma and controls influx and outflow of substances (Figure 10). The cell plasma contains a nucleus and various organelles. Organelles include the flagella, the chloroplasts, the Golgi apparatus, the mitochondrion, the vacuole, the pyrenoid, the contractile vacuole and the endoplasmic reticulum (Tomaselli 2013). The heart of cell is the nucleus, which contains the genetic information of the cell. In the complex structure of a microalgae cell, each organelle has a specific function. The flagella are enclosed in the plasma membrane and serve as a simple means of cell locomotion through the cultural medium. The photosynthesis takes place in the chloroplasts. The Golgi apparatus is a synthesis site for many proteins and polysaccharides and is responsible for the secretion of the cell wall (Borowitzka *et al.* 2018). Another organelle is the mitochondrion, which produces adenosine triphosphate (ATP) that is essential for photosynthesis. This organelle contains also the respiratory apparatus. The vacuole can perform very different tasks, such as the storage of proteins or ions. The pyrenoid is used for the storage of carbon dioxide and, therefore, is very important for the photosynthesis. The contractile vacuole is a regulatory organelle, which collects fluid from the surrounding medium or rejects fluid to this medium. Finally, the endoplasmic reticulum balances the protein content in the cell.

Two steps characterize microalgae reproduction: the interphase and the mitosis. In the interphase, the size of the mother cell doubles so that two daughter cells can develop. Meanwhile, two identical deoxyribonucleic acid (DNA) profiles are formed and the number of organelles and components of the cell increases. During the mitosis, the mother cell is then halved and two new cells are formed (Tomaselli 2013).

3.3.2 Microalgae production modes

Today, microalgae cultivation mostly occurs under photoautotrophic conditions. This means that inorganic carbon, the main carbon source, is assimilated by the cells throughout photosynthesis. The microalgae can also be cultivated under heterotrophic conditions. Here, the cells use organic carbon for their growth, such as glucose or acetate, which eliminates the light requirement for photosynthesis. As no consideration is made for light availability, the configuration of the reactors is easier, and the reactors do not need to be thin. Since biomass concentration is higher in these systems, the harvesting costs are reduced. Biomass production rates and lipid content are also higher and organic carbon from wastewater could be removed (Brennan and Owende 2010).

Finally, the mixotrophic conditions are a mix between the photoautotrophic and the heterotrophic conditions. Indeed, several microalgae species can use photosynthesis to convert inorganic carbon and simultaneously assimilate organic compounds. As growth is not totally dependent from light availability, biomass losses during night or in case of too low light intensity are avoided. Compared to photoautotrophic conditions, higher growth rates are reported for this cultivation mode. Nevertheless, they are lower compared to the heterotrophic mode (Whitton *et al.* 2015).

No full-scale plant using heterotrophic or mixotrophic conditions has been developed. Indeed, some challenges remain. A limited number of microalgae species can grow under heterotrophic conditions and it is very difficult to quantify the adequate amount of organic substrate to be supplied. Indeed, a too high amount leads to growth inhibition. Additional costs due to the organic substrate are also incurred and the supply of organic matter can lead to contamination or competition with other microorganisms (Khan *et al.* 2016). In the present thesis, the experimental work is entirely conducted under photoautotrophic conditions.

3.3.3 Environmental conditions for microalgae production

Environmental factors, such as nutrient supply, light conditions, temperature and pH are essential for the development of microalgae cells. These factors influence the quantitative and qualitative composition of the cells (Borowitzka *et al.* 2018).

3.3.3.1 Nutrients

For their growth, microalgae mostly use carbon, nitrogen and phosphor (Grobbelaar 2013). The Redfield ratio empirically determined by Redfield in 1934 states an average stoichiometric composition of $C_{106}N_{16}P_1$. The nutritional composition of a culture medium should approximate this ratio (Choi and Lee 2015).

Carbon corresponds to about 50 % of the microalgae biomass. Carbon dioxide in the atmosphere is not sufficient for high production rates (Grobbelaar 2013). Therefore, pure CO_2, flue gas or a mixture of air and CO_2 is introduced into the culture. Throughout the rising of the gas bubbles, mixing of the culture can simultaneously be achieved.

Besides carbon, nitrogen is the most important source of nutrients and represents about 10 % of the biomass. In the cells, it is found in the form of amino acids and proteins. It is also an intermediate product of cell metabolism and plays an important role in the structure of nucleotides, such as ATP,

the energy carrier of cells (Borowitzka *et al.* 2018). Ammonium, nitrate, nitrite or urea can be used, the preferred form for microalgae being ammonium.

Although phosphorus represents only 1 % of the biomass or even less, this compound is indispensable for the synthesis of DNA and is also a component of the ATP (Kim 2015). The lipid and carbohydrate contents of microalgae are also dependent on the supply of phosphorus (Richmond and Hu 2013). The preferred form of phosphorus for the microalgae is orthophosphate.

Hence, phosphorus and nitrogen must be added into the culture medium. For this purpose, commercial synthetic fertilizers or wastewater effluents can be used.

3.3.3.2 Micronutrients

Approximately 30 inorganic elements are useful or even crucial for microalgae growth. These include magnesium, calcium, iron and sulfur. In addition, many trace elements, like boron, manganese, zinc or cobalt play an important role (Grobbelaar 2013). In particular, iron plays an important role for photosynthesis, nitrogen fixation and DNA synthesis (Hu 2013).

3.3.3.3 Temperature

Since most biological processes are enzymatic processes that are temperature-dependent, this environmental factor is essential for microalgae growth. Therefore, temperature must be kept in the range of 16 - 27 °C (Chmiel 2011). According to Singh and Singh (2015), in a temperature range from 15 to 30 °C, high production rates can be observed for most species. Outside this range, the culture can be lost.

3.3.3.4 pH

The pH is a primary environmental factor, as it strongly influences the biochemical reactions in the microalgae cells. Extreme pH values lead to a cessation of cellular processes and culture collapse (Yen *et al.* 2019). A value comprised between 6.5 and 7.5 is generally aimed at. The pH naturally tends to increase during cultivation. Indeed, protons from the dissociation of water are used to transport nitrate and phosphate ions through the membrane, leading to the production of hydroxyl radicals in the culture medium (Whitton *et al.* 2015). However, pH increases lead to indirect nitrogen and phosphorus removal and should be avoided. Hence, at a pH value higher than 7, ammonium is converted into ammonia gas, which is consequently volatized and stripped out of the solution. At high pH values, phosphorus precipitates with metal ions and is not available anymore for the microalgae. These pH increases are usually counterbalanced by the supply of CO_2 into the culture medium.

3.3.3.5 Light exposure

Light exposure should be constant, since, otherwise, the cells must constantly adapt to the new environmental conditions. However, this is impossible in full-scale plants irradiated by the sunlight. Figure 11 represents the dependence of microalgae growth on light exposure. In absence of light, the photoautotrophic microalgae can not grow. In the limitation phase, the specific growth rate increases almost linearly with the light intensity, until it reaches a maximum in the light saturation phase. This means that, for optimal growth, it is necessary to maintain the light intensity in the light saturation

range. When the light intensity is too high, growth rates decline and, in extreme cases, growth can even stop (Yen *et al.* 2019).

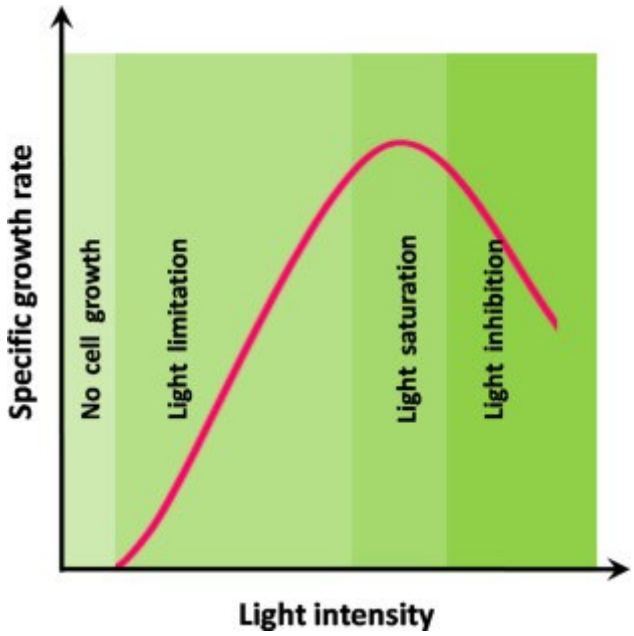

Figure 11: Specific growth rate according to light intensity during microalgae cultivation under photoautotrophic conditions (Yen et al. 2019)

In the present study, in lab-scale experiments, light intensity was measured as the photosynthetic photon flux density (PPFD), which is defined by the number of photons in the 400 - 700 nm waveband incident per unit time on a unit surface. For full-scale experiments, the irradiance, which is the amount of radiation per unit time and per unit area on a receiving surface from all directions, was used. The data was delivered by sensors or meteorological institutions.

3.3.4 Nutrient remediation mechanisms in a microalgae cell

The three main nutrients carbon, nitrogen and phosphorus are assimilated into biomass by the microalgae cells following different interconnected biochemical pathways (Figure 12). In case of a high pH value in the culture medium, nitrogen and phosphorus are also indirectly removed throughout formation of ammonia gas and precipitation respectively. These different pathways are summarized in this section.

The light is absorbed by the pigment chlorophyll a in the thylakoid, where the light-dependent cycle of the photosynthesis takes place. While water is reduced to oxygen, ATP and NADH are produced. During the light-independent Calvin cycle, carbon dioxide is reduced and, using the previously formed ATP and NADH, carbohydrates like glucose are produced. During this cycle, NADH is oxidized in $NADP^+$ and ATP is hydrolyzed in adenosine diphosphate (ADP) and inorganic phosphate (Pi).

During the light-dependent cycle, $NADP^+$ is reduced in NADH and the ADP is regenerated in ATP. The inorganic forms of nitrogen (nitrates, nitrites and ammonium) are transported across the cell membrane in the preferred form of ammonium. Nitrates and nitrites are reduced to ammonium with the help of the nitrate reductases and protons transported across the cell membrane. This reduced form of nitrogen is then assimilated into amino acids for the formation of proteins. Nitrogen can also be indirectly removed in case of the alkalization of the culture medium. In this case, ammonia gas and water are produced and the gas is stripped from the solution.

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Phosphate is transported across the cell membrane in the preferred form of HPO_4^{2-} and assimilated into nucleotides and nucleic acids. It is to be noticed that nitrogen compounds are necessary for the assimilation of phosphorus, as nucleotides are also composed of a nitrogenous base. Excess phosphate can be stored in the form of polyphosphate granules in the vacuole of the cell. Organic phosphorus can also be transported across the cell membrane and transformed into phosphate by the phosphatases. When the culture medium becomes alkaline, phosphorus can be indirectly removed throughout precipitation with metal ions (Whitton *et al.* 2015).

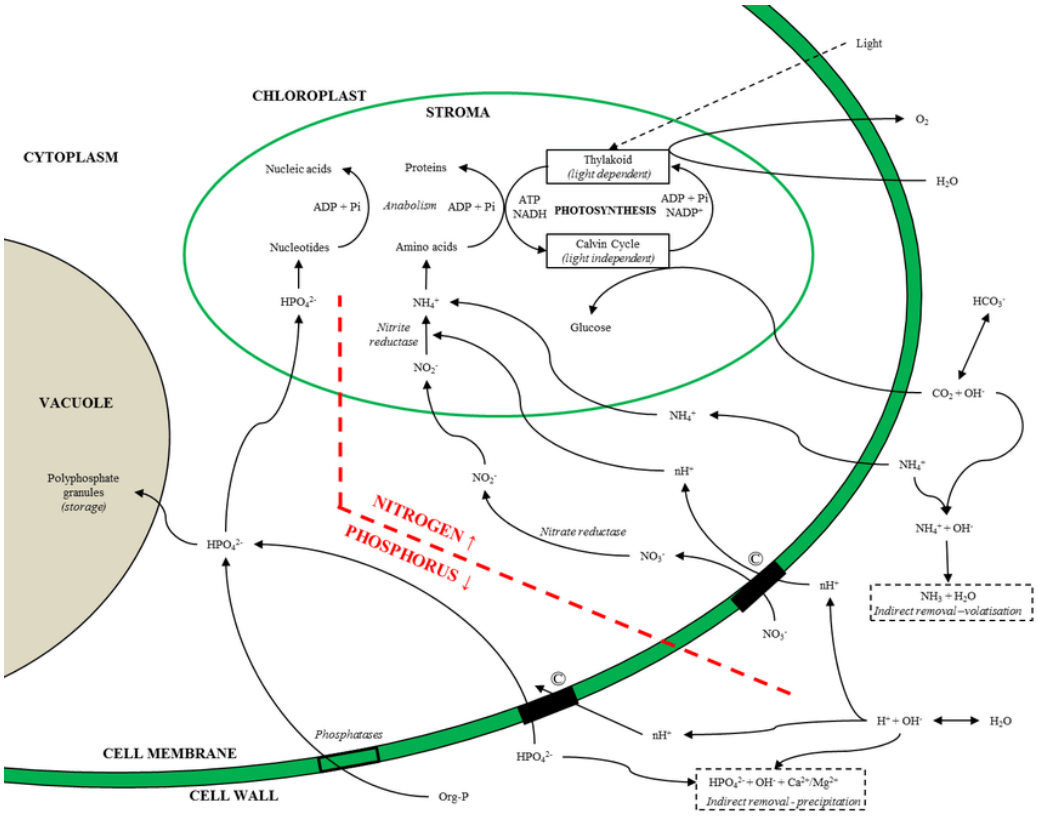

Figure 12: Biochemical pathways for nutrient assimilation and indirect nitrogen and phosphorus removal during microalgae cultivation (Whitton et al. 2015)

3.3.5 Microalgae growth in batch and continuous processes

The simplest and cheapest cultivation method for microalgae is the batch cultivation. At the beginning of the batch, nutrients are added into the culture. This culture grows under fixed parameters until the harvesting takes place (Lee and Shen 2013). Growth under batch conditions is divided into four phases: the lag phase, the exponential phase, the stationary phase, and the death phase (Figure 13).

During the lag phase, the microalgae cells first need to adapt to their new environmental conditions (culture medium, nutrients, temperature, light), which causes a delay for the starting of their growth. Once the cells have adapted to these new conditions, the growth happens exponentially. This phase

usually ends due to a limitation of nutrients availability. Thereafter, a decrease of the production rates is observed.

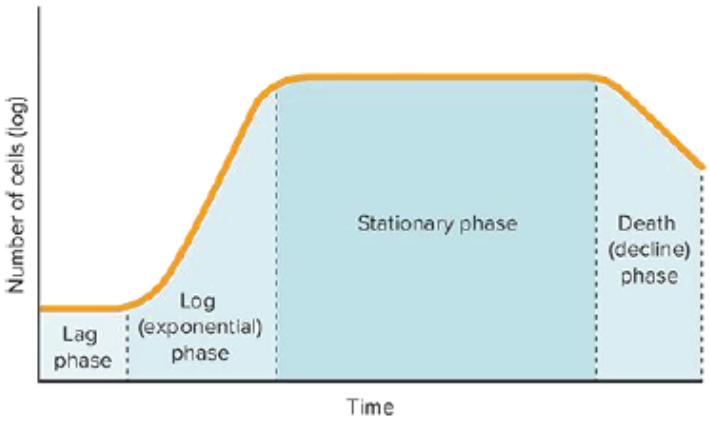

Figure 13: The four phases of microalgae growth in batch processes (Molecular Devices 2019)

The stationary phase is characterized by a constant biomass concentration. Newly formed and dying cells bend in equilibrium. The last phase, called the death phase, shows a decrease of the biomass concentration, meaning that more cells die than new ones are formed. Such behavior occurs when the environmental conditions do not meet the requirements of the microalgae, such as too high or too low temperatures, contamination by bacteria, inadequate light, or when the nutrients contained in the culture medium have been consumed (Kim 2015).

While batch cultivation is easy to implement and cheap, continuous cultivation offers better perspectives related to the production rates but requires more effort from a technical point of view. With this method, fresh medium is added continuously or at certain intervals. At the same time as culture medium addition, microalgae are harvested and a certain amount remains in the culture (Lee and Shen 2013). This method ensures that the cells are always in the exponential phase and leads to enhanced biomass production rates.

In order to quantify microalgae growth, the two main parameters used are the biomass production rate (BPR) and the specific growth rate μ. While the BPR describes the daily volumetric microalgae biomass production, μ is calculated during the exponential phase. It defines the fraction of increase in biomass over a unit time and is inversely proportional to the doubling time of the cells**.** Contrary to the parameter BPR, the calculation of μ avoids the influence of biomass concentration in the culture medium and can be useful to compare the performance of two different systems (Richmond and Hu 2013). For outdoor full-scale applications, the photosynthesis efficiency (PE), that describes the fraction of light energy converted into microalgal biomass, is also an essential parameter.

3.3.6 Application of microalgae

The different applications of microalgal biomass are represented in Figure 14 and detailed in the following section.

3.3.6.1 Biodiesel production

Biodiesels are derivatives of oil crops and biomass that are used in conventional diesel engines. After lipids extraction, a reaction between triglycerides and alcohol, called transesterification, converts the

lipids into monoesters. While biodiesel from oil crops (sugar cane, sugar beet, sugar maize) and lignocellulosic agriculture residues represents the 1st and 2nd generation of biodiesel, microalgae biodiesel is the 3rd generation. This biodiesel has similar chemical and physical properties to crude oil diesel and cumulates numerous advantages (Brennan and Owende 2010):

- As it comes from biomass, it is a renewable diesel.
- Fewer levels of particulates, carbon monoxides, hydrocarbons and SO_x are emitted.
- Up to 78 % less of CO_2 is emitted compared to petroleum diesel.
- Compared to the 1st and 2nd generation biodiesel, there is no food competition or competition for the use of arable land and higher CO_2 fixation rates are achieved.
- Microalgae cells can double in periods of 3.5 h. Microalgae can be produced all year round.

However, numerous drawbacks avoid the expansion of the use of biodiesel from microalgae. Microalgae cultivation needs high light availability and high photosynthetic efficiencies to reach high production rates. Furthermore, cultivation parameters must be optimized to enhance the lipids content in the cells, which represents a severe challenge. Although the technology is technically viable, nowadays, the biodiesel from microalgae are not competitive because of the high energy costs for cultivation and harvesting. Indeed, some authors report that the energy required for biodiesel production is six times higher than the energy recovered (Murphy *et al.* 2015). Nevertheless, the announced depletion of crude oil could lead to an increase of the economic suitability of microalgae production for biodiesel purposes.

3.3.6.2 Anaerobic digestion

Anaerobic digestion of microalgae is a promising alternative to biodiesel production. If the lipids content of microalgae is lower than 40 % (Pragya *et al.* 2013), anaerobic digestion from microalgae has a superior energy balance and offers a higher energy recovery. Compared to the cultivation of microalgae for biodiesel purposes, less effort is needed since the production of cells with high-lipids content is not the main aim. Moreover, the energy needed for harvesting is lower than for biodiesel production, as the microalgae do not need to be totally dried. Indeed, sedimentation and concentration of the biomass in a settler are sufficient for anaerobic process purposes (Sialve *et al.* 2009).

Although anaerobic digestion of microalgae cumulates numerous advantages, previous researches on the topic are very scarce and several challenges remain. First, biodegradation of the organic matter can be difficult because of the resistant cell wall as well as the biochemical composition of microalgae, which often presents an unfavorable carbon/nitrogen ratio. The cellular protein content leads to ammonia release, which is extremely toxic for the anaerobic microorganisms present in the digester. Finally, the high sodium content of microalgae can also be inhibitory for an anaerobic process. Nevertheless, the use of pretreatments and the co-digestion with another substrate can counterbalance these problems encountered. Pretreatments can disintegrate the most refractory organic fraction of the cell, making it more accessible to the anaerobic microorganisms. Co-digestion leading to more favorable carbon/nitrogen ratios also shows efficient results, as for example the co-digestion of microalgal biomass with paper waste (Yen and Brune 2007).

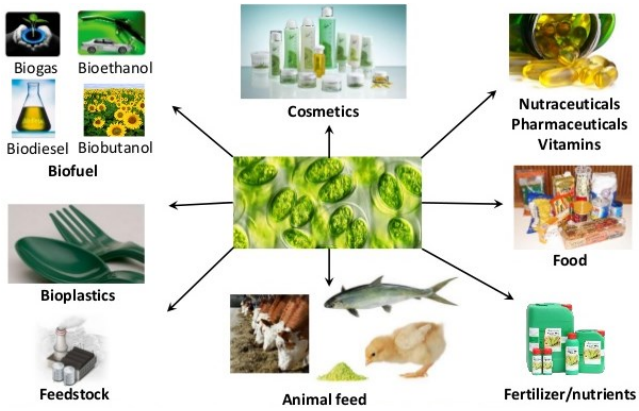

Figure 14: The different applications of microalgal biomass (Funk 2019)

3.3.6.3 Combustion

Combustion of microalgae is also a possible application. For instance, this is defined by the burning in presence of air and at a temperature of more than 800 °C of the biomass, leading to a conversion of the chemical energy stored in the cells into hot gases. First researches indicate that higher costs would be generated compared to coal fuel. Indeed, since the moisture content must be less than 50 %, the costs for harvesting and drying are comparatively high. The literature about this topic is still very scarce. However, the co-combustion of coal and microalgae could lead to lower greenhouse gas and pollutant emissions (Brennan and Owende 2010).

3.3.6.4 Biofertilizers

Literature about fertilizers made from microalgae is likewise scarce. According to Coppens *et al.* (2016) and Pulz and Gross (2004), the use of fertilizers from microalgae cumulates following advantages:

- These fertilizers have the potential to prevent nutrient losses throughout the gradual nutrient release.
- They promote water storage in soils and contribute to surface solidification against erosion in arid regions.
- They contain trace elements and compounds that promote plant growth, such as phytohormones, vitamins, carotenoids, amino-acids and antifungal substances.

For instance, compared to a commercial synthetic fertilizer, a similar growth of tomatoes was reported with a fertilizer made from microalgae. The fruit quality was even increased throughout higher sugar and carotenoid content. However, fertilizer production from microalgae is environmentally viable only if wastewater effluents are used as culture medium. For health reasons, the accumulation of toxins in the biomass still needs to be precisely assessed (Usher *et al.* 2014).

3.3.6.5 Commercial applications

A wide range of biochemical applications of microalgae for food, medicals or animal feed can be found. The bigger market for microalgae production is the human food and food supplements. Although microalgae have been considered to solve the world food shortages, they are eaten as it only in few Asian and African countries. On the contrary, health supplements containing microalgae are much more widespread because of their high protein, vitamin and iron contents (Milledge 2011).

The use of microalgae for animal feed represents 30 % of the global microalgae production, with the main application for aquaculture. Pharmaceutical industries use the pigments contained in the microalgae, as well as polyunsaturated fatty acids, whose the human body lacks an enzyme to synthesize them. Finally, carotenoids, which are substances converted in vitamin A by the body, are used for food colorants or pharmaceutics. Cosmetics contain also microalgae substances like pigments (Milledge 2011).

3.3.7 Microalgae cultivation at full-scale

3.3.7.1 Cultivation processes

At full-scale, microalgae cultivation can be conducted in several different systems. Indeed, the most efficient way from an economic and environmental point of view has not been stated yet.

The cultivation systems can be divided into closed and open systems and into suspended and non-suspended biomass systems (Whitton *et al.* 2015) (Figure 15).

Figure 15: Schema of the different cultivation systems for microalgae production (Whitton et al. *2015)*

The open systems can be open vessels, natural water, inclined surface devices or raceway ponds. The most popular system is the high rate algal pond (HRAP). In this system, the raceway open-pond is mixed using a paddle wheel, which enables circulation of the culture and prevents from biomass settlement. They are usually 20 - 60 cm deep, have a biomass concentration of up to 1 $g \cdot L^{-1}$ and a HRT comprised between 4 and 10 days (Whitton *et al.* 2015). The related daily biomass production reported to the irradiated area BPR_{α} usually ranges 10 - 25 $g \cdot m^{-2} \cdot d^{-1}$ (Murphy *et al.* 2015). Open systems cumulate the advantages of simple maintenance and being cheap. However, no pH, temperature or mixing monitoring is possible. As a direct consequence, the temperature of the culture medium can be very low in the cold seasons and strongly negatively affect the production rates. Furthermore, high losses of sparged CO_2 and water are reported, as well as the regular contamination of the culture medium, leading to low BPRs or even the loss of the culture.

In closed systems, the disadvantages found in open systems are not present, as these systems permit more control of pH, temperature, mixing and represent a barrier for potential pathogens. Moreover, it permits the cultivation of monocultures, which is not the case in open systems. Increased BPR_{α} ranging 35 - 40 $g \cdot m^{-2} \cdot d^{-1}$ can be reached and the biomass concentration can be up to 2 $g \cdot L^{-1}$ (Murphy *et al.* 2015). However, the infrastructures are much more complex and consequently expensive. The closed systems are mainly to find under the form of photobioreactors (PBRs), which enclose the culture in a series of narrows (tubular PBRs) or panels (flat panel PBRs). Throughout pumping and degassing, the culture medium can circulate in the PBRs. Because of the high construction and operational costs, this system is typically used for the production of high value products. Advantages and disadvantages of open and closed systems are resumed in Table 4.

Table 4: Advantages and disadvantages of open and closed systems for microalgae production (Murphy et al. 2015)

Cultivation system	Advantages	Disadvantages
Open	• cheap • simple maintenance • simple scale-up	• high risk of contamination • water losses throughout evaporation • light limitation by high depth • lower BPR and biomass concentration • higher HRT • no pH and temperature control
Closed	• good control of the cultivation parameters • reduced risk of contamination • cultivation of monocultures possible • increased BPR and biomass concentration • lower HRT	• infrastructures more complex • expensive • difficult scale-up

While the HRAPs and the PBRs are systems with suspended biomass, the use of systems with non-suspended biomass is not yet widespread. The most common is the matrix-immobilization, where the microalgae are encapsulated in a hydrophilic polymer. This leads to better BPR_{α} (80 - 100 $g \cdot m^{-2} \cdot d^{-1}$) and biomass concentrations of up to 3.3 $g \cdot L^{-1}$ and offers protection against contamination. However, the access to CO_2 and nutrients for the microalgae cells stays problematic, making this system not yet suitable for industrial applications (Whitton *et al.* 2015). Figure 16 shows examples of full-scale plants.

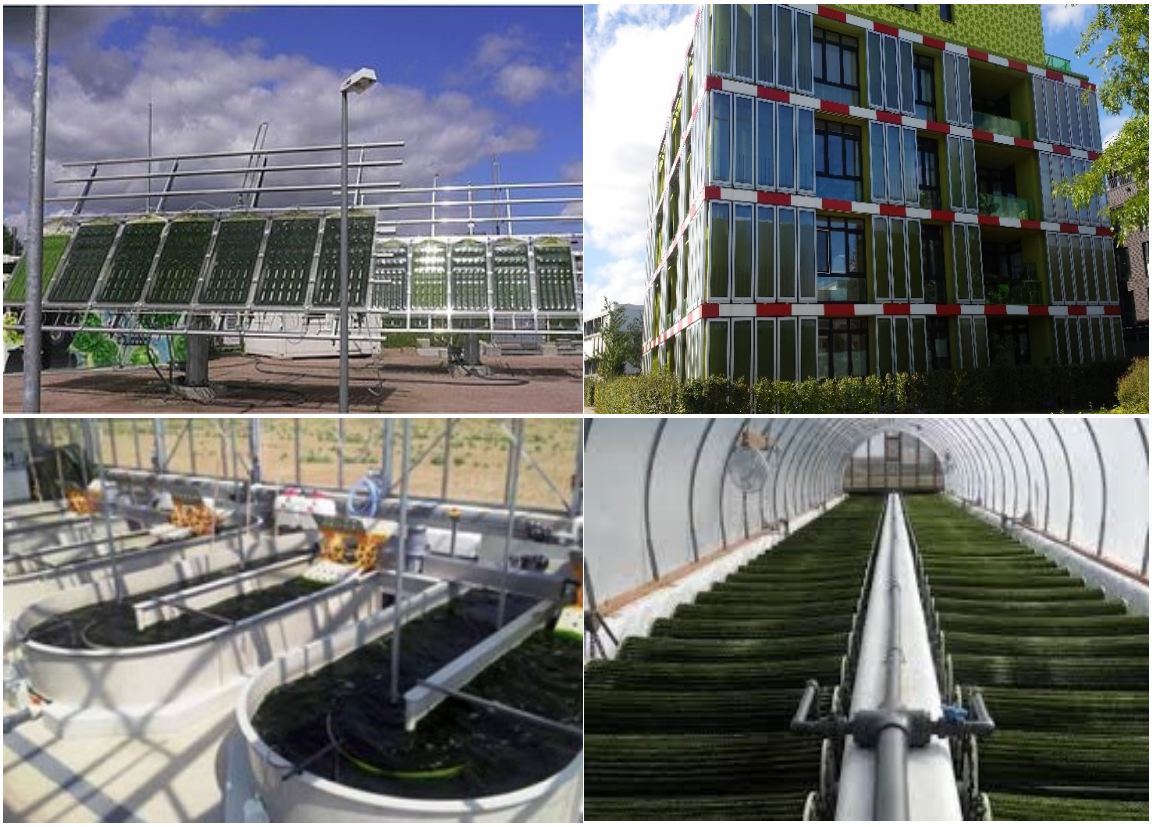

Figure 16: Examples of cultivation systems at full-scale - at the top left: tubular photobioreactors; at the top right: flat-panel photobioreactors; at the bottom left: high rate algal ponds (© Elad Zohar, Erber Future Business GmbH); at the bottom right: matrix immobilization (©Jiri Kopecky, institute of Microbiology, Trebon)

3.3.7.2 Harvesting

Once the microalgae have been cultivated, the biomass must be separated from the liquid phase in the so-called harvesting step. For suspended biomass, because of the low biomass concentration as well as the low size and density of microalgae, this represents a great challenge. Hence, a high amount of energy is required, which usually represents between 20 % and 30 % of the total production costs (Whitton *et al.* 2015). As for microalgae cultivation, the most efficient harvesting method still needs to be found. Typically, centrifugation is used. This method is the most reliable and efficient but stays very expensive. A filtration process, which has the same advantages and disadvantages, can also be considered. Sedimentation is a cheap process but does not achieve reliable results. Dissolved air flotation is defined by the raising of microalgal biomass to the surface of a basin throughout attachment of air bubbles to the biomass, which enhances its flotation characteristics. Once the microalgae have accumulated on the surface, they can be easily removed. This solution is reliable but expensive. Finally, flocculation after addition of a metal coagulant positively charged can be used. However, the use of coagulants can be problematic for microalgae reuse. The use of non-suspended biomass systems automatically leads to a decrease of the harvesting costs. Nonetheless, the challenges related to these systems for cultivation must still be taken up (Whitton *et al.* 2015).

3.3.8 Advantages of the use of microalgae for wastewater treatment

Microalgae production also represents a very appealing technology for wastewater treatment. The numerous advantages related to this use are presented in the following section:

- Using microalgae, nitrogen and phosphorus can be entirely and simultaneously removed without the addition of any chemical product. Usually, in municipal WWTPs, nitrogen is transformed to nitrogen gas throughout the nitrification and denitrification process. Phosphorus is precipitated separately by the means of metal salts and eliminated after decantation (Gregorio *et al.* 2010; Li and Brett 2012; Beuckels *et al.* 2015). The increase of chemicals use due to this phosphorus elimination method in WWTPs has led to an increase of the infrastructure and transport needs and causes health and safety problems. In particular, small communities are affected by the rise of chemicals use and its consequences (Whitton *et al.* 2015). In addition, these precipitation reactions do not allow phosphorus recycling and a large amount of sludge is produced, requiring post-treatments. Hence, compared to municipal WWTPs, nitrogen and phosphorus removal throughout microalgae cultivation is very attractive.
- Most microalgae grow under photoautotrophic conditions, and, therefore, do not need an external organic carbon source. During photosynthesis, they instead use carbon dioxide, one of the main gases responsible for the global warming, as carbon source (Oswald and Golueke 1960). Thus, using flue gas, wastewater treatment by the means of microalgae reduces the carbon footprint of the wastewater process (Arbib *et al.* 2013) and the greenhouse gas emissions (Gouveia *et al.* 2016). The use of microalgae for nutrient degradation presents a total balance of -0.2 kg $CO_2 \cdot m^{-3}$ treated wastewater instead of +0.6 kg $CO_2 \cdot m^{-3}$ treated wastewater with a conventional wastewater treatment plant using an activated sludge process and nitrification/denitrification as nitrogen removal process. At the same time, throughout the photosynthesis process, 17 kg $O_2 \cdot kg^{-1}$ TN_{rem} are produced, which leads to an improvement of the water quality.

- From the perspective of the microalgae industry, coupling wastewater treatment and microalgae cultivation enables to save water and fertilizer based on limited resources and to decrease the production costs. Because of the phosphorus depletion, this nutrient will probably become more and more expensive in the coming decades. The generalized replacement of commercial fertilizer for microalgae production will also reduce the green gas emissions of microalgae culture, given that the production of 1 kg of fertilizer generates the emission of 3 kg of carbon dioxide (Fertilizers Europe 2011). Moreover, the costs related to fresh water and fertilizers represent up to 50 % of the production costs in full-scale plants (Singh and Das 2014).
- Trace organic micropollutants can be removed using microalgae for wastewater treatment (Whitton *et al.* 2015).

Hence, wastewater treatment using microalgae cultivation has numerous advantages and currently appears to be the only environmentally friendly technology that can completely remove ammonium and phosphorus in a single reactor without the addition of environmentally harmful chemical products. Simultaneously, this leads to the production of biomass that can be used onsite for energy purposes (anaerobic degradation of microalgae) or be sold with an economic benefit (pigments, fatty acids).

3.3.9 Species studied in the present thesis

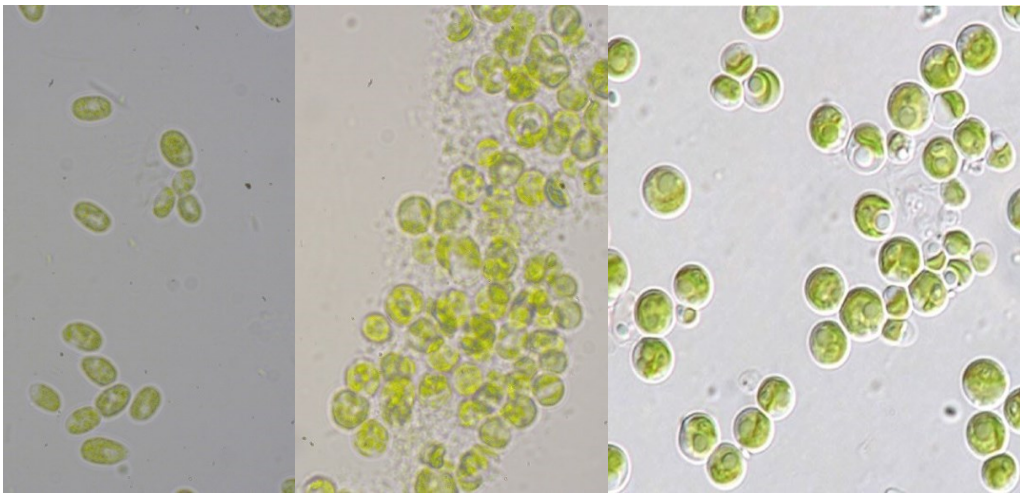

Figure 17: Species studied in the present thesis - from left to right: Acutodesmus obliquus, Chlorella vulgaris and Chlorella sorokiniana (Salbitani and Carfagna 2017)

In the present work, three different microalgae species are studied: *Acutodesmus obliquus*, *Chlorella vulgaris* and *Chlorella sorokiniana* (Figure 17). *Acutodesmus obliquus*, formerly called *Scenedesmus obliquus* is a unicellular eukaryotic plant of the *Chlorophyta* strain. The elliptic single cells of *Acutodesmus obliquus* tend to organize into associations of a few cells. The cell size of *Acutodesmus obliquus* varies between 2 and 10 µm (Tomaselli 2013). The species of the genus *Chlorella* likewise belong to the *Chlorophyta* strain (Richmond and Hu 2013). They possess ovoid cells and a thin cell wall. Their size ranges between 2 and 10 µm (Lizzul *et al.* 2018). *Chlorella* is the most important species for microalgae production and is essentially sold as health food (Richmond and Hu 2013).

4 Materials and Methods

4.1 AnMBR operation

The following paragraphs describe the AnMBR operation in detail. The flow diagram of the system is shown in Figure 19.

4.1.1 AnMBR reactor

The AnMBR reactor (Figure 18) consists of a thermally insulated stainless steel reactor composed of two separate chambers. The upper chamber has a total filling volume of about 850 liters and the lower one of about 50 liters. While the upper larger chamber contains the sludge, the lower chamber is used to heat or cool the upper chamber with a target temperature of 37 °C in the reactor. Therefore, a thermostat (VarioCool VC1200, Lauda, Germany, Figure 18) was used throughout the whole plant operating time except between December 2017 and May 2018. Because of an electrical failure of the thermostat, hot water from the thermostat was replaced during this period by hot water from a heat exchanger of the heat cycle of BIQ-The Algae House.

A pH- and temperature sensor (EGA143-VP, Meinsberg, Germany) is used to record and control the pH value and the temperature of the sludge in the reactor. Likewise, two pressure sensors (BD Sensors, Germany) are installed in the fluid phase and in the gas phase respectively. They allow monitoring the sludge level and the overpressure in the reactor. When the overpressure in the gas area has reached a value of 25 mbar, the pressure relief valve opens and the biogas phase flows off via a gasmeter (TG0.5, Ritter, Germany), whereby the biogas production can be quantified.

Figure 18: AnMBR reactor and thermostat

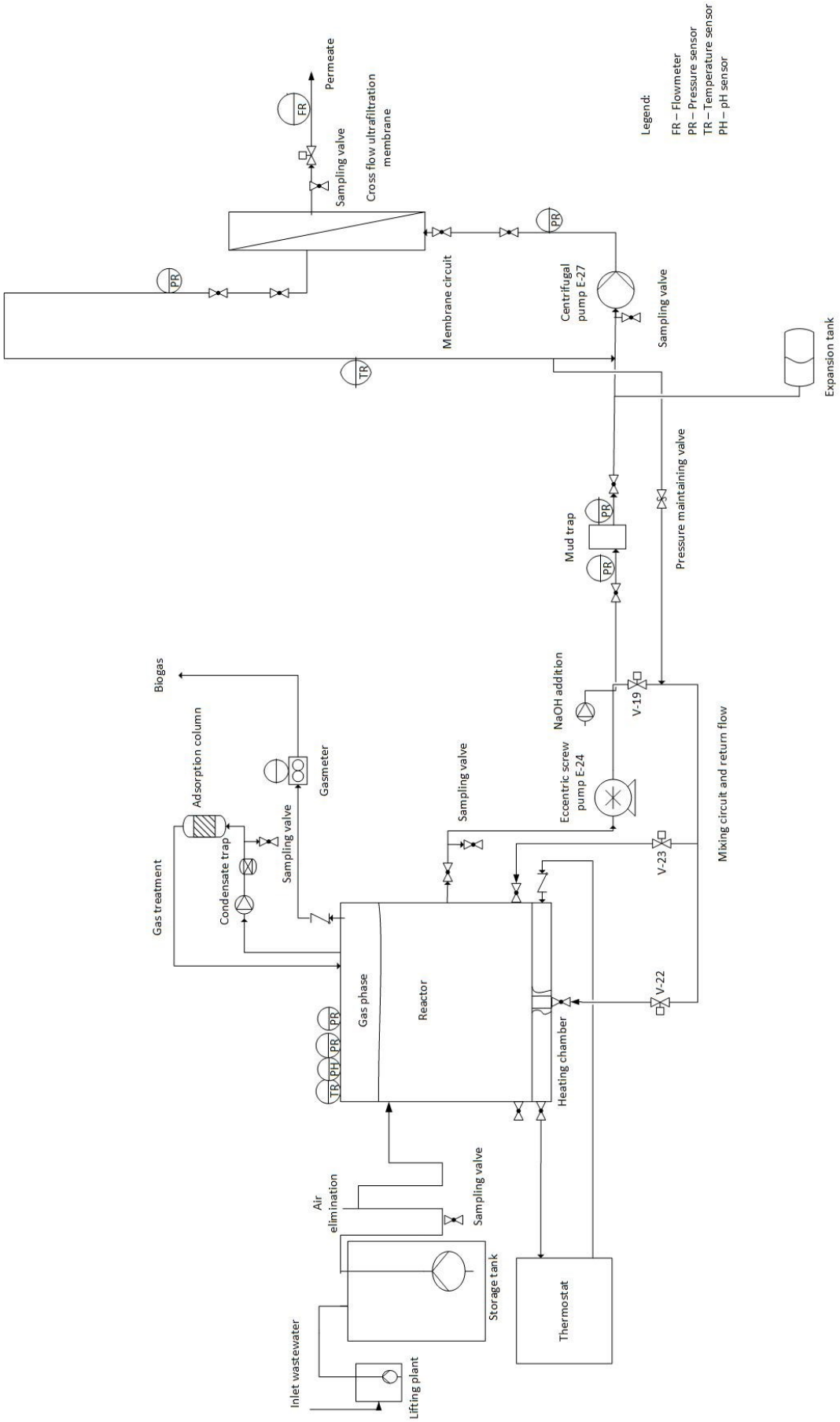

Figure 19: AnMBR flow diagram

4.1.2 Wastewater supply

Between April 2017 and May 2018, domestic wastewater was taken directly from the sewage pipeline of the residential building. Therefore, a Y-branch (45 °) was installed in the pipeline. Since the pipe runs vertically in this section, a semicircular sheet was placed into the branch and extended to the main flow (Figure 20). The collected sewage flew to a 9 L lifting plant (Sololift2WC3, Grundfos, Denmark), which shredded larger solid particles. Due to regular clogging of the sheet and the lifting plant due to toilet paper accumulation, the sheet was temporally shortened in December 2017 and a sieve with wire was erected on the semicircular surface. Since May 2018, the whole domestic wastewater produced in the residential building is directed by the means of a new sewage pipeline to the lifting plant. When the lifting plant is full, the wastewater flows through a Y-branch to the sewer (Figure 21).

The lifting plant is connected to a plastic storage tank (230 L filling capacity, Figure 21). To prevent any overrun, an intermediate level regulator (until May 2018) or a float switch (from May 2018) prevents any overrun of the storage tank. A sewage handling pump placed inside the storage tank automatically turns on at low fluid level or pressure of the gas phase in the reactor, whereby fresh domestic wastewater flows into the reactor. A siphon upstream of the reactor prevents any air supply into the reactor.

Figure 20: Wastewater supply between April and May 2018 - left: lifting pump, sewage pipeline, Y-branch and semicircular sheet - right: shortened semicircular sheet with sieve

Figure 21: Left: 230-L plastic storage tank - right: new sewage pipeline from May 2018

4.1.3 AnMBR operation in the automatic mode

In the automatic mode, the AnMBR operation is divided into three phases, which automatically run repetitive one after the other. A complete cycle includes the following steps: mixing of the reactor, filtration and sludge circulation between the membrane circuit and the reactor.

Reactor mixing aims the homogenization of the biomass in the reactor and is performed by circulating the sludge from the middle area into the lower area of the reactor. For this purpose, valves V-19 and V-23 (for sludge return on the side) or V-22 (for a sludge return through the bottom of the reactor in the middle area) open and the eccentric screw pump E-24 (F9, Stübbe, Germany) turns on. At the beginning of each cycle, depending on the daily permeate requirement, the permeate volume obtained so far and the filtration duration of the previous cycle, reactor mixing duration is automatically calculated.

During the filtration phase, valves V-19 and V-23 are closed and valve V-22 is opened. The eccentric screw pump E-24 feeds the sludge into the membrane circuit, past a 1 mm mud trap (Kwerk, Germany), which protects the membrane from blockages, and builds up sufficient pressure in the membrane circuit. This operating pressure in the membrane circuit is regulated by a pressure-maintaining valve, which opens and let the sludge flow into the reactor by pressure higher than the operating pressure. Subsequently, the centrifugal pump E-27 (E27, Ebara, Japan), which operates sludge circulation in the membrane circuit, is turned on, and the valve V-13, through which the permeate runs, is opened. When the permeate is not sampled, it flows into a storage tank. In normal automatic operation, permeate removal is set to 2 L or 6 L per cycle. In this project, a 0.31 m^2 cross-flow ultrafiltration membrane module (Bioflow, PVDF, Berghof, Germany) composed of 18 tubes with an inside diameter of 8 mm

was used. With an average pore size of 30 nm, the membrane permits to retain the bacteria and viruses but is permeable to salts and colloids.

During the sludge circulation between the membrane circuit and the reactor, the valve V-13 is closed and the pumps E-24 and E-27 continue to run. Thereby, the sludge in the membrane circuit flows back into the reactor, which avoids biomass accumulation in the membrane circuit. Figure 22 shows the main components of the AnMBR pilot plant.

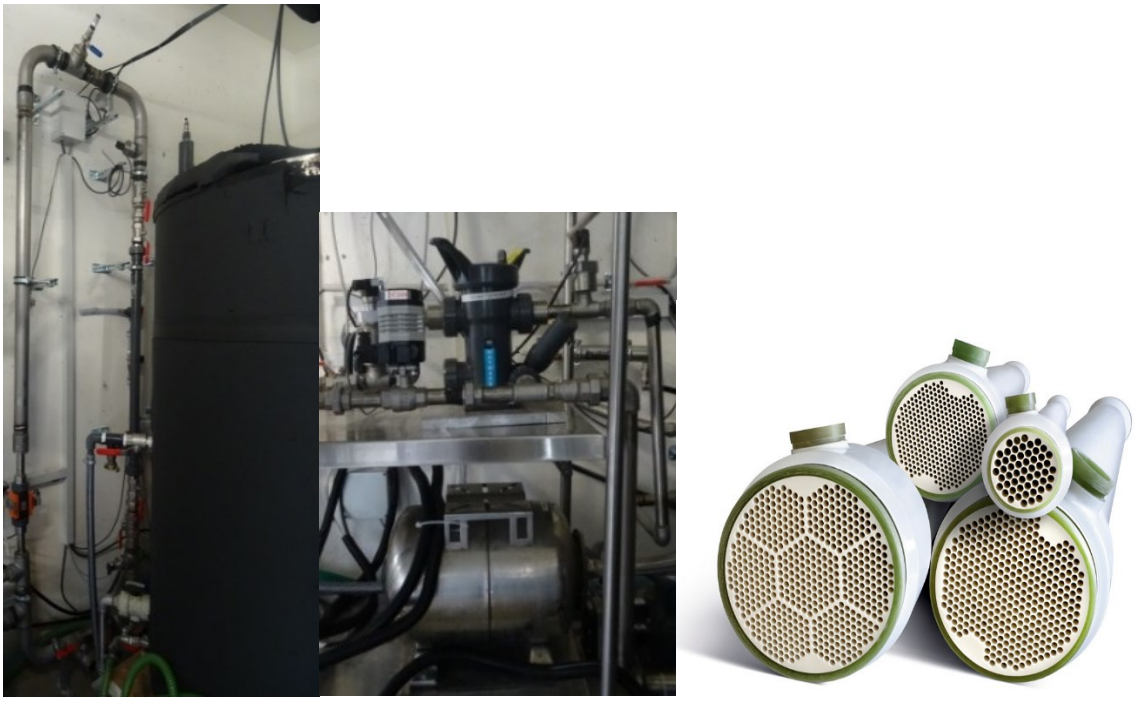

Figure 22: Main components of the AnMBR pilot-plant - left: AnMBR reactor and membrane circuit; middle: valve V-19 and mud trap (top) and expansion tank (bottom); right: example of Bioflow membranes from Berghof (Berghof Group 2013)

4.1.4 Further components and regulation processes

Hydrogen sulfide (H_2S) contained in the gas phase is continuously treated. For this purpose, the biogas circulates in a closed circuit through an adsorption column based on iron hydroxide granulate (Ferrosorp Sk, HegoBiotec, Germany, Figure 23). A condensate trap installed in front of the adsorption column and the heating and thermal insulation of the adsorption column avoid condensate in the gas circuit. H_2S is adsorbed and iron (III) sulfide is formed according to the following equation:

$$2\ Fe(OH)_3 + 3\ H_2S \rightarrow Fe_2S_3 + 6\ H_2O \quad (1)$$

For pH regulation, sodium hydroxide (NaOH) is regularly fed into the reactor during the mixing cycles using a peristaltic pump with an aim value of 7. In addition, an expansion tank placed in front of the membrane circulation circuit permits to compensate the potential pressure fluctuations. Four pressure sensors placed in front of and after the membrane and the mud trap (P1, P2, P3 and P4 in the flow diagram) (BD sensors, Germany), a temperature sensor in the membrane circuit (Titec, Germany) and a flowmeter on the permeate side (Kobold, Germany) complete the measuring technology of the system. The measurement data acquisition, control and online monitoring of the entire system is carried out via a Compact RIO system (cRIO-9024, National Instruments, Germany, Figure 23 and Figure 24).

The initial filling and seeding of the reactor were carried out with 550 L digested sludge from the municipal wastewater treatment plant Köhlbrandhöft in Hamburg, Germany. The sludge stayed in the reactor at a neutral pH of 7.2 and a temperature of 32.8 °C during one month before the continuous operation of the AnMBR with regular wastewater feeding began.

Figure 23: Left: adsorption column - right: Compact Rio system

4.1.5 Sample taking

Samples from wastewater after the storage tank, sludge between the reactor and the pump E-24 and permeate just after the membrane were usually taken between once and three times a week. Gas was generally sampled every two weeks.

4.1.6 Membrane cleaning

As membrane performance is not the main focus on this thesis, the steps for membrane cleaning are summarized in the Appendix 1.

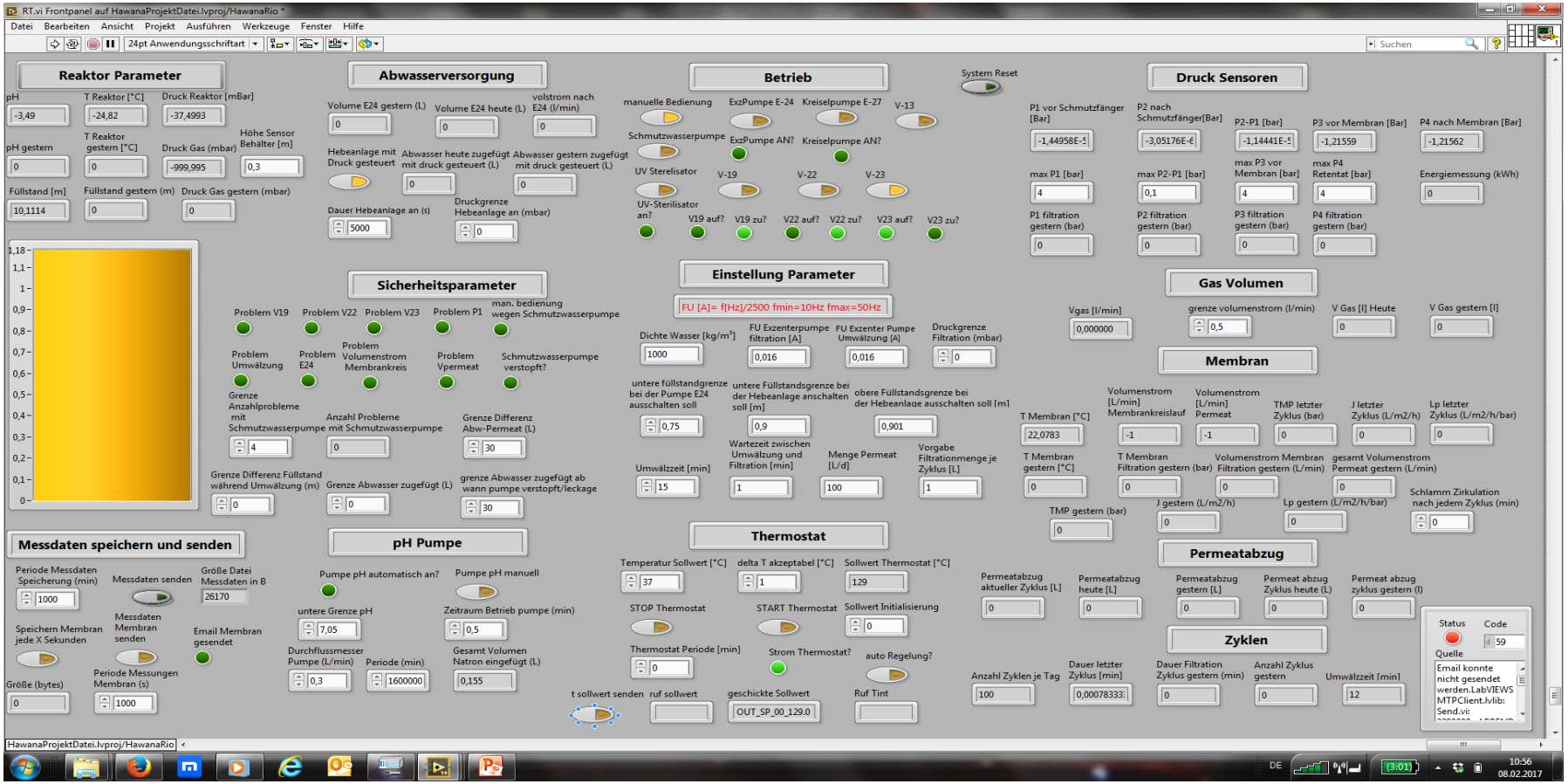

Figure 24: Online monitoring of the AnMBR pilot-plant throughout the software Labview from National Instruments

4.2 Microalgae cultivation

4.2.1 Experimental setup at the University of Hamburg

The experimental setup consists of nine autoclaved tubular pipes with a capacity of each 350 mL (Figure 25). The temperature in the tubes is kept constant using a water bath at 25 °C (Thermostat DC40-K40, Haake, Germany). Artificial lighting takes place via five neon tubes (Philipps, Master TL-D 58 W/840), which gave a total PPFD of 446 ± 44 µmol·s^{-1}·m^{-2}. A continuous circulation of air and carbon dioxide (1 L·min^{-1}·$L^{-1}_{culture}$) with a carbon dioxide concentration from 4 % to 5 % is used as carbon source for the microalgae. To this purpose, after its passage throughout a filter, the gas flows along a glass pipe placed at the back of each tubular pipe down to the bottom of each tubular pipe containing the culture medium. This also ensures the homogenization of the culture medium. As the plugs placed at the top of each tubular pipe are gas permeable, the gas contained in the gas phase of the tubular pipes can escape into the room. At the beginning of the experiment, the pH was set to 7.5. Each day, the pH was controlled and adjusted with NaOH in the case of acidification of the culture medium (pH < 7).

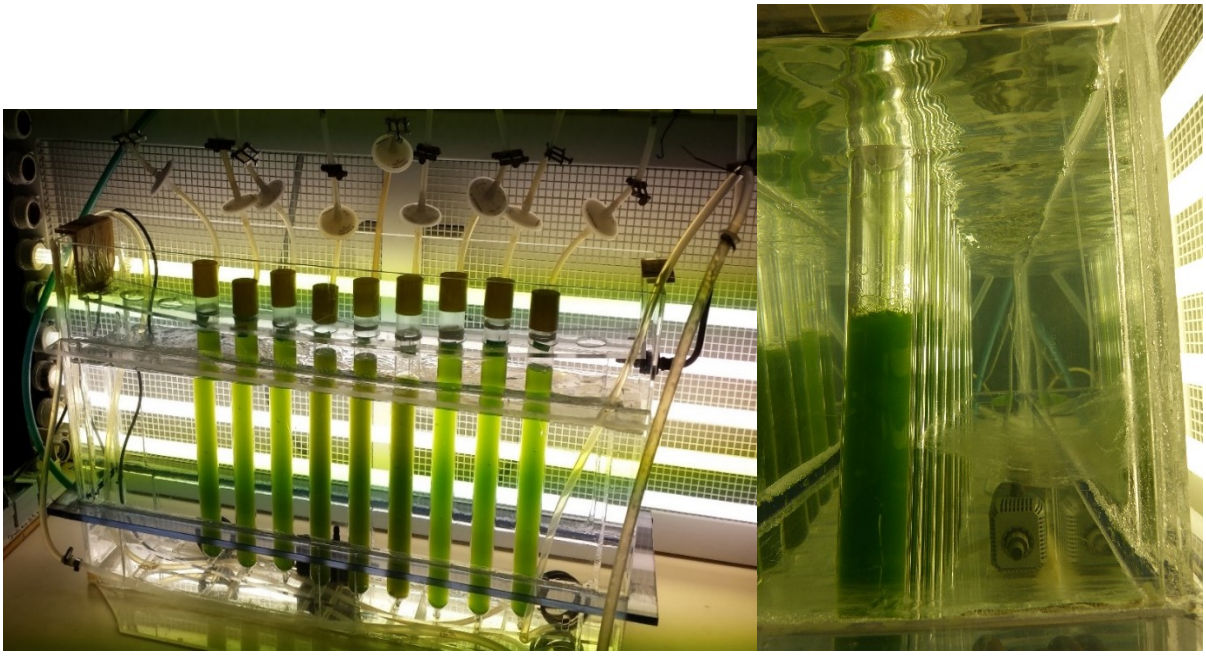

Figure 25: Experimental setup for microalgae cultivation at the University of Hamburg

4.2.2 Experimental setup at the Technical University of Berlin

The microalgae are cultured in 1-L bottles, which are fumigated with CO_2 (0.05 L·min^{-1}·$L^{-1}_{culture}$) and room air (0.95 L·min^{-1}·$L^{-1}_{culture}$) (Figure 26). Depending on the experiments, three, six or nine bottles are used. A continuous mixture of the culture medium is achieved using magnetic stirrers. Two LED daylight tubes (Naturnah, 26 W, Germany) imitate the sun daylight and give a PPFD of 270 ± 36 µmol·s^{-1}·m^{-2}. This is comparable to the illumination conditions during the first lab scale experiment at the University of Hamburg (in average 260 µmol·s^{-1}·m^{-2} during 24 hours). The temperature of the cultures is not regulated and corresponds to room temperature (between 20 and 23 °C).

Figure 26: Experimental setup for microalgae cultivation at the TU Berlin

4.2.3 Experimental setup at the outdoor pilot-plant Hamburg-Reitbrook

Two separately operated outdoor glass flat photobioreactors (PBRs) were used for full-scale experiments (see Figure 27, Figure 28 and Table 5). During the first experiment in fall 2017, the first PBR had a cultivation volume capacity of 28 L, an illuminated area of 1.26 m^2 and a culture volume/illuminated area ratio of 22 $L \cdot m^{-2}$. With the tubes, which are required to circulate the culture medium between the outdoor PBR and the indoor process components, and the sedimentation tank, where the permeate is filled, the total volume of the first line (L1) amounted to 66 L. A circulating eccentric screw pump (Seepex GmbH, Germany) with a flow rate of 4.0 $L \cdot min^{-1}$ ensured a continuous circulation of the culture medium. The second PBR had a cultivation volume capacity of 31 L, an illuminated area of 3.12 m^2 and a cultivation volume/illuminated area ratio of 10 $L \cdot m^{-2}$. The total volume of the second line (L2) reached 72 L. The circulating pump of the same model as L1 was operated at a speed of 4.5 $L \cdot min^{-1}$. Before the second experiment in summer 2018 began, the PBR from L1 was replaced with a PBR of the same model as L2. The tubes length between the outdoor PBRs and the inside pumps and sedimentation tanks was reduced so that L1 and L2 total volumes amounted to 52 L and 57 L respectively. During the second experiment, the circulating pumps were set to 2.0 $L \cdot min^{-1}$ and 1.0 $L \cdot min^{-1}$ in L1 and L2 respectively in order to prevent flooding of the culture at the top of the PBRs in the return flow.

During both experiments, the PBRs were continuously fed with flue gas from a cogeneration plant (about 8 % CO_2) with a volume flow of approximatively 1 $L \cdot L^{-1}_{reactor} \cdot min^{-1}$. In order to ensure an optimum temperature of the culture medium, the PBRs were heated or cooled using a heat exchanger by temperatures lower than 20 °C or higher than 25 °C. Since flue gas can lead to very low pH levels, NaOH was automatically added with a pH target of 7. A programmable logic controller (PLC, Siemens) ran the process and the pH and temperature measurements were saved every five minutes. The irradiance was recorded by the weather station Hamburg-Bergedorf and sensors placed on the roof of BIQ-The Algae House during the first and the second experiment respectively. The data was given in $W \cdot m^{-2}$ and were converted into $\mu mol \cdot s^{-1} \cdot m^{-2}$ for further comparisons with the literature and the lab-scale experiments (Lang *et al.* 1981). Once a week, a compressed air purge aiming at the dissolution of biofouling in the pipes was performed.

Figure 27: Microalgae cultivation in the outdoor pilot-plant Hamburg-Reitbrook - left: Glass flat panel photobioreactors of L1 (left) and of L2 (right) - right: Glass flat panel photobioreactor of L2

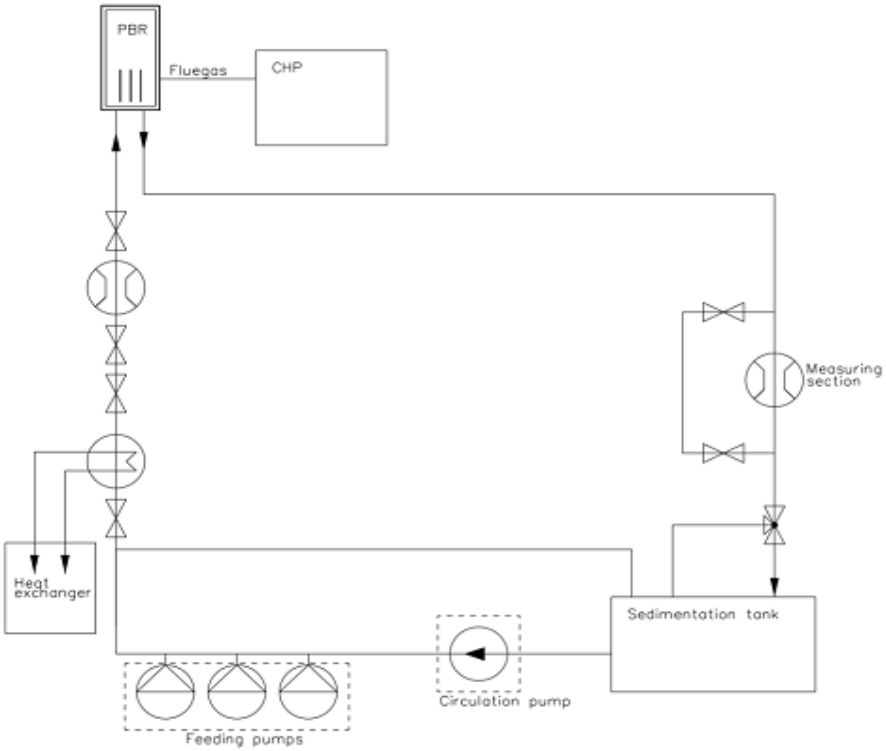

Figure 28: Flow diagram of microalgae cultivation in the outdoor pilot-plant Hamburg-Reitbrook

Table 6 summarizes the main parameters of the three different setups used in this thesis.

Table 5: Main parameters for microalgae cultivation in Line 1 and Line 2 in the pilot-plant Hamburg-Reitbrook

Parameter	1st experiment fall 2017		2nd experiment summer 2018	
	Line 1	Line 2	Line 1	Line 2
Total volume (L)	66	72	52	57
PBR volume (L)	28	31	31	31
Illuminated area (m^2)	1.26	3.12	3.12	3.12
Cultivation volume/illuminated area of the PBR ($L \cdot m^{-2}$)	22	10	10	10
Volume/illuminated area of the line ($L \cdot m^{-2}$)	52	23	17	18
Volume PBR/Volume line (%)	42	43	60	54
Circulation flow ($L \cdot min^{-1}$)	4.0	4.5	2.0	1.0
Flue gas composition	8 % CO_2, 9 % O_2, 83 % N_2			

Table 6: Summary of the experimental setups and the main parameters related to microalgae cultivation at the University of Hamburg, at the TU Berlin and at the outdoor pilot-plant Hamburg-Reitbrook

Parameter	University of Hamburg	TU Berlin	Pilot-plant Hamburg-Reitbrook
Cultivation material	9 tubular pipes	3, 6 or 9 bottles	2 outdoor flat panel photobioreactors
Culture volume (L)	0.350	1	between 52 and 72
Temperature (°C)	25	Ambient air	not constant - PBR heated or cooled by temperatures lower than 20 °C or higher than 25 °C
PPFD ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	446 ± 44	270 ± 36	depending on the weather conditions
Light: dark period (h)	14:10	24:0	depending on season
pH	target of 7.5 - adjusted with NaOH in case of acidification (pH < 7)	target of 7 - adjusted with NaOH in case of acidification (pH < 6.5)	target of 7 - adjusted with NaOH in case of acidification (pH < 7)
CO_2 addition ($L \cdot min^{-1} \cdot L^{-1}_{culture}$)	0.05	0.05	0.05

4.2.4 Organization of the experimental work

4.2.4.1 Microalgae cultivation at the University of Hamburg with *Acutodesmus obliquus*

The experiment conducted at the University of Hamburg aimed at the comparison of microalgae growth and nutrient degradation in a permeate culture and in a synthetic culture medium with similar initial nutrient concentrations. As synthetic culture media always contained micronutrients that are necessary for cell growth, this first experiment also aimed at the comparison of microalgae growth and nutrient degradation in a permeate culture without additional micronutrients and in a permeate culture with additional micronutrients. The experiment generally aimed to find out if permeate could replace a synthetic culture medium in the microalgae industry.

To this purpose, three repeated batch tests were carried out in triplicates with the following culture media (three pipes for each culture medium):

- (A) Permeate from the AnMBR.
- (B) Permeate enriched with micronutrients and EDTA. Using magnesium sulfate ($MgSO_4 \cdot 7H_2O$), manganese (II) chloride ($MnCl_2 \cdot 4H_2O$) and iron (II) sulfate ($FeSO_4 \cdot 7H_2O$), 7 $mg \cdot L^{-1}$ of Mg, 5 $mg \cdot L^{-1}$ of Mn, 25 $mg \cdot L^{-1}$ of Fe and 23 $mg \cdot L^{-1}$ of S were added. The chelating agent EDTA was added with an initial concentration of 8 $mg \cdot L^{-1}$. The concentrations were chosen to ensure that the micronutrients would not become limiting factors during the experiments. Hence, they were similar or higher than in the commercial fertilizer and the culture media usually used for microalgae culture. EDTA was added following the composition of the KC medium (Kessler and Czygan 1970).
- (C) The commercial fertilizer Ferty Basis 1 (Planta Düngemittel GmbH, Germany) was used as control for the experiment. As it does not contain a nitrogen source, nitrogen was added in the form of ammonium nitrate (NH_4NO_3). The commercial fertilizer was diluted with distilled water and NH_4NO_3 added so that TN and TP initial concentrations were similar to those of the cultures (A) and (B). Based on the composition of the commercial fertilizer, the initial micronutrient concentration amounted to 6 $mg \cdot L^{-1}$ of Mg, 25 $mg \cdot L^{-1}$ of S, 0.4 $mg \cdot L^{-1}$ of Fe and 0.1 $mg \cdot L^{-1}$ of Mn. Fe was present in the commercial fertilizer as chelate from DTPA and EDDHA and Mn as chelate from EDTA.

The permeate used in this experiment was sampled only once and stored at 4 °C. For this experiment, 20 mL of the species *Acutodesmus obliquus* from the microalgae collection MCZH-SVCK of the University of Hamburg was used as pre-culture medium. The pre-culture was cultivated in a 2-L bottle with the commercial fertilizer Ferty Basis 1 and potassium nitrate as nitrogen source under a continuous lighting of 331.7 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Before beginning the pre-culture, the culture medium was autoclaved and pH was adjusted to 7.5. After 7 days, the pre-culture was centrifuged during 10 minutes at 4,000 rpm (Thermo scientific, USA). The biomass was then washed with distilled water and centrifuged again to obtain algae cells free of nutrients contained in the culture medium. The microalgae cells obtained were subsequently added at equal mass into the nine pipes and inoculated with the culture media. In order to avoid the formation of foam, 10 µL of silicon anti-foaming agent (Silifoam SD882, Wacker, Germany) was also added in each pipe. At the end of each batch, the content of the three pipes of a same culture medium was mixed together in a 2-L bottle and centrifuged during 10 min at 4,000 rpm. Aiming an initial biomass concentration of approximately 0.5 $g \cdot L^{-1}$, the biomass was divided in the same three pipes and inoculated with the corresponding culture medium.

4.2.4.2 Microalgae cultivation at the TU Berlin with *Acutodesmus obliquus*

The experiments conducted with *Acutodesmus Obliquus* in the laboratories of the TU Berlin aimed at the confirmation of the results obtained with the permeate (A) and the enriched permeate (B) during the experiment at the University of Hamburg. During these experiments, the goal was also to find out which micronutrients caused the differences observed between both culture media. To this purpose, three different experiments were carried out. In the experiment (1), permeate (A) and permeate enriched with micronutrients and EDTA (B) were simultaneously cultivated in triplicates (three bottles for each culture medium) throughout two repeated batches. Using magnesium chloride, manganese

(II) chloride, iron (II) sulfate and EDTA, the micronutrients and the chelating agent concentrations were the same as the experiment conducted at the University of Hamburg (Table 7).

Table 7: Summary of the experimental work conducted at lab-scale with Acutodesmus obliquus

Parameter	Experiment in the University of Hamburg	Experiment (1) TU Berlin	Experiment (2) TU Berlin	Experiment (3) TU Berlin
Duration (d)	18	14	4	7
Culture medium	(A) permeate (B) permeate + Mg, Mn, Fe, S and EDTA (C) a synthetic culture medium using Ferty Basis 1 and ammonium nitrate as TP and TN source respectively	(A) permeate (B) permeate + Mg, Mn, Fe, S and EDTA	(D) culture (A) from the end of (1) enriched with Mn and EDTA on day 0 and Fe and S on day 2 (E) culture (A) from the end of (1) enriched with Mg and EDTA on day 0 and Mn on day 2	first five days: (A) permeate after 5 days: enrichment with Mg, Mn, Fe, S and EDTA in 3 bottles and with Fe, S and EDTA in the other 3 bottles
Number of successive batches carried out	3	2	1	1
Permeate used	permeate sampled one week before the start of the experiment and stored at 4 °C between each batch	permeate sampled one week before the start of the experiment and stored at 4 °C between both batches	-	permeate sampled a few days before the start of the experiment
Inoculation medium	pre-culture based on 20 mL of *Acutodesmus obliquus* from the microalgae collection MCZH-SVCK of the University of Hamburg	*Acutodesmus obliquus* culture of L2 from the end of the repeated batch tests conducted in fall 2017 at the pilot-plant Hamburg-Reitbrook	-	culture (A) from the end of the 2nd batch of the experiment (1)

At the end of the second batch test of (1), about one-third of the culture medium (A) was stored in the refrigerator for further experiments and the other two-thirds were divided into two triplicates for the experiment (2). On day 0 of (2), $MnCl_2 \cdot 4H_2O$ and EDTA were added into three bottles (culture medium (D)) and $MgCl_2 \cdot 7H_2O$ and EDTA into the three other bottles (culture medium (E)). After 2 days of cultivation, $FeSO_4 \cdot 7H_2O$ was added into the culture medium (D) and $MnCl_2 \cdot 4H_2O$ into the culture medium (E).

The experiment (3) aimed to confirm the results obtained in (2). A culture composed of new permeate and stored microalgae culture (A) from the end of the experiment (1) was started in triplicates. On

Day 5 of (3), the culture was divided into six bottles. $MgCl_2 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$ and EDTA were added in one triplicate and $FeSO_4 \cdot 7H_2O$ and EDTA in the other triplicate.

Micronutrient addition at the beginning of the experiments (2) and (3) was similar to the experiment (1).

At the beginning of the experiment (1), the *Acutodesmus obliquus* culture of L2 from the end of the repeated batch tests conducted in fall 2017 at the pilot-plant Hamburg-Reitbrook was used for the inoculation. It means that the culture had already been cultivated with permeate during 4 batches. For the inoculation at the beginning of each experiment and between each batch, the microalgae were not centrifuged. Instead of that, the content of the three bottles of the same culture medium was mixed together in a 2-L bottle and reused for the next batch and the same culture medium. This aimed at the imitation of the experimental conditions in the pilot-plant Hamburg-Reitbrook. Moreover, new permeate was sampled and used for each batch experiment during the experiment (1) and each further experiment.

4.2.4.3 Microalgae cultivation at the TU Berlin with *Chlorella vulgaris*

As during the first experiment conducted at the University of Hamburg, the cultivation of *Chlorella vulgaris* aimed at the investigation of cell growth and nutrient degradation in permeate culture. Indeed, this species is the most important for microalgae production and is used very often for nutrient removal of wastewater streams. Comparisons with a synthetic culture medium were also made in order to know if the permeate is suitable for the replacement of synthetic culture media used for microalgae production. The goal was also to find out if the observations made with permeate cultures and *Acutodesmus obliquus* during the previous experiments are confirmed and to compare the performance obtained with both species. The investigation of the microalgae species *Chlorella vulgaris* was divided into three experiments (Table 8).

Three successive batch tests were performed during the experiment (1). During the 1st batch test of (1), three different culture media were simultaneously cultivated in triplicates (3 bottles for each culture medium). The culture media were:

- (A) permeate
- (B) a synthetic culture medium using the fertilizer Ferty Basis 1 and ammonium sulfate as TP and TN source respectively
- (C) a synthetic culture medium using Ferty Basis 1 and ammonium nitrate as TP and TN source respectively

Ammonium sulfate, ammonium nitrate and the fertilizer Ferty Basis 1 were diluted with tap water to obtain in (B) and (C) similar TP and TN initial concentrations to the permeate (A). As ammonium addition into a synthetic culture medium leads to great pH decrease, the use of ammonium nitrate was preferred in the experiment conducted with *Acutodesmus obliquus* in the University of Hamburg. However, as TN in the permeate is mostly composed of ammonium, the use of a TN source exclusively composed of ammonium would be useful for further comparisons of the permeate (A) with a synthetic culture medium. Therefore, the use of ammonium sulfate was investigated in order to compare the pH decreases in the culture media (B) and (C) and decide if ammonium sulfate could replace ammonium nitrate in the next batch experiments. During the 2nd and the 3rd batch tests, microalgae cultivation with the permeate (A) and the synthetic culture medium using ammonium sulfate as TN

source (B) was performed. Within the experiment (1), the same permeate P1 was used. Between the batch tests, P1 was stored in the fridge at 4 °C.

Table 8: Summary of the experimental work conducted at lab-scale with Chlorella vulgaris

Parameter	Experiment (1)	Experiment (2)	Experiment (3)
Duration (d)	28	4	22
Culture medium	(A) permeate (B) a synthetic culture medium using Ferty Basis 1 and ammonium sulfate as TP and TN source respectively (C) a synthetic culture medium using Ferty Basis 1 and ammonium nitrate as TP and TN source respectively (only for the 1st batch test)	(D) culture (A) from the end of the 3rd batch test of the experiment (1) enriched with $FeSO_4 \cdot 7H_2O$ and EDTA (E) culture (A) from the end of the 3rd batch test of the experiment (1) enriched with $FeCl_2 \cdot 4H_2O$ and EDTA	(A) permeate (B) a synthetic culture medium using Ferty Basis 1 and ammonium sulfate as TP and TN source respectively
Number of successive batch tests	3	1	2
Permeate used	P1 – stored at 4 °C between each batch	-	1st batch: P2 2nd batch: P3
Inoculation medium	pre-culture based on 20 mL of *Chlorella vulgaris* from the microalgae collection MCZH-SVCK of the University of Hamburg	-	(A): the culture (A) from the end of the 2nd batch test of the experiment (1) (B): culture (B) from the end of the 3rd batch test of the experiment (1)

The microalgae culture (A) from the end of the 3rd batch test of (1) was further investigated for the experiment (2). The content of the three bottles of (A) was mixed together and divided into six bottles. While $FeSO_4 \cdot 7H_2O$ and EDTA were added into three bottles (culture medium (D)), iron (II) chloride ($FeCl_2 \cdot 4H_2O$) and EDTA were added into the three other bottles (culture medium (E)). The concentration of Fe and EDTA added in the experiment (2) was similar to that during the previous experiments conducted with *Acutodesmus obliquus*.

During the experiment (3), the permeate (A) and the control culture with ammonium sulfate as TN source (B) were investigated again during two batch tests. While the microalgae of the end of the 3rd batch test of (1) were used as inoculum for the culture medium (B), the culture of the end of the 2nd

batch test of (1) was preferred for the permeate (A). The fresh sampled permeates P2 and P3 were used for the 1st and 2nd batch tests respectively.

20 mL of *Chlorella vulgaris* from the microalgae collection MCZH-SVCK of the University of Hamburg was used as inoculum for the pre-culture. This was performed at ambient temperature and a continuous PPFD of 270 ± 36 µmol·s^{-1}·m^{-2}. A concentration of 4 g·L^{-1} of the synthetic fertilizer Ferty Basis 1 and 3.85 g·L^{-1} of potassium nitrate was obtained by dilution of these two compounds with tap water. Before the inoculation with the microalgae strain, the culture medium was autoclaved. Then, it was cultivated in a 1-liter bottle during 10 days. After 10 days, a new pre-culture was begun in three 1-L bottles. Therefore, 200 mL of the culture obtained was diluted with 800 mL of tap water. After 7 days, the content of the three bottles was centrifuged during 20 min at 6,000 rpm in order to obtain biomass free from the nutrients still contained in the synthetic culture medium. Thereafter, the microalgae cells were divided in equal mass into the nine bottles and mixed with the related culture medium.

At the beginning of the pre-culture and of each batch test as well as in case of the observation of foam formation during the cultivation, 20 µL of silicon anti-foaming agent (Carl Roth GmbH, Germany) was added into the bottles. During an experiment, at the end of each batch test, the content of the three bottles related to the same culture medium was mixed together in a 2-L bottle and reused for the next batch and the same culture medium.

4.2.4.4 Microalgae cultivation at the TU Berlin with *Chlorella sorokiniana*

One of the main goals of the experiments conducted with *Chlorella sorokiniana* was to investigate if the microalgae cells are able to assimilate the nutrients and to grow in a culture medium defined by comparatively high nutrient concentrations. Indeed, nutrient concentrations between the experiments conducted with *Chlorella vulgaris* and the beginning of the experiments conducted with *Chlorella sorokiniana* significantly increased. As a reminder, the difficulty for the microalgae cells to assimilate the nutrients increases with increasing nutrient concentrations in the culture medium (Su *et al.* 2012). These experiments also aimed at the comparison of the performance achieved in a permeate culture and in a synthetic culture medium. Finally, a comparison of the performance obtained with *Acutodesmus obliquus*, *Chlorella vulgaris* and *Chlorella sorokiniana* was aimed. The investigation of the microalgae species *Chlorella sorokiniana* was divided into four experiments.

During the experiment (1), two successive batch tests were performed in triplicates with the same permeate P1 from 06.06.18 as culture medium (culture (A)).

The experiment (2) is a prolongation of the experiment (1). For this purpose, the culture from the end of the experiment (1) was equally divided into six bottles. In the culture medium (D), no chemicals were supplied, which means that the permeate culture medium from the experiment (1) was further cultivated. In the culture medium (E), KNO_3 was supplied at the beginning of the experiment with a concentration of approximately 0.4 g·L^{-1}.

During the two successive batch tests of the experiment (3), two different culture media were simultaneously cultivated in triplicates. The culture media were:

- (A) permeate
- (B) permeate enriched with $(NH_4)_2SO_4$

For the 1st batch of (3), the pre-culture was used as inoculum and the permeate was P2 from 31.07.18. The culture from the end of the 1st batch was used for the 2nd batch. In addition, the permeate P3 from 14.08.18 with a similar nutrient concentration to P2 was used for this 2nd batch. $(NH_4)_2SO_4$ was added at a concentration of 0.37 and 0.33 $g \cdot L^{-1}$ on day 0 and 13 respectively during the 1st batch of (3). During the 2nd batch, 0.31 and 0.60 $g \cdot L^{-1}$ were supplied on day 0 and 8 respectively.

Table 9: Summary of the experimental work conducted at lab-scale with Chlorella sorokiniana

Parameter	Experiment (1)	Experiment (2)	Experiment (3)	Experiment (4)
Duration (d)	19	4	37	44
Culture medium	(A) permeate	(D) culture (A) from the end of the 2nd batch test of the experiment (1) (E) culture (A) from the end of the 2nd batch test of the experiment (1) enriched with KNO_3	(A) permeate (B) permeate enriched with $(NH_4)_2SO_4$	(A) permeate – supply of micronutrients on day 17 in the 1st batch and on day 9 in the 2nd batch (C) a synthetic culture medium using Ferty Basis 1 and ammonium sulfate as TP and TN source respectively
Number of successive batch tests	2	1	2	2
Permeate used	P1 from 06.06.18	-	1st batch: P2 from 31.07.18 2nd batch: P3 from 14.08.18	1st batch: P3 from 14.08.18 2nd batch: P2 from 31.07.18
Inoculation medium	pre-culture based on 10 mL of *Chlorella sorokiniana* from outdoor experiments in the pilot-plant Hamburg-Reitbrook	-	1st batch: Pre-culture based on 10 mL of *Chlorella sorokiniana* from outdoor experiments in the pilot-plant Hamburg-Reitbrook 2nd batch: culture from the end of the 1st batch of (3)	1st batch: culture from the end of the 2nd batch of (3) 2nd batch: culture from day 17 of the 1st batch of (4) before supply of micronutrients into the permeate (A)

In the experiment (4), two different culture media were simultaneously cultivated in triplicates during two successive batch tests:

- (A) permeate
- (C) a synthetic culture medium using Ferty Basis 1 and $(NH_4)_2SO_4$ as TP and TN source respectively

$(NH_4)_2SO_4$ and the fertilizer Ferty Basis 1 were diluted with tap water to obtain in (C) similar initial TP and TN concentrations to the permeate (A). In the permeate (A), the micronutrients Mg, Mn, Fe, S and the chelating agent EDTA were supplied on day 17 and 9 of the 1st and 2nd batch test respectively. The micronutrients were added with similar concentrations to the experiments conducted with *Acutodesmus obliquus* and EDTA concentration amounted to 0.45 $g \cdot L^{-1}$. For the 1st batch of (4), the microalgae from the end of the 2nd batch test of (3) were used as inoculum. In the following batch, the culture from day 17 of the 1st batch of (4) before supply of micronutrients into the permeate (A) was preferred. The permeates P3 and P2 were used for the 1st and 2nd batch test respectively. The experimental conditions of these four experiments are resumed in Table 9.

10 mL of *Chlorella sorokiniana* from outdoor experiments in the pilot-plant Hamburg-Reitbrook was used as inoculum for the pre-culture. As for the previous experiments in the TU Berlin, this was performed at ambient temperature and a continuous PPFD of 270 ± 36 $\mu mol \cdot s^{-1} \cdot m^{-2}$. First, the microalgae were cultivated in a 50-mL bottle with tap water and a concentration of 4 $g \cdot L^{-1}$ of the synthetic fertilizer Ferty Basis 1 and 1 $g \cdot L^{-1}$ of potassium nitrate. Before the inoculation with the microalgae strain, the culture medium was autoclaved. As the biomass concentration reached 0.5 $g \cdot L^{-1}$, the microalgae were then cultivated in a 150-mL bottle. Finally, from a biomass concentration of 2.9 $g \cdot L^{-1}$, the culture was transferred in a 1-L bottle and the microalgae further grew under the same conditions. After a total of 10 days, a concentration of 2.3 $g \cdot L^{-1}$ was reached. Before the beginning of the experiment (1) and (3), a part of the pre-culture was centrifuged during 20 min at 6,000 rpm in order to obtain biomass free from the nutrients still contained in the synthetic culture medium. The rest of the pre-culture was stored in the fridge at a temperature of 4 °C.

At the beginning of the pre-culture and of each batch test as well as in case of the observation of foam formation during the cultivation, 20 µL of silicon anti-foaming agent (Carl Roth GmbH, Germany) were added into the bottles. Within an experiment, at the end of each batch test, the content of the three bottles related to the same culture medium were mixed together in a 2-L bottle and reused for the next batch and the same culture medium.

4.2.4.5 Outdoor full-scale experiments in Hamburg-Reitbrook with *Acutodesmus obliquus*

The outdoor full-scale experiments in the pilot-plant Hamburg-Reitbrook aimed at the following investigations:

- Comparison of growth and nutrient degradation rates at full-scale with the results obtained at lab-scale and the literature for full-scale pilot plants
- Assessment of the influence of weather conditions
- Comparison of the final nutrient concentrations with the German and European regulations
- Assessment of the suitability of permeate for microalgae cultivation at full-scale

During the two full-scale experiments conducted at the pilot-plant Hamburg-Reitbrook, the microalgae were always cultivated with permeate without additional elements. At the beginning of the first batch

experiment in fall 2017, L1 was inoculated with microalgae of the species *Acutodesmus obliquus*, which had been cultivated continuously during several months in PBRs of BIQ-The Algae House with synthetic fertilizer and flue gas. The first batch was only conducted with L1. The culture of L2 was started at the beginning of the second batch of L1 and inoculated with the culture medium of L1 from the end of the first batch. In summer 2018, cultures in L1 and L2 were simultaneously started and inoculated with *Acutodesmus obliquus* that has been cultivated during one month with synthetic fertilizer in the same PBR (Table 10).

Before the start of the first experiment, the PBRs were cleaned with water and hydrogen peroxide during two days. For the second experiment, the PBRs were only cleaned with water during one hour, as a long cleaning process had been carried out a few days before. After the emptying of the line and a compressed air purge to remove the remaining microalgae in the pipes, permeate, microalgae and 1 mL of silicon anti-foaming agent (Silifoam SD882, Wacker, Germany) were added using the sedimentation tanks. Between each batch, the procedure was the following: the cultivation lines were emptied and a compressed air purge was carried out. Then, the new sampled permeate and microalgae from the previous batch were added to begin a new batch.

While four and three batches were conducted with L1 and L2 respectively during the experiment in fall 2017, six and five batches were carried out with L1 and L2 respectively in the experiment in summer 2018.

Table 10: Summary of the experimental work conducted at the outdoor full-scale plant with Acutodesmus obliquus

Parameter	**1st experiment fall 2017**		**2nd experiment summer 2018**	
	Line 1	**Line 2**	**Line 1**	**Line 2**
Culture medium	Permeate	Permeate	Permeate	Permeate
Inoculation medium	*Acutodesmus obliquus* from BIQ-The Algae House cultivated with synthetic fertilizer and flue gas	*Acutodesmus obliquus* from the end of the first batch in L1	*Acutodesmus obliquus* cultivated in the pilot-plant Hamburg-Reitbrook with synthetic fertilizer and flue gas	*Acutodesmus obliquus* cultivated in the pilot-plant Hamburg-Reitbrook with synthetic fertilizer and flue gas
Inoculation volume (L)	14	15	2	2
Microalgae culture kept as inoculate for the following batch (L)	10 - 20	10 - 20	6 - 10	6 - 10
Number of successive batches carried out	4	3	6	5
Experiment dates	28.09.2017-07.11.2017	09.10.2017-07.11.2017	07.06.2018-13.07.2018	07.06.2018-09.07.2018

4.3 Analysis

4.3.1 Nutrient determination

The parameters NH_4-N, TN, PO_4-P, TP, COD and BOD_5 were determined using standard cuvette tests (Hach Lange, Germany). Cuvette tests are ready-to-use reagent packages for photometric analysis and cover a widely range of water and wastewater applications. Each parameter and each measuring range possesses its own specific procedure. In summary, a certain volume of the sample, if necessary diluted with distilled water, and eventually additional reagents are pipetted in the cuvette. By heating the cuvette (TN, TP and COD) or at room temperature (NH_4-N, PO_4-P and BOD_5), a chemical reaction leads to a coloration of the solution. At the end of the procedure, the concentration of the sample is automatically read using a spectrophotometer (Lasa 100, Hach Lange, Germany).

More details about the procedure can be seen in the Appendix 2.

4.3.2 Total solids (TS) and volatile solids (VS) determination

Porcelain dishes are used for TS and VS determinations. Using a precision balance (Sartorius, Germany), the initial amount of sample is calculated by weighing the empty dish and the dish with the sample. Then, the ceramic dish is dried in a drying oven (Heraeus, Germany) at a temperature of 105 °C. After one day, the dish is cooled in a desiccator and weighed. For VS determination, the dried dish is burned in a muffle furnace (Heraeus, Germany) for 2 hours at 550 °C. After cooling in the desiccator, the dish is weighed again. The following calculations are used (APHA 2005):

$$TS = 1000 * \frac{W_{total} - W_{dish}}{W_{sample} - W_{dish}} [g \cdot kg^{-1}] \quad (2) \qquad VS = TS * \frac{W_{total} - W_{volatile}}{W_{total} - W_{dish}} [g \cdot kg^{-1}] \quad (3)$$

with

W_{total}: weight of dish and sample after drying [g], W_{dish}: weight of empty dish [g], W_{sample}: initial weight of dish and sample [g] and $W_{volatile}$: weight of dish and sample after burning [g].

A triplicate determination is performed for each sample.

4.3.3 Microalgae concentration determination

To determine the microalgal biomass concentration, the samples were vacuum filtered with 0.45 µm cellulose membrane filters (Sartorius, Germany) during the experiment at the University of Hamburg and with glass microfiber filters (LLG, Germany) during the other experiments. The filters were dried in a drying oven (Heraeus, Germany) for 24 hours at 105 °C. Using a precision balance (Sartorius, Germany), the biomass concentration C is calculated as follows:

$$C = \frac{W_{total} - W_{filter}}{V_{sample}} [g \cdot L^{-1}] \quad (4)$$

with

W_{total}: weight of the dried sample [g], W_{filter}: initial weight of the filter [g] and V_{sample}: sample volume [L].

4.3.4 Parameters related to wastewater supply

The hydraulic retention time **HRT** is calculated as follows:

$$HRT = \frac{V_R * N}{V_w} [d] \ (5)$$

V_R: average sludge volume in the reactor during an experimental period [L], V_w: supplied volume of wastewater during an experimental period [L] and N: number of days of the experimental period [d].

The organic loading rate OLR is obtained following the equation:

$$OLR = \frac{V_w * C_{COD,w}}{V_R * N} [g\ COD \cdot m^{-3} \cdot d^{-1}] \ (6)$$

with

V_w: supplied volume of wastewater during an experimental period [L], $C_{COD,w}$: COD concentration in wastewater [g·L^{-1}], V_R: sludge volume in the reactor [m^3] and N: number of days of the experimental period [d].

The food to microorganism ratio F/M is defined as:

$$F/M = \frac{V_w * C_{COD,w}}{m_{VS,R} * N} [kg\ COD \cdot kg^{-1}\ VS \cdot d^{-1}] \ (7)$$

with

V_w: supplied volume of wastewater during an experimental period [L], $C_{COD,w}$: COD concentration in wastewater [kg·L^{-1}], $m_{VS,R}$: average VS mass in the reactor [kg] and N: number of days of the experimental period [d].

4.3.5 Parameters related to COD removal

The COD removal efficiency RE_{COD} of the AnMBR process is calculated as follows:

$$RE_{COD} = 100 * \frac{C_{COD,w} * V_w - C_{COD,p} * V_p}{C_{COD,w} * V_w} [\%] \ (8)$$

with

$C_{COD,w}$: COD concentration in wastewater during an experimental period [g·L^{-1}], V_w: supplied volume of wastewater during an experimental period [L], $C_{COD,p}$: COD concentration in permeate during an experimental period [g·L^{-1}] and V_p: discharged volume of permeate during an experimental period [L].

The COD biological removal efficiency $RE_{COD,b}$, which only takes into account the COD that is biologically removed, is calculated as follows:

$$RE_{COD,b} = 100 * \frac{C_{COD,w} * V_w - C_{COD,p} * V_p + m_{COD,R_B} - m_{COD,R_E}}{C_{COD,w} * V_w} [\%] \ (9)$$

with

m_{COD,R_B}: COD mass in the reactor at the beginning of an experimental period [g], m_{COD,R_E}: COD mass in the reactor at the end of an experimental period [g], $C_{COD,w}$: COD concentration in wastewater during an experimental period [g·L^{-1}], V_w: supplied volume of wastewater during an experimental

period [L], $C_{COD,p}$: COD concentration in permeate during an experimental period [g·L^{-1}] and V_p: discharged volume of permeate during an experimental period [L].

4.3.6 Parameters related to biogas production and composition

To determine the biogas composition, gas samples are collected in 0.5-liter plastic gasbags between the membrane pump and the adsorption column of the gas circuit. CH_4, CO_2, O_2, nitrogen (N_2), hydrogen (H_2) and nitrous oxide (N_2O) are measured by gas chromatography (GC) (Agilent 6890 HP GC Series). The methane volume V_{CH4} produced in the pilot-plant is calculated as follow:

$$V_{CH4} = V * \alpha_{CH4} \text{ [L] (10)}$$

with

V: experimental biogas volume known by reading the gasmeter [L] and α_{CH4}: CH_4 proportion determined by gas chromatography.

For direct comparison with the literature, the biogas volume under normal conditions V_0 is calculated. According to the VDI Directive 4630 (Verein Deutscher Ingenieure 2014), the following empirical formula is applied:

$$V_0 = V * T_0 * \frac{p - p_{water}}{p_o * T} \text{ [L] (11) with } p_{water} = 6.11213 * e^{\frac{17.5043 * (T - 273.15)}{T - 31.95}} \text{ [hPa] (12)}$$

with

V: experimental biogas volume known by reading the gasmeter [L], p: pressure in the gasmeter (1013 hPa), p_{water}: water vapor pressure [hPa], p_o: pressure under standard conditions (1013 hPa), T_0: temperature under standard conditions (273,15 K) and T: temperature in the reactor [K].

4.3.7 Parameters related to methane yields and sludge production

The methane yield Y_{CH_4} is determined using the following equation:

$$Y_{CH_4} = \frac{V_{0,CH_4}}{C_{COD,w} * V_w} \text{ [m}^3 \text{ CH}_4\text{·kg}^{-1} \text{ COD] (13)}$$

with

V_{0,CH_4}: methane production under standard conditions during an experimental period [m^3], $C_{COD,w}$: COD concentration in wastewater during an experimental period [kg·m^{-3}] and V_w: supplied volume of wastewater during an experimental period [m^3].

The methane production relative to the COD mass that is removed $V_{0,CH_{4COD_{rem}}}$ is calculated under standard conditions as follows:

$$V_{0,CH_{4COD_{rem}}} = 100 * \frac{Y_{CH_4}}{RE_{COD}} \text{ [m}^3 \text{ CH}_4\text{·kg}^{-1} \text{ COD}_{rem}\text{] (14)}$$

with

Y_{CH_4}: methane yield [m^3 CH_4·kg^{-1} COD] and RE_{COD}: COD removal efficiency [%].

The specific biomethane production $\boldsymbol{SBP}$, which relates to the daily amount of biogas produced per volume unit of reactor, is defined as:

$$\boldsymbol{SBP = \frac{V_{0,CH_4}}{V_R * N}\ [m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}]\ (15)}$$

with

$\boldsymbol{V_{0,CH_4}}$: methane production under standard conditions during an experimental period [m^3], $\boldsymbol{V_R}$: sludge volume in the reactor [m^3] and $\boldsymbol{N}$**:** number of days of the experimental period [d].

The biosolids production $\boldsymbol{BSP}$, which describes the accumulation of organic compounds in the reactor, is calculated as follows:

$$\boldsymbol{BSP = 100 * \frac{m_{VS,R_E} - m_{VS,R_B}}{C_{COD,w} * V_w * RE_{COD}}\ [kg\ VS \cdot kg^{-1}\ COD_{rem}]\ (16)}$$

with

$\boldsymbol{m_{VS,R_B}}$**:** VS concentration in the reactor at the beginning of an experimental period [kg], $\boldsymbol{m_{VS,R_E}}$: VS concentration in the reactor at the end of an experimental period [kg], $\boldsymbol{C_{COD,w}}$: COD concentration in wastewater during an experimental period [$kg \cdot m^{-3}$], $\boldsymbol{V_w}$: supplied volume of wastewater during an experimental period [m^3] and $\boldsymbol{RE_{COD}}$**:** COD removal [%].

4.3.8 Dissolution of methane in the permeate

The methane loss throughout dissolution in the sludge and then in the permeate is estimated using Henry's law. The Henry constant for methane is first calculated according to Helgeson (1967):

$$\boldsymbol{log_{10}\ (K_H) = \frac{2370.40}{T} - 16.33 + 0.0185 * T\ (17)}$$

with

$\boldsymbol{K_H}$**:** average Henry constant during an experimental period [$mol \cdot L^{-1} \cdot bar^{-1}$] and $\boldsymbol{T}$**:** average temperature during an experimental period [K].

The concentration of methane gas dissolved in the permeate $\boldsymbol{C_{CH_4}}$ is determined as follows:

$$\boldsymbol{C_{CH_4} = K_H * p_{CH_4}\ [mol \cdot m^{-3}]\ (18)}$$

with

$\boldsymbol{K_H}$**:** average Henry constant during an experimental period [$mol \cdot m^{-3} \cdot bar^{-1}$] and $\boldsymbol{p_{CH_4}}$**:** average partial pressure of methane in the gas phase during an experimental period [bar].

The molar amount of methane gas $\boldsymbol{n_{CH_4}}$ that leaves the reactor dissolved in the permeate is calculated using the following equation:

$$\boldsymbol{n_{CH_4} = C_{CH_4} * V_p\ [mol]\ (19)}$$

with

$\boldsymbol{C_{CH_4}}$: average concentration of methane gas dissolved in the permeate [$mol \cdot m^{-3}$] and $\mathbf{V_p}$**:** volume of permeate discharged during an experimental period [m^3].

The volume of methane gas V_{CH_4} lost through dissolution in the permeate is obtained using the ideal gas law:

$$\boldsymbol{V_{CH_4} = \frac{n_{CH_4} * R * T}{p}\ [m^3]\ (20)}$$

with

$\boldsymbol{n_{CH_4}}$**:** molar amount of methane gas leaving the reactor during an experimental period [mol], $\boldsymbol{R}$**:** Gas constant (8.314 J·mol^{-1}·K^{-1}), $\boldsymbol{T}$**:** average temperature during an experimental period [K] and $\boldsymbol{p}$**:** pressure in the gasmeter (1.013*10^5 Pa).

4.3.9 Volume variations in the AnMBR during an experimental period

During an experimental period, the sludge level in the reactor can vary. This has an influence on the pressure in the gas phase and, therefore, on the gas volume read on the gasmeter. In case of sludge level differences between the beginning and the end of an experimental period, a balance of the gas phase must be performed to calculate the effective produced gas volume. For this purpose, the biogas is assumed as an ideal gas. Knowing the temperature and the pressure in the gas phase at the beginning and at the end of the experimental period, the molar fluctuation in the gas phase $\boldsymbol{\Delta n}$ during the experimental period is calculated as follows:

$$\boldsymbol{\Delta n = \frac{1}{R} * \left(\frac{p_E * V_E}{T_E} - \frac{p_B * V_B}{T_B}\right)\ [mol]\ (21)}$$

with

$\boldsymbol{R}$**:** gas constant (8.314 J·mol^{-1}·K^{-1}), $\boldsymbol{p_B}$: pressure in the gas phase at the beginning of an experimental period [Pa], $\boldsymbol{p_E}$: pressure in the gas phase at the end of an experimental period [Pa], $\boldsymbol{V_B}$: gas phase volume at the beginning of an experimental period [m^3], $\boldsymbol{V_E}$ **:** gas phase volume at the end of an experimental period [m^3], $\boldsymbol{T_B}$: temperature in the reactor at the beginning of an experimental period [K] and $\boldsymbol{T_E}$ **:** temperature in the reactor at the end of an experimental period [K].

Using the ideal gas law, this molar fluctuation is converted to a volume fluctuation $\boldsymbol{\Delta V}$. For this purpose, the temperature over the experimental period is averaged:

$$\boldsymbol{\Delta V = \frac{\Delta n * R * T}{p}\ [m^3]\ (22)}$$

with

$\boldsymbol{\Delta n}$**:** molar fluctuation during an experimental period [mol], $\boldsymbol{R}$**:** gas constant (8.314 J·mol^{-1}·K^{-1}), $\boldsymbol{T}$**:** average temperature during an experimental period [K] and $\boldsymbol{p}$**:** pressure in the gasmeter (1.013*10^5 Pa).

Finally, the effective produced gas volume $\boldsymbol{V}$ is calculated using:

$$\boldsymbol{V = V_{read} + \Delta V\ [L]\ (23)}$$

with

$\boldsymbol{V_{read}}$**:** biogas volume read on the gasmeter during an experimental period [L] and $\boldsymbol{\Delta V}$**:** volume fluctuation during an experimental period [L].

4.3.10 Parameters relative to microalgae culture at a lab-scale

The volumetric biomass production rate $\boldsymbol{BPR}$ and the specific growth rate $\boldsymbol{\mu}$ are determined as follows:

$$\boldsymbol{BPR = \frac{C_f - C_i}{t_f - t_i}\ [g \cdot L^{-1} \cdot d^{-1}]\ (24)} \qquad \mu = \frac{ln\,(C_f/C_i)}{t_f - t_i}\ [d^{-1}]\ (25)$$

with

$\boldsymbol{C_i}$: initial microalgal biomass concentration [g·L^{-1}], $\boldsymbol{C_f}$: final microalgal biomass concentration [g·L^{-1}], $\boldsymbol{t_i}$: first day of the experiment [d] and $\boldsymbol{t_f}$**:** last day of the experiment [d].

The nutrient removal efficiency $\boldsymbol{RE}$ and removal capacity $\boldsymbol{RC}$ are calculated using the following equations:

$$\boldsymbol{RE = \frac{C_i - C_f}{C_i} * 100\ [\%]\ (26)} \qquad \boldsymbol{RC = \frac{C_i - C_f}{t_f - t_i}\ [g \cdot L^{-1} \cdot d^{-1}]\ (27)}$$

with

$\boldsymbol{C_i}$: initial nutrient concentration [g·L^{-1}], $\boldsymbol{C_f}$: final nutrient concentration [g·L^{-1}], $\boldsymbol{t_i}$: first day of the experiment [d] and $\boldsymbol{t_f}$**:** last day of the experiment [d].

4.3.11 Parameters relative to microalgae culture at full-scale

During the outdoor microalgae experiments at full-scale, the microalgae biomass production rate $\boldsymbol{BPR_a}$ and the nutrient removal capacity $\boldsymbol{RC_a}$ are calculated referring to the illuminated area of the PBR:

$$\boldsymbol{BPR_a = \frac{(C_f - C_i) * V_{PBR}}{(t_f - t_i) * A_{PBR}}\ [g \cdot m^{-2} \cdot d^{-1}]\ (28)}$$

with

$\boldsymbol{C_i}$: initial microalgal biomass concentration [g·L^{-1}], $\boldsymbol{C_f}$: final microalgal biomass concentration [g·L^{-1}], $\boldsymbol{t_i}$: first day of the experiment [d], $\boldsymbol{t_f}$**:** last day of the experiment [d], $\boldsymbol{V_{PBR}}$: volume of the line containing the PBR [L] and $\boldsymbol{A_{PBR}}$: illuminated area of the PBR [m^2].

The parameter $\boldsymbol{RC_a}$ is calculated as follows:

$$\boldsymbol{RC_a = \frac{(C_i - C_f) * V_{PBR}}{(t_f - t_i) * A_{PBR}}\ [g \cdot m^{-2} \cdot d^{-1}]\ (29)}$$

with

$\boldsymbol{C_i}$: initial nutrient concentration [g·L^{-1}], $\boldsymbol{C_f}$: final nutrient concentration [g·L^{-1}], $\boldsymbol{t_i}$: first day of the experiment [d], $\boldsymbol{t_f}$**:** last day of the experiment [d], $\boldsymbol{V_{PBR}}$: volume of the line containing the PBR [L] and $\boldsymbol{A_{PBR}}$: illuminated area of the PBR [m^2]

During the lab-scale experiments, the light conditions are expressed as photosynthetic photon flux density $\boldsymbol{PPFD}$, defined as the number of photons in the 400 - 700 nm waveband incident per unit time on a unit surface. During the full-scale experiments, the light conditions are expressed as solar

irradiance E_e. In order to allow comparisons between the light conditions, the following conversion relative to an artificial daylight source is applied (Lang *et al.* 1981):

$$E_e = \frac{PPFD}{2.1} \; [W \cdot m^{-2}] \; (30)$$

with

$PPFD$: photosynthetic photon flux density [µmol·s^{-1}·m^{-2}]

The photosynthetic efficiency PE, which is defined as the proportion of light energy converted into chemical energy during photosynthesis, is calculated using the following equation (Soletto *et al.* 2008):

$$PE = \frac{r * H}{E_e * A} * 100 \; [\%] \; (31)$$

with

r: daily biomass growth [g·d^{-1}], H: enthalpy of dry biomass (21.01 kJ·g^{-1}), E_e: solar irradiance [kJ·d^{-1}·m^{-2}] and A: illuminated surface [m^2].

4.3.12 Standard deviation

A standard deviation from the mean value is calculated when more than two measurement values are taken into account:

$$s = \sqrt{\frac{1}{n} * \sum_{i=1}^{n} (x_i - \overline{x})^2} \; (32)$$

with x_i: measurement value i, $\overline{x}$: mean value and n: number of measurement values.

4.4 Temporal development and main goals of the experimental work

The operation of the AnMBR was divided into two main periods. The period I (PI) was comprised between the 19.04.17 and the 19.02.18. Mid-march 2017, before the beginning of process operation, the reactor was seeded with 400 L of digested sludge from the WWTP Köhlbrandhöft, Hamburg. Until mid-April, the sludge was continuously circulated in the reactor and wastewater was regularly added. On the first day of PI, the sludge volume amounted to approximately 620 L in the reactor. Because of several technical problems between April and June 2017, only the results from the 28.06.17 are shown in this work.

Due to the sudden defect of the eccentric screw pump E24, the AnMBR operation was stopped from the 20.02.18 until the 09.04.18. Before the new start of the plant, the sheet that was set in the Y-branch of the wastewater pipeline was removed due to regular clogging. As both the wastewater volumes obtained and wastewater nutrient composition were consequently extremely low, large works on the wastewater pipelines were conducted in the BIQ at the beginning of May 2018. They aimed to supply the lifting plant with the whole domestic wastewater produced in the residential building (see section 4.1.2). The start of the period II (PII) on the 09.05.18 coincides with the start of wastewater supply using this new wastewater pipeline. Then, the AnMBR ran until the 15.10.18.

Table 11: Temporal division of the AnMBR operation

Period	**PI**						**PII**									
Phase	1	2			3		1	2		3		4		5	
Subphase	-	2a	2b	2c	3a	3b	-	2a	2b	3a	3b	4a	4b	5a	5b
Duration	21	86			62		13	30		53		29		33	

Table 12: Summary of the main goals of the experimental work

Experimental work	**Main goals**
AnMBR operation (full-scale)	• Assessment of the performance of the AnMBR process in relation to wastewater composition, COD removal, COD and BOD_5 concentrations in the permeate, biogas composition, methane production and the recovery of the main nutrients NH_4-N and PO_4-P in the permeate for microalgae cultivation • Search for the optimal operating parameters of the plant: HRT, OLR, F/M ratio • Comparison of the results obtained with conventional and decentralized WWTPs
Acutodesmus obliquus cultivation at the University of Hamburg (lab-scale)	• Comparison of microalgae growth and nutrient removal in the permeate culture and in a synthetic culture medium characterized by similar initial nutrient concentrations • Comparison of microalgae growth and nutrient removal in the permeate culture and in the permeate culture supplied with additional micronutrients • Comparison of the results with the literature • Comparison of the final nutrient concentrations with the German and European regulations
Acutodesmus obliquus cultivation at the TU Berlin (lab-scale)	• Confirmation of the differences observed between the permeate culture and the permeate culture enriched with additional micronutrients during the previous experiment • Investigation of the responsible micronutrients causing these differences • Comparison of the results with the literature • Comparison of the final nutrient concentrations with the German and European regulations
Chlorella vulgaris cultivation at the TU Berlin (lab-scale)	• Comparison of microalgae growth and nutrient removal in the permeate culture and in a synthetic culture medium • Confirmation of the observations made with permeate cultures and *Acutodesmus obliquus* during the previous experiments • Investigation of the causes of the differences observed between the different culture media • Comparison of the results with the literature • Comparison of the final nutrient concentrations with the German and European regulations
Chlorella sorokiniana cultivation at the TU Berlin (lab-scale)	• Assessment of the suitability of permeate characterized by increased nutrient concentrations for microalgae growth and nutrient assimilation • Comparison of growth and nutrient removal rates with a synthetic culture medium • Comparison of the results of the permeate culture with the results obtained with *Chlorella vulgaris* and *Acutodesmus obliquus* • Comparison of the results with the literature • Comparison of the final nutrient concentrations with the German and European regulations
Acutodesmus obliquus cultivation at the outdoor pilot-plant Hamburg-Reitbrook (full-scale)	• Comparison of growth and nutrient degradation rates at full-scale with the results obtained at lab-scale and the literature for full-scale pilot-plants • Assessment of the influence of weather conditions • Comparison of the final nutrient concentrations with the German and European regulations • Assessment of the suitability of permeate for microalgae cultivation at full-scale

The main periods PI and PII are divided into 3 and 5 phases respectively, which are defined by different operation parameters as the HRT or the OLR. These phases are also divided into subphases. The phases and subphases are detailed in Table 11.

Figure 29 represents the temporal development of the experiments. While the two main periods of the AnMBR operation are represented in the arrow, the different experiments conducted with the microalgae are represented around the arrow. It is to be noticed that the first experiment conducted with *Acutodesmus obliquus* in the University of Hamburg is not associated with a main period of the AnMBR operation. Indeed, in the time of this experiment, the anaerobic process was not stable due to multiple leakages. Finally, Table 12 summarizes the different aims of the experimental work.

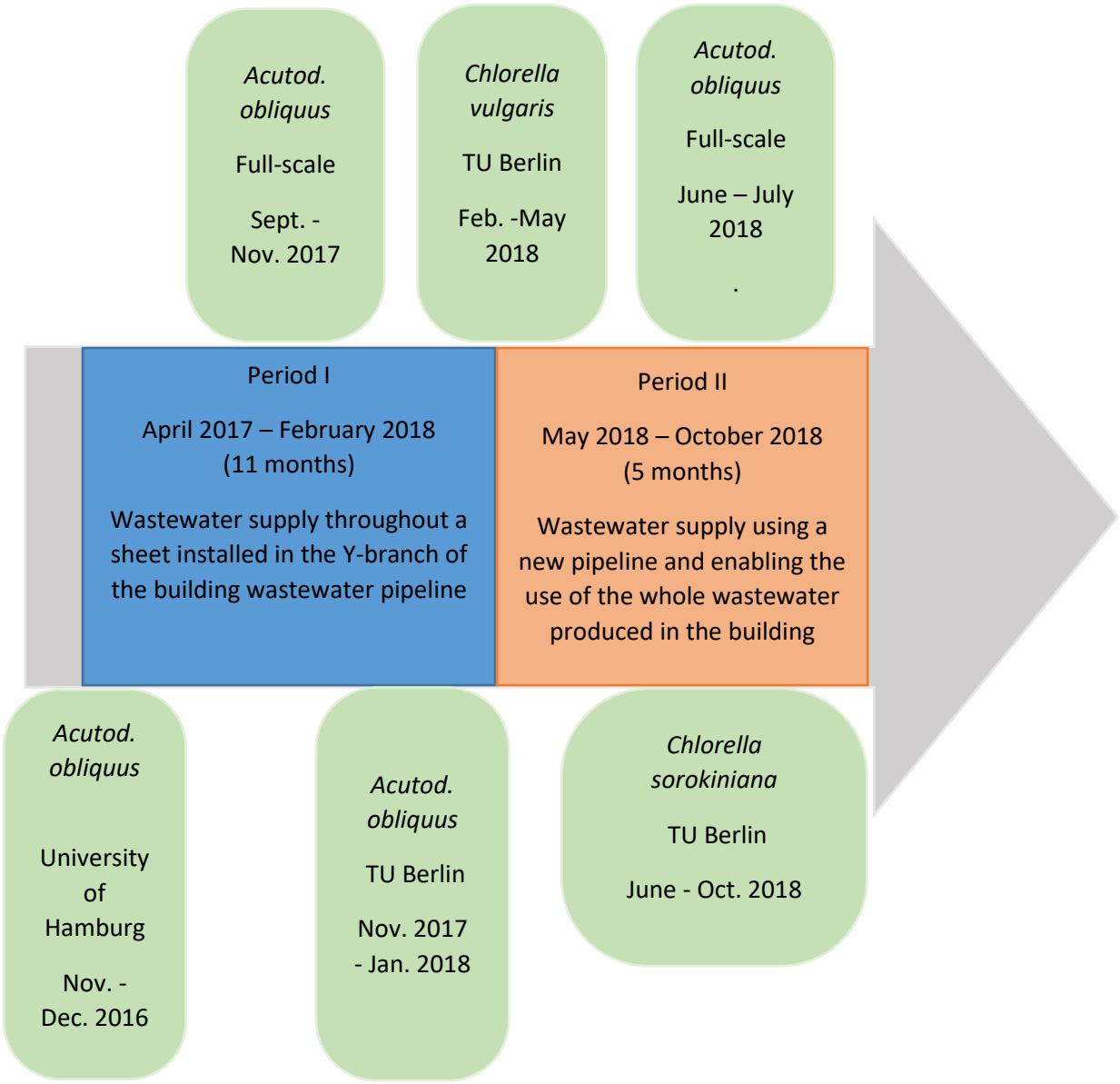

Figure 29: Temporal development of the experimental work of this thesis

5 Results and Discussion

5.1 AnMBR operation

5.1.1 Representability of the experimental results

In the following, the performance of the AnMBR process with regard to wastewater composition, biogas composition, COD removal, COD and BOD_5 concentrations in the permeate, as well as methane production is discussed. The related data is based on 50-mL samples of wastewater, sludge from the reactor and permeate that were taken once a week during PI and three times a week during PII. The gas phase was usually sampled every other week. Since the biogas composition is usually not subject to strong fluctuations within two weeks under stable operation, the measured biogas composition is considered to be representative of the process.

The sludge composition changed only stepwise in case of supply of very high-strength or low-strength wastewater, since the reactor volume was much higher than the daily wastewater volume supplied (HRT varying between 4 and 9 days). Moreover, as the reactor was continuously mixed, the measured values of the sludge composition are also considered representative of the system.

On the contrary, the experimental values related to wastewater composition are considered to be relatively inaccurate. Since the lifting pump was directly connected to the sewage pipeline of the house, very different wastewater streams were obtained, depending on whether it was wastewater from the shower, from the kitchen or from the toilets. Likewise, permeate composition was subject to large fluctuations and no linear course could be determined. Nevertheless, permeate composition is estimated to be more accurate than wastewater composition.

As an example, the development of the COD concentration in the wastewater, the sludge and the permeate during PI and PII is shown in Figure 30 and Figure 31. While the COD value in the sludge had few fluctuations and a nearly constant increasing course, the COD concentration in the wastewater and in the permeate was subject to larger fluctuations with several peak values. In comparison to the errors caused by the random sampling, the errors due to the measuring technique (standard cuvette tests, gasmeter, gas chromatography and TS and VS determinations) were considered very low.

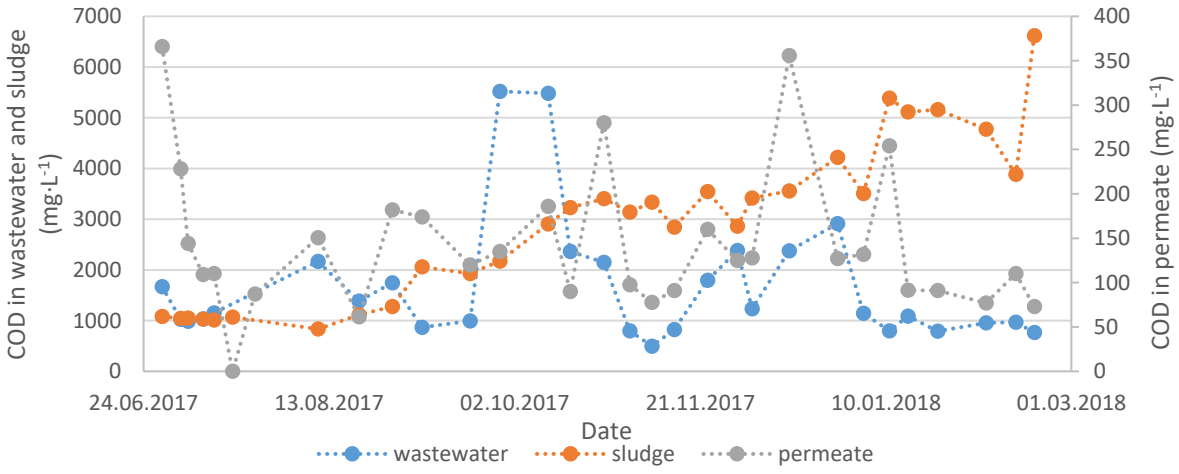

Figure 30: COD concentration in the wastewater, the sludge and the permeate during PI

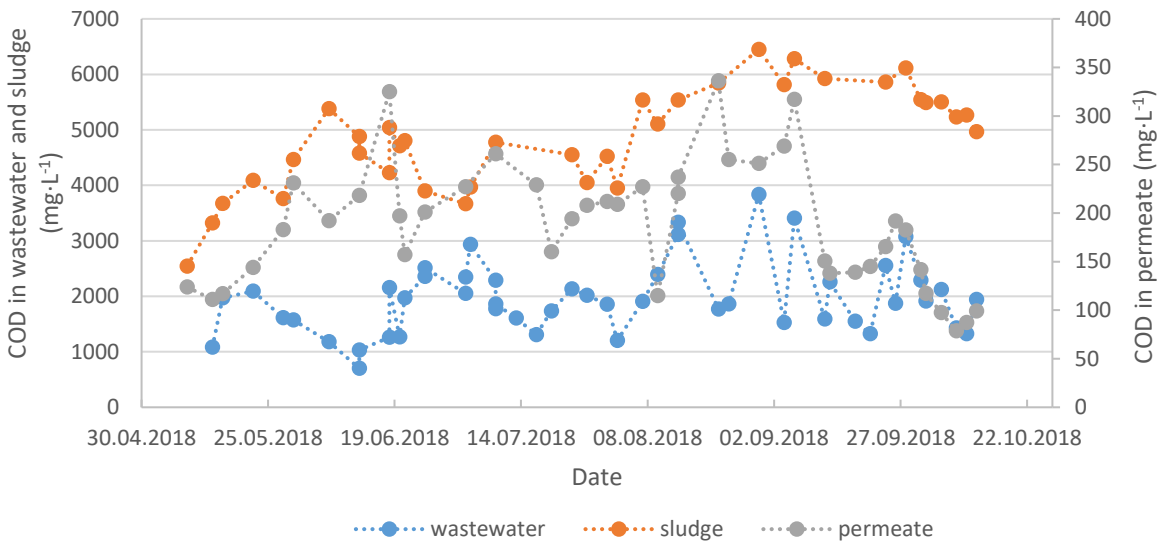

Figure 31: COD concentration in the wastewater, the sludge and the permeate during PII

5.1.2 Wastewater composition during the period I and the period II

Table 13: Wastewater composition during PI and PII

Parameter	Period I (PI)			Period II (PII)			Variation between PI and PII medians (%)
	Mean value	Median	Measuring range	Mean value	Median	Measuring range	
TS (g·kg^{-1})	1.41 ± 0.63	1.26	0.66 - 3.69	1.36 ± 0.36	1.28	0.80 - 2.38	**+1.6**
VS (g·kg^{-1})	0.98 ± 0.61	0.86	0.24 - 3.28	0.99 ± 0.40	0.90	0.34 - 2.27	**+4.7**
COD (g·L^{-1})	1.92 ± 1.23	1.74	0.50 - 6.17	1.97 ± 0.64	1.91	0.70 - 3.84	**+9.8**
BOD_5 (mg·L^{-1})	698 ± 272	699	240 - 1300	698 ± 259	611	357 - 1161	**-13**
COD/BOD_5	2.29 ± 0.70	2.02	1.21 - 3.47	3.30 ± 1.41	3.04	1.33 - 6.51	**+51**
TN (mg·L^{-1})	127 ± 49	120	57.6 - 293	142 ± 33	137	72.0 - 225	**+14**
TP (mg·L^{-1})	21.7 ± 10.1	21.1	7.50 - 42.4	33.5 ± 11.1	33.6	10.0 - 55.6	**+59**

Wastewater composition over PI and PII is summarized in Table 13. Concerning the parameters TS and VS, wastewater composition was very similar in both periods. While COD and TN showed a low increase of 10 % and 14 % respectively, the ratio COD/BOD_5 and TP had a significant increase of 51 % and 59 % respectively. Simultaneously, BOD_5 decreased by 13 %. The strong increase of TP concentration combined with the low increase of TN concentration may be due to the increase of feces proportion in the wastewater. Indeed, during PI, it was observed that profounder the semicircular sheet was set in the sewage pipe and the higher suspended solids proportion in the wastewater was. However,

because of regular blockages, this semicircular sheet was usually not set profound in the Y-branch. According to Boutin and Eme (2016), TN/TP in mixed wastewater (sum of the contributions of blackwater and greywater) amounts to 4, while TN/TP in urine and TN/TP in feces amount to 13 and 2 respectively. During PI, TN/TP was relatively high and reached 6. In PII, TN/TP decreased to a value of 4. This attests that, contrary to PI, all the feces flew to the lifting pump during PII. Nevertheless, according to the data of Boutin and Eme (2016), this increase of feces proportion in the wastewater should have led to an increase of 55 % and 39 % of the BOD_5 and COD concentrations respectively, as well as a significant increase of TS and VS concentrations. Several reasons could explain the relatively low increase of TS, VS and COD concentration and the decrease of BOD_5 concentration.

First, a decrease of toilet paper proportion could explain these opposing results, as this item brings a high contribution for these four parameters. This hypothesis is very plausible, as the residential building is composed of a few apartments and the change of only one habitant leads to fluctuations of wastewater composition, as each person possesses its own characteristics for wastewater production. This hypothesis was verified, as the lifting pump was very often clogged by toilet paper during PI. This has never happened during PII. Hence, the presence of the semi-circular sheet and the Y-branch during PI influenced wastewater composition throughout the retaining of toilet paper.

The simultaneously increase of TS, VS and COD concentrations and decrease of BOD_5 concentration might be the formation of a thick organic layer in the upper part of the plastic storage tank during PII, as well as the presence of flies, larva and earthworms in the tank. This phenomenon accentuated from July 2018, which coincides with the significant increase of the ratio COD/BOD_5. In the organic layer, a part of the organic matter was retained. In addition, the flies, larva and earthworms probably degraded a part of the organic matter, as it would be the case in an activated sludge process for example. This could explain the significant increase of the ratio COD/BOD_5.

Indeed, according to Rosenwinkel *et al.* (2015), if the ratio COD/BOD_5 approximates 2, the wastewater is defined as easily biodegradable, as it was the case during PI. For higher values, as in PII (median of 3.04), wastewater is not easily biodegradable. This high ratio obtained in PII would confirm the hypothesis that the wastewater was partially degraded in the storage tank. This has rarely happened during PI, because the wastewater volumes obtained were always too low and the HRT of the wastewater in the storage tank amounted to only a few hours. On the contrary, during PII, as much more wastewater as needed was received in the storage tank, the HRT of wastewater there amounted to several days and the organic layer, the flies, the larva and the earthworms could easily further grow.

The significant influence of the presence of the storage tank on the wastewater composition was proved by a two-days experiment. Figure 32 shows the fluctuations of COD concentration in wastewater on the 31.07.18 and 01.08.18 over 5 hours and 4 hours respectively. On the 31.07.18, during the experiment, the reactor was fed with 178 L of wastewater. During the five hours, the lifting pump upstream the storage tank was powered off, so that the storage tank was empty after the last measurement (31.07.18 16:15). During the evening, the lifting pump was powered back on. During the 4 hours measurements on the 01.08.18, the lifting pump was also powered back off and the reactor was supplied with a volume of 118 L of wastewater. The experiment aimed to observe the hypothetic differences between the composition of wastewater that had settled during several days in the storage tank (31.07.18) and fresh wastewater that stayed only a few hours in the storage tank (01.08.18). The goal was also to observe the differences in wastewater composition in case of the lowest part of the storage tank or the upper part corresponding to the thick organic layer is supplied into the reactor.

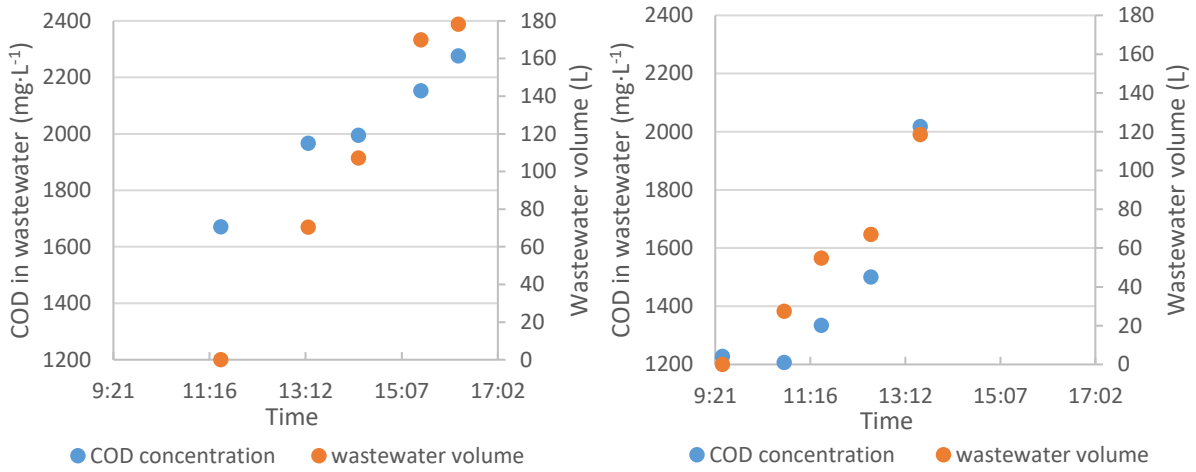

Figure 32: COD concentration in wastewater and wastewater volume supplied into the reactor during a two-days experiment - left: on the 31.07.2018; right: on the 01.08.2018

While the COD in wastewater amounted to between 1.7 and 2.3 g·L^{-1} on the 31.07.18, it was usually comprised in the range 1.2 - 1.5 g·L^{-1} on the 01.08.18. More precisely, on the 31.07.18, as the storage tank emptied, COD concentration in wastewater increased. Since the sewage pump is set on the lower part of the tank, as the emptying progressed, the thick organic layer always came nearer to the sewage pump. This probably caused the strong COD increase. On the contrary, the values in the range 1.2 - 1.5 g·L^{-1} on the 01.08.18 are more representative for the composition of the wastewater that is received at the lifting station, since the wastewater only stayed one night in the storage tank. The last measurement showing a COD concentration of 2.0 g·L^{-1} is due to the influence of the upper organic layer. Indeed, the storage tank was almost empty for the last measurement and the organic layer was not totally removed the day before.

Finally, the very large measuring ranges reached for each parameter attest to the continual fluctuations of wastewater composition. These fluctuations were expected, as it is a real plant connected to the domestic wastewater network. Depending on which wastewater stream (greywater, urine or feces) flows in the pipeline, the composition of wastewater received in the lifting pump varies. As explained above, these fluctuations were emphasized by the presence of the 230-L plastic storage tank. Moreover, because of the presence of the flies, earthworms and larva as well as the formation of the thick organic layer in the upper part of the tank, the storage tank was regularly cleaned with tap water or emptied in the sewers of the building. Therefore, the content of the storage tank was diluted, which led to occasional lower values related to wastewater composition, particularly in the summer 2018 (Figure 31).

5.1.3 Comparison of wastewater composition with literature for municipal wastewater

In Table 14, the wastewater composition obtained during PI and PII is first compared with literature values for municipal wastewater at the input of WWTPs. The mean values of the measured parameters (TS, VS, COD, BOD_5, TN and TP) were 18 % to 124 % higher than the literature values for high-strength domestic wastewater (Rawat *et al.* 2011). Therefore, domestic wastewater of the HAWANA project was classified as high-strength. This can be explained by the fact that the literature values indicate

wastewater composition at the input of a WWTP. Hence, these values are related to wastewater that has been transported through sewers to the WWTP and that has been possibly mixed with low-strength industrial wastewater, rainwater and street cleaning water. Moreover, during transport in the sewers, a part of the organic matter can be degraded or solids settle in the pipes (Brebbia and Kungolos 2007). This results in lower COD, TS and VS concentrations at the input of the WWTP.

Table 14: Comparison of wastewater composition during PI and PII with literature

Parameter	Median HAWANA during the period I	Median HAWANA during the period II	Composition of municipal wastewater (Rawat *et al.* 2011)			Composition of decentralized domestic wastewater (DWA 2008)
			Low-strength	medium	High-strength	
TS ($g \cdot kg^{-1}$)	1.26	1.28	0.350	0.720	1.200	1.52
VS ($g \cdot kg^{-1}$)	0.86	0.90	0.185	0.365	0.600	1.10
COD ($g \cdot L^{-1}$)	1.74	1.91	0.250	0.500	1.000	1.07
BOD_5 ($mg \cdot L^{-1}$)	699	611	110	220	400	393
TN ($mg \cdot L^{-1}$)	120	137	20	40	85	118
TP ($mg \cdot L^{-1}$)	21	34	4	8	15	18.3

5.1.4 Comparison of wastewater composition with literature for decentralized WWTPs

In order to verify the plausibility of the wastewater composition obtained during the HAWANA project, this composition was compared with data from the German Association for Water Management, Wastewater and Waste related to existing decentralized WWTPs with wastewater source separation (urine, feces and greywater) (DWA 2008). For each wastewater source and each parameter, using the daily volume flows and loads, the domestic wastewater composition was calculated (Table 14). Except for the COD, BOD_5 and TP (PII) HAWANA values, which were 63 - 79 %, 55 - 78 % and 86 % higher than the DWA values, all other parameters were close to the DWA values. This shows that the wastewater used in this project has similar properties compared to other existing decentralized WWTPs. The differences observed with the DWA data are normal, as wastewater composition is dependent on several parameters, as flush volume, frequency of the use of cleaning products, alimentation, amount of toilet paper used, presence of food leftovers, etc.

5.1.5 Nutrient recovery for microalgae cultivation throughout the AnMBR process

Table 15 shows the average concentrations of TN, NH_4-N, TP and PO_4-P for the periods June 2017 - February 2018 and May 2018 - October 2018 in the wastewater, reactor and permeate. This comparison aims to examine if a total recovery of the macronutrients contained in the wastewater is possible for microalgae growth through the AnMBR process. During PI, while TN concentration in the wastewater averaged 116 $mg \cdot L^{-1}$, the average TN concentration in the permeate reached 115 $mg \cdot L^{-1}$. This attests a complete recovery of the nutrient nitrogen in the permeate. With 99 $mg \cdot L^{-1}$, the mean value of NH_4-N in the permeate was higher than in the wastewater effluent (63 $mg \cdot L^{-1}$) and was

explained by the mineralization of organic nitrogen during the anaerobic process. Likewise, PO_4-P concentration was higher in permeate than in wastewater due to the mineralization of organic phosphorus (11 mg·L^{-1} in wastewater and 15 mg·L^{-1} in permeate). In total, 87 % of TP in wastewater was recovered in the permeate. Between May and October 2018, TN recovery was likewise almost complete and achieved 95 %. Nevertheless, TP recovery decreased and only reached 79 %. This could be explained by the slower mineralization of this nutrient due to the strong increase of TP concentration in wastewater. Hence, it was proved that the AnMBR pilot-plant is suitable for nutrient recovery aiming microalgae growth.

Table 15: Nutrient recovery throughout the AnMBR process

Parameter	June 2017 – February 2018				May 2018 – October 2018			
	Wastewater	Reactor	Permeate	Nutrient recovery (%)	Wastewater	Reactor	Permeate	Nutrient recovery (%)
TN (mg·L^{-1})	116 ± 45	241 ± 65	115 ± 37	99	142 ± 33	279 ± 43	135 ± 30	95
NH_4-N (mg·L^{-1})	63 ± 19	104 ± 40	99 ± 36	-	67 ± 19	101 ± 23	97 ± 26	-
TP (mg·L^{-1})	17 ± 6	34 ± 9	15 ± 5	87	34 ± 11	72 ± 19	27 ± 9	79
PO_4-P (mg·L^{-1})	11 ± 5	16 ± 5	15 ± 6	-	25 ± 7	25 ± 6	23 ± 7	-

5.1.6 Performance of the AnMBR pilot-plant

For both the experimental periods PI and PII, the times in which stable operation of the plant was achieved are subdivided into several phases corresponding to specific HRTs and OLRs (see section 4.4). It is to be noticed that during the entire phase 3 of PI, the temperature in the reactor ranged between 31.1 and 33.9 °C. These temperatures are up to 6 °C lower than the target temperature of 37 °C. These lower temperatures were caused by the defect of the thermostat and the heating of the reactor by means of a heat exchanger of the BIQ heat cycle as a temporary solution.

In the following, both the mean values and the medians are represented for the parameters that are dependent on the wastewater, sludge and permeate composition. Here, the use of the median is particularly relevant, since the presented data involves random samples and samples of wastewater covering a very wide range of COD concentration. In this case, there are more often outliers. As the mean value is very sensitive to such outliers, the results related to the medians are compared in the following section. Furthermore, while the results including the subphases are presented in the Appendix 3, for more clarity, only the results obtained during the entire phases 1 to 3 during the period I and 1 to 5 during the period II are represented in the form of tables in the following.

5.1.6.1 Period I

5.1.6.1.1 Phases 1 and 2

The phases 1 and 2 are characterized by temperatures approaching the target temperature of 37 °C (Table 16). Phase 1 (28.06.17 - 18.07.17) corresponds to a high HRT of 9.2 days. Between the 30.08.17 and 23.11.17 (phase 2), it comes to a much lower HRT of 5.1 days. This phase is divided into three subphases (subphase 2a, 2b and 2c), each corresponding to HRTs of 5.1 days, 4.2 days and 5.4 days. In total, the phase 2 lasted 86 days, which enabled the collection of more data.

At a HRT of 9.2 days (phase 1), the median related to the COD concentration in the permeate amounted to 144 mg·L^{-1}. This is in the upper range of the COD medians obtained in permeate during the phases and subphases 2, 2a, 2b and 2c (97 - 156 mg·L^{-1}). These increased values during the phase 1 can be explained by the startup of the AnMBR operation, as this phase was considered as an adaptation phase of the biocenosis to the wastewater.

Table 16: AnMBR performance during the phases 1 to 3 of PI - italic: average value - bold: median value

Parameter	Phase 1 28.06. - 18.07.		Phase 2 30.08. - 23.11.		Phase 3 19.12 - 19.02	
Duration (d)	21		86		62	
HRT (d)	9.2		5.1		4.8	
Temperature (°C)	37.2		35.9		32.8	
pH	6.8		6.3		6.1	
COD in wastewater (g·L^{-1})	*1.17*	**1.03**	*2.09*	**1.74**	*1.31*	**0.97**
VS in sludge (g·kg^{-1})	*0.77*	**0.76**	*1.61*	**1.62**	*2.48*	**2.40**
COD in permeate (mg·L^{-1})	*191*	**144**	*145*	**135**	*146*	**110**
COD removal (%)	*85*	**88**	*93*	**93**	*88*	**89**
COD biological removal (%)	*82*	**83**	*87*	**86**	*66*	**60**
CH_4 content (%)	80		69		71	
OLR (kg COD·m^{-3}·d^{-1})	*0.13*	**0.11**	*0.41*	**0.34**	*0.27*	**0.21**
F/M ratio (kg COD·kg^{-1} VS·d^{-1})	*0.16*	**0.15**	*0.25*	**0.21**	*0.11*	**0.09**
SBP (m^3 CH_4·m^{-3}·d^{-1})	0.023		0.048		0.029	
Methane yield (m^3 CH_4·kg^{-1} COD)	*0.18*	**0.20**	*0.12*	**0.14**	*0.11*	**0.14**
Methane production (m^3 CH_4·kg^{-1} COD_{rem})	*0.21*	**0.23**	*0.13*	**0.14**	*0.12*	**0.15**
BSP (kg VS· kg^{-1} COD_{rem})	*0.10*	**0.11**	*0.02*	**0.03**	*0.11*	**0.13**

During the experimental subphase 2b, a permeate concentration of 135 $mg \cdot L^{-1}$ was achieved with the shortest HRT of 4.2 days and an OLR of 1.30 kg $COD \cdot m^{-3} \cdot d^{-1}$. In comparison, a COD concentration of 135 $mg \cdot L^{-1}$ was achieved in the permeate over the entire experimental phase 2 with a HRT of 5.1 days and an OLR of 0.34 kg $COD \cdot m^{-3} \cdot d^{-1}$. These results show that despite an increase in the OLR during the subphase 2b, the pilot-plant was not overloaded and the quality of the permeate did not change. Therefore, in this temperature range, the HRT could be further reduced and the OLR further increased, which would allow higher daily treated wastewater volumes or smaller reactor volumes.

As VS in sludge regularly increased between the beginning of the phase 1 and the end of the phase 2, the parameters OLR and F/M ratios were not proportional. Overall, the F/M ratios amounted to between 0.08 and 0.84 kg $COD \cdot kg^{-1}$ $VS \cdot d^{-1}$. The highest F/M ratio was achieved with 0.84 kg $COD \cdot kg^{-1}$ $VS \cdot d^{-1}$ in the experimental phase 2b, which coincides with the shorter residence time of 4.2 days and the higher OLR of 1.30 kg $COD \cdot m^{-3} \cdot d^{-1}$.

Overall, a very high COD removal amounting to between 88 and 97 % was reached. The higher COD removal of 97 % was achieved with a HRT of 4.2 days (subphase 2b). This higher COD removal did not lead to a better permeate quality, as a very high COD concentration of 5.48 $g \cdot L^{-1}$ characterized the wastewater during the subphase 2b. During the other phases, lower COD removal values were determined with lower COD concentrations ranging 0.82 - 1.74 $mg \cdot L^{-1}$ in the feed.

The COD biological removal showed heterogeneous results (74 - 96 %). The relatively low values obtained during the phases 2a and 2c attest that high quantities of the supplied organic matter were not degraded by the biologic process but instead of that accumulated in the reactor. During the period 2b, COD removal and COD biological removal were identic (96 %), which proves the efficiency of the anaerobic process during this phase.

During the phases 1 and 2, CH_4 content in the gas phase regularly decreased, amounting to 81 % in the phases 1 and 2a and only to 61 % in the subphase 2c. The highest SBP of 0.068 m^3 $CH_4 \cdot m^{-3} \cdot d^{-1}$ was also achieved with the shortest HRT of 4.2 days during the phase 2b. As a comparison, at a HRT of 5.1 days (phase 2), the SBP amounted to 0.048 m^3 $CH_4 \cdot m^{-3} \cdot d^{-1}$. The methane yield amounted to 0.05 and 0.14 m^3 $CH_4 \cdot kg^{-1}$ COD during the subphase 2b and the entire phase 2 respectively. The lower value during the subphase 2b is explained by the fact that the process can not completely degrade the organic substances by higher loading rates because of less contact time between the substrate and the microorganisms.

The experimental subphase 2b showed the highest SBP with simultaneously a high OLR and a low COD concentration in the permeate. Therefore, this period can be considered as a reference for a proper AnMBR operation at a temperature approximating 37 °C. Nevertheless, these results are only based on a 19-days observation, which was characterized by extremely high COD concentration in wastewater. Hence, the results of the entire phase 2, which regroups data of an 86-days operation, seem more accurate. During this entire phase 2, the permeate quality and COD removal were also excellent but the HRT and the SBP were respectively slightly higher and lower compared to the subphase 2b.

These results obtained during the subphase 2b and the entire phase 2 attest for an efficient process as well as a good quality of the effluent. Consequently, the OLR should be increased in order to achieve a higher SBP, which is the most important parameter relating to methane production in full-scale applications, as it is related to the volume of reactor used. Nevertheless, the OLR must not be too high,

since a too high quantity of organic matter supplied could lead to an inhibition of the anaerobic process. Since simultaneously COD concentration in the wastewater decreased and the wastewater volumes available in the BIQ were too low, a further reduction of the HRT and an increase of the OLR under the same temperature conditions were not possible.

5.1.6.1.2 Phase 3

Phase 3 was also subdivided into two subphases characterized by HRT of 4.3 days (subphase 3a) and 5.6 days (subphase 3b). The HRT over the entire duration of the 62-days experimental phase 3 was 4.8 days. During this phase, lower reactor temperatures in the range of 31.1 - 33.9 ° C were achieved.

During the phase 3, a significant influence of the F/M ratio on the COD concentration in the permeate was observed. With a F/M ratio of 0.06 kg COD·kg^{-1} VS·d^{-1} (subphase 3b), a high-quality permeate with a COD concentration median of 84 mg·L^{-1} was achieved. In contrast, with a doubled F/M ratio of 0.12 kg COD·kg^{-1} VS·d^{-1} (subphase 3a), a 55 % higher COD concentration of 130 mg·L^{-1} was determined. However, in both cases, these values are representative for a good permeate quality and respect the requirements related to German small WWTPs.

Compared to the experimental phase 2, lower OLRs (0.16 - 0.26 kg COD·m^{-3}·d^{-1}) and F/M ratios were achieved. The reasons were the lower COD concentrations in wastewater (0.87 - 1.12 mg·L^{-1}) and the higher VS concentrations in the sludge (2.19 - 2.64 g·kg^{-1}) during the phase 3. Simultaneously, despite high values of 88 % to 91 %, COD removal was lower than during the phase 2 (88 - 97 %). Moreover, during the phase 3, the COD biological removal was relatively low (60 %). This shows that an important proportion of the organic matter supplied to the reactor only accumulated in the sludge instead of being degraded. While this parameter was still high during the subphase 3a (72 %), it only amounted to 34 % during the subphase 3b. The decrease of both COD removal and COD biological removal despite the relatively low OLR and F/M ratio was surely due to the temperature in the reactor. Indeed, the lower average temperature as well as the temperature drop from 35 °C to 25 °C within three weeks in November negatively affected the biocenosis in the reactor.

During the experimental phase 3, the CH_4 content in the gas phase was stable at 71 - 72 %. Like in the phase 2, the highest SBP of 0.039 m^3 CH_4·m^{-3}·d^{-1} was obtained at the lowest HRT of 4.3 days and the highest OLR of 0.26 kg COD·m^{-3}·d^{-1}. This corresponded also to the highest methane yield of 0.15 m^3 CH_4·kg^{-1} COD. Compared to the phase 2, the methane yield obtained in the phase 3 was similar and the SBP was relatively lower.

In the phase 3, the subphase 3a appears as the reference period. During this subphase, the highest values related to the daily volumes of wastewater treated and the parameter SBP were obtained. Despite the higher OLR and F/M ratio, COD concentration in the permeate stayed at a relatively low median of 130 mg·L^{-1}, which assures a good quality of the permeate. However, as for the experimental subphase 2b, in order to know if the requirements for small WWTPs with regard to the COD concentration in the permeate can be met in the long term under these temperature and OLR conditions, the relating low HRT of 4.3 days should be set for a longer observation time.

5.1.6.1.3 Summary of Period I

In summary, it can be assumed that with a temperature approximating 37 °C, the optimal OLR and the optimal F/M ratio amount to 1.30 kg COD·m^{-3}·d^{-1} and 0.84 kg COD·kg^{-1} VS·d^{-1} respectively for this system. These parameters coincide with the highest SBP of 0.068 m^3 CH_4·m^{-3}·d^{-1}. In addition, the COD concentration median of 135 mg·L^{-1} in the permeate meets the legal requirements for small WWTPs.

However, the limited wastewater volumes available combined with the decrease of COD concentration in wastewater from the end of October 2017 made an increase of the OLR and the F/M ratio as well as a reduction of the HRT impossible. Hence, the optimal OLR and F/M ratio related to this system could be higher, as well as the optimal HRT could be lower.

At a lower temperature range of 31.1 - 33.9 °C, the optimal conditions were reached during the experimental subphase 3a, which was characterized by an OLR of 0.26 kg COD·m^{-3}·d^{-1} and a F/M ratio of 0.09 kg COD·kg^{-1} VS·d^{-1}, as well as the highest temperature of 33.9 °C. These conditions led to COD concentrations in the permeate of 130 mg·L^{-1}, which comply with the legal requirements regarding small WWTPs. The corresponding SBP amounted to 0.039 m^3 CH4·m^{-3}·d^{-1}.

5.1.6.2 Period II

5.1.6.2.1 Phase 1

Phase 1 directly started after the installation of the new sewage pipeline system in the residential building (see section 4.4). As a reminder, before the start of PII, the AnMBR plant was not operated during 1.5 month between the end of February and the beginning of April 2018. Then, between the beginning of April and the beginning of May, the plant was unregularly fed with extremely low-strength wastewater. Consequently, in the beginning of PII, the anaerobic process was in a "restart" phase and was characterized by a weak biocenosis and a relatively low CH_4 content of 65 % in the gas phase. This phase lasted only 13 days because of the sudden blockage of the membrane caused by a defect of the mud trap. In addition, the temperature reached a high average value of 41 °C due to the defect of the thermostat.

During this phase 1, with a COD concentration of 120 mg·L^{-1}, an excellent permeate quality was reached (Table 17). This was due to the high COD removal of 94 %, which was the best over the entire period PII. However, this excellent COD removal was mostly caused physically by the membrane, since the COD biological removal only reached 48 %. This led to a high BSP of 0.84 kg VS·kg^{-1} COD_{rem}. These results show that the process was still not optimal and more time was needed to reactivate the microorganisms contained in the sludge. During the phase 1, the OLR and the F/M ratio amounted to 0.26 kg COD·m^{-3}·d^{-1} and 0.10 kg COD·kg^{-1} VS·d^{-1} respectively. Moreover, because of a defect of the gasmeter, no parameter relating to biomethane production was calculated.

5.1.6.2.2 Phase 2

Likewise, during the phase 2, no gas volume measurement was possible. This phase lasted 30 days, which corresponds to 7 times the HRT of 4.2 days. This means that the results of this phase are representative for a long-term operation of the plant. During this phase, the CH_4 content in the gas phase stabilized at 72 % and, compared to the phase 1, wastewater was 19 % less concentrated. The increase of the daily volume of wastewater supplied to the system led to an increase of 23 % and 30 % of the OLR (0.32 kg COD·m^{-3}·d^{-1}) and the F/M ratio (0.10 kg COD·kg^{-1} VS·d^{-1}) respectively. This presumably led to the lower COD removal of 86 % and the higher COD concentration of 195 mg·L^{-1} in the permeate. In parallel, the biological COD removal reached a higher value of 74 %, showing that the anaerobic process had restarted.

The phase 2 was divided into two subphases 2a and 2b. In the subphase 2a, with 188 mg·L^{-1} COD in the permeate and 87 % COD removal, the characteristics of the process were slightly better. This was probably due to the slightly lower OLR (0.32 kg COD·m^{-3}·d^{-1}) and F/M ratio (0.13 kg COD·kg^{-1} VS·d^{-1}).

However, the most important difference was the biological COD removal, which reached 62 % and 85 % respectively during the subphases 2a and 2b. Since the COD removal also amounted to 85 % during the subphase 2b and the CH_4 content stabilized, it was considered that the restart phase after the 3 months of shutdown of the plant had ended.

5.1.6.2.3 Phase 3

Table 17: AnMBR performance during the phases 1 to 5 of PII - italic: average value - bold: median value

Parameter	Phase 1 10.05. - 22.05.		Phase 2 23.05. - 21.06.		Phase 3 22.06. – 13.08.		Phase 4 14.08. – 11.09.		Phase 5 12.09. – 14.10.	
Duration (d)	13		30		53		29		33	
HRT (d)	7.5		4.2		5.7		5.6		7.0	
Temperature (°C)	41.0		37.6		37.0		36.7		36.2	
pH	6.4		6.5		6.7		6.64		6.5	
COD in wastewater ($g \cdot L^{-1}$)	*1.71*	**1.97**	*1.48*	**1.42**	*2.07*	**2.02**	*2.55*	**2.49**	*1.95*	**1.93**
VS in sludge ($g \cdot kg^{-1}$)	*2.69*	**2.69**	*2.38*	**2.38**	*2.81*	**2.68**	*3.37*	**3.36**	*2.86*	**2.81**
COD in permeate ($mg \cdot L^{-1}$)	*124*	**120**	*206*	**195**	*202*	**209**	*254*	**253**	*133*	**139**
COD removal (%)	*93*	**94**	*86*	**86**	*90*	**89**	*90*	**89**	*93*	**92**
COD biological removal (%)	*40*	**48**	*75*	**74**	*87*	**86**	*87*	**86**	*102*	**102**
CH_4 content (%)	65		72		76		74		73	
OLR ($kg\ COD \cdot m^{-3} \cdot d^{-1}$)	*0.23*	**0.26**	*0.36*	**0.34**	*0.37*	**0.36**	*0.46*	**0.45**	*0.28*	**0.28**
F/M ratio ($kg\ COD \cdot kg^{-1}\ VS \cdot d^{-1}$)	*0.09*	**0.10**	*0.15*	**0.14**	*0.13*	**0.13**	*0.14*	**0.13**	*0.10*	**0.10**
SBP ($m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}$)	-		-		0.057		0.058		0.038	
Methane yield ($m^3\ CH_4 \cdot kg^{-1}$ COD)	-	**-**	-	**-**	*0.16*	**0.16**	*0.13*	**0.13**	*0.14*	**0.14**
Methane production ($m^3\ CH_4 \cdot kg^{-1}\ COD_{rem}$)	-	**-**	-	**-**	*0.18*	**0.19**	*0.15*	**0.15**	*0.13*	**0.14**
BSP ($kg\ VS \cdot kg^{-1}\ COD_{rem}$)	*1.16*	**0.84**	*0.00*	**0.00**	*0.03*	**0.03**	*0.00*	**0.00**	*-0.05*	**-0.05**

Because of the relatively high COD concentrations in the permeate during the subphase 2b and the increasing COD concentrations in wastewater, the daily target permeate volume was decreased from 120 $L \cdot d^{-1}$ to 100 $L \cdot d^{-1}$ at the beginning of the phase 3. Consequently, the HRT increased and reached 5.7 days. However, due to the extremely high COD concentration of 2.02 $g \cdot L^{-1}$ compared to the phase 2, the OLR and the F/M ratio were similar to phase 2 and amounted to 0.36 kg $COD \cdot m^{-3} \cdot d^{-1}$ and 0.13 kg $COD \cdot kg^{-1}\ VS \cdot d^{-1}$ respectively.

COD removal amounted to 89 % and was slightly higher than in the phase 2. The biological COD removal was also very high and reached 86 %, which definitely attests the optimal anaerobic conditions in the reactor. The CH_4 content in the gas phase was also slightly higher than during the phase 2 and amounted to 76 %. During this phase, the SBP, methane yield and methane production amounted to respectively 0.057 $m^3 CH_4 \cdot m^{-3} \cdot d^{-1}$, 0.16 $m^3 CH_4 \cdot kg^{-1}$ COD and 0.19 $m^3 CH_4 \cdot kg^{-1} COD_{rem}$.

Despite the high COD removal, the COD concentration in the permeate was comparatively high and reached 209 $mg \cdot L^{-1}$. Here, it must be taken into consideration that two canals of the membrane were damaged at the end of the phase 3, which negatively influenced the COD concentration in the permeate. Considering the measurements of soluble COD which were done additionally on the permeate samples, COD concentration in permeate over the entire period should amount to 186 $mg \cdot L^{-1}$ with an undamaged membrane.

The subphases 3a and 3b were characterized by similar parameters, except for the VS concentration in sludge that was much higher in the subphase 3b, as well as the biological COD removal and the F/M ratio that were relatively lower during the subphase 3b. Likewise, better SBP (0.060 $m^3 CH_4 \cdot m^{-3} \cdot d^{-1}$), methane yield (0.17 $m^3 CH_4 \cdot kg^{-1}$ COD) and methane production (0.23 $m^3 CH_4 \cdot kg^{-1} COD_{rem}$) were reached during the subphase 3b.

5.1.6.2.4 Phase 4

At the end of the phase 3, the two identified damaged canals were sealed with adhesive resin. After this work on the membrane, a target value of 100 $L \cdot d^{-1}$ permeate was further applied. This aimed to see if a better COD concentration could be reached using an undamaged membrane and to observe if the process could adapt to the relatively high supply of organic matter. During this phase, a similar HRT to phase 3 (5.6 days) was reached. Nevertheless, the 23 % higher COD concentration of 2.49 $g \cdot L^{-1}$ led to a much higher OLR of 0.45 kg $COD \cdot m^{-3} \cdot d^{-1}$. Because of the simultaneous increase of VS concentration in sludge, the F/M ratio stayed similar at 0.13 kg $COD \cdot kg^{-1}$ $VS \cdot d^{-1}$.

During this phase 4, similar COD removal and COD biological removal of 89 % and 86 % respectively were reached. Despite the very good efficiency of the process, permeate quality deteriorated. The COD concentration in permeate amounted to 253 $mg \cdot L^{-1}$, which was presumably due to the significant increase of the OLR. A similar CH_4 content and SBP of 74 % and 0.058 $m^3 CH_4 \cdot m^{-3} \cdot d^{-1}$ respectively were achieved. However, the methane yield and methane production significantly decreased, reaching only 0.13 $m^3 CH_4 \cdot kg^{-1}$ COD and 0.15 $m^3 CH_4 \cdot kg^{-1} COD_{rem}$.

The phase 4 was divided into 2 subphases. The subphase 4a was characterized by a low HRT of 4.8 days and, due to technical problems, the subphase 4b was characterized by a much higher HRT of 7.5 days. During the subphase 4a, with 3.12 $g \cdot L^{-1}$, the COD concentration in wastewater was at its highest value over the entire period PII. This led to the highest OLR and F/M ratio of 0.65 kg $COD \cdot m^{-3} \cdot d^{-1}$ and 0.19 kg $COD \cdot kg^{-1}$ $VS \cdot d^{-1}$. This increase had a direct influence on the methane yield and methane production values, which reached the lowest values over PII (0.08 $m^3 CH_4 \cdot kg^{-1}$ COD and 0.09 $m^3 CH_4 \cdot kg^{-1} COD_{rem}$). Likewise, despite the higher organic matter supply, the SBP reached a low value of 0.052 $m^3 CH_4 \cdot m^{-3} \cdot d^{-1}$. This shows that the organic matter supply was too important during the subphase 4a, leading to an incomplete degradation of the organic matter supplied and sludge accumulation.

On the contrary, the parameters related to biogas production were higher during the subphase 4b. In this subphase, the biological COD removal was extremely high and even higher than the COD removal,

which comes from the degradation of organic matter accumulated during the subphase 4a. Nevertheless, as the subphase 4b lasted only 12 days, these results may be inaccurate.

5.1.6.2.5 Phase 5

The daily target permeate volume was decreased from 100 $L \cdot d^{-1}$ to 80 $L \cdot d^{-1}$ and aimed a lower supply of organic matter and a better permeate quality. With 1.93 $g \cdot L^{-1}$ and 2.81 $g \cdot kg^{-1}$, the COD concentration in wastewater and the VS concentration in the sludge were similar to the phase 3. The decreased wastewater supply led to a higher HRT of 7.0 days and a reduced OLR and F/M ratio of 0.28 kg $COD \cdot m^{-3} \cdot d^{-1}$ and 0.10 kg $COD \cdot kg^{-1}$ $VS \cdot d^{-1}$ respectively. This had a direct influence on the COD removal, which was with 92 % the second highest COD removal over PII. Because of this higher removal and the decrease of COD concentration in wastewater, an excellent permeate quality with a COD concentration of 139 $mg \cdot L^{-1}$ was reached. However, the SBP logically decreased and only amounted to 0.038 m^3 $CH_4 \cdot m^{-3} \cdot d^{-1}$. The BSP achieved a negative value of -0.05 kg $VS \cdot kg^{-1}$ COD_{rem} that was caused by the decrease of VS concentration in the sludge contained in the reactor. This was probably due to the increase of the HRT, which enabled the anaerobic degradation of organic matter that had accumulated in the reactor. Simultaneously, the methane yield and methane production amounted to 0.14 m^3 $CH_4 \cdot kg^{-1}$ COD and 0.14 m^3 $CH_4 \cdot kg^{-1}$ COD_{rem} respectively.

5.1.6.2.6 Summary of Period II

Over the period PII, the phases 1 and 5 appear as references, as they are characterized by an excellent permeate quality, which is the main aim of the pilot-plant. However, theses phase are also characterized by a high HRT of 7 days and a low SBP of 0.038 m^3 $CH_4 \cdot m^{-3} \cdot d^{-1}$ (phase 5), which are both key parameters for the economic viability of the process.

In the future operation of the plant, a situation similar to the phase 4, and particularly to the subphase 4a, has to be avoided. Indeed, despite the higher OLR during this phase, the SBP was lower than in other phases, which is characteristic for a hyperacidity of the milieu caused by the supply of a too high quantity of organic matter. In the phases 2 and 3, with intermediate OLR and F/M ratio of 0.34 - 0.36 kg $COD \cdot m^{-3} \cdot d^{-1}$ and 0.13 - 0.14 kg $COD \cdot kg^{-1}$ $VS \cdot d^{-1}$, a high SBP of 0.057 m^3 $CH_4 \cdot m^{-3} \cdot d^{-1}$ and an average COD concentration in permeate of approximately 200 $mg \cdot L^{-1}$ were achieved. Hence, when an excellent permeate quality is not the main aim, theses phases with the according OLR and F/M ratio should be privileged.

5.1.6.3 Summary of the performance achieved during Period I and Period II

Figures 33 to 36 resume the performance of the AnMBR process reached during the eight entire experimental periods for the parameters COD concentration in wastewater, HRT, OLR, COD removal, SBP and COD concentration in permeate. First, the great standard deviations of COD concentration in wastewater can be seen in Figure 33. These amounted to between 21 % and 82 % of the COD concentration in wastewater. As it was already mentioned in the sections 5.1.1 and 5.1.3, these comparatively high standard deviations are normal in wastewater treatment. Indeed, wastewater composition varies according to the origin of the stream. However, these high standard deviations strongly influence the reliability of the parameters depending on COD concentration in wastewater, like the parameters OLR and COD removal. During PII 2, PII 3, PII 4 and PII 5, the mean value of COD concentration in wastewater was close to the median. Nevertheless, during the other experimental phases, because of several peak values, the difference between the mean value and the median represented between 12 % and 25 % of the median value. The use of the median enables to decrease

the influence of these outliers. Overall, while PII 3 and PII 4 were characterized by COD concentrations in wastewater higher than 2 $g \cdot L^{-1}$, these reached less than 1.5 $g \cdot L^{-1}$ during PI 1, PI 3 and PII 2.

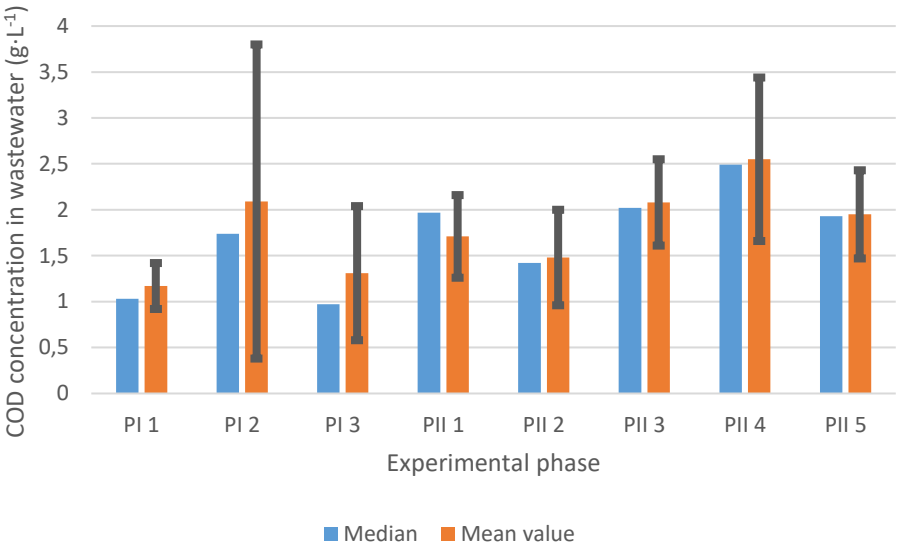

Figure 33: Variation of COD in wastewater during PI and PII (median, mean value and standard deviation)

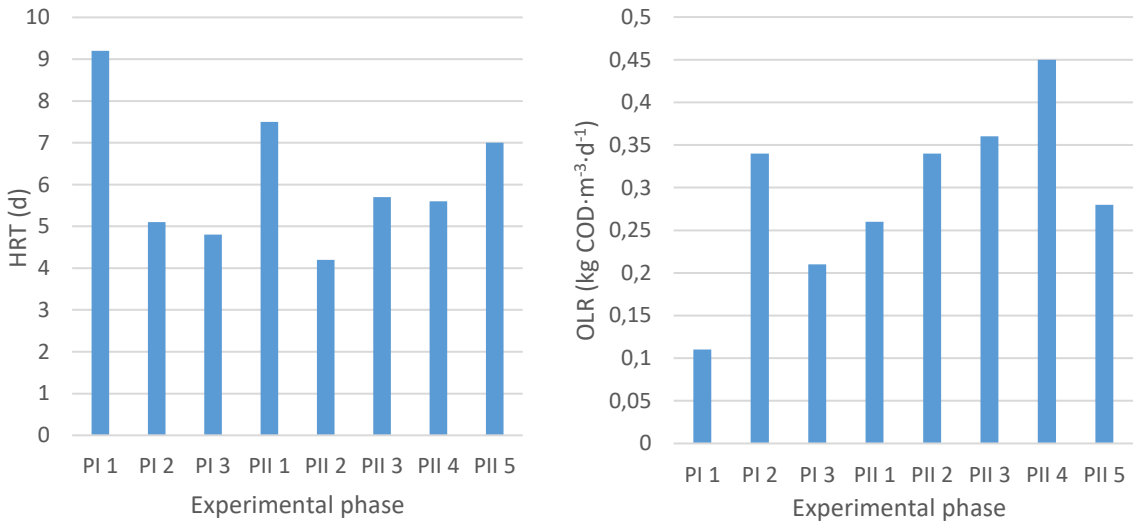

Figure 34: Variation of HRT (left) and OLR (right) during PI and PII (median)

Figure 34 clearly shows that the parameters HRT and OLR were not proportional due to the strong variations of the COD concentration in wastewater. During PI, despite similar HRTs during the phases 2 and 3, the OLR was approximately 62 % higher during the phase 2. In addition, starting from the phase 3 of PII, the diagram clearly shows that the increase of the HRT aiming a decrease of the OLR did not work for the phases 3 and 4. Indeed, despite the increase of the HRT, the OLR further increased in the phases 3 and 4 of PII. In total, the HRT ranged between 4 and 9 days, the OLR reached its lowest value during PI 1 (0.11 kg $COD \cdot m^{-3} \cdot d^{-1}$) and its highest value during PII 4 (0.45 kg $COD \cdot m^{-3} \cdot d^{-1}$).

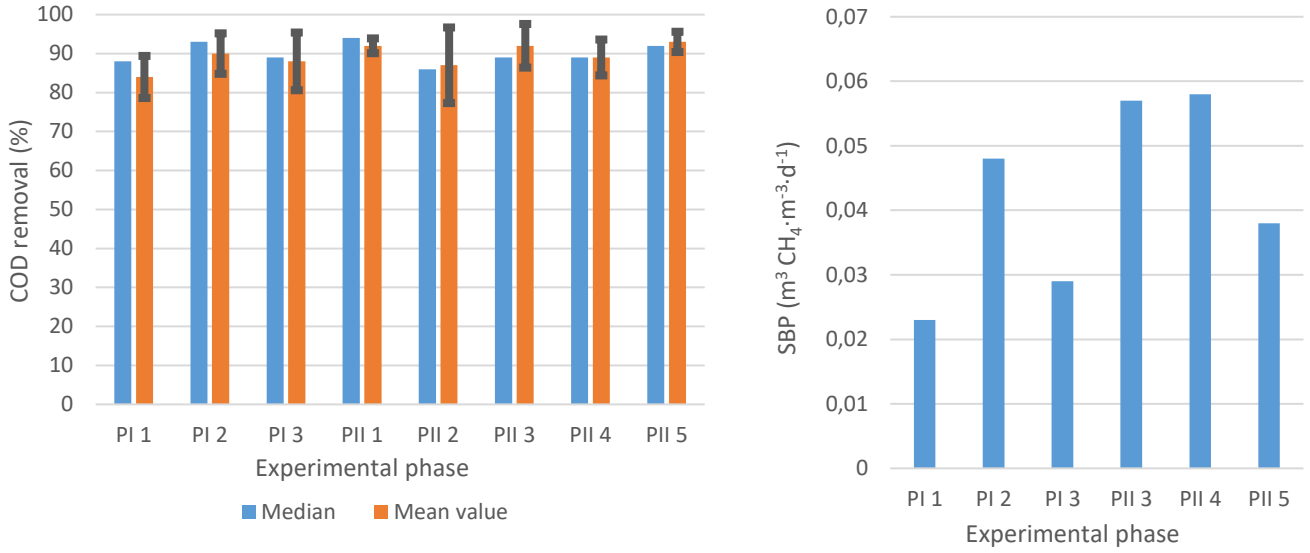

Figure 35: Variation of COD removal (median, mean value and standard deviation - left) and SBP (median - right) during PI and PII

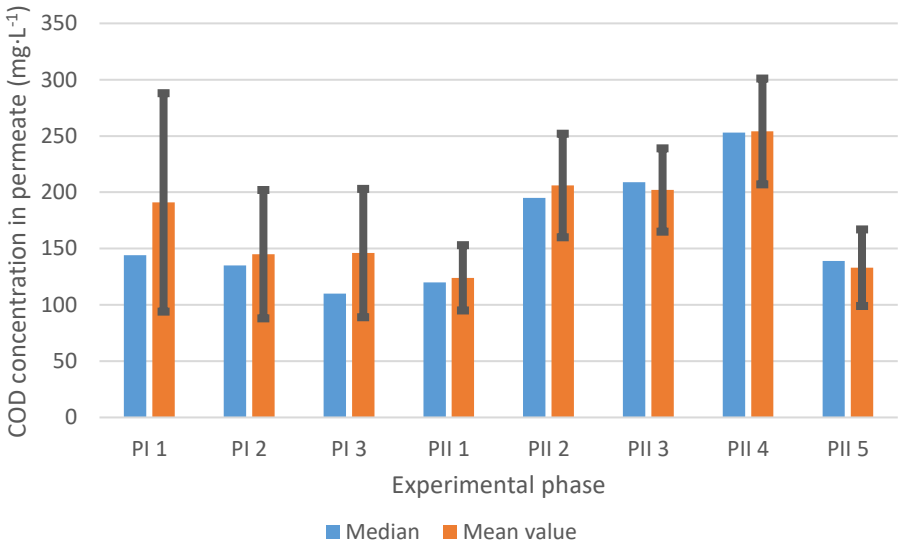

Figure 36: Variation of COD in permeate during PI and PII (median, mean value and standard deviation)

Concerning the parameter COD removal (Figure 35), the median was calculated using the median of COD concentration in wastewater and permeate over a whole experimental phase as well as the total volume of wastewater supplied in the reactor and the total volume of the permeate. For the mean value, COD removal was calculated on each sampling day and the standard deviation represents the fluctuations of COD removal between the different sampling days. Overall, both values were very close and the standard deviation ranged between 2 % and 10 %. Nevertheless, the value related to the median of COD concentration in wastewater and permeate over an entire experimental phase seems here more accurate. Indeed, on a sampling day, the permeate is not obtained from the wastewater that is sampled on this day, but from wastewater that first stayed a few days in the reactor. Hence, the mean value calculated is less precise.

Using the median, COD removal above 90 % was achieved during PI 2, PII 1 and PII 5. During these 3 phases, as well as during PI I and PI 3, the COD concentration in permeate was less than 150 $mg \cdot L^{-1}$

(Figure 36) and respected the German requirements for the discharge of wastewater streams from small WWTPs into the water bodies. Nevertheless, as for COD concentration in wastewater, the strong variations of COD concentration in permeate lead to high standard deviations. These ranged between 10 % and 51 % of the value of the COD concentration in permeate. Finally, the highest values related to SBP were obtained during PI 2, PII 3 and PII 4 (Figure 35). Hence, the reference phase in the present work is PI 2, which is simultaneously characterized by a high COD removal, a high SBP and a low COD concentration in the permeate. During PII, the phase 5 can be seen as the reference. However, during PII 5, the OLR and the SBP were not as high as during PI 2, probably because of the weak biocenosis after the 3 months of shutdown of the plant combined with the absence of filling of new seed sludge into the reactor. Moreover, PII was characterized by extremely high COD concentration in the wastewater, which probably influences the performance of the AnMBR pilot-plant.

Compared to the literature (Table 18), CH_4 proportions above the literature values of 25 - 75 % were reached between July and the end of September 2017 (PI). At the same time, the CO_2 levels were 22 to 67 % lower than indicated in the literature. It was assumed that the reactor had a low hydrogen partial pressure, which favored the formation of CH_4 from CO_2 and hydrogen. From October 2017 until the end of PI, CH_4 and CO_2 contents were in the upper range of the literature values. Except for the measurements conducted on the 20.12.17 and 14.02.18 (3.4 and 2.4 % respectively), O_2 concentration was always within the average literature values (< 2 %). This was not the case for N_2, whose content is reported in the literature as < 2 %.

Similar behaviors were observed during PII. Starting from the end of May 2018, CH_4 content was always in the upper range of the literature values. Like during PI, CO_2 content was up to 38 % lower than the lowest value indicated in the literature. While N_2 content during PII was also similar to PI, O_2 was up to 60 % lower during PII, which guaranties more optimal anaerobic conditions.

Table 18: Comparison of biogas composition with literature (FNR 2016)

Component	Literature (%)	HAWANA PI (%)	HAWANA PII (%)
CH_4	25 - 75	60 - 84	71 - 80
CO_2	24 - 45	8 - 35	15 - 24
N_2	< 2	2 - 16	2 - 12
O_2	< 2	0.3 - 3	0.2 – 1.2

Overall, the potential of the HAWANA AnMBR pilot-plant in terms of biogas composition has been demonstrated. At a high CH_4 content, the subsequent gas treatment before biogas use may be cheaper, because less CO_2 has to be separated. One possibility to reuse the biogas is the decentralized generation of heat and electricity, for example in a combined heat and power plant (CHP). The injection of CH_4 into the house's gas network or the production of cold by means of adsorption or absorption processes are other possible applications.

5.1.6.4 Correlations between the main parameters characterizing the AnMBR pilot-plant

In this section, correlations between the main parameters characterizing an AnMBR process are investigated. First, the influence of the HRT, the wastewater composition and the VS concentration in sludge on the OLR and the F/M ratio is studied. Then, correlations are established for the parameters

that are of main interest for the process: the quantity of biomethane produced (parameter SBP), the efficiency of the process (COD removal) and the effluent quality (COD concentration in the permeate). The values related to the subphase 2b of the period 1 are not taken into consideration because of the comparatively extremely high COD concentration in the influent (5.48 $mg \cdot L^{-1}$). Using these correlations, this study aims to predict and plan the future operation of decentralized AnMBR processes.

5.1.6.4.1 OLR and F/M ratio

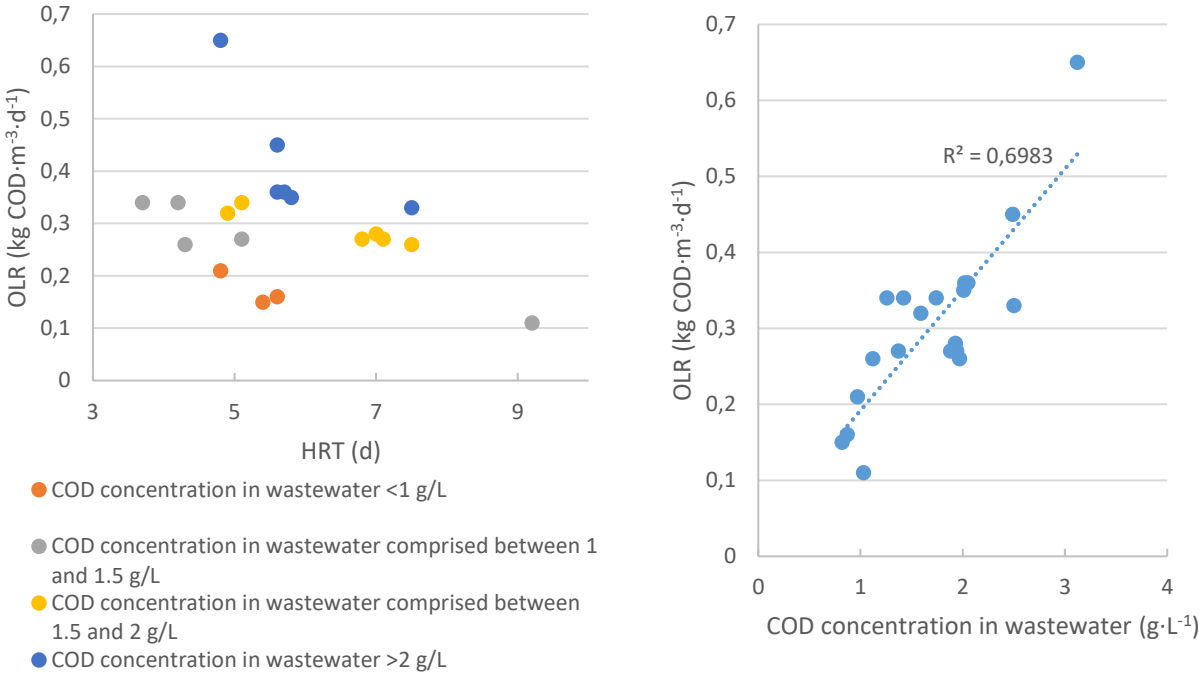

Figure 37: Variation of OLR according to HRT (left) and COD concentration in wastewater (right) during PI and PII

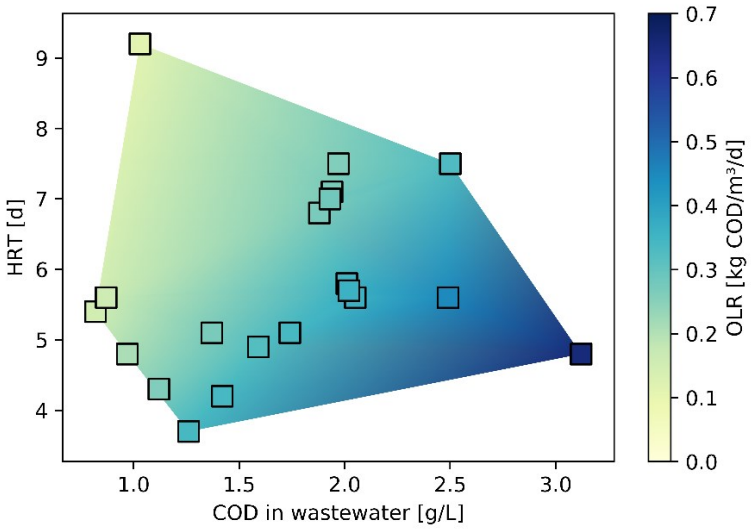

Figure 38: Variation of OLR according to both HRT and COD concentration in wastewater during PI and PII

First, the dependence of the OLR to other parameters is studied. Indeed, since the process is more profitable with higher amounts of wastewater treated, the OLR is one of the most important parameters of the process. Here, contrary to what was expected, a correlation with the HRT could not

be found (Figure 37). This was explained by the significant and constant fluctuations of wastewater composition. However, a linear correlation with a coefficient of determination R^2 of 0.70 can be draw between the COD concentration in wastewater and the OLR.

These results underline the main difficulty of this work, which was to correctly adapt the OLR to the need of the process. The OLR is usually set by accordingly adapting the HRT. However, here, since the dependence of the OLR to the HRT was extremely low and the COD concentration in wastewater could not be known instantly, this was extremely difficult. Since the fluctuations in the wastewater composition were much higher in the present work than in most conventional or decentralized WWTPs, this difficulty was even greater. This example shows the challenge to move from a lab-scale process to a pilot-plant process. Figure 38 summarizes the variations of the parameter OLR as a function of both the wastewater composition and the HRT. Such graphic may be helpful for the future operation of AnMBR processes in decentralized WWTPs.

During the experimental phases, it was also tried to adapt the F/M ratio in order to study the behavior of the system to changes of this parameter. Indeed, the F/M ratio characterizes the system more accurately than the OLR, as this parameter takes into consideration both the daily supply of organic matter and the sludge concentration in the reactor. To accordingly set the F/M ratio represented a further challenge, as this parameter depends on both the OLR and the VS concentration in sludge, which are fluctuating parameters.

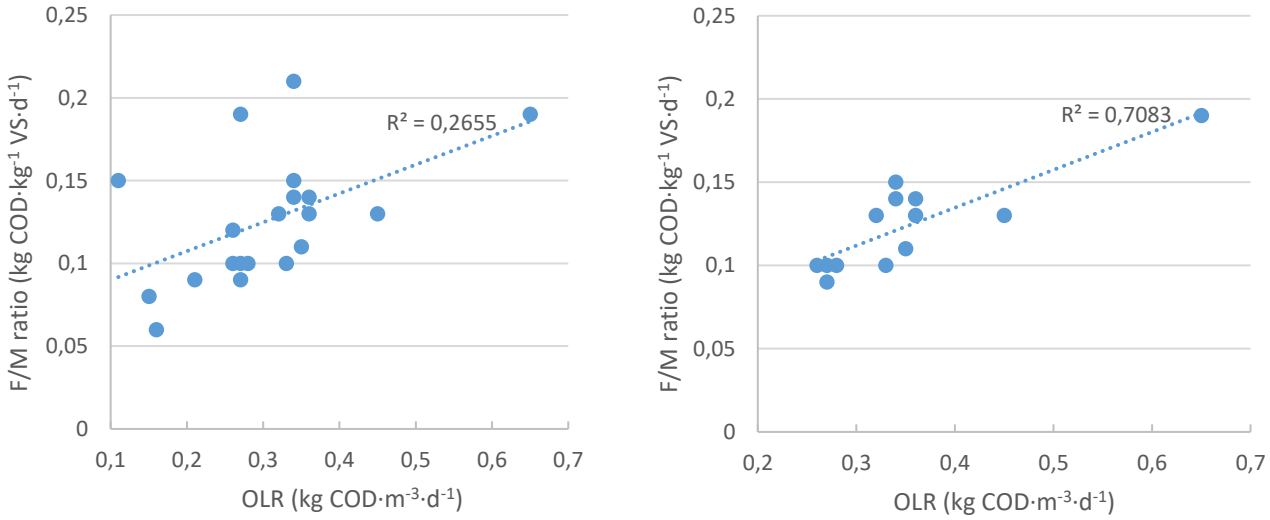

Figure 39: Variation of the F/M ratio according to the OLR during both PI and PII (left) and only PII (right)

The coefficient of determination R^2 of the linear correlation between the F/M ratio and the OLR only amounted to 0.27 (Figure 39), presumably because of the increase of the VS concentration in sludge from 0.76 $g \cdot L^{-1}$ at the beginning of PI to 3.36 $g \cdot L^{-1}$ at the end of PII. However, by separating PI and PII, an adapted correlation ($R^2 = 0.71$) was found between the F/M ratio and the OLR during PII. Related to PI, the F/M ratio was as much dependent on the OLR ($R^2 = 0.43$) as on the VS concentration in sludge ($R^2 = 0.45$) (Figure 40). Figure 41 summarizes the F/M ratio as a function of both the OLR and the VS in sludge during PI and PII.

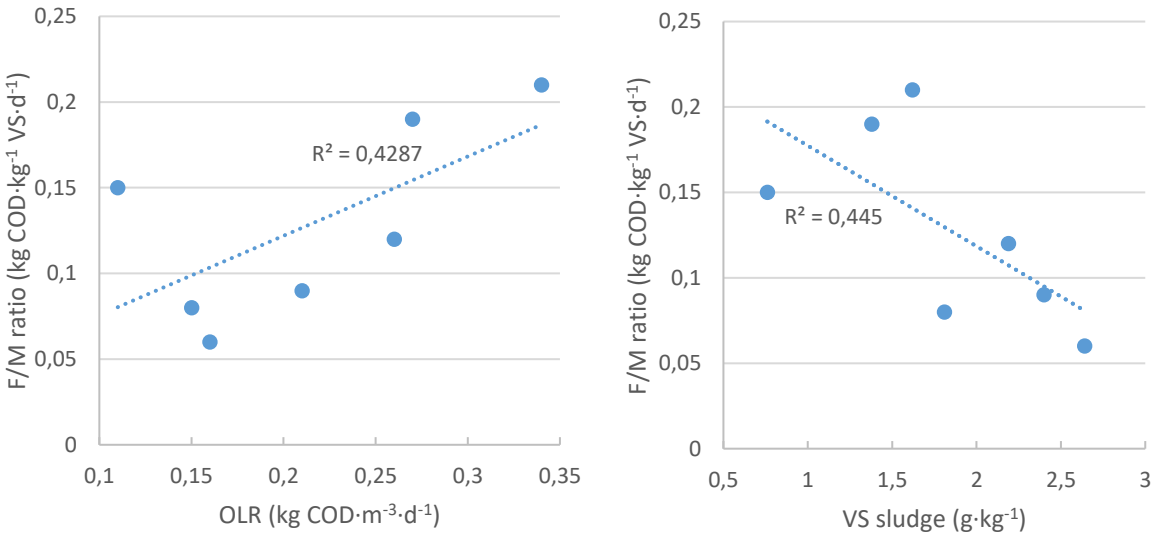

Figure 40: Variation of the F/M ratio according to the OLR (left) and VS concentration in sludge (right) during PI

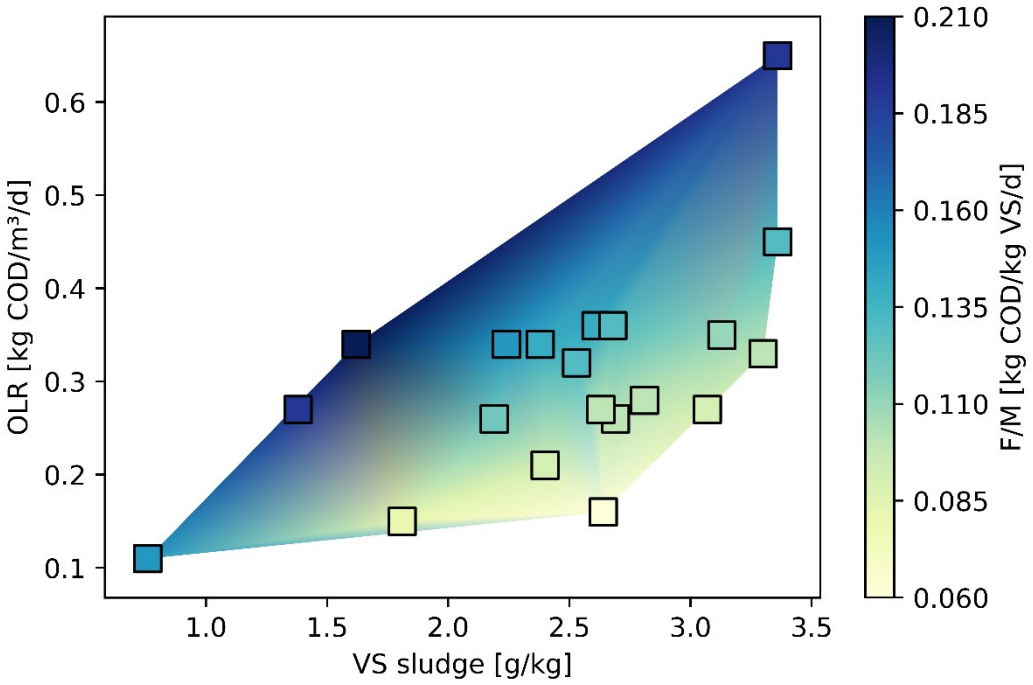

Figure 41: Variation of the F/M ratio according to both the OLR and VS concentration in sludge during PI and PII

5.1.6.4.2 Methane production (parameter SBP)

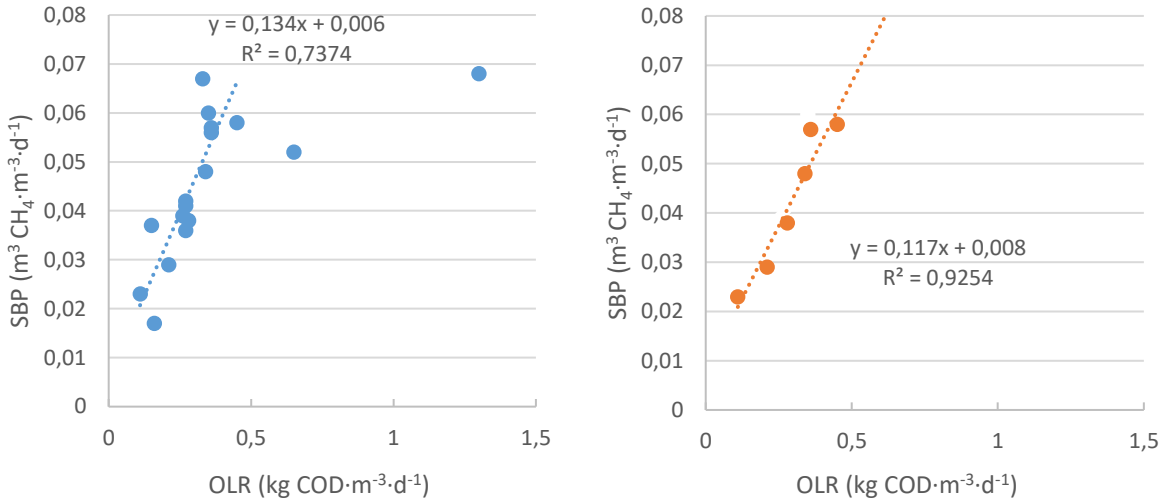

Figure 42: Variation of the SBP according to the OLR during all experimental phases and subphases of PI and PII (left) and all the entire experimental phases of PI and PII (right)

Up to an OLR value of 0.45 kg COD·m^{-3}·d^{-1}, a linear correlation was found between the SBP and the OLR (Figure 42). With values much higher than 0.45 kg COD·m^{-3}·d^{-1}, the related SBP was lower or only slightly higher than the SBP reached at an OLR of 0.45 kg COD·m^{-3}·d^{-1}. This phenomenon is well described in the literature. Figure 43 shows that, starting from an OLR value to be determined, the SBP slowly decreases and then significantly decreases, meaning an oversupply of organic matter and leading to the acidification and the crash of the anaerobic process. As the SBP is defined by the amount of methane produced per volume unit of reactor and per day, it is the most important parameter related to biogas production from an economic point of view. If the emphasis is put on the methane production, the AnMBR plant should be operated in the rated range comprised between an OLR of approximately 0.35 and 0.45 kg COD·m^{-3}·d^{-1}. In addition, taking only into consideration the entire experimental phases 2 and 3 of PI and 3 to 5 of PII, the coefficient of determination R^2 amounted to 0.93, which shows that entire phases characterized by longer experimental times are more accurate.

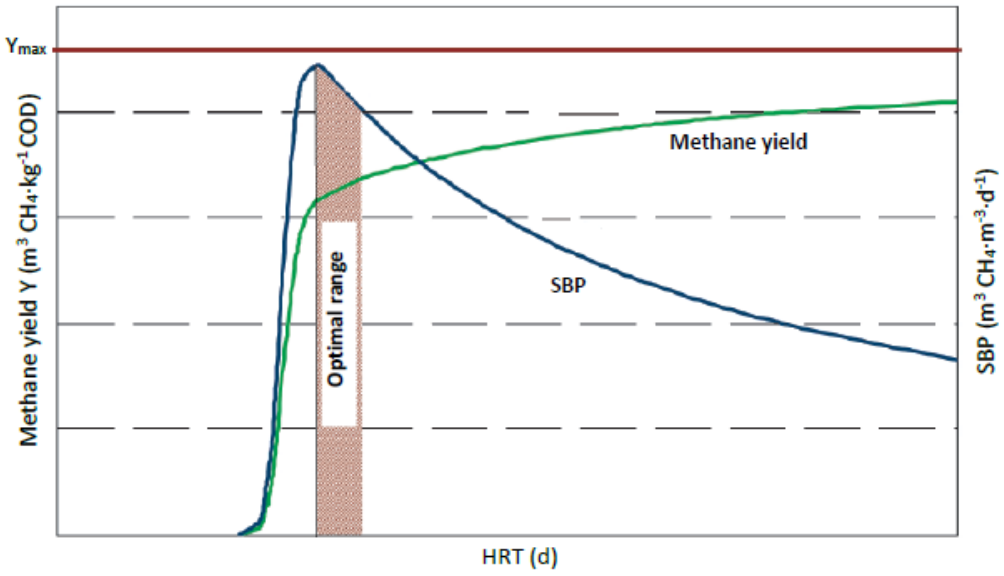

Figure 43: SBP and methane yield according to the OLR (Linke 2006)

5.1.6.4.3 Efficiency of the process (COD removal)

When both the periods PI and PII are taken into consideration, no correlation can be established between the COD removal and the HRT, the OLR or the F/M ratio. Only an inaccurate correlation between the COD removal and the COD concentration in wastewater could be found ($R^2 = 0.16$). This correlation shows increasing COD removal by increasing COD concentration in wastewater (Figure 44). Indeed, the particulate part of the COD increased with increasing COD concentrations in wastewater. This led to a higher COD retention due to the membrane, also when the biological COD removal was not optimal.

Here, it was expected to observe a dependence of the COD removal on the F/M ratio. Starting from a value to be determined, COD removal should decrease with increasing F/M ratios, as the contact time with microorganisms is too low for an optimal biodegradation. Nevertheless, this phenomenon was not observed (Figure 45). It is to be noticed that similar observations were made taking into consideration the biological COD removal as a function of the COD concentration in wastewater, the HRT, the OLR and the F/M ratio.

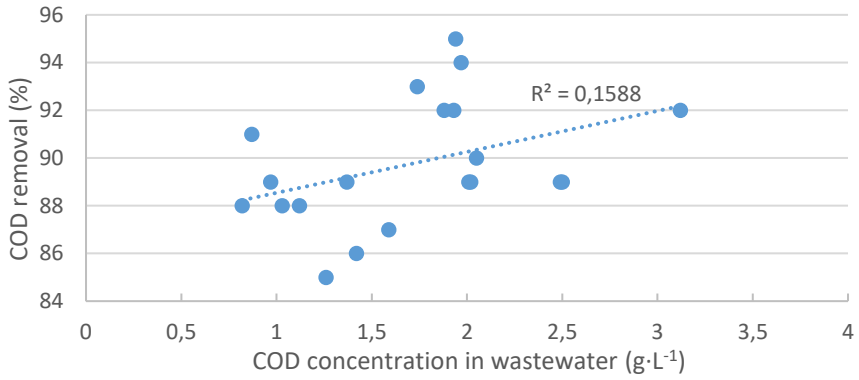

Figure 44: COD removal according to COD concentration in wastewater during PI and PII

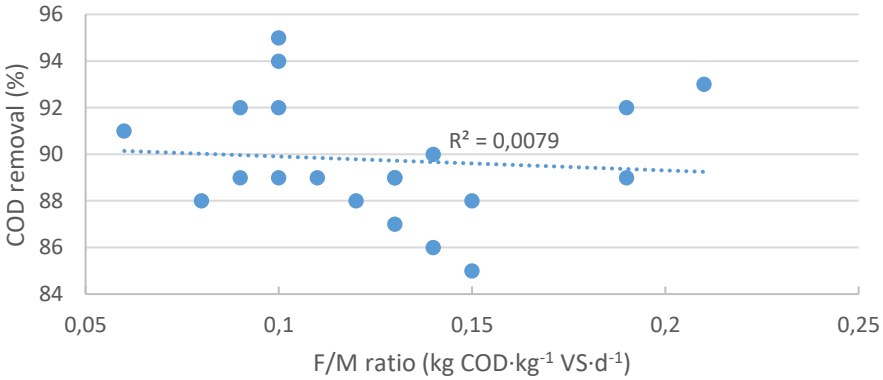

Figure 45: COD removal according to the F/M ratio during PI and PII

Related to PII, a correlation was found between the COD removal and the HRT (Figure 46). A linear regression with a coefficient of determination R^2 of 0.58 was established and showed increasing COD removal with increasing HRT. Taking into consideration only the entire phases 1, 2, 3, 4 and 5 of PII, which are characterized by longer experimental time meaning more accurate results, the coefficient of determination R^2 amounts to 0.99. This shows a perfect correlation between the COD removal and the HRT. Furthermore, COD removal as a function of both the COD concentration in wastewater and the HRT clearly shows the difficulty to precisely predict the efficiency of the process (Figure 47).

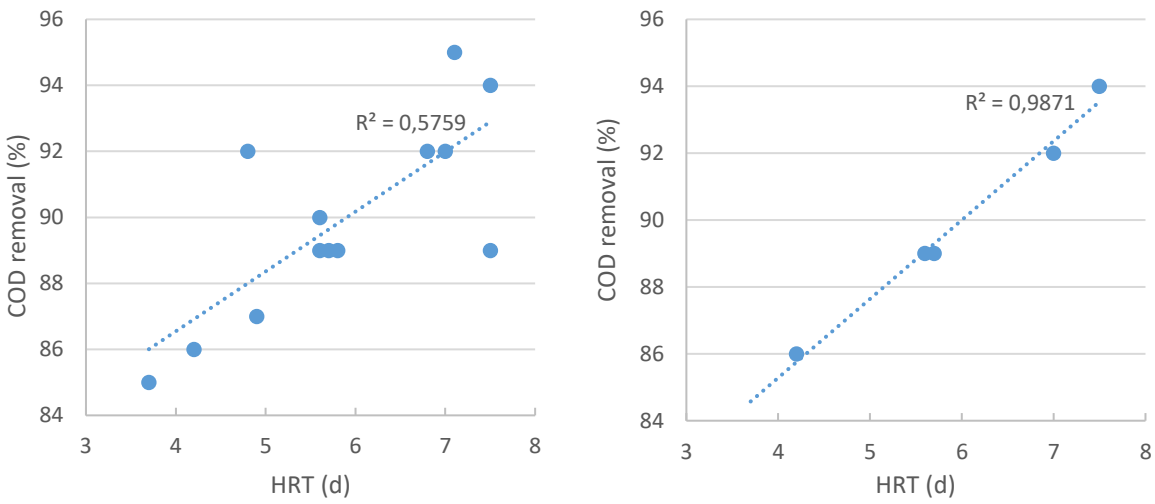

Figure 46: COD removal according to the HRT during all the experimental phases and subphases of PII (left) and all the experimental phases of PII (right)

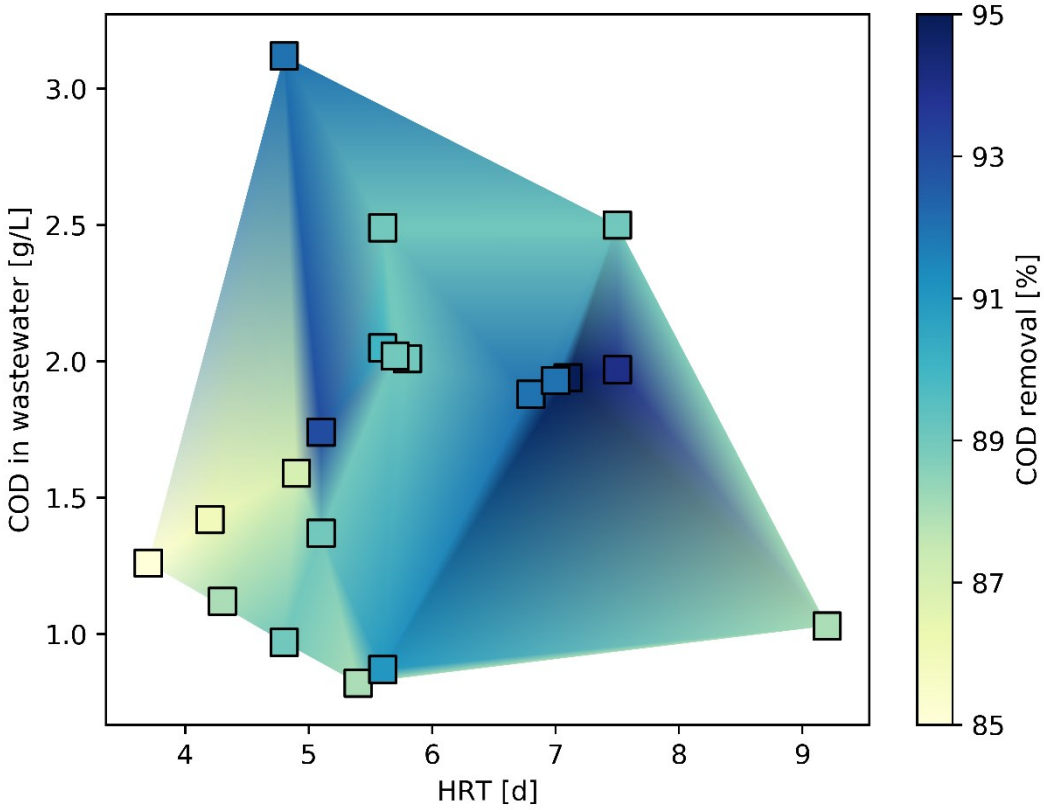

Figure 47: COD removal according to both the HRT and COD concentration in wastewater during PI and PII

5.1.6.4.4 Effluent quality (COD concentration in the permeate)

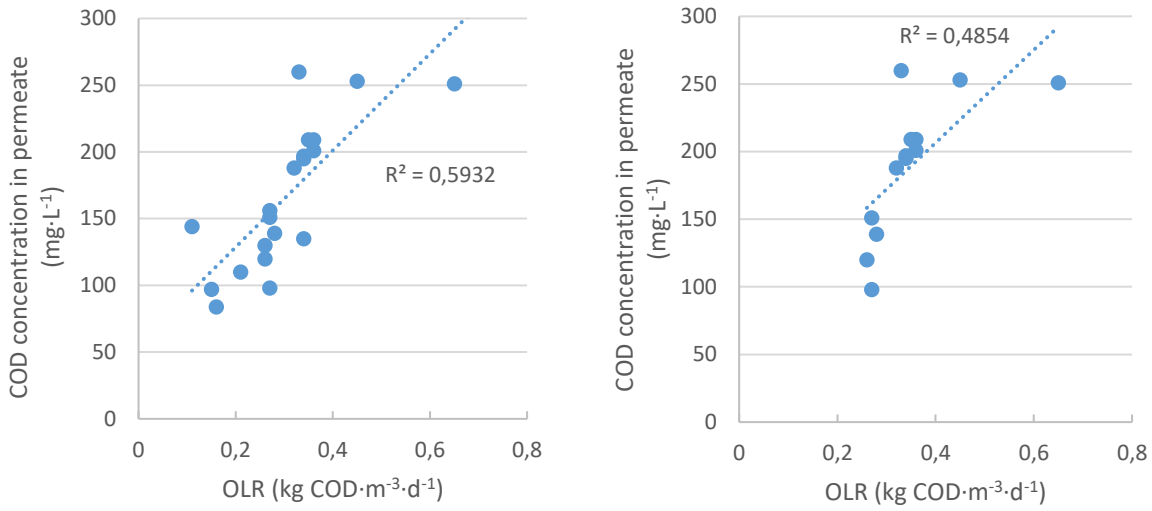

Figure 48: COD concentration in permeate according to the OLR during both PI and PII (left) and only during PII (right)

Taking into consideration both periods PI and PII, a first linear correlation was found between the COD concentration in the permeate and the OLR (R^2 = 0.59 - Figure 48). Logically, the COD concentration in the permeate increased with increasing OLR values. Separating PI and PII, no adequate correlation could be established for PI. This may be due to the short experimental times of each phase, as well as the limited measurements points compared to PII. Relating to PII, a linear correlation was established with a R^2 amounting to 0.49.

A dependence of the COD concentration in the permeate on the COD concentration in the wastewater was established with a similar coefficient of determination (R^2 = 0.52 – see Figure 49). Hence, higher COD concentrations are obtained in the permeate when the wastewater is higher concentrated. Indeed, increasing COD concentrations in wastewater lead to more organic matter supply into the reactor and less time for substrate degradation by the biocenosis present in the sludge.

However, no accurate regression was established between the COD concentration in the permeate and the F/M ratio. For the same reasons as thus mentioned for the parameter COD removal (section 5.1.6.4.3), a linear regression may have been expected. In addition, for the dependence of the COD concentration in the permeate on the HRT, a correlation could be found only for the entire experimental phases 1, 2, 3, 4 and 5 of PII (R^2 = 0.49 – see Figure 50). Logically, it shows the decrease of the COD concentration in the permeate with increasing HRTs. This is explained by the longer contact time with the microorganisms present in the reactor for the biodegradation of the substrate as well as the non-accumulation of VFA (soluble COD) caused by a too fast acidogenesis due to the oversupply of organic matter. Figure 51 represents the effluent quality as a function of both the OLR and the COD concentration in wastewater. For future operations of the AnMBR, this graphic can be used to find in which ranges a permeate quality respecting the requirements for small WWTPs in Germany is reached. In this case, a good effluent quality is obtained for OLR values up to 0.3 kg COD·m^{-3}·d^{-1} and COD concentrations in wastewater up to 2 g·L^{-1}.

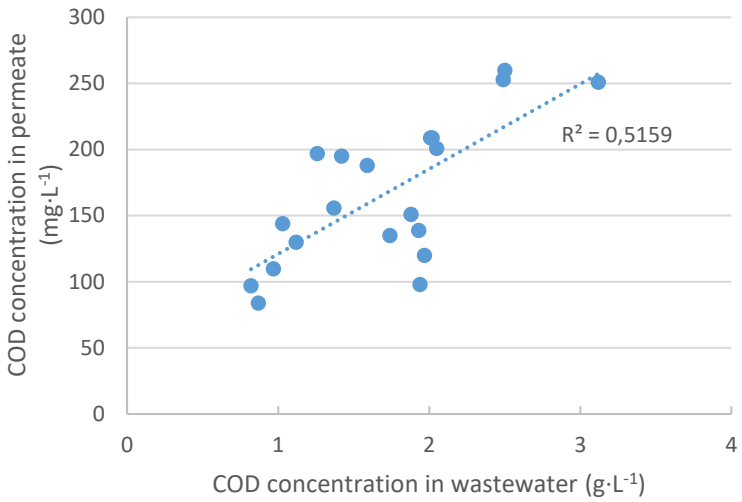

Figure 49: COD concentration in permeate according to COD concentration in wastewater during PI and PII

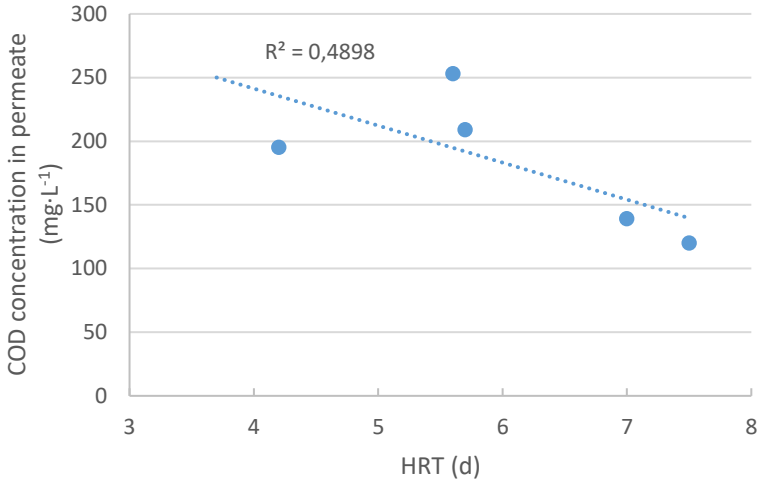

Figure 50: COD concentration in permeate according to the HRT during the entire experimental phases of PII

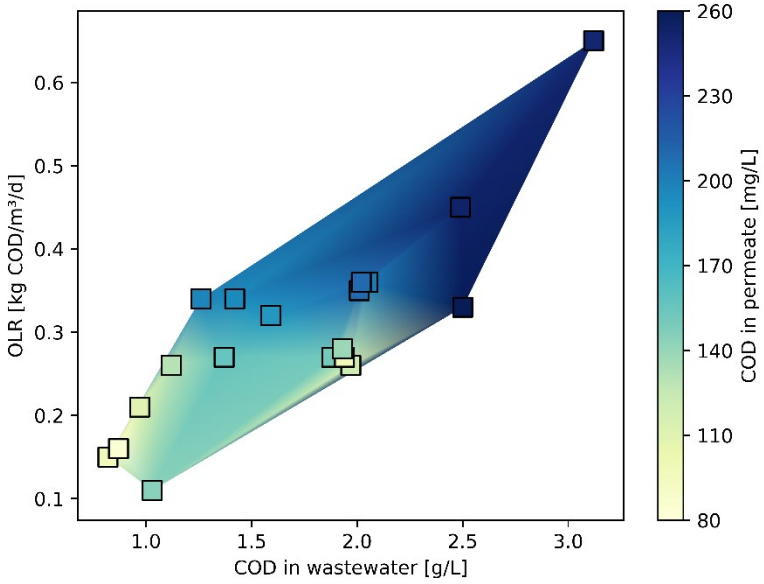

Figure 51: COD concentration in permeate according to both the OLR and COD concentration in wastewater during PI and PII

5.1.6.4.5 Future operation of the AnMBR process

Once these correlations are known, decisions concerning the future operation of decentralized AnMBR processes can be made. First, it must be decided if the emphasis is put on the biomethane production (parameter SBP) or on the effluent quality (COD concentration in permeate). As mentioned in the section 5.1.6.4.2, if the emphasis is put on the biomethane production, the daily production of methane per volume unit of reactor will be at its highest level for an OLR comprised between 0.35 and 0.45 kg $COD \cdot m^{-3} \cdot d^{-1}$. In this case, the OLR should be accordingly monitored using the correlations found between this parameter and the HRT and COD concentration in wastewater.

According to the correlations established between the COD concentration in the permeate and the OLR, in the rated range for methane production, the COD concentration in the permeate would amount to between 183 and 219 $mg \cdot L^{-1}$. Hypothesizing that the COD concentration in the effluent stays stable after the microalgae process (see section 5.2.6.3), these values are above the requirements for discharge of wastewater effluents in the water bodies in Germany. Therefore, two options can be envisaged. The first one is to stay at an OLR value in the rated range for methane production (parameter SBP). In this case, a post-treatment to respect the COD requirements for discharge into the water bodies should be implemented after the microalgae process. In order to polish the effluent, this post-treatment may be a filtration process (nanofiltration membrane), an ozonation process or an adsorption process.

The second option is to decrease the OLR. According to the correlations established in this work, to respect the requirements for the WWTPs of the size category 1 (maximum COD concentration of 150 $mg \cdot L^{-1}$), the OLR should be reduced to a value of 0.26 kg $COD \cdot m^{-3} \cdot d^{-1}$. Compared to the rated range for the parameter SBP, this represents a loss of methane production of 33 to 42 %. This second option is the most practical for small decentralized WWTPs like in BIQ, where the simplicity and the compactness of the process combined with an efficient wastewater treatment are the main aims.

5.1.7 Performance of the AnMBR pilot-plant compared with small decentralized WWTPs

Table 19 compares COD and BOD_5 removal obtained in the present work with various small decentralized WWTPs. For the COD data related to the AnMBR, the measuring ranges during PI and PII are considered. As fewer BOD_5 measurements were performed, the data is related to the entire periods PI and PII respectively. Most plants presented here are treating domestic wastewater or blackwater from residential buildings and dormitories.

In the literature studies, compared to the present thesis, COD and BOD_5 concentrations in wastewater are extremely low. In particular, blackwater levels amount to 406 $mg \cdot L^{-1}$ and 1218 $mg \cdot L^{-1}$ (Atasoy *et al.* 2007, Abdel Shafy *et al.* 2009), while Graaff *et al.* (2010) report COD values for untreated and vacuum evacuated blackwater in the range of 7.7 to 9.8 $g \cdot L^{-1}$. The lower values related to blackwater composition are explained by the use of pre-treatments (UASB reactor and sieve). In Abegglen *et al.* (2008), COD concentration in the effluent amounts to 562 $mg \cdot L^{-1}$ and is much lower than the average COD concentration in decentralized wastewater plants (see Table 1). This is due to the use of domestic wastewater after primary clarification. In Fernandes *et al.* (2013), Moussavi *et al.* (2010) and Tanaka *et al.* (2006), because of the drainage system, wastewater may have been diluted with rainwater, which was never the case in the project HAWANA. Overall, while COD concentration in wastewater

amounts to between 179 and 1218 mg·L^{-1} in the compared literature, it achieved values comprised between 970 and 2090 mg·L^{-1} in the present work.

The HRTs of the literature listed above range from 1 day to 18 days. The average HRTs of 4.2 to 7.5 days in the project HAWANA are higher than in the three MBR processes and the septic tank but remain much lower than in the wetlands.

Table 19: Comparison of COD and BOD_5 removal in the AnMBR process with small decentralized WWTPs in the literature

Type of WWTP	Wastewater stream	COD			BOD_5			HRT (d)	Author
		Removal (%)	Influent (mg·L^{-1})	Effluent (mg·L^{-1})	Removal (%)	Influent (mg·L^{-1})	Effluent (mg·L^{-1})		
Constructed wetland	blackwater with a UASB reactor as pre-treatment (Egypt)	83.5	406.1	67	86.4	183.8	25	10	Abdel Shafy *et al.* (2009)
Constructed wetland	domestic wastewater (dormitory, Sri Lanka)	40.8	178.9	105.9	65.7	54.2	18.6	18	Tanaka *et al.* (2013)
Sequencing batch reactor (SBR)	domestic wastewater (residential building, Brazil)	83	260	44	-	-	-	-	Fernandes *et al.* (2013)
MBR	blackwater after sieving 6 mm (dormitory, Turkey)	96	1218	42	98	406	8	1.5	Atasoy *et al.* (2007)
MBR	domestic wastewater after primary clarification (four-person house, Switzerland)	93	562	38	-	-	-	3	Abegglen *et al.* (2008)
MBR	Domestic wastewater of a 250-residents district (Germany)	95 - 96	848 - 1031	43 - 45	-	-	-	1	Stüber and Lüdicke (2010)
Septic tank	domestic wastewater (residential building, Iran)	77	243	58	85	178	26	1	Moussavi *et al.* (2010)
AnMBR	domestic wastewater (residential building, Germany)	86 - 94	970 - 2490	110 - 253	99	698 700	9 8	4 - 9	HAWANA

The septic tank process reached a lower COD removal of 77 % with a HRT of 1 day. This still allowed a very low COD concentration of 58 $mg \cdot L^{-1}$ in the effluent because of the low-strength wastewater. The MBR processes in Atasoy *et al.* (2007) and Stüber and Lüdicke (2010) achieved a very high COD removal of 96 % and 95 - 96 % respectively as well as very low COD concentrations ranging between 42 $mg \cdot L^{-1}$ and 45 $mg \cdot L^{-1}$ in the effluent despite the very short residence times of less than 1.5 day. Compared to the AnMBR pilot-plant, the significant differences in the HRT are explained by the process, which was not anaerobic but aerobic. The MBR process in Abegglen *et al.* (2008) operated with a residence time of 3 days achieved a COD removal of 93 %. However, the feed concentration was much lower than in the present work, which explains the very low effluent concentration of 38 $mg \cdot L^{-1}$.

Except for the constructed wetland in Tanaka *et al.* (2013), all small decentralized WWTPs show high (77 %) to very high (93 - 96 %) COD removal. With COD removal ranging 86 - 94 % during the experimental phases, the results of the present work are in the upper range of literature values. The effluent COD concentration of the HAWANA plant (110 - 253 $mg \cdot L^{-1}$) is very high compared to the other WWTPs, but pollutant charges are generally much weaker in the literature. With regard to the BOD_5 concentration, the HAWANA facility reached an extremely high removal of 99 %. Thus, it exceeded all values described in the literature (65.7 - 98 %). In addition, with 9 and 8 $mg \cdot L^{-1}$ during PI and PII respectively, the effluent concentration was lower than in the other plants (8 - 26 $mg \cdot L^{-1}$).

Overall, compared to other small decentralized WWTPs, the AnMBR pilot-plant shows very high COD and BOD_5 removal, which represents the main advantage of this process over other small decentralized WWTPs. However, the AnMBR process was less efficient in terms of HRT. This disadvantage could be avoided using sludge with a higher VS concentration, which would mean a higher and more active microorganisms population.

5.1.8 Performance of the AnMBR pilot-plant compared with AnMBR treating domestic wastewater in the literature

A comparison with other AnMBRs used for domestic wastewater treatment is needed to further evaluate the performance of the present AnMBR pilot-plant. In a review, Shin and Bae (2018) report COD feed concentrations ranging 275 - 1462 $mg \cdot L^{-1}$ (Table 20). In the present work, the COD feed concentration comprised between 970 $mg \cdot L^{-1}$ and 2490 $mg \cdot L^{-1}$ is in the upper range of these literature values.

Ozgun *et al.* (2013) report in another review COD removal of 52 % to 99 %. In comparison, the COD removal in the present work is in the upper range of these values (86 - 94 %). Shin and Bae (2018), who studied more modern and advanced pilot-plants, report a COD removal of 85 % to 91 %. Here, the results of the HAWANA process are in the same order of magnitude. In addition, the COD concentration in the permeate of 110 $mg \cdot L^{-1}$ to 253 $mg \cdot L^{-1}$ is in the upper range of the literature values ranging 36 - 225 $mg \cdot L^{-1}$ (Shin and Bae 2018). The difference with the literature is explained by the more concentrated wastewater streams in the BIQ.

The HRT of the present AnMBR is higher than in most of the literature. While Ozgun *et al.* (2013) mention HRTs between 0.8 h and 5 d, Shin and Bae (2018) report HRTs of less than 1.5 d. According to these two reviews, the OLR is usually in the range of 0.23 - 2 kg $COD \cdot m^{-3} \cdot d^{-1}$, with maximum values of 12.5 kg $COD \cdot m^{-3} \cdot d^{-1}$. In the present work, the OLR amounted to between 0.21 and 0.45 kg $COD \cdot m^{-3} \cdot d^{-1}$. Hence, these values are in the lower range of the literature values.

In addition, TS concentration in the sludge amounting to between 0.6 and 80 $g \cdot L^{-1}$ is reported in the literature. In the present work, the TS values comprised between 2.2 and 4.4 $g \cdot L^{-1}$ are also in the lower range of these results. This comparatively low TS concentration means that the process had to be operated at a lower OLR in order to achieve similar F/M ratios. With a much higher TS concentration in the sludge, it is to assume that much higher OLRs meaning lower HRTs would have been reached. For this purpose, the AnMBR plant should have been operated longer in order to accumulate enough particulate matter in the reactor. In order to faster reach high TS content in the sludge, more seed sludge from the municipal WWTP could have been added into the reactor before the start of AnMBR operation. However, as the available wastewater volume in the BIQ was limited before the work on the pipelines in May 2018, such OLR values would not have been reached and the reactor would have been underfed. Hence, a lower TS content enabled here higher F/M ratios and permitted to investigate which conditions are optimal for the AnMBR operation. Of course, for a commercialized full-scale application, higher TS ranges should be chosen.

With regard to methane production, Ozgun *et al*. (2013) and Shin and Bae (2018) report values ranging from 0.07 to 0.31 $m^3 \cdot kg^{-1}$ COD_{rem}. The results of the present work are in the lower range of the literature (0.14 - 0.19 $m^3 \cdot kg^{-1}$ COD_{rem}). Due to the stoichiometry, the theoretical methane production amounts to 0.35 $m^3 \cdot kg^{-1}$ COD_{rem} . However, a small part of the methane dissolved in the sludge and got lost in the permeate, as no methane recovery system was installed. In addition, McCarty *et al.* (2011) report that up to 15 % of the sustaining energy in COD is lost during the biodegradation process. The reason for this is, on the one hand, the conversion of higher-energy components, such as carbohydrates, into methane (8 %). On the other hand, 7 % of the energy is used to build up the cells of the anaerobic microorganisms.

In the experimental phase 2 of PI, according to McCarty *et al.* (2011) and the fact that 86 % of the COD supplied into the reactor was biodegraded during the anaerobic process, a value of about 0.26 $m^3 \cdot kg^{-1}$ COD_{rem} was expected. As 8 % of the methane produced was lost throughout dissolution in the permeate, the “real” methane production approximated 0.15 $m^3 \cdot kg^{-1}$ COD_{rem}. The difference between both values can be explained by the following reason. As described in Section 5.1.1, the fluctuations in wastewater composition were very high. Because wastewater was sampled only once a week during PI, the results presented in this work are not accurate and the exact amount of COD added into the reactor is unknown.

The low methane production of 0.15 $m^3 \cdot kg^{-1}$ COD_{rem} during the experimental phase 3 of PI can be explained by the lower biodegradation of the substrate (60 % of the COD supplied). Therefore, only 68 % of the removed COD was biodegraded. The 32 % that were not biodegraded were retained by the membrane and accumulated in the reactor. With regard to the theoretical stoichiometric value and with 15 % energy loss in the biological conversion of COD to methane (McCarty *et al.* 2011), a value of 0.19 $m^3 \cdot kg^{-1}$ COD_{rem} was expected in the phase 3. This is in the same order of magnitude as the measured results (0.18 $m^3 \cdot kg^{-1}$ COD_{rem} when the dissolved methane volume is taken into account).

Table 20: Comparison of the AnMBR performance with AnMBRs used for domestic wastewater treatment in the literature

Parameter	Review Ozgun *et al*. (2013)	Review Shin and Bae (2018)	Project HAWANA – medians of the experimental phases 2 and 3 of PI and the phases 1 to 5 of PII
COD in wastewater ($mg \cdot L^{-1}$)	-	275 - 1462	970 - 2490
COD removal (%)	52 - 99	85 - 91	86 - 94
COD in permeate ($mg \cdot L^{-1}$)	-	36 - 225	110 - 253
HRT	0.8 h - 5 d	2.2 - 33 h	4.2 d – 7.5 d
OLR ($kg\ COD \cdot m^{-3} \cdot d^{-1}$)	0.23 - 12.5	0.4 - 2.5	0.21 - 0.45
TS in sludge ($g \cdot L^{-1}$)	0.5 - 80	0.6 - 32	2.2 – 4.4
Methane production ($m^3 \cdot kg^{-1}\ COD_{rem}$)	0.062 - 0.27	0.07 - 0.31	0.14 - 0.19
BSP ($kg\ VS \cdot kg^{-1}\ COD_{rem}$)	-	0.05 - 0.46	0.00 – 0.84

During the phases 3 to 5 of PII, between 6.5 and 7.8 % of the produced biogas was lost due to its dissolution in the permeate, which represented over an entire experimental phase between 58 and 115 L of biomethane. Hence, during these three phases, the "real" methane production amounted to 0.20, 0.16 and 0.15 $m^3 \cdot kg^{-1}$ COD_{rem} respectively. Because of energy loss in the biological conversion of COD to methane, methane production of 0.26, 0.26 and 0.30 $m^3 \cdot kg^{-1}$ COD_{rem} were expected. Hence, the methane production measured during the experimental phases was 23 % to 50 % lower than these theoretical values. These significant differences were also explained by the high fluctuations of wastewater composition, which makes difficult to know the exact amount of COD removed by the process. Moreover, it could be possible that more than 15 % of the sustaining energy in COD was lost during the biodegradation process. Indeed, each biological process has its own specificities and this value of 15 % is only a general value. In addition, low mass transfer conditions that lead to oversaturation could have caused a higher amount of dissolved methane in the sludge and in the permeate (Rongwong *et al.* 2018).

In summary, the COD removal of the AnMBR plant was with 86 - 94 % in the upper range of the literature, while the OLR (0.21 - 0.45 kg $COD \cdot m^{-3} \cdot d^{-1}$), the TS concentration in the reactor (2.2 - 4.4 $g \cdot L^{-1}$) and the methane production (0.14 - 0.19 $m^3 \cdot kg^{-1}$ COD_{rem}) were in the lower literature range. The domestic wastewater used in the present work was highly concentrated, which makes a direct comparison with the literature difficult. However, the values presented here can be used as a reference if a decentralized anaerobic wastewater treatment in size up to 30 PE in combination with a membrane filtration unit comes into consideration.

5.1.9 Performance of the AnMBR pilot-plant compared with German conventional WWTPs

In this section, the performance of the AnMBR in terms of COD removal and COD concentration in the effluent is compared with the performance of approximately 5,000 WWTPs in Germany (DWA 2010). In the present work, compared to these WWTPs, a very similar COD removal was reached. While

between 92 % and 95 % of the influent COD is removed in the German WWTPs, using the AnMBR process, COD removal ranged between 86% and 94 %. In particular, COD removal reaches 92 % in the WWTPs of the size category 1 that correspond to small WWTPs. Hence, during optimal operation, the COD removal in the AnMBR and a small conventional WWTP is the same. This represents a strong advantage for the development of AnMBR systems as small WWTPs in the near future. Compared to conventional WWTPs, with a value of 99 %, BOD_5 removal was slightly better in the present work. Despite the very high-strength wastewater used, a BOD_5 concentration of only 8 to 9 $mg \cdot L^{-1}$ characterized the permeate.

5.2 Microalgae cultivation

5.2.1 Microalgae cultivation at laboratory scale

5.2.1.1 *Acutodesmus obliquus* cultivation at the University of Hamburg

As a reminder, this first experiment aimed at the comparison of growth and nutrient removal in a permeate culture and in a synthetic culture medium. As synthetic culture media always contained micronutrients, microalgae growth and nutrient degradation in a permeate culture without additional micronutrients and in a permeate culture with additional micronutrients were also investigated.

5.2.1.1.1 Initial nutrient concentration

The initial NH_4-N, TN, TP and biomass concentrations are shown in Table 21. In the permeate (A) and the enriched permeate (B), they respectively ranged 73.7 - 98.6 $mg \cdot L^{-1}$, 97.2 - 126.2 $mg \cdot L^{-1}$ and 13.6 - 14.6 $mg \cdot L^{-1}$. TN and TP initial concentrations in the control culture (C) were similar to (A) and (B). As ammonium nitrate was used as nitrogen source in the control culture (C), NH_4-N concentration in (C) amounted in average to 49 % of the NH_4-N concentration in (A) and (B).

Table 21: Initial nutrient and biomass concentrations in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C)

Batch test	Duration (d)	Initial NH_4-N concentration ($mg \cdot L^{-1}$)			Initial TN concentration ($mg \cdot L^{-1}$)			Initial TP concentration ($mg \cdot L^{-1}$)			Initial biomass concentration ($g \cdot L^{-1}$)		
		A	B	C	A	B	C	A	B	C	A	B	C
1st batch	8	73.7 ± 13.7	73.7 ± 13.7	39.0 ± 4.1	97.2 ± 4.2	97.2 ± 4.2	110.1 ± 6.2	14.0 ± 0.6	14.0 ± 0.6	11.5 ± 0.6	0.294 ± 0.016	0.388 ± 0.025	0.257 ± 0.012
2nd batch	5	97.2 ± 10.7	97.2 ± 10.7	45.6 ± 4.4	122.2 ± 0.3	122.2 ± 0.3	111.0 ± 0.2	13.6 ± 0.5	13.6 ± 0.5	12.0 ± 3.5	0.694 ± 0.002	0.832 ± 0.004	0.671 ± 0.004
3rd batch	5	98.6 ± 2.0	98.6 ± 2.0	46.2 ± 3.3	126.2 ± 1.0	126.2 ± 1.0	106.8 ± 0.3	14.6 ± 1.8	14.6 ± 1.8	12.4 ± 4.9	0.664 ± 0.014	0.774 ± 0.019	0.653 ± 0.014

5.2.1.1.2 pH

During the 1st batch test, the cultures (A) and (B) showed a low pH decrease. This value started from 7.6 and fell to respectively 6.7 and 6.3 in the permeate (A) and the enriched permeate (B) after one day (Figure 52). Until day 5, these low fluctuations were similar every day. Starting from day 5, the pH values related to the cultures (A) and (B) systematically ranged 7.1 - 8.4, with an average of 7.6 in (A) and 7.9 in (B). As these values are optimal for microalgae growth, no pH adjustment was needed starting from day 5. On the contrary, extreme decreases of pH were observed with the commercial fertilizer (C) as culture medium. These fluctuations were the most severe on the 1st day of each batch test, falling within the range 3 - 4. In the course of each batch test, the fluctuations became lower and lower. Nonetheless, except between day 5 and day 8, it was needed to adjust the pH every day. This was presumably due to lacking buffer ions in the deionized water combined with the addition of ammonium nitrate into the culture.

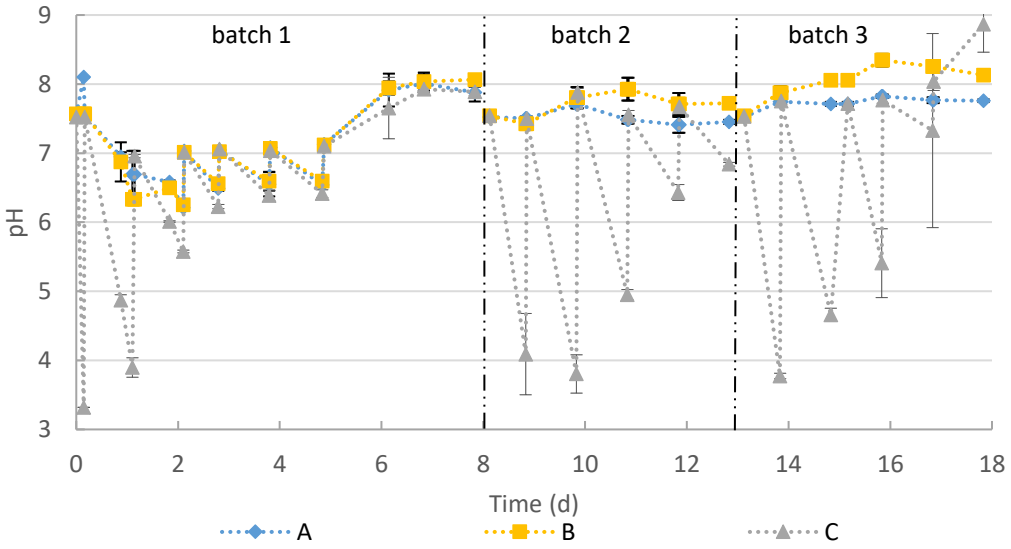

Figure 52: pH in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C)

5.2.1.1.3 TN and NH_4-N

During the 1st batch test, four days were required to remove 93 % of TN in the permeate (A) and the enriched permeate (B) (Figure 53 and Table 22). During the 2nd batch test, it also took four days to remove 96 % TN in (A) and (B). However, during the 3rd batch test, the microalgae in the culture (A) could no longer completely assimilate this macronutrient. After five days, while 96 % TN had already been removed from the culture (B), RE_{TN} in the permeate (A) only achieved 58 %. At the same time, in the control culture (C), RE_{TN} reached 93 %, 98 % and 96 % after 8, 5 and 5 days respectively.

The significant decrease of TN uptake starting from the 3rd batch test was confirmed by NH_4-N uptake results (Appendix 4). During the first two batch tests, NH_4-N was completely assimilated in each culture. Nevertheless, during the 3rd batch, while RE_{NH4-N} reached 100 % in the cultures (B) and (C), only 59 % of NH_4-N was removed from the permeate (A). In this culture, in the course of the repeated batches, the final NH_4-N concentration amounted to 0.12, 0.13 and 40.7 $mg \cdot L^{-1}$. In the cultures (B) and (C), this nutrient remained only in very low concentrations. These varied between 0.07 and 0.11 $mg \cdot L^{-1}$ in the enriched permeate (B) and between 0.05 and 0.16 $mg \cdot L^{-1}$ in the synthetic culture medium (C).

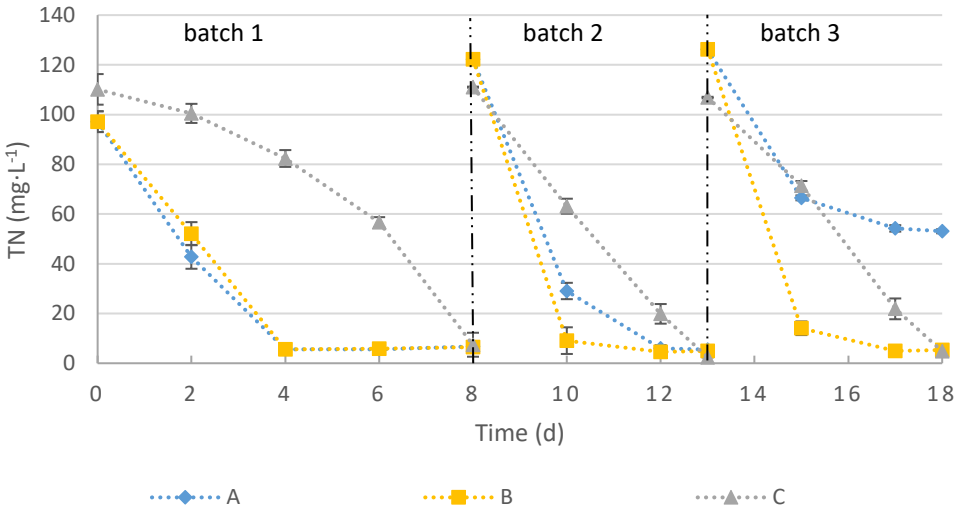

Figure 53: TN concentration in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C)

The RC parameter confirmed that, despite similar RE_{TN} in the enriched permeate (B) and the synthetic culture medium (C), TN uptake happened faster in (B). Indeed, in average, RC_{TN} amounted to 27 $mg \cdot L^{-1} \cdot d^{-1}$ in (B) and was 49 % higher than in (C). In the permeate (A), this parameter decreased from 23 to 15 $mg \cdot L^{-1} \cdot d^{-1}$, what was in line with the decrease of TN removal efficiency.

Table 22. Final TN and NH_4-N concentrations, RE_{TN}, RE_{NH4-N} and RC_{TN} in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C)

Batch test	Final TN concentration ($mg \cdot L^{-1}$)			RE_{TN} (%)			RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)			Final NH_4-N concentration ($mg \cdot L^{-1}$)			RE_{NH4-N} (%)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1st batch	6.77 ± 0.52	6.54 ± 0.18	7.45 ± 4.82	93	93	93	23	23	13	0.12 ± 0.03	0.10 ± 0.00	0.05 ± 0.02	100	100	100
2nd batch	5.51 ± 0.34	4.89 ± 0.10	2.43 ± 0.04	96	96	98	23	29	22	0.13 ± 0.02	0.11 ± 0.05	0.16 ± 0.01	100	100	100
3rd batch	53.1 ± 0.8	5.30 ± 0.25	4.76 ± 1.84	58	96	96	15	30	20	40.7 ± 1.3	0.07 ± 0.03	0.13 ± 0.01	59	100	100

5.2.1.1.4 TP

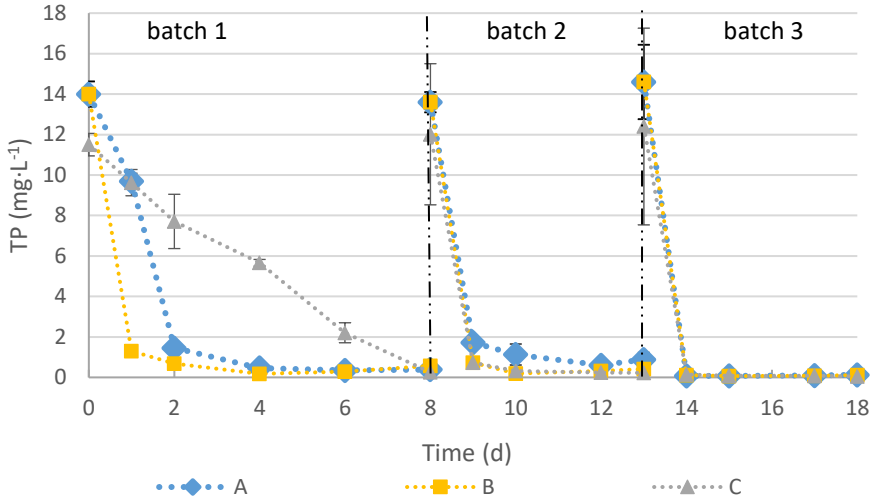

Figure 54: TP concentration in the permeate (A), the permeate enriched with micronutrients (B) and the synthetic commercial fertilizer (C) - The curve (A) is voluntarily represented in a thicker way, otherwise it is not visible in the part of the figure representing the 3rd batch test.

Table 23: Final TP concentration, RE_{TP} and RC_{TP} in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C)

Batch test	Final TP concentration ($mg \cdot L^{-1}$)			RE_{TP} (%)			RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)		
	A	B	C	A	B	C	A	B	C
1st batch	0.38 ± 0.07	0.57 ± 0.06	0.26 ± 0.04	97	96	98	3.4	3.5	1.4
2nd batch	0.88 ± 0.34	0.41 ± 0.05	0.22 ± 0.10	94	97	98	3.2	6.7	5.8
3rd batch	0.12 ± 0.08	0.11 ± 0.04	0.05 ± 0.00	99	99	100	15	15	12

Over the three batches, RE_{TP} reached 97 %, 94 % and 99 % in the permeate (A) and 96 %, 97 % and 99 % in the enriched permeate (B) (Figure 54 and Table 23). At the end of each batch, TP was present only in very low concentration levels that varied from 0.11 to 0.88 $mg \cdot L^{-1}$. These results were similar to the results obtained with the control culture (C), where final TP concentrations ranging 0.05 - 0.26 $mg \cdot L^{-1}$ and RE_{TP} amounting to 98 %, 98 % and 100 % were reached. TP was entirely assimilated within four days, four days and one day in (A) and four days, two days and one day in (B). The time required to completely remove TP from the control culture (C) was the same as for the culture (B), except during the 1st batch where eight days were needed in (C). RC_{TP} significantly increased in the course of the three batches. While this parameter amounted to 3.4, 3.5 and 1.4 $mg \cdot L^{-1} \cdot d^{-1}$ in (A), (B) and (C) respectively during the 1st batch test, it ranged 12 - 15 $mg \cdot L^{-1} \cdot d^{-1}$ in the 3rd batch test. This was presumably due to two different reasons. First, during the 1st batch test, the microalgae cells needed to adapt to a new culture medium. Once the cells have adapted to the permeate, nutrient assimilation was faster. This was also observed for TN in (B) and (C) between the 1st and the 2nd batch tests. Secondly, in the course of the 2nd batch test, between day 10 and day 13, the microalgae cells stay during 3 days without phosphorus supply, as TP concentrations were very low in the culture medium.

This could have led to the phenomenon of luxury uptake at the beginning of the 3rd batch test. Indeed, if the microalgae are submitted to a phosphorus deficiency in the culture medium for a certain time, as soon as this nutrient is available again, larger TP amounts than necessary for the metabolic processes can be absorbed into the cells and stored (Cuellar-Bermudez *et al.* 2017).

5.2.1.1.5 Microalgae growth

The 1st batch test showed a similar growth between the permeate (A) and the permeate enriched with micronutrients (B), which respectively reached a biomass concentration of 2.32 $g \cdot L^{-1}$ and 2.45 $g \cdot L^{-1}$ after 8 days (see Figure 55 and Table 24).

Compared to (A) and (B), the microalgae cells of the synthetic culture medium (C) slowly grew and reached a final concentration of 1.36 $g \cdot L^{-1}$. During this 1st batch, the cultures (A), (B) and (C) achieved a BPR of 0.253 $g \cdot L^{-1} \cdot d^{-1}$, 0.258 $g \cdot L^{-1} \cdot d^{-1}$ and 0.138 $g \cdot L^{-1} \cdot d^{-1}$.

During the 2nd batch test, the cultures (A) and (C) showed a similar growth and reached after 5 days a final biomass concentration of 2.03 $g \cdot L^{-1}$ and 2.02 $g \cdot L^{-1}$ respectively. The related BPR amounted to 0.268 $g \cdot L^{-1} \cdot d^{-1}$ in (A) and 0.269 $g \cdot L^{-1} \cdot d^{-1}$ in (C). The enriched permeate (B) performed a much better growth and reached a final biomass concentration of 2.91 $g \cdot L^{-1}$ and a BPR of 0.417 $g \cdot L^{-1} \cdot d^{-1}$. These values were respectively 43 % and 56 % higher than in (A).

During the 3rd batch test, the differences between the permeate (A) and the enriched permeate (B) increased. The culture (B) continued to grow well, reaching a biomass productivity of 0.400 $g \cdot L^{-1} \cdot d^{-1}$. In contrast, microalgae growth in the permeate (A) was almost non-existent. The BPR reached 0.105 $g \cdot L^{-1} \cdot d^{-1}$, representing only 26% of the BPR obtained in the culture (B). The control culture (C) reached a final concentration of 1.79 $g \cdot L^{-1}$ and ranged between the final biomass concentrations of (A) and (B), which amounted respectively to 1.19 and 2.77 $g \cdot L^{-1}$.

The maximum value of the specific growth rate μ_{max} reached its highest values during the 1st batch for the permeates (A) and (B) (0.58 d^{-1} in (A) and 0.47 d^{-1} in (B)). This was probably due to the lower initial biomass concentration, which reached to more light availability for the microalgae cells. Compared to (B), the slightly higher value in (A) during the 1st batch test was also attributed to the lower initial biomass concentration in (A). In (A) and (B), μ_{max} reached in the 2nd and the 3rd batch tests values comprised between 0.26 and 0.30 d^{-1}.

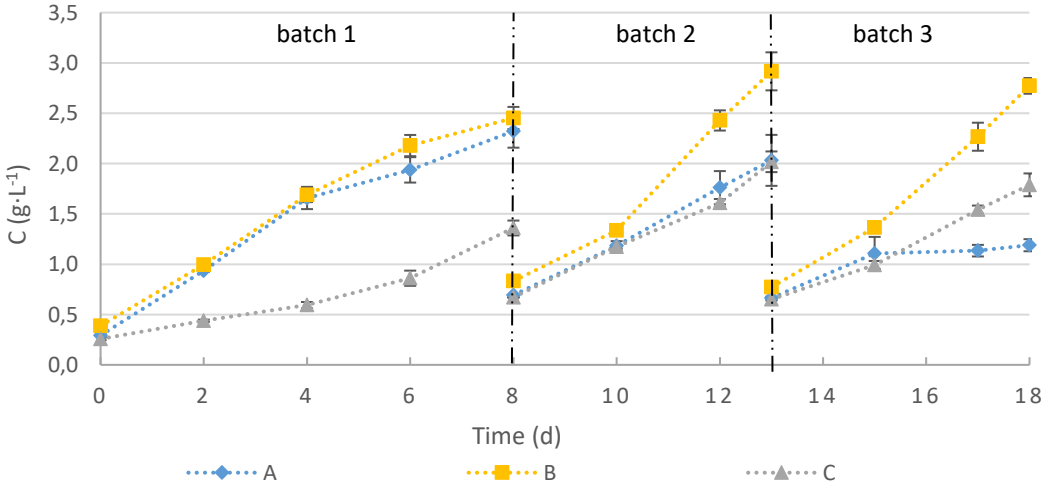

Figure 55: Biomass concentration in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C)

Table 24: Final biomass concentration, BPR and μ_{max} in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C)

Batch test	Final biomass concentration ($g \cdot L^{-1}$)			BPR ($g \cdot L^{-1} \cdot d^{-1}$)			μ_{max} (d^{-1})		
	A	B	C	A	B	C	A	B	C
1st batch	2.32 ± 0.16	2.45 ± 0.11	1.36 ± 0.07	0.25	0.26	0.14	0.58	0.47	0.27
2nd batch	2.03 ± 0.25	2.91 ± 0.19	2.02 ± 0.10	0.27	0.42	0.27	0.27	0.30	0.28
3rd batch	1.19 ± 0.06	2.77 ± 0.08	1.79 ± 0.11	0.11	0.40	0.23	0.26	0.28	0.22

5.2.1.2 *Acutodesmus obliquus* cultivation at the TU Berlin

During the three experiments conducted in the laboratories of the TU Berlin, the cultures temperature amounted to 23 ± 1 °C and, without regulation, the pH remained stable at 7.0 ± 0.1.

5.2.1.2.1 Experiment (1)

The experiment (1) aimed at the confirmation of the differences observed between the permeate (A) and the enriched permeate (B) during the previous experiment at the University of Hamburg and related to TN assimilation and biomass growth. NH_4-N, TN, TP and biomass concentrations in the permeate (A) and the permeate enriched with micronutrients (B) at the beginning of the two successive batch tests are shown in Table 25. Due to the fluctuation of permeate composition, TP and TN initial concentrations were in average 67 % and 17 % higher in the 2nd batch test than in the 1st batch test. Compared to the experiment conducted at the University of Hamburg, the nutrient concentrations in the beginning of the 2nd batch test of the experiment (1) were similar.

Table 25: Initial nutrient concentrations in the permeate (A) and the permeate enriched with micronutrients (B) during the experiment (1)

Batch test	Duration (d)	Initial NH_4-N concentration ($mg \cdot L^{-1}$)		Initial TN concentration ($mg \cdot L^{-1}$)		Initial TP concentration ($mg \cdot L^{-1}$)		Initial biomass concentration ($g \cdot L^{-1}$)	
		A	B	A	B	A	B	A	B
1st batch	9	67.9 ± 2.0	65.9 ± 1.1	87.5 ± 2.9	85.1 ± 1.8	9.33 ± 0.8	9.33 ± 0.8	0.13 ± 0.01	0.23 ± 0.01
2nd batch	5	93.2 ± 3.4	93.4 ± 1.5	101.5 ± 0.5	100.5 ± 3.2	16.6 ± 0.6	14.4 ± 0.5	0.25 ± 0.08	0.45 ± 0.10

During the 1st batch, very similar results were obtained in both culture media. RE_{TN}, $RE_{NH4\text{-}N}$ and RE_{TP} amounted to 95 %, 100 % and 98 % in (A) and 92 %, 100 % and 99 % in (B) (see Figure 56, Table 26 and Table 27). Starting from the 2nd batch test, a significant decrease of TN uptake was observed in the permeate (A). While the microalgae in the permeate enriched with micronutrients (B) assimilated 91 % of TN and 100 % of NH_4-N, RE_{TN} and $RE_{NH4\text{-}N}$ in (A) only reached 58 % and 71 %. After 2 days of cultivation, TN and NH_4-N concentrations stayed constant in (A) at approximately 40 and 30 $mg \cdot L^{-1}$ respectively. Hence, the experiment (1) confirmed that the microalgae were not able to completely assimilate TN and NH_4-N in the course of successive batch tests with permeate (A) as culture medium.

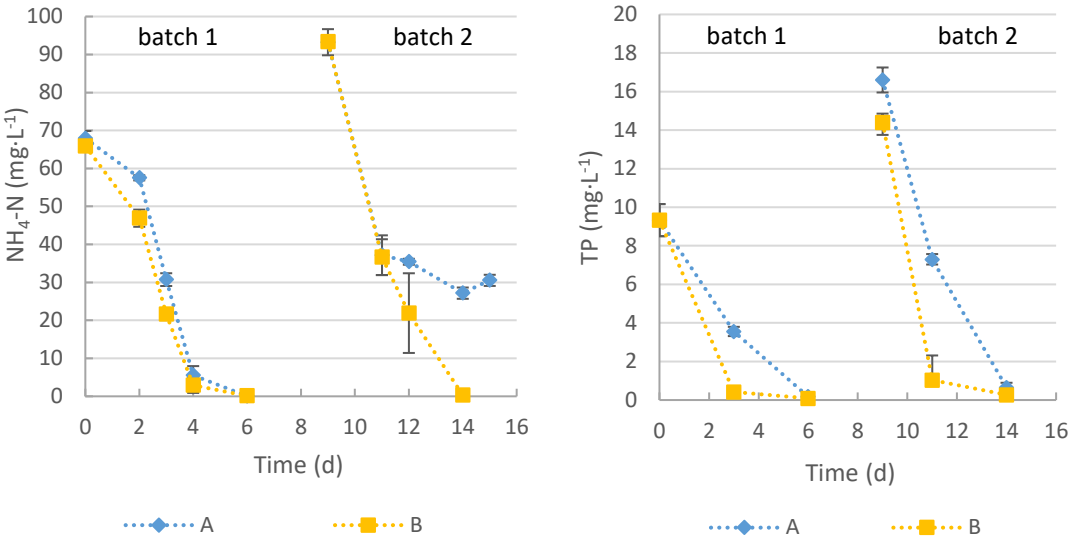

Figure 56: NH_4-N (left) and TP (right) concentrations in the permeate (A) and the permeate enriched with micronutrients (B) during the experiment (1)

Table 26: Final TN and NH_4-N concentrations, RE_{TN}, RE_{NH4-N} and RC_{NH4-N} in the permeate (A) and the permeate enriched with micronutrients (B)

Batch test	TN final concentration ($mg \cdot L^{-1}$)		RE_{TN} (%)		NH_4-N final concentration ($mg \cdot L^{-1}$)		RE_{NH4-N} (%)		RC_{NH4-N} ($mg \cdot L^{-1} \cdot d^{-1}$)	
	A	B	A	B	A	B	A	B	A	B
1st batch	4.3 ± 0.2	7.09 ± 0.3	95	92	0.08 ± 0.02	0.12 ± 0.06	100	100	11	11
2nd batch	42.7 ± 3.3	9.3 ± 43.3	58	91	27.2 ± 1.5	0.27 ± 0.16	71	100	13	19

In relation to the nutrient assimilation rate, RC_{NH4-N} amounted to 11 $mg \cdot L^{-1} \cdot d^{-1}$ in (A) and 13 $mg \cdot L^{-1} \cdot d^{-1}$ in (B) during the 1st batch test. In the 2nd batch test, with RC_{NH4-N} values of 13 and 19 $mg \cdot L^{-1} \cdot d^{-1}$ in (A) and (B) respectively, NH_4-N was assimilated faster. In (A), the slightly faster NH_4-N uptake despite the decrease of RE_{NH4-N} is remarkable. It shows that this macronutrient could be assimilated faster than in the 1st batch test during 2 days. After this time, the microalgae could suddenly not assimilate NH_4-N anymore.

As in the first laboratory experiment conducted at the University of Hamburg, TP was entirely removed in both culture media over the two batch tests (on average 97 % in (A) and 99 % in (B)). The biomass concentration in (A) and (B) amounted to 2.00 and 2.17 $g \cdot L^{-1}$ respectively at the end of the 1st batch test and 1.49 and 1.63 $g \cdot L^{-1}$ at the end of the 2nd batch test (Figure 57). These differences were not caused by a slower growth during the 2nd batch test but by the lower duration of the batch test.

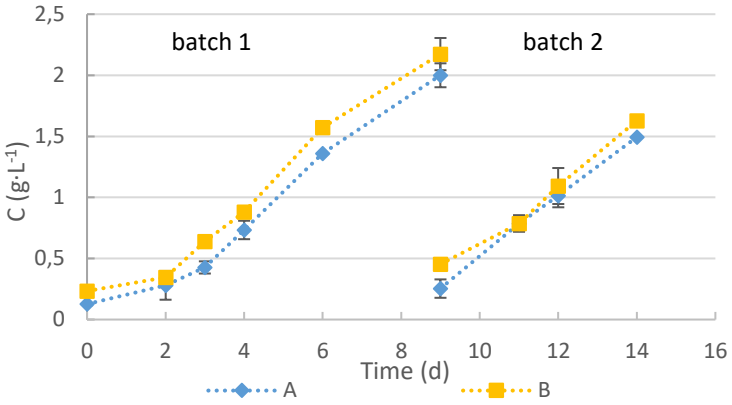

Figure 57: Biomass concentration in the permeate (A) and the permeate enriched with micronutrients (B) during the experiment (1)

Table 27: Final biomass and TP concentrations, BPR, μ_{max}, RE_{TP} and RC_{TP} in the permeate (A) and the permeate enriched with micronutrients (B)

Batch test	Final biomass concentration ($g \cdot L^{-1}$)		BPR ($g \cdot L^{-1} \cdot d^{-1}$)		μ_{max} (d^{-1})		TP final concentration ($mg \cdot L^{-1}$)		RE_{TP} (%)		RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)	
	A	B	A	B	A	B	A	B	A	B	A	B
1st batch	2.00 ± 0.10	2.17 ± 0.13	0.21	0.22	0.54	0.61	0.18 ± 0.03	0.09 ± 0.01	98	99	1.5	8.9
2nd batch	1.49 ± 0.09	1.63 ± 0.15	0.25	0.23	0.57	0.33	0.66 ± 0.24	0.27 ± 0.12	96	98	3.2	6.7

Contrary to the previous experiment, despite the decrease of TN assimilation in (A), only little difference in BPR was observed during the 2nd batch test between (A) and (B). This reached 0.25 $g \cdot L^{-1} \cdot d^{-1}$ in (A) and 0.23 $g \cdot L^{-1} \cdot d^{-1}$ in (B). Hence, TN stock was still sufficient in the microalgae cells of (A) to ensure an optimal growth. Nevertheless, with longer cultivation times or the start of a 3rd batch test, larger differences in biomass production between both culture media would have been certainly observed.

Relating to μ_{max}, values ranging 0.54 - 0.61 d^{-1} were achieved, except during the 2nd batch in the permeate enriched with micronutrients (B), where μ_{max} only reached 0.33 d^{-1}. This difference was probably due to the higher initial biomass concentration in the 2nd batch (experimental error at the beginning of the 2nd batch). Hence, less light was available for each cell, leading to a lower specific growth rate.

5.2.1.2.2 Experiment (2)

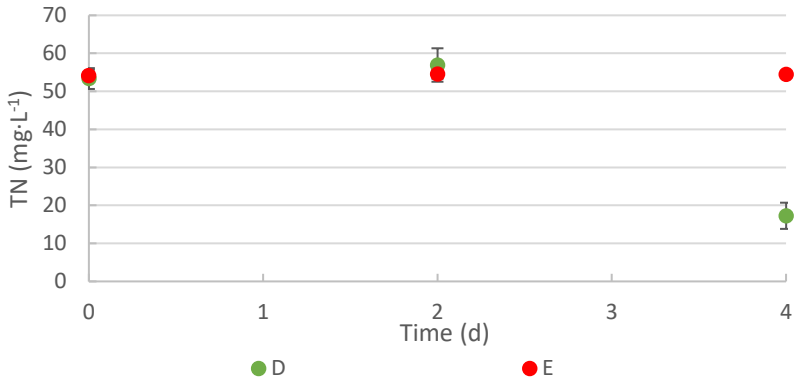

Figure 58: TN concentration in the culture (A) from the end of the experiment (1) enriched with Mn and EDTA on day 0 and Fe and S on day 2 (culture medium (D)) and with Mg and EDTA on day 0 and Mn on day 2 (culture medium (E)) during the experiment (2)

The experiment (2) aimed to find out which micronutrients are responsible for the differences in TN assimilation observed during the previous experiments at the University of Hamburg and at the TU Berlin. The microalgae culture (A) from the end of the experiment (1) was used for the experiment (2) and enriched with micronutrients as defined in the section 4.2.4.2. After two days, TN remained constant at a concentration of 57 $mg \cdot L^{-1}$ in permeate with additional Mn and EDTA (D) and 55 $mg \cdot L^{-1}$ in the permeate with additional Mg and EDTA (E) (Figure 58). After four days, TN remained stable at 55 $mg \cdot L^{-1}$ in (E) despite the addition of Mn on day 2. At the same time, TN concentration decreased by 68 % after the addition of Fe and S to the culture medium (D). In this experiment, it appeared that the addition of iron (II) sulfate ensures an improved TN removal from the permeate. This could explain the differences in TN assimilation observed between the permeate (A) and the enriched permeate (B) in the previous experiments.

5.2.1.2.3 Experiment (3)

The experiment (3) aimed to confirm the results obtained during the experiment (2) and to examine whether Fe and S were the limiting micronutrients in the permeate or whether the combination of Mn, Fe and S was responsible for TN uptake in the culture medium (D). Therefore, a culture containing new permeate inoculated with microalgae from the culture medium (A) of the experiment (1) was begun. Starting from the third day, this new culture could not assimilate TN anymore and a final constant concentration of 41.3 $mg \cdot L^{-1}$ was reached (Table 28). On the fifth day, the content of the three bottles was divided into six bottles and the different micronutrients were added as defined in the section 4.2.4.2. After two more days, in each culture medium, NH_4-N reached very low concentrations (Figure 59).

At the same time, while TN achieved a final concentration of 14.8 $mg \cdot L^{-1}$ in the culture enriched with Fe, S, Mn, Mg and EDTA, a final concentration of 12.1 $mg \cdot L^{-1}$ was reached in the permeate enriched with Fe, S and EDTA. This indicates that only the addition of iron (II) sulfate was responsible for the great amelioration of TN uptake in the permeate culture. In contrast, it was confirmed that the enrichment of permeate with Mg and Mn does not lead to a better TN removal. However, the final biomass concentration was slightly higher in permeate enriched with Fe, S, Mn, Mg and EDTA (4.8 $g \cdot L^{-1}$) than in permeate enriched with Fe, S and EDTA (3.9 $g \cdot L^{-1}$). As a result, the two micronutrients Mg and Mg presumably lead to a slightly better growth.

Table 28: Initial and final NH_4-N, TN and biomass concentrations during the experiment (3) with permeate as culture medium and addition of Fe, Mg, Mn, S and EDTA after 5 days in one triplicate and addition of Fe, S and EDTA after 5 days in another triplicate

Culture medium	Duration (d)	NH_4-N concentration ($mg \cdot L^{-1}$)		TN concentration ($mg \cdot L^{-1}$)		Biomass concentration ($g \cdot L^{-1}$)	
		initial	final	initial	final	initial	final
Permeate	5	62.1 ± 7.6	30.5 ± 1.6	104.3 ± 1.7	41.3 ± 1.9	0.20 ± 0.03	1.51 ± 0.07
Addition of Fe, Mg, Mn, S and EDTA after 5 days	2	23.5 ± 1.4	0.073 ± 0.001	-	14.8 ± 1.6	1.58 ± 0.08	4.77 ± 0.49
Addition of Fe, S and EDTA after 5 days	2	24.2 ± 3.2	0.067 ± 0.017	-	12.1 ± 0.8	1.73 ± 0.12	3.90 ± 0.37

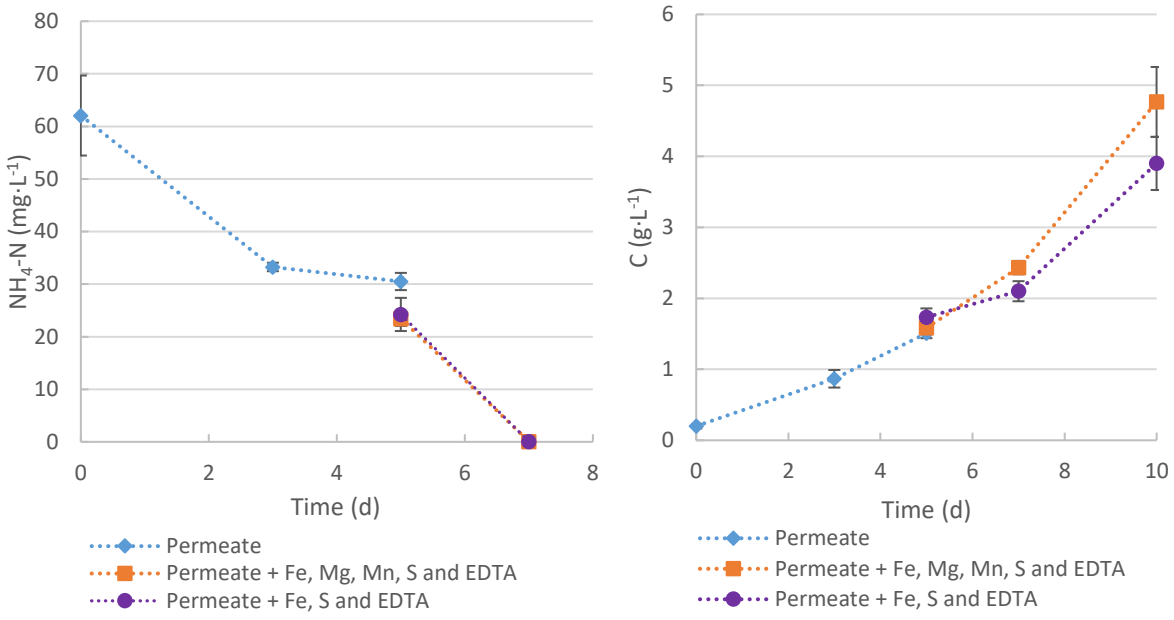

Figure 59: NH_4-N concentration (left) and biomass concentration (right) during the experiment (3) with permeate as culture medium and addition of Fe, Mg, Mn, S and EDTA after 5 days in one triplicate and addition of Fe, S and EDTA after 5 days in another triplicate

5.2.1.3 *Chlorella vulgaris* cultivation at the TU Berlin

As a reminder, the investigation of the species *Chlorella vulgaris* aimed at the observation of cell growth and nutrient assimilation in the permeate culture and the comparison with the performance obtained with a synthetic culture medium. The goal was also to find out if the results obtained with permeate cultures and *Acutodesmus obliquus* during the previous experiments are confirmed and to compare the performance obtained with both species.

5.2.1.3.1 Initial NH_4-N, TN, TP and biomass concentrations

Table 29: Initial nutrient and biomass concentrations in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) in the experiments (1) and (3)

Batch test	Duration (d)	Initial NH_4-N concentration ($mg \cdot L^{-1}$)			Initial TN concentration ($mg \cdot L^{-1}$)			Initial TP concentration ($mg \cdot L^{-1}$)			Initial biomass concentration ($g \cdot L^{-1}$)		
		A	B	C	A	B	C	A	B	C	A	B	C
1st batch of (1)	13	66.5 ± 2.5	82.2 ± 4.6	39.1 ± 0.3	68.7 ± 1.1	82.1 ± 2.8	82.9 ± 0.8	7.77 ± 0.35	7.19 ± 0.27	7.19 ± 0.27	0.19 ± 0.10	0.03 ± 0.01	0.07 ± 0.01
2nd batch of (1)	9	54.2 ± 0.8	64.5 ± 3.6	-	59.2 ± 0.6	65.2 ± 4.3	-	6.43 ± 0.06	7.50 ± 0.09	-	0.30 ± 0.00	0.34 ± 0.04	-
3rd batch of (1)	6	56.2 ± 0.7	58.0 ± 0.8	-	78.6 ± 3.2	74.5 ± 6.3	-	7.56 ± 0.03	7.69 ± 0.09	-	0.15 ± 0.06	0.18 ± 0.06	-
1st batch of (3)	9	81.3 ± 2.8	84.5 ± 1.2	-	84.2 ± 5.6	98.1 ± 3.3	-	7.89 ± 0.04	7.34 ± 0.09	-	0.19 ± 0.09	0.19 ± 0.04	-
2nd batch of (3)	13	78.9 ± 1.3	81.7 ± 2.5	-	81.5 ± 3.1	85.7 ± 0.6	-	7.19 ± 0.07	6.47 ± 0.08	-	0.10 ± 0.00	0.10 ± 0.01	-

The NH_4-N, TN, TP and biomass concentrations at the beginning of each batch of the experiments (1), and (3) are reported in Table 29. The initial concentrations of the experiment (2) are not represented, as the culture with permeate (A) from the end of the 3rd batch of the experiment (1) was used for this experiment. Throughout the three batches of (1), the initial NH_4-N, TN and TP concentrations in the permeate (A) and the control culture (B) respectively ranged 54.2 - 84.2 $mg \cdot L^{-1}$, 59.2 - 82.1 $mg \cdot L^{-1}$ and 6.43 - 7.77 $mg \cdot L^{-1}$. During the experiment (3), TN amounted to between 79.9 $mg \cdot L^{-1}$ and 98.1 $mg \cdot L^{-1}$. In average, the initial TN concentration in the culture (A) during (3) was 20 % higher than in the experiment (1). In contrast, TP remained constant and ranged 6.47 - 7.89 $mg \cdot L^{-1}$ during the experiment (3).

5.2.1.3.2 pH

As for the experiments conducted with *Acutodesmus obliquus*, pH stayed constant in the permeate (A) at 7.1 ± 0.3 throughout the three batches and without pH regulation (Appendix 5). This confirmed the well-adapted buffer capacity of the permeate for microalgae cultivation. In contrast, the pH in the control cultures (B) and (C) regularly needed to be regulated by addition of NaOH. Indeed, the pH reached values down to 4.3 in (B) and 4.9 in (C). As the pH needed to be adjusted in both culture media and there were only slight differences between the pH course in (B) and (C), the culture medium (B) with ammonium sulfate as TN source was further employed for the next batches. Indeed, as this TN source is constituted only of ammonium, it facilitates the comparisons with permeate, which is also predominantly composed of ammonium. During the 2nd and 3rd batch test of (1), the pH needed to be

corrected only once during each batch. Throughout the 1st, 2nd and 3rd batch, the pH amounted to 6.5 ± 0.7, 7.0 ± 0.5 and 6.8 ± 0.4 in the control culture (B).

During the experiment (2), by addition of two different iron salts in the permeate (A), the pH stayed constant at a neutral level without pH regulation. In the experiment (3), a neutral pH of 6.9 ± 0.1 was measured in (A). Simultaneously, in the control culture (B), pH decreased down to values of 5.3. Throughout the two batches of (3), the pH was corrected 5 times and an average pH of 6.7 ± 0.7 and 6.7 ± 0.5 respectively was achieved.

5.2.1.3.3 Experiment (1)

5.2.1.3.3.1 TN and NH_4-N

During the first two batch tests, in each culture medium, NH_4-N was totally assimilated by the microalgae (Figure 60). At the end of each batch, only very low concentrations varying in the range of 0.052 - 0.073 $mg \cdot L^{-1}$ were measured (Table 30). Simultaneously, between 91 % and 94 % of TN was removed and final concentrations were always equal or less than 5.2 $mg \cdot L^{-1}$. Starting from the 3rd batch test, great differences were observed between the permeate (A) and the control culture (B). While TN and NH_4-N removal amounted to 92 % and 100 % respectively in (B), only 61 % of TN and 52 % of NH_4-N were assimilated in the permeate (A) within 6 days. This corresponded to final TN and NH_4-N concentrations of 30.8 $mg \cdot L^{-1}$ and 26.8 $mg \cdot L^{-1}$ respectively. Hence, as for the microalgae species *Acutodesmus obliquus*, a significant decrease of TN assimilation by the microalgae species *Chlorella vulgaris* was observed in the permeate (A) in the course of the successive batch tests.

Furthermore, $RC_{NH4\text{-}N}$ was the highest during the 2nd batch test, where it amounted to 18 $mg \cdot L^{-1} \cdot d^{-1}$ and 21 $mg \cdot L^{-1} \cdot d^{-1}$ in (A) and (B) respectively. On the contrary, this parameter achieved 4.9 $mg \cdot L^{-1} \cdot d^{-1}$ in (A) during the 3rd batch test, which only corresponded to 27 % of $RC_{NH4\text{-}N}$ in (A) during the 2nd batch. While 6 days were needed to remove this nutrient during the 1st batch, only 4 days were needed in the 2nd batch in (A) and (B) and in the 3rd batch test in (B).

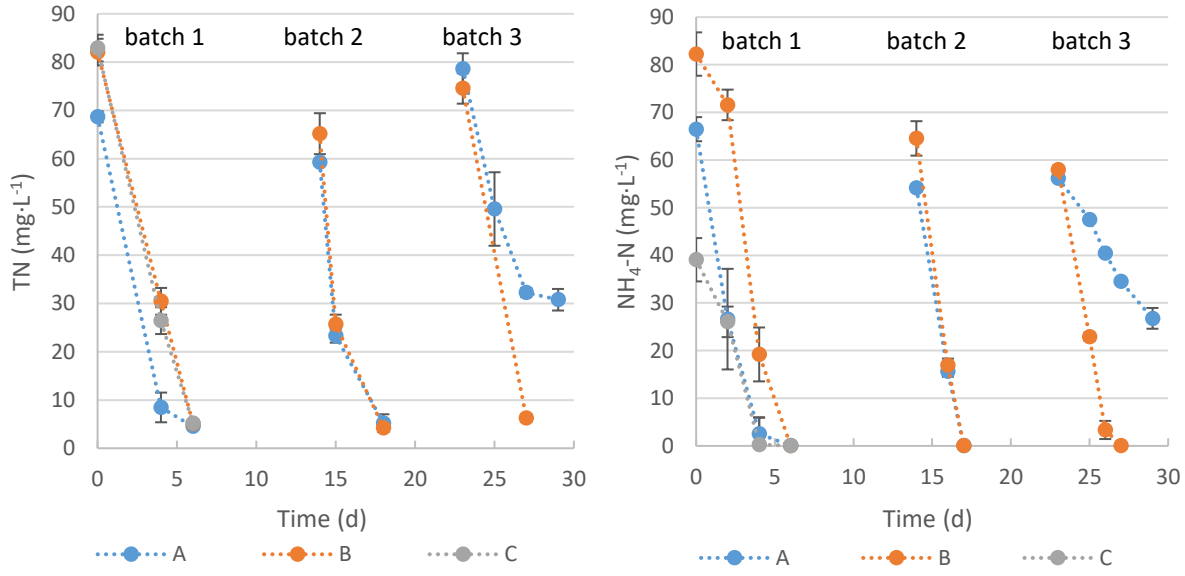

Figure 60: TN (left) and NH_4-N (right) concentrations in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) during the experiment (1)

Table 30: Final TN and NH_4-N concentrations, RE_{TN}, RE_{NH4-N} and RC_{NH4-N} in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) during the experiment (1)

Batch test	Final TN concentration ($mg \cdot L^{-1}$)			RE_{TN} (%)			Final NH_4-N concentration ($mg \cdot L^{-1}$)			RE_{NH4-N} (%)			RC_{NH4-N} ($mg \cdot L^{-1} \cdot d^{-1}$)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1st batch	4.51 ± 0.28	5.09 ± 0.36	5.20 ± 0.93	93	94	94	0.052 ± 0.002	0.067 ± 0.009	0.064 ± 0.033	100	100	100	11	14	6.5
2nd batch	5.21 ± 1.83	4.19 ± 0.82	-	91	94	-	0.064 ± 0.009	0.073 ± 0.013	-	100	100	-	18	21	-
3rd batch	30.8 ± 2.2	6.25 ± 0.82	-	61	92	-	26.8 ± 2.2	0.079 ± 0.015	-	52	100	-	4.9	14	-

5.2.1.3.3.2 TP

The nutrient TP was totally removed in each culture medium throughout the three batches (Figure 61). RE_{TP} achieved values between 95 and 99 % and final concentrations equal or less than 0.37 $mg \cdot L^{-1}$ were systematically achieved (Table 31). Hence, as for the microalgae species *Acutodesmus obliquus*, the reduction in TN assimilation in the permeate (A) did not lead to a decrease of TP removal. However, during the 3rd batch corresponding to the significant decrease of TN uptake in the permeate culture (A), TP assimilation in (A) was much slower than during the other batches. RC_{TP} reached a value of 1.2 $mg \cdot L^{-1} \cdot d^{-1}$. This corresponded only to 63 % and 32 % of the values achieved in the 1st and 2nd batch test.

In contrast, in the control culture (B), RC_{TP} increased by 118 % in the course of the successive batch tests and reached a value of 3.7 $mg \cdot L^{-1} \cdot d^{-1}$ in the last batch. Furthermore, at the beginning of the 2nd and 3rd batch test, a decrease of TP concentration of 46 % and 63 % respectively was observed in the culture medium (B) a few minutes after inoculation with the microalgae. This fast phosphorus removal was explained by the so-called luxury uptake. In the 1st and the 2nd batch test, after TP complete removal, the microalgae were further cultivated during 10 days and 7 days respectively. This aimed to better and longer observe potential differences in biomass production between the different culture media. Hence, the microalgae were facing a lack of TP during several days. Nevertheless, the phenomenon of luxury uptake was only slightly observed in the permeate (A) during the 2nd batch test and not observed at all during the last batch test. Why luxury uptake occurred in the synthetic culture medium (B) and only slightly in the permeate (A) is unclear.

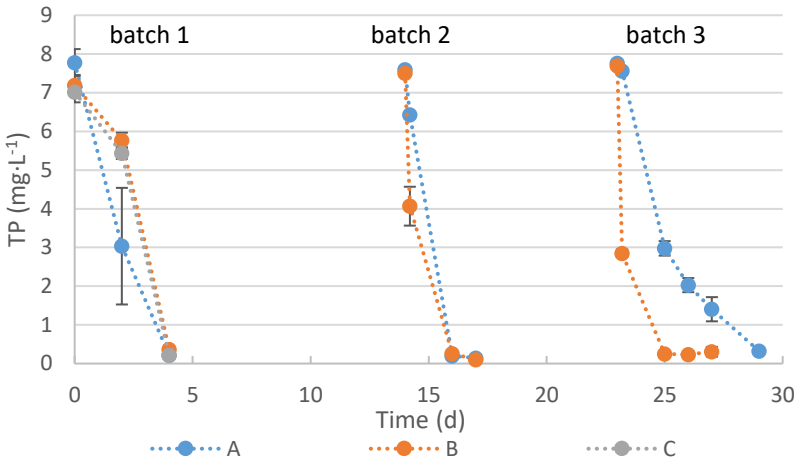

Figure 61: TP concentration in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) during the experiment (1)

Table 31: Final TP concentration, RE_{TP} and RC_{TP} in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) during the experiment (1)

Batch test	TP final concentration (mg·L^{-1})			RE_{TP} (%)			RC_{TP} (mg·L^{-1}·d^{-1})		
	A	B	C	A	B	C	A	B	C
1st batch	0.22 ± 0.01	0.37 ± 0.07	0.22 ± 0.01	97	95	97	1.9	1.7	1.7
2nd batch	0.14 ± 0.03	0.10 ± 0.02	-	98	99	-	3.7	3.6	-
3rd batch	0.33 ± 0.07	0.30 ± 0.13	-	96	96	-	1.2	3.7	-

5.2.1.3.3.3 Microalgae growth

During the 1st batch test, the microalgae similarly grew in the three culture media, reaching final concentrations amounting to between 3.03 and 3.13 g·L^{-1} (Figure 62 and Table 32). However, μ_{max} was 40 % higher in (B) than in (A). This was caused by the 84 % lower initial biomass concentration in (B). Starting from the 2nd batch test, the differences between the permeate (A) and the control culture (B) continuously increased. At the end of the 2nd and 3rd batch test, the final concentration in (A) only amounted to 55 % and 45 % respectively of that in (B). In the 2nd batch, as the differences in the biomass concentration development occurred 3 days after inoculation, μ_{max} was similar in (A) and (B). However, in the 3rd bath, μ_{max} was with 0.96 d^{-1} 48 % higher in (B) than in (A), which attests for the better microalgae cell health in the control culture (B).

In contrast, the BPR in (A) slightly increased from 0.22 to 0.25 g·L^{-1}·d^{-1} between the 1st and the 2nd batch test, and then decreased to 0.18 g·L^{-1}·d^{-1} in the 3rd batch. In the control culture (B), the BPR increased by 100 % between the 1st and 2nd batch test. This could be due to a long adaptation time needed at the beginning of the 1st batch test, especially caused by the great pH decreases up to a value of 4.3 on day 1. Indeed, the biomass concentration stagnated in (B) and (C) between day 0 and day 2 and microalgae growth really began starting from day 2. Furthermore, the initial biomass concentration amounted to 0.34 g·L^{-1} in the 2nd batch test in (B), that is to say 11 times the initial biomass concentration in the 1st batch test. In this case, also if the duplication time of the microalgae cells is

similar in the 1st and 2nd batch test, more biomass per unit of time will be produced if the initial biomass concentration is higher.

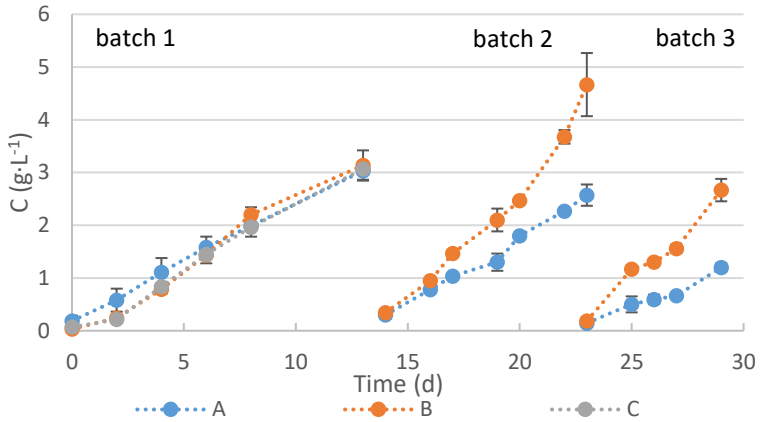

Figure 62: Biomass concentration in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) during the experiment (1)

Table 32: Final biomass concentration, BPR and μ_{max} in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) during the experiment (1)

Batch test	Final biomass concentration ($g \cdot L^{-1}$)			BPR ($g \cdot L^{-1} \cdot d^{-1}$)			μ_{max} (d^{-1})		
	A	B	C	A	B	C	A	B	C
1st batch	3.03 ± 0.17	3.13 ± 0.29	3.07 ± 0.05	0.22	0.24	0.23	0.60	1.00	0.57
2nd batch	2.57 ± 0.20	4.67 ± 0.60	-	0.25	0.48	-	0.48	0.51	-
3rd batch	1.20 ± 0.08	2.67 ± 0.21	-	0.18	0.41	-	0.65	0.96	-

5.2.1.3.4 Experiment (2)

In the experiment (1), the main results obtained in the lab-scale experiments by cultivating *Acutodesmus obliquus* with permeate (A) were confirmed with the species *Chlorella vulgaris*. Hence, the significant reduction observed in TN removal and in biomass production in the course of the successive batch tests was not strain specific to the species *Acutodesmus obliquus* and would have probably been observed with any species.

Fe is one of the most important micronutrients for microalgal biomass production and is especially a key element in TN assimilation (Naito *et al.* 2005; Brorowitzka *et al.* 2016). Consequently, it was assumed that this micronutrient played a very important function in the great increase of TN removal observed by addition of iron (II) sulfate into the permeate in the experiments (2) and (3) conducted with *Acutodesmus obliquus* at the TU Berlin (Figure 58 and Figure 59).

In order to verify this hypothesis, the culture (A) was divided into two triplicates at the end of the 3rd batch test of the experiment (1) conducted with *Chlorella vulgaris*. As defined in the section 4.2.4.3, iron (II) chloride and iron (II) sulfate were added into the different triplicates. In both triplicates, NH_4-N concentration was very similar and equal or less than 0.58 and 0.01 $mg \cdot L^{-1}$ after two and four days respectively (Figure 63). After two days, TN reached a concentration of 6.97 $mg \cdot L^{-1}$ in the permeate

enriched with iron (II) sulfate (E) and 7.47 mg·L^{-1} in the permeate enriched with iron (II) chloride (D). Hence, these similar results show that only the lack of Fe was responsible for the decrease in TN removal observed in the permeate cultures (A) during the experiments conducted with *Acutodesmus obliquus* and *Chlorella vulgaris*.

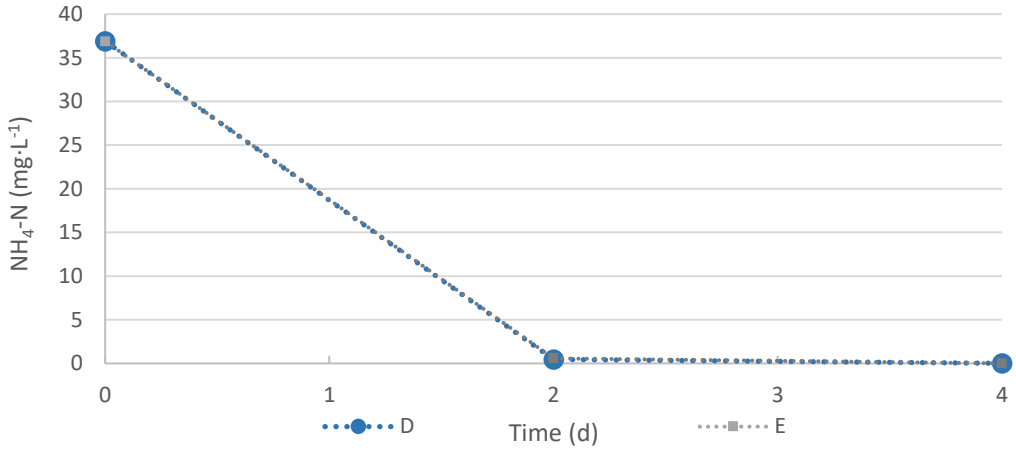

Figure 63: NH_4-N concentration during the experiment (2) by addition of iron (II) chloride (culture medium (D)) and iron (II) sulfate (culture medium (E)) into the permeate culture (A) from the end of the experiment (1)

5.2.1.3.5 Experiment (3)

5.2.1.3.5.1 TN and NH_4-N

At the beginning of the experiment (3), while the microalgae culture (B) from the end of the 3rd batch of (1) was used for the control culture (B), the culture with permeate (A) from the end of the 2nd batch test of (1) was used as inoculum for the new culture (A). As new permeates P2 and P3 were employed for the 1st and 2nd batch test of (3), the use of this inoculum aimed to observe if TN and NH_4-N would again not be totally assimilated in the permeate culture (A), as it happened during the 3rd batch of (1).

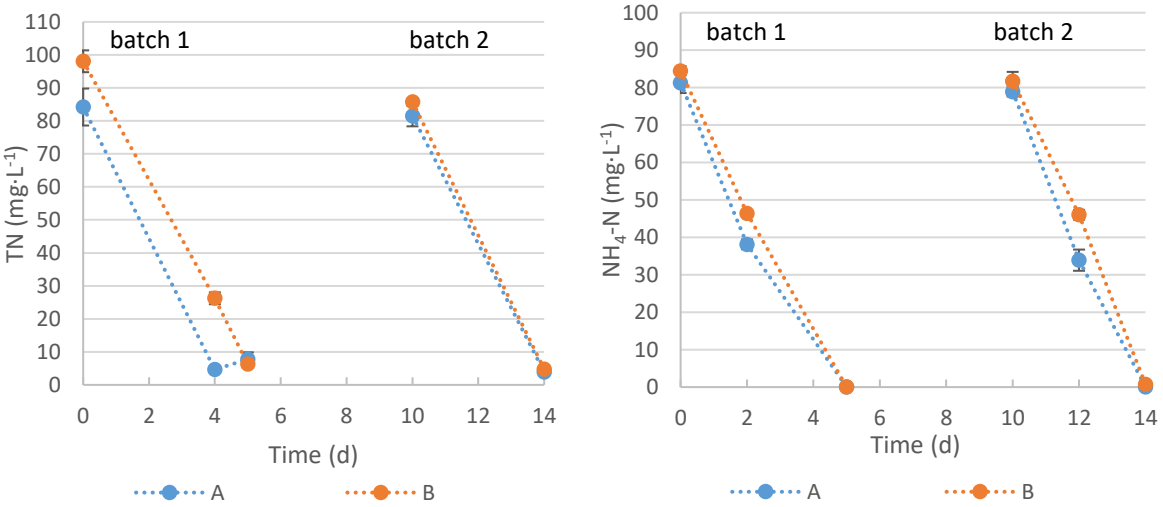

Figure 64: TN (left) and NH_4-N concentrations (right) in the permeate (A) and the synthetic culture medium enriched with ammonium sulfate (B) during the experiment (3)

Contrary to the previous experiments, TN and NH_4-N were totally removed from the culture (A) (Figure 64). In both batches in (A), after 4 days, NH_4-N was only present in very low concentrations and the TN final concentration amounted to 4.6 and 3.9 mg·L^{-1} respectively (Table 33). This was in the order of

magnitude of the final concentrations reached in (B). TN uptake occurred even faster in (A) than in (B) during the 1st batch, probably because of the inconstant pH values in (B). Throughout the two batch tests, in the permeate (A), TN and NH_4-N removal achieved 95 % and 100 % respectively.

This assumedly means that, contrary to the experiment (1), no lack of iron in the microalgae cells occurred. To explain why the use of different permeates led to a different assimilation of this nutrient, iron was measured in the different permeates (Table 34).

While iron concentration amounted to 0.31 $mg \cdot L^{-1}$ in the permeate P1 used during the three batches of (1), it amounted to 0.50 and 0.46 $mg \cdot L^{-1}$ in the permeates P2 and P3 used during the experiment (3). Hence, compared to the experiment (1), iron concentration was 61 % and 48 % higher during (3). This could explain the great differences obtained in TN and NH_4-N uptake during the experiments conducted with *Chlorella vulgaris*.

Table 33: Final TN and NH_4-N concentrations, RE_{TN}, RE_{NH4-N} and RC_{NH4-N} in the permeate (A) and the synthetic culture medium enriched with ammonium sulfate (B) during the experiment (3)

Batch test	TN final concentration ($mg \cdot L^{-1}$)		RE_{TN} (%)		NH_4-N final concentration ($mg \cdot L^{-1}$)		RE_{NH4-N} (%)		RC_{NH4-N} ($mg \cdot L^{-1} \cdot d^{-1}$)	
	A	B	A	B	A	B	A	B	A	B
1st batch	4.6 ± 0.0	6.3 ± 1.0	95	94	0.04 ± 0.00	0.04 ± 0.01	100	100	20	17
2nd batch	3.9 ± 0.4	4.8 ± 0.5	95	94	0.06 ± 0.00	0.79 ± 0.52	100	99	25	25

Table 34: Fe concentration in the three different permeates used for the experimental work conducted with the species Chlorella vulgaris

Permeate	P1 (experiment 1)	P2 (1st batch of (3))	P3 (2nd batch of (3))
Fe ($mg \cdot L^{-1}$)	0.31	0.50	0.48

5.2.1.3.5.2 TP

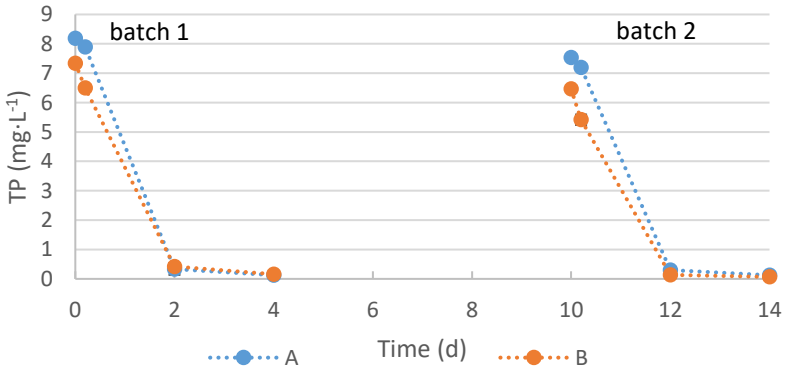

Figure 65: TP concentration in the permeate (A) and the synthetic culture medium enriched with ammonium sulfate (B) during the experiment (3)

As for the previous experiments, throughout the two batches of the experiment (3), TP was entirely removed from the permeate (A), reaching an average RE_{TP} of 95 % (Figure 65 and Table 35). Simultaneously, RC_{TP} ranged 3.4 - 3.8 $mg \cdot L^{-1} \cdot d^{-1}$ in (A). Both parameters were very similar to the values

reached in (B). Throughout the two batches, final TP concentrations equal or less than 0.16 mg·L^{-1} were achieved.

5.2.1.3.5.3 Microalgae growth

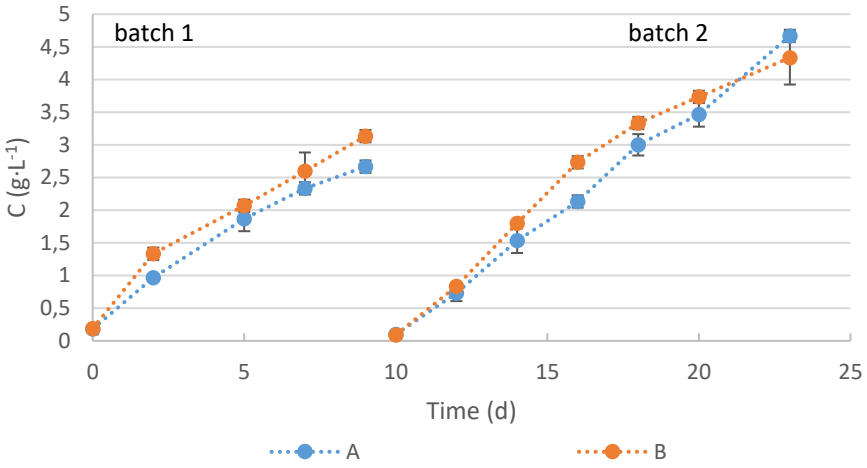

Figure 66: Biomass concentration in the permeate (A) and the synthetic culture medium enriched with ammonium sulfate (B) during the experiment (3)

During this experiment, microalgae growth showed quite similar results (Figure 66). During the 1st batch test, the biomass production was slightly higher in the control culture (B) and a concentration of 3.13 g·L^{-1} and a BPR of 0.33 g·L^{-1}·d^{-1} were reached. At the same time, in the permeate (A), both parameters were 15 % lower. During the 2nd batch test, microalgae growth was similar during the first two days and then, the microalgae in (B) grew slightly faster until day 20. Starting from this day, the biomass concentration was similar in (A) and (B) and amounted to 4.67 and 4.33 g·L^{-1} respectively after 12 days of cultivation. The BPRs were also very similar and reached 0.35 and 0.33 g·L^{-1}·d^{-1} in (A) and (B). Likewise, μ_{max} in (A) and (B) was similar and reached values comprised between 0.88 and 1.11 d^{-1}.

Table 35: Final biomass and TP concentrations, BPR, μ_{max}, RE_{TP} and RC_{TP} in the permeate (A) and the permeate enriched with ammonium sulfate (B) during the experiment (3)

Batch Test	Final biomass concentration (g·L^{-1})		BPR (g·L^{-1}·d^{-1})		μ_{max} (d^{-1})		Final TP concentration (mg·L^{-1})		RE_{TP} (%)		RC_{TP} (mg·L^{-1}·d^{-1})	
	A	B	A	B	A	B	A	B	A	B	A	B
1st batch	2.67 ± 0.09	3.13 ± 0.09	0.28	0.33	0.88	0.99	0.13 ± 0.00	0.13 ± 0.02	98	98	3.8	3.5
2nd batch	4.67 ± 0.09	4.33 ± 0.41	0.35	0.33	0.99	1.11	0.16 ± 0.03	0.07 ± 0.01	98	99	3.4	3.2

5.2.1.4 *Chlorella sorokiniana* cultivation at the TU Berlin

5.2.1.4.1 Initial NH_4-N, TN, TP and biomass concentrations

The nutrient and biomass concentrations at the beginning of each batch are reported in Table 36 and Table 37. Throughout the two batches of (1), the initial NH_4-N, TN and TP concentrations in the permeate (A) respectively ranged 77.3 - 82.0 $mg \cdot L^{-1}$, 91.2 - 104 $mg \cdot L^{-1}$ and 15.6 - 17.0 $mg \cdot L^{-1}$. During the experiments (3) and (4), due to an extreme change of wastewater composition caused by the new sewage pipeline system in the residential building, nutrient concentration in the permeate doubled. While TN amounted to between 138 $mg \cdot L^{-1}$ and 190 $mg \cdot L^{-1}$, TP achieved values ranging between 35.3 $mg \cdot L^{-1}$ and 38.3 $mg \cdot L^{-1}$. Overall, the TN/TP ratio in the permeate amounted to between 3.7 and 6.7. Throughout the supply of KNO_3 at the beginning of the experiment (2) and considering TN and TP concentrations at the beginning of the 2nd batch of (1), a TN/TP ratio of 10.1 was achieved in the experiment (2). During the 1st batch of (3), supply of $(NH_4)_2SO_4$ on day 0 and day 13 led to TN/TP ratios of 7.1 and 8.9 respectively. In the 2nd batch test, the addition of this nitrogen source on day 0 and day 8 enabled to obtain a TN/TP ratio of 5.9 and 10.4 respectively.

Table 36: Initial nutrient concentrations, biomass concentration and TN/TP ratio in the permeate (A), the permeate enriched with a nitrogen source (B) and the synthetic culture medium (C) during the experiments (1), (3) and (4)

Batch test	Initial NH_4-N concentration ($mg \cdot L^{-1}$)			Initial TN concentration ($mg \cdot L^{-1}$)			Initial TP concentration ($mg \cdot L^{-1}$)			Initial biomass concentration ($g \cdot L^{-1}$)			TN/TP ratio		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1st batch of (1)	82.0 ± 1.0	-	-	91.2 ± 6.1	-	-	17.0 ± 0.2	-	-	0.11 ± 0.02	-	-	5.4	-	-
2nd batch of (1)	77.3 ± 1.2	-	-	104 ± 4	-	-	15.6 ± 0.4	-	-	0.33 ± 0.06	-	-	6.7	-	-
1st batch of (3)	131 ± 2	227 ± 2	-	190 ± 4	268 ± 21	-	38.3 ± 1.1	37.5 ± 0.1	-	0.12 ± 0.00	0.09 ± 0.02	-	5.0	7.1/ 8.9[1]	-
2nd batch of (3)	141 ± 10	208 ± 3	-	151 ± 4	217 ± 6	-	35.3 ± 0.4	36.5 ± 0.6	-	0.21 ± 0.08	0.23 ± 0.04	-	4.3	5.9/ 10[1]	-
1st batch of (4)	142 ± 2	-	180 ± 2	159 ± 13	-	184 ± 4	35.3 ± 0.7	-	32.6 ± 0.2	0.25 ± 0.02	-	0.29 ± 0.02	4.5	-	5.6
2nd batch of (4)	127 ± 0	-	160 ± 2	138 ± 3	-	170 ± 4	37.3 ± 0.4	-	29.9 ± 0.1	0.27 ± 0.05	-	0.37 ± 0.05	3.7	-	5.7

[1]: Initial TN/TP ratio considering the supplementary addition of a nitrogen source on day 13 in the 1st batch of (3) and on day 8 in the 2nd batch of (3)

Table 37: Initial nutrient concentrations, biomass concentration and TN/TP ratio in the permeate from the end of the experiment (1) (culture medium (D)) and in the permeate from the end of the experiment (1) enriched with KNO_3 (culture medium (E)) during the experiment (2)

Batch test	Initial TN concentration ($mg \cdot L^{-1}$)		Initial TP concentration ($mg \cdot L^{-1}$)		Initial biomass concentration ($g \cdot L^{-1}$)		TN/TP ratio in (E)	
	D	E	D	E	D	E	Beginning of (2)	Considering TN and TP concentration at the beginning of the 2nd batch of (1) + the addition of KNO_3 at the beginning of (2)
Experiment (2)	10.6 ± 2.8	63.8 ± 10.0	3.50 ± 0.10	3.57 ± 0.06	2.33 ± 0.07	2.31 ± 0.04	17.9	10.1

5.2.1.4.2 pH

The pH in the permeate (A) stayed at a neutral level without pH regulation. Over the four experiments, pH amounted to 7.0 ± 0.2 in (A). During the experiment (3), the average pH in the permeate enriched with nitrogen (B) reached 6.9 ± 0.3 without regulation. During the 44 days of the experiment (4), pH in the commercial fertilizer (C) needed to be regulated 13 times because of too low pH levels. Overall, pH amounted to 6.7 ± 0.5 in (C).

5.2.1.4.3 Experiment (1)

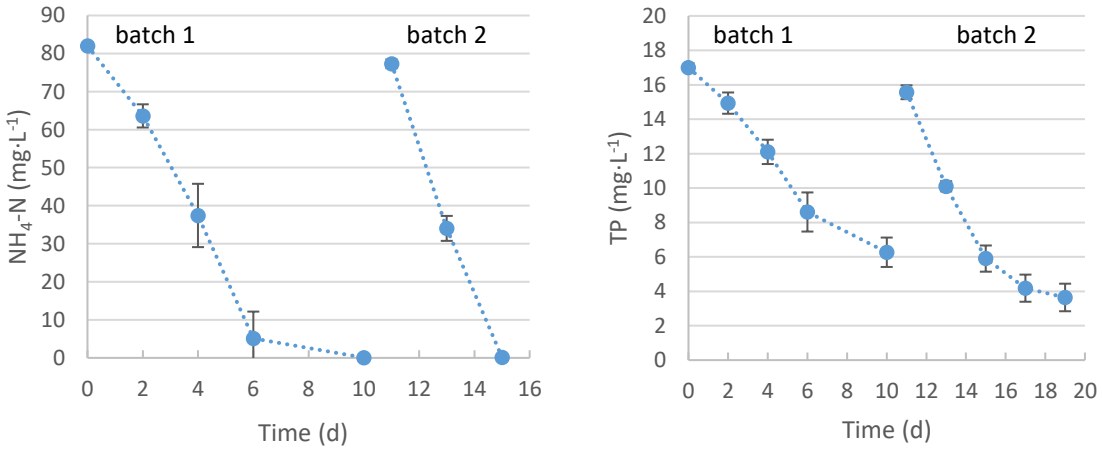

Figure 67: NH_4-N (left) and TP (right) concentrations in the permeate (A) during the experiment (1)

Table 38: Final TN and NH_4-N concentrations, RE_{TN}, $RE_{NH4\text{-}N}$ and RC_{TN} in the permeate (A) during the experiment (1)

Batch test	Final TN concentration ($mg \cdot L^{-1}$)	RE_{TN} (%)	RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)	Final NH_4-N concentration ($mg \cdot L^{-1}$)	$RE_{NH4\text{-}N}$ (%)
1st batch	9.62 ± 5.24	89	8.16	0.07 ± 0.02	100
2nd batch	8.65 ± 1.03	92	23.9	0.13 ± 0.01	100

The main goal of this experiment was the investigation of nutrient removal and microalgae growth in the permeate culture defined by higher nutrient concentrations than in the previous experiments. As

a reminder, the difficulty for the microalgae cells to assimilate the nutrients increases with increasing nutrient concentrations in the culture medium (Su *et al.* 2012).

Over the two batch tests, NH_4-N was totally assimilated with final concentrations ranging between 0.07 mg·L^{-1} and 0.13 mg·L^{-1} (Figure 67). TN removal amounted to between 89 % and 92 % and the final concentrations were less than 10 mg·L^{-1} (Table 38). Compared to the 1st batch, TN removal was 3 times faster during the 2nd batch test. This was probably caused by the good adaptation of the microalgae to the permeate. However, during the 1st and 2nd batch, only 63 % and 76 % respectively of TP was assimilated by the microalgae. TP removal started to decrease and stagnate as long as no more NH_4-N was available in the culture medium. Therefore, the main hypothesis to explain the incomplete TP removal was a too low TN/TP ratio in the permeate. Despite the incomplete TP assimilation, the microalgae grew well and reached a final concentration of 2.60 g·L^{-1} and 2.12 g·L^{-1} respectively (Figure 68). The related BPRs amounted to 0.25 and 0.22 g·L^{-1}·d^{-1} respectively (Table 39).

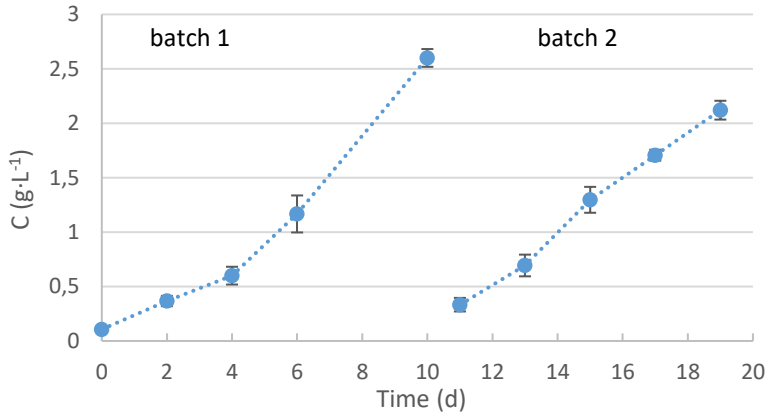

Figure 68: Biomass concentration in the permeate (A) during the experiment (1)

Table 39: Final biomass and TP concentrations, BPR, μ_{max}, RE_{TP} and RC_{TP} in the permeate (A) during the experiment (1)

Batch test	Final TP concentration (mg·L^{-1})	RE_{TP} (%)	RC_{TP} (mg·L^{-1}·d^{-1})	Final biomass concentration (g·L^{-1})	BPR (g·L^{-1}·d^{-1})	μ_{max} (d^{-1})
1st batch	6.27 ± 0.85	63	1.07	2.60 ± 0.08	0.25	0.62
2nd batch	3.64 ± 0.80	76	1.49	2.12 ± 0.09	0.22	0.37

5.2.1.4.4 Experiment (2)

The experiment (2) aimed to investigate if the supply of a nitrogen source would lead to a better assimilation of TP. For this purpose, the culture from the end of (1) was divided into six bottles as defined under Section 4.2.4.4. While the permeate culture was further cultivated as it in (D), KNO_3 was added in the culture medium (E). Hence, in (E), TN concentration on day 0 amounted to 64 mg·L^{-1}.

While TP could not be assimilated in (D), TP concentration achieved 0.63 mg·L^{-1} after only 2 days in (E) (Figure 69). Compared to TP concentration at the beginning of the 2nd batch of (1), this represented an almost complete TP removal of 96 %. In addition, TN final concentration amounted to 20 mg·L^{-1} in (E). The hypothesis worked out after the experiment (1) was here verified. TP could not be totally assimilated during (1) because of the too fast consumption of the nitrogen source contained in the permeate and the unfavorable TN/TP ratio in the permeate. However, the adjustment of the TN/TP ratio in the permeate culture medium can lead to both a complete TN and TP removal. Despite the

non-assimilation of TP in (D), the microalgae continued to grow and reached a final biomass concentration of 3.43 g·L^{-1}. Due to the assimilation of both TP and the additional TN source, the growth was much better in (E) and a final concentration of 4.39 g·L^{-1} was achieved.

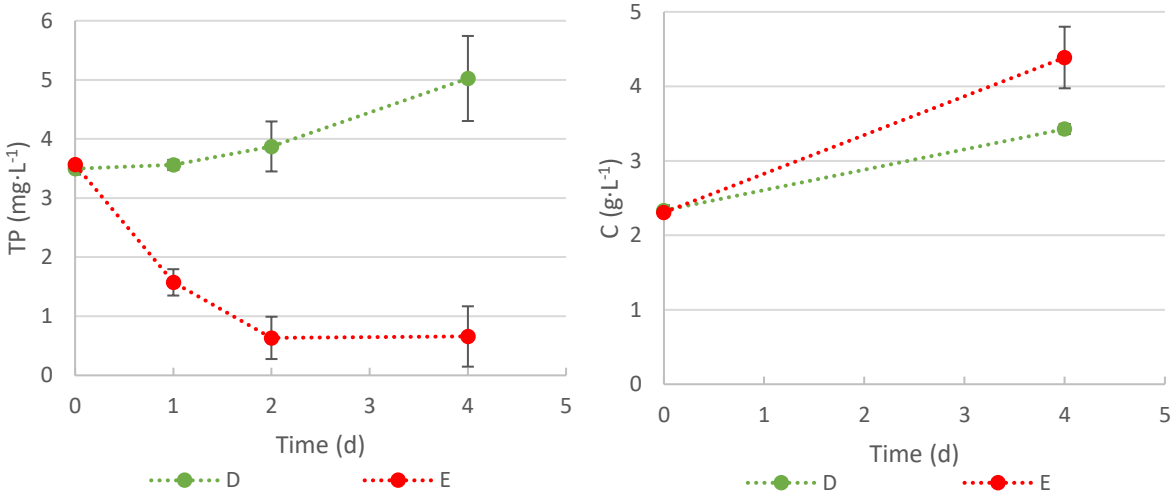

Figure 69: TP concentration (left) and biomass concentration (right) in the permeate from the end of the experiment (1) (culture medium (D)) and in the permeate from the end of the experiment (1) enriched with KNO_3 (culture medium (E)) during the experiment (2)

5.2.1.4.5 Experiment (3)

The experiments (3) had two main objectives. First, it was the first time during the project that such extremely high nutrient concentrations were achieved in the permeate. Hence, one aim was to determine if the microalgae are able to grow in this permeate and to assimilate the nutrients. Indeed, with increasing nutrient concentrations in the culture medium, nutrient assimilation becomes more and more difficult (Su *et al.* 2012).

The second aim was to find the optimum TN/TP ratio with which both TN and TP are assimilated. Indeed, the experiment (1) showed that, with the permeate used for this experiment, ratios of 5.4 and 6.7 are too low and led to the incomplete uptake of TP. On the contrary, a ratio of 10.1 is too high, as a TN concentration of 20 mg·L^{-1} remained in (E) at the end of the experiment (2).

During the 1st batch of (3), the TN/TP ratio amounted to 5.0 in (A) and 7.1 in (B). In these culture media, NH_4-N was totally assimilated within 7 days and 13 days respectively (Figure 70). Until day 13, no significant difference was observed in TP removal despite additional nitrogen supply (Figure 71). On day 13, TP concentration amounted to 17.4 mg·L^{-1} and 20.1 mg·L^{-1} in (A) und (B) respectively. However, the microalgae grew better in presence of additional nitrogen. On day 13, the biomass concentration achieved 4.7 g·L^{-1} and 6.9 g·L^{-1} respectively in (A) and (B) (Figure 72). This represents a significant difference but, in both cases, the microalgae grew very well and reached extremely high biomass concentrations. Likewise, μ_{max} was slightly higher in (B) (0.95 d^{-1}) than in (A) (0.80 d^{-1}).

Considering the initial TN and TP concentrations on day 0, the supply of additional $(NH_4)_2SO_4$ on day 13 corresponded to a TN/TP ratio of 8.9. Within 3 days, NH_4-N concentration in (B) decreased from 71 mg·L^{-1} to 0.29 mg·L^{-1}. During this time, TP concentrations slightly decreased in both (A) and (B). Although the uptake rate was higher in (B), no significant differences were observed between both culture media. Unfortunately, since the culture volumes became too low in the 1-L bottles due to the regular sampling, the batch test could not be further conducted.

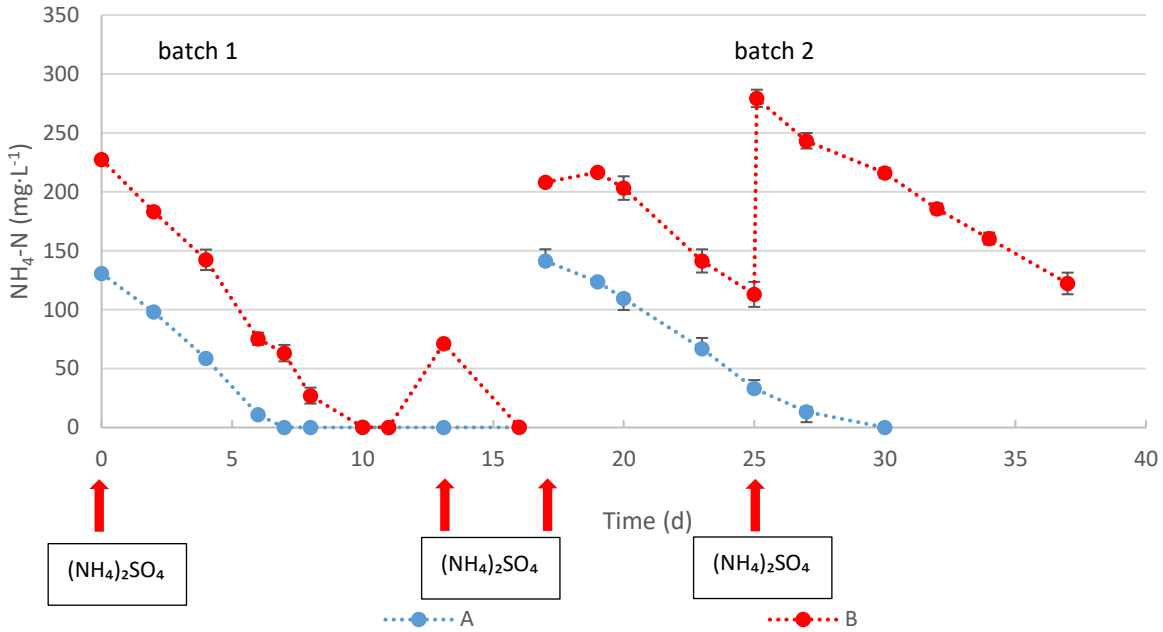

Figure 70: NH_4-N concentration in the permeate (A) and the permeate enriched with $(NH_4)_2SO_4$ (B) during the experiment (3)

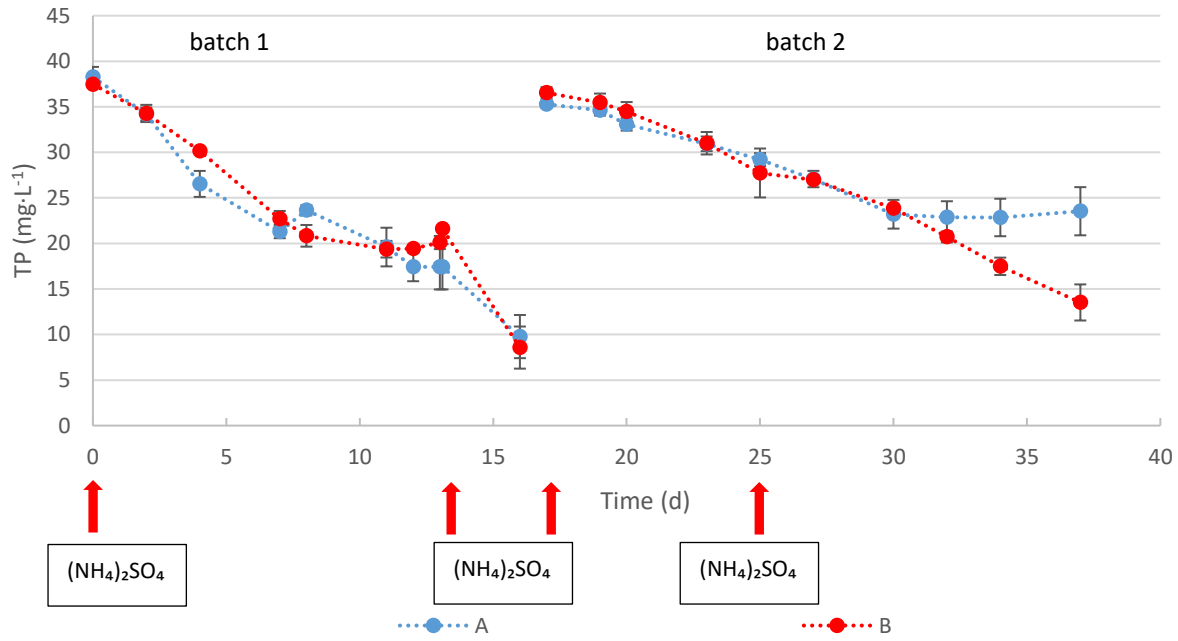

Figure 71: TP concentration in the permeate (A) and the permeate enriched with $(NH_4)_2SO_4$ (B) during the experiment (3)

In the 2nd batch test, until day 25, the difference of TN/TP ratio (4.3 in (A) and 5.9 in (B)) did not lead to any difference in TP removal and biomass concentration. However, the addition of $(NH_4)_2SO_4$ into (B) corresponding to a TN/TP ratio of 10.4 on day 25 led to a much better TP removal in this culture medium starting from day 32. This difference related to TP removal was first observed when NH_4-N contained in the permeate (A) was very low, which led to a stagnation of TP removal in (A).

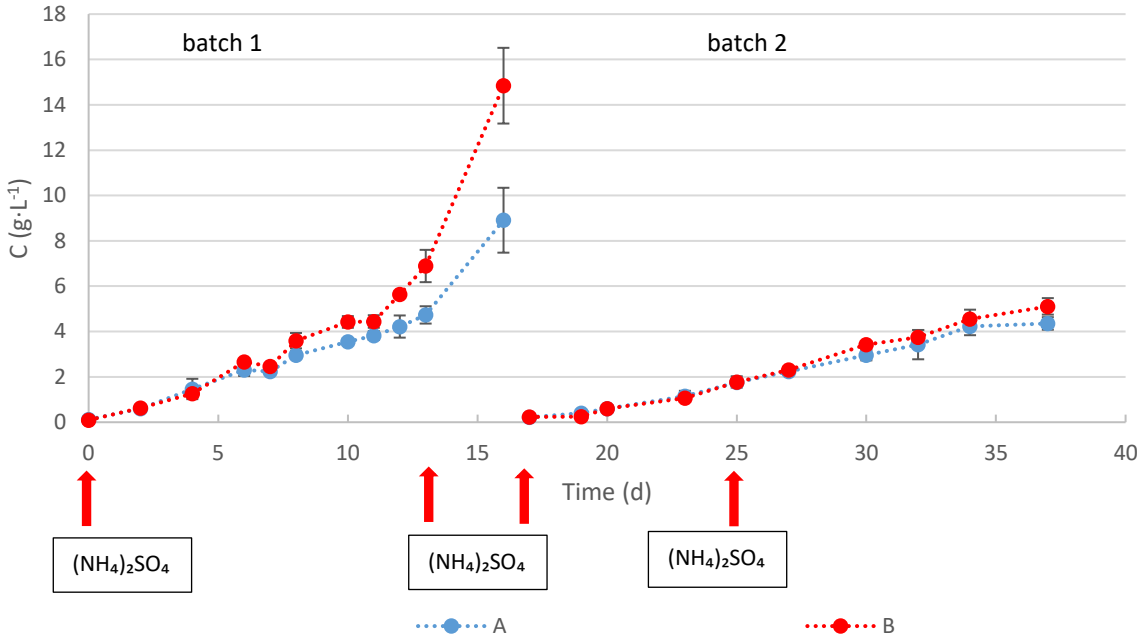

Figure 72: Biomass concentration in the permeate (A) and the permeate enriched with $(NH_4)_2SO_4$ (B) during the experiment (3)

This confirms the hypothesis that *Chlorella sorokiniana* can only assimilate TP in presence of a nitrogen source in the permeate. Because of the too low volumes of culture medium reached in the 1-L bottles, the experiment was ended on day 36. This corresponded to a final TP concentration of 23.5 $mg \cdot L^{-1}$ in (A) and 13.5 $mg \cdot L^{-1}$ in (B) as well as a TP removal of 33 % and 63 % in (A) and (B) respectively. Final biomass concentrations of 4.4 and 5.1 $g \cdot L^{-1}$ were achieved in (A) and (B) respectively. Compared to the 1st batch, this represented only low differences between both culture media. However, while TP concentration would have surely further stagnated in the permeate (A), NH_4-N and TP would have presumably been entirely consumed on day 42 or 43 in (B). The final biomass concentration in (B) would have been accordingly much higher than in (A).

Table 40 and Table 41 resume the performance of microalgae culture during the experiment (3). Two further observations were done. First, RC_{TN} was much higher in (B) than in (A), attesting that TN uptake was faster with higher initial TN concentrations. Compared to other experiments conducted with *Chlorella sorokiniana*, *Chlorella vulgaris* or *Acutodesmus obliquus*, very high BPRs were achieved in the 1st batch of (3), including for the permeate (A) (0.54 and 0.92 $g \cdot L^{-1} \cdot d^{-1}$ in (A) and (B) respectively).

Table 40: Final TN and NH_4-N concentrations, RE_{TN}, RE_{NH4-N} and RC_{TN} in the permeate (A) and the permeate enriched with $(NH_4)_2SO_4$ (B) during the experiment (3)

Batch Test	Final TN concentration ($mg \cdot L^{-1}$)		RE_{TN} (%)		RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)		Final NH_4-N concentration ($mg \cdot L^{-1}$)		RE_{NH4-N} (%)	
	A	B	A	B	A	B	A	B	A	B
1st batch	22.7 ± 2.8	27.5 ± 2.3	88	92	10.4	19.1	0.13 ± 0.01	0.29 ± 0.05	100	100
2nd batch	10.5 ± 1.4	137 ± 6	94	64	7.0	12.1	0.14 ± 0.02	122 ± 9	100	67

Table 41: Final TP and biomass concentrations, RE_{TP}, RC_{TP}, BPR and μ_{max} in the permeate (A) and the permeate enriched with $(NH_4)_2SO_4$ (B) during the experiment (3)

Batch test	Final TP concentration ($mg \cdot L^{-1}$)		RE_{TP} (%)		RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)		Final biomass concentration ($g \cdot L^{-1}$)		BPR ($g \cdot L^{-1} \cdot d^{-1}$)		μ_{max} (d^{-1})	
	A	B	A	B	A	B	A	B	A	B	A	B
1st batch	9.8 ± 2.4	8.6 ± 2.3	74	77	1.8	1.8	8.91 ± 1.43	14.8 ± 1.7	0.54	0.92	0.80	0.95
2nd batch	23.5 ± 2.6	13.5 ± 2.0	33	63	0.93	1.2	4.36 ± 0.28	5.11 ± 0.36	0.21	0.24	0.41	0.92

However, in the 2nd batch, the BPRs significantly decreased and amounted to 0.21 and 0.24 $g \cdot L^{-1} \cdot d^{-1}$ in (A) and (B) respectively. Hence, these values are similar to previous experiments of the present project. Nevertheless, considering the first 13 days of the 1st batch, BPRs of 0.35 and 0.52 $g \cdot L^{-1} \cdot d^{-1}$ respectively were achieved in (A) and (B). After this, a significant increase was observed within three days. This increase could have been caused by fluid evaporation combined with the extremely low culture medium volume remaining in the bottles. Hence, the BPRs of 0.35 and 0.52 $g \cdot L^{-1} \cdot d^{-1}$ respectively in (A) and (B) seem more accurate. Nevertheless, these BPR values are likewise much higher than in the previous experiments of the present work.

5.2.1.4.6 Experiment (4)

The experiment (4) aimed to investigate if the same observations concerning the difficulty to remove TP without the additional supply of a nitrogen source could be done with a synthetic culture medium having similar nutrient composition to the permeate. Hence, the main goal of this experiment was to observe if similar BPRs, TP and TN removal could be achieved cultivating simultaneously *Chlorella sorokiniana* with the permeate (A) and a commercial fertilizer (C).

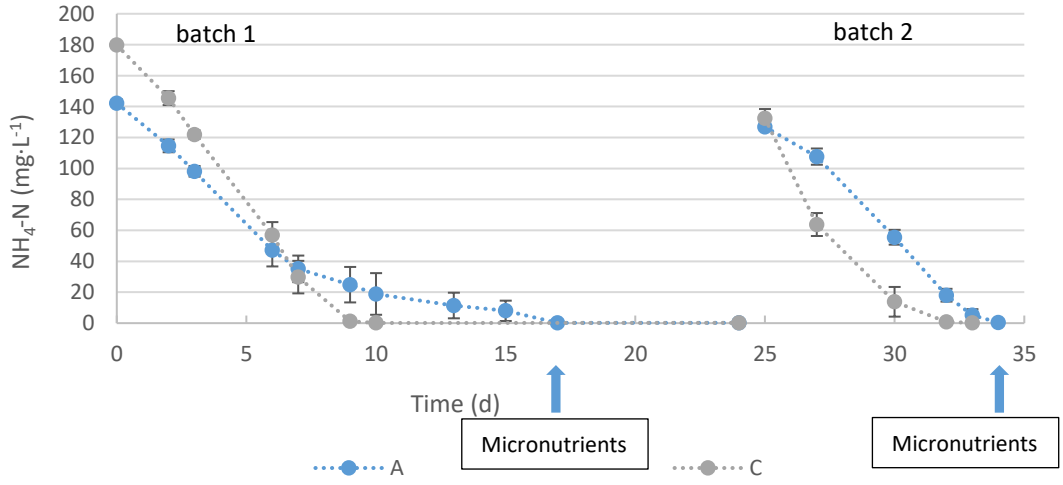

Figure 73: NH_4-N concentration in the permeate (A) and the synthetic culture medium (C) during the experiment (4) - Addition of Mg, Mn, Fe, S and EDTA into (A) on day 17 and day 34

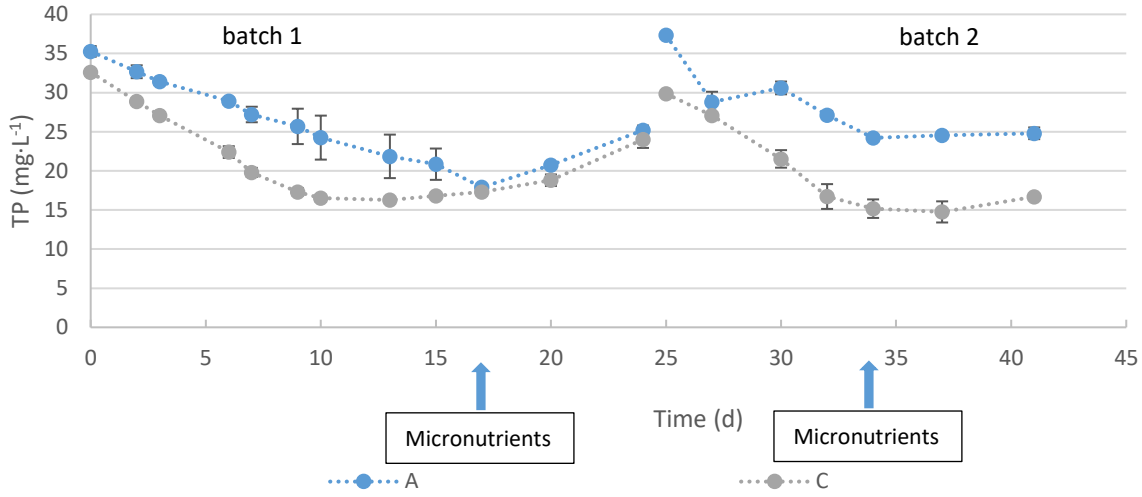

Figure 74: TP concentration in the permeate (A) and the synthetic culture medium (C) during the experiment (4) - Addition of Mg, Mn, Fe, S and EDTA into (A) on day 17 and day 34

During the first batch, NH_4-N was completely assimilated after 17 and 10 days in the permeate (A) and the commercial fertilizer (C) respectively (Figure 73). This corresponded to TN uptake rates of 8.7 and 13.4 $mg \cdot L^{-1} \cdot d^{-1}$ respectively (Table 42). The faster TN removal in (C) was confirmed during the 2nd batch test, where the TN uptake rate amounted to 14.3 and 18.0 $mg \cdot L^{-1} \cdot d^{-1}$ in (A) and (C) respectively.

In the 1st batch test, TP removal in (A) and (C) was similar and amounted to 49 % and 50 % respectively (Figure 74 and Table 43). During the 2nd batch test, TP removal was slightly higher in the commercial fertilizer (51 %) than in the permeate (35 %). However, these values were very low and TP removal stagnated in both culture media starting from day 34. Moreover, the addition of micronutrients in (A) on day 17 during the 1st batch and on day 34 during the 2nd batch did not influence TP removal. Overall, after the 1st and the 2nd batch tests, TP final concentrations amounted to 17.9 and 24.2 $mg \cdot L^{-1}$ in the permeate (A) and 16.3 and 14.8 $mg \cdot L^{-1}$ in the commercial fertilizer (C).

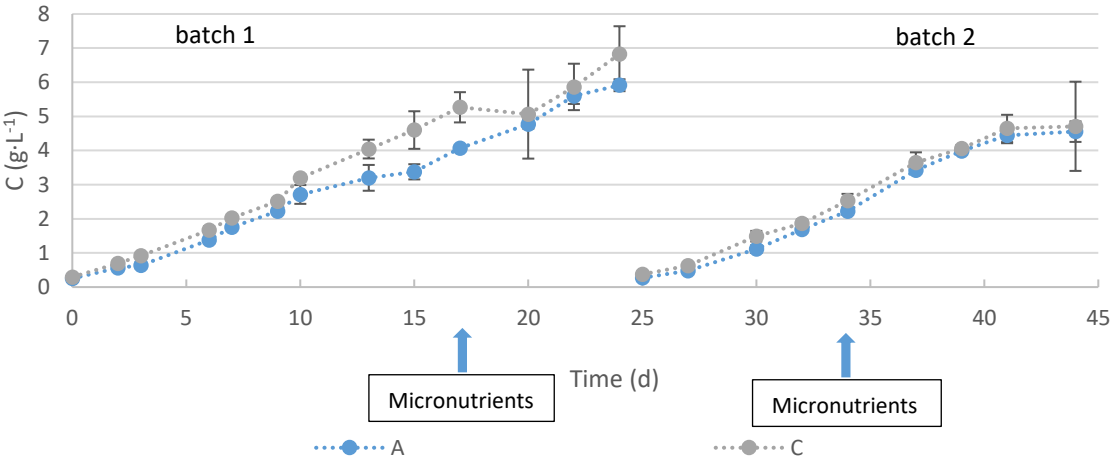

Figure 75: Biomass concentration in the permeate (A) and the synthetic culture medium (C) during the experiment (4) - Addition of Mg, Mn, Fe, S and EDTA into (A) on day 17 and day 34

Compared to the permeate (A), *Chlorella sorokiniana* grew slightly better in the commercial fertilizer (C) (Figure 75). While the BPRs related to (A) amounted to 0.24 and 0.26 $g \cdot L^{-1} \cdot d^{-1}$ in the 1st and 2nd batch tests, this parameter achieved values of 0.27 $g \cdot L^{-1} \cdot d^{-1}$ during both batches in (C). Here, a significant influence of the addition of micronutrients in (A) was observed in the 1st batch. Starting from day 10,

the gap between the two curves representing (A) and (C) gradually increased. However, after micronutrient addition on day 17, the gap decreased and similar biomass concentrations were even achieved on day 20 and day 22. In addition, the BPR amounted to 0.22 $g \cdot L^{-1} \cdot d^{-1}$ before micronutrient addition and increased up to 0.26 $g \cdot L^{-1} \cdot d^{-1}$ after micronutrient addition. Nevertheless, the positive influence of micronutrient addition on microalgae growth in the permeate (A) was not clearly observed during the 2nd batch, so that no definitive conclusion could be drawn. Anyway, since very high biomass concentrations were reached in the permeate (A) without micronutrient addition in the experiments (1) and (3), the statement of the influence of micronutrients addition on microalgae growth did not represent the main aim of this experiment. Likewise, μ_{max} was similar in the permeate (A) and in the control culture (C), where it amounted to 0.40 - 0.43 d^{-1} in the 1st batch and 0.29 d^{-1} in the 2nd batch. Hence, it confirmed the similar performance between (A) and (C).

Overall, this experiment proved that the incomplete TP removal in the permeate (A) was not due to a lack of micronutrients in the permeate (A). Moreover, as the microalgae were not able to efficiently remove TP from the commercial fertilizer (C), the incomplete TP removal in the permeate (A) was not due to the permeate itself or eventual pollutants contained in the permeate. On the contrary, as it was suggested at the end of the experiment (3), this was only caused by an unfavorable TN/TP ratio.

Table 42: Final TN and NH_4-N concentrations, RE_{TN}, RE_{NH4-N} and RC_{TN} in the permeate (A) and in the synthetic culture medium during the experiment (4)

Batch Test	Final TN concentration ($mg \cdot L^{-1}$)		RE_{TN} (%)		RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)		Final NH_4-N concentration ($mg \cdot L^{-1}$)		RE_{NH4-N} (%)	
	A	B	A	B	A	B	A	B	A	B
1st batch	11.2 ± 0.8	9.49 ± 0.88	93	95	8.7	13.4	0.18 ± 0.02	0.12 ± 0.04	100	100
2nd batch	9.36 ± 1.59	7.70 ± 1.80	93	95	14.3	18.0	0.38 ± 0.15	0.20 ± 0.09	100	100

Table 43: TP and biomass final concentrations, RE_{TP}, RC_{TP}, BPR and μ_{max} in the permeate (A) and in the synthetic culture medium (C) during the experiment (4)

Batch Test	TP final concentration ($mg \cdot L^{-1}$)		RE_{TP} (%)		RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)		Final biomass concentration ($g \cdot L^{-1}$)		BPR ($g \cdot L^{-1} \cdot d^{-1}$)		μ_{max} (d^{-1})	
	A	C	A	C	A	C	A	C	A	C	A	C
1st batch	17.9 ± 0.1	16.3 ± 0.3	49	50	1.0	1.3	5.91 ± 1.17	6.82 ± 0.82	0.24	0.27	0.40	0.43
2nd batch	24.2 ± 0.32	14.8 ± 1.3	35	51	1.5	1.3	4.36 ± 0.28	5.11 ± 0.36	0.26	0.27	0.29	0.29

5.2.2 Microalgae cultivation at full-scale with outdoor flat photobioreactors

The two experiments conducted in fall 2017 and summer 2018 in the outdoor full-scale pilot-plant Hamburg-Reitbrook aimed at the comparison of microalgae growth and nutrient removal with the values obtained at lab-scale during the experiments conducted with *Acutodesmus obliquus* at the

University of Hamburg and at the TU Berlin. Since it is an outdoor pilot-plant, the influence of weather conditions needed also to be assessed. This work at lab-scale must demonstrate if the use of permeate is suitable for microalgae cultivation and nutrient assimilation at full-scale.

5.2.2.1 Abiotic conditions

The temperature, pH and light conditions during the 1st experiment in fall 2017 and the 2nd experiment in summer 2018 are summarized in Table 44. Because of the defect of the storage function of the control system, the temperature and pH data of the 2nd and the 3rd batch tests in fall 2017 is missing. Nevertheless, no abnormality of the temperature or the pH value was observed in the related samples.

During the 1st experiment in fall 2017, the average temperatures achieved values ranging 16.8 - 20.4 °C. The PBRs are heated by temperatures lower than 20 °C. However, during the 4th batch test, the heating capacity of the heat exchanger seemed to be not enough to achieve an average temperature of 20 °C, probably because of too low ambient temperatures. These averaged 12.6 °C during the 1st batch test and only 9.7 °C during the 4th batch test.

Figure 76 shows the development of temperature and pH during the 4th batch test. Within the days characterized by the highest light conditions like the 29.10.17, 30.10.17 or 06.11.17, the temperature differences in the PBRs were huge and could represent a stress factor for the microalgae. For example, the temperature in L2 amounted to approximately 26.5 °C at 02:00 PM on 06.11.17 and only 12 °C at 02:00 AM during the following night (outdoor temperature of approximately 2 °C). On days with a low PPFD, like the 31.10.17, these differences between day and night temperatures in the PBRs were not significant.

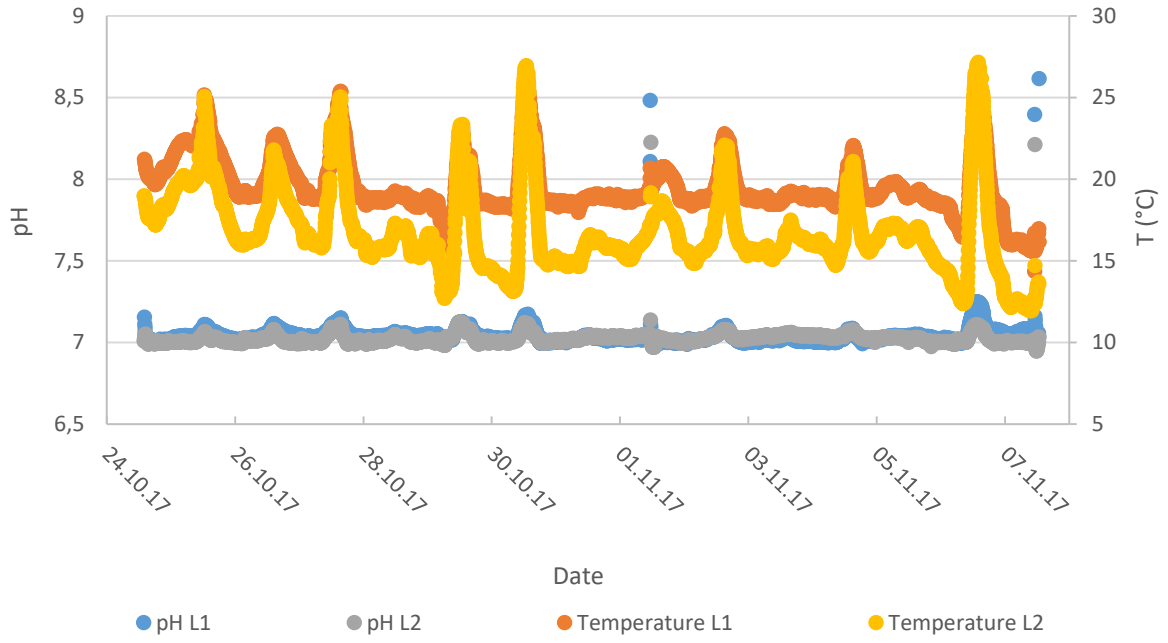

Figure 76: Temperature and pH in L1 and L2 during the 4th batch test of the fall season experiment

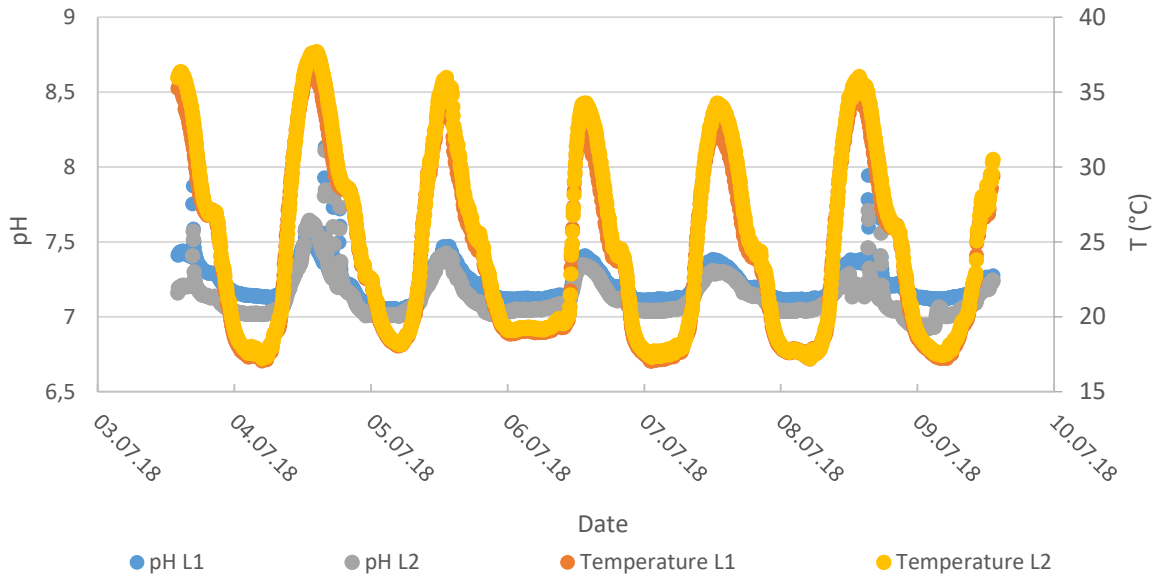

Figure 77: Temperature and pH in L1 and L2 during the 5th batch test of the summer season experiment

During the 2nd experiment in summer 2018, the average temperatures in the PBRs were higher and comprised between 20.3 and 26.3 °C. The standard deviation was also much higher and achieved up to 6 °C. For example, during the 5th batch, despite the cooling of the PBRs starting from a temperature of 25 °C, the PBRs temperature reached values up to 37 °C during the day (Figure 77). Since temperatures of 17 - 18 °C were achieved during the night, this represents a temperature variation of up to 20 °C within 12 hours.

During both experiments, the average pH values ranged 7.0 - 7.6 and were closed to the target pH of 7.0. The highest pH values obtained during the 1st batch in fall 2017 and the 4th batch in summer 2018 are explained by a technical problem affecting flue gas supply into the PBRs for three days and one day respectively and conducting to pH values up to 11.

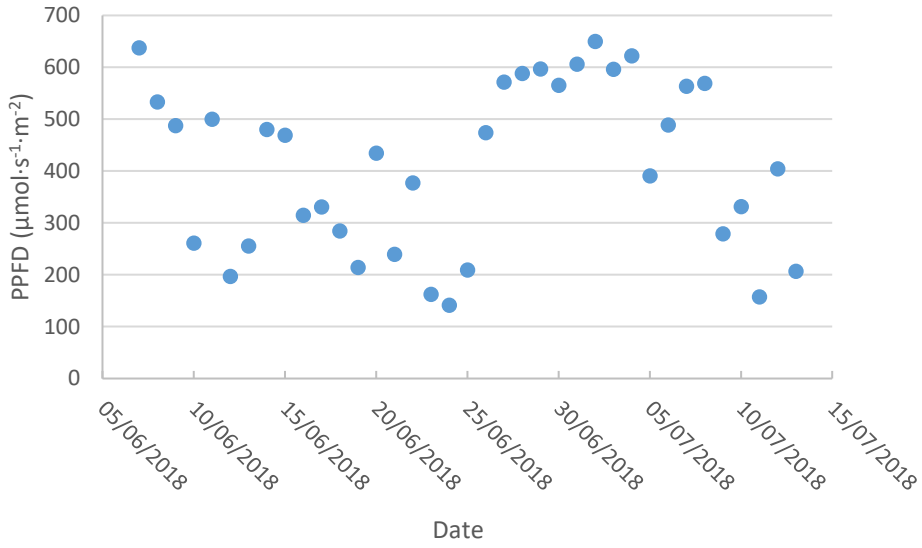

Figure 78: PPFD during the experiment in the summer season

The 1st experiment in fall 2017 was characterized by decreasing light conditions. During the four batch tests, the related average PPFD amounted to 150, 126, 90 and 81 µmol·s^{-1}·m^{-2}. In summer 2018, the

light conditions were comparatively very good and the average PPFD during the six batch tests ranged between 263 and 567 µmol·s^{-1}·m^{-2}. Compared to the 1st batch of the experiment in fall 2017, this represents a PPFD increase of 81 % to 293 %. During this 2nd experiment in summer 2018, the 2nd, 3rd and 6th batch tests were characterized by the lowest light conditions with PPFD of 328, 263 and 287 µmol·s^{-1}·m^{-2} respectively. The highest light conditions were reached during the 4th and 5th batch tests (567 and 544 µmol·s^{-1}·m^{-2}). Within a batch test, depending on the daily weather, great differences were reached. The standard deviation ranged 46 - 75 µmol·s^{-1}·m^{-2} during the 1st experiment and 95 - 133 µmol·s^{-1}·m^{-2} during the 2nd experiment (Figure 78). This represents up to 57 % and 42 % of the average PPFD during the 1st and the 2nd experiment respectively.

Table 44: PPFD, pH and temperature during the two outdoor experiments in the fall season and in the summer season

Experiment	Batch test	PPFD (µmol·s^{-1}·m^{-2})	T in L1 (°C)	pH in L1	T in L2 (°C)	pH in L2
Fall 2017	**1st batch 28.09. - 09.10.**	150 ± 75	20.4 ± 2.2	7.5 ± 1.1	-	-
	2nd batch 09.10. - 17.10.	126 ± 59	defect of the storage function of the control system			
	3rd batch 17.10. - 24.10.	90 ± 51	defect of the storage function of the control system			
	4th batch 24.10. - 07.11.	81 ± 46	19.5 ± 1.8	7.0 ± 0.1	16.8 ± 2.7	7.0 ± 0.0
Summer 2018	**1st batch 07.06. - 12.06.**	448 ± 131	25.9 ± 4.3	7.1 ± 0.3	26.3 ± 4.8	7.0 ± 0.3
	2nd batch 12.06. – 19.06.	328 ± 109	23.1 ± 3.9	7.1 ± 0.1	23.4 ± 4.3	7.1 ± 0.1
	3rd batch 19.06. - 25.06.	263 ± 110	20.3 ± 3.7	7.1 ± 0.1	20.5 ± 3.9	7.1 ± 0.1
	4th batch 25.06. - 03.07.	567 ± 133	24.7 ± 6.0	7.6 ± 1.3	25.1 ± 6.4	7.5 ± 1.1
	5th batch 03.07. - 09.07.	544 ± 116	24.0 ± 5.8	7.2 ± 0.1	24.6 ± 6.3	7.1 ± 0.1
	6th batch 09.07. - 13.07.	287 ± 95	22.1 ± 4.5	7.2 ± 0.1	-	-

5.2.2.2 1st experiment in the fall season

5.2.2.2.1 Nutrient removal

5.2.2.2.1.1 NH_4-N

During the 1st experiment in fall 2017, the nutrient NH_4-N was totally removed (99 % on average - Figure 79 and Table 45). At the end of three of the four batch experiments, NH_4-N was only present at very low concentrations (0.049 - 0.74 $mg \cdot L^{-1}$). The 3rd batch experiment in L1 represents an exception, as the final NH_4-N concentration amounted to 2.89 $mg \cdot L^{-1}$. However, it is assumed that a complete ammonium removal would have been achieved if the experiment had run one or two more days. Instead of that, it was decided to begin the 4th batch test before the onset of winter and the shutting down of both lines.

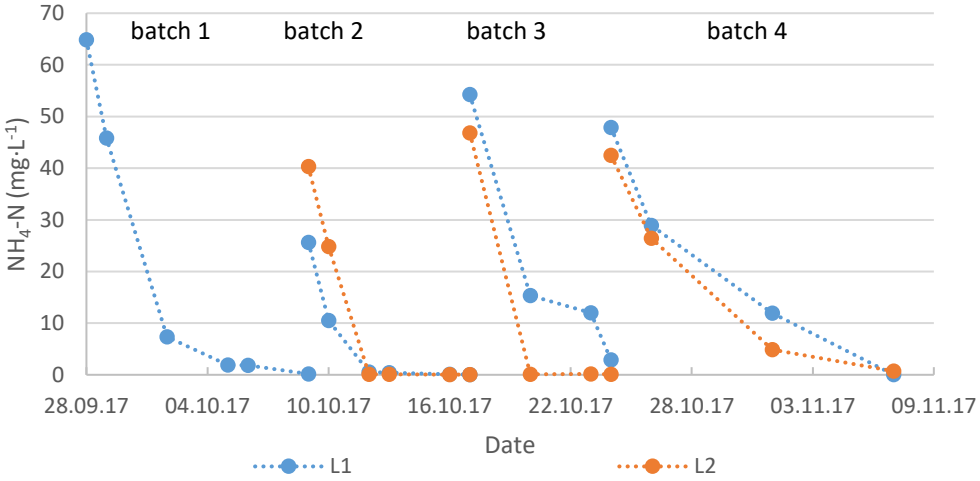

Figure 79: NH_4-N concentration in L1 and L2 during the full-scale experiment in the fall season

Table 45: Initial concentration, final concentration, RE and RC related to NH_4-N in L1 and L2 during the full-scale experiment in the fall season

Parameter	L1				L2		
	1st Batch	2nd Batch	3rd Batch	4th Batch	2nd Batch	3rd Batch	4th Batch
Initial concentration ($mg \cdot L^{-1}$)	64.9	25.7	54.3	47.9	40.3	46.9	42.5
Final concentration ($mg \cdot L^{-1}$)	0.147	0.048	2.89	0.049	0.043	0.067	0.74
RE (%)	100	100	95	100	100	100	98
RC ($mg \cdot L^{-1} \cdot d^{-1}$)	5.9	8.4	7.3	3.4	13	16	3.0

During the 2nd and 3rd batch tests, NH_4-N removal was faster in L2 than in L1. While $RC_{NH4\text{-}N}$ achieved 13.4 and 16 $mg \cdot L^{-1} \cdot d^{-1}$ in L2, it only amounted to 8.4 and 7.3 $mg \cdot L^{-1} \cdot d^{-1}$ in L1. This represented respectively 27 % and 47 % of $RC_{NH4\text{-}N}$ in L2. These results indicate that the lower cultivation volume/illuminated area ratio of the PBR of L2 enables much faster nutrient assimilation due to the enhanced light availability. This tendency was not confirmed during the 4th batch test, probably

because the average temperature in L2 was approximately 3 °C below that of L1. Moreover, the light conditions were already limited, so that, compared to the previous batch experiments, only very low $RC_{NH4\text{-}N}$ were achieved (3.65 and 2.98 $mg \cdot L^{-1} \cdot d^{-1}$ in L1 and L2 respectively).

5.2.2.2.1.2 TN

TN removal reached an average of 74 ± 24 % over all four batch tests (Figure 80 and Table 46). Unlike NH_4-N, these results indicate an incomplete TN removal and strong variations within the batch tests. While more than 90 % were removed in three batch experiments and final TN concentrations below 7 $mg \cdot L^{-1}$ were achieved, only 46, 67 and 36 % of TN were removed during the 3rd batch test in L1, the 4th batch test in L1 and the 4th batch test in L2. TN final concentrations amounted to 35, 21 and 37 $mg \cdot L^{-1}$ respectively. These relatively high TN final concentrations despite the total assimilation of NH_4-N were explained by higher NO_3-N and NO_2-N concentrations in the culture medium. Final NO_3-N concentrations reached 10, 16 and 32 $mg \cdot L^{-1}$ respectively, while the NO_2-N value at the end of the 3rd batch test in L1 amounted to 14 $mg \cdot L^{-1}$. In comparison, NO_3-N and NO_2-N values lower than 2 $mg \cdot L^{-1}$ were achieved in the batch tests that achieved a TN removal greater than 90 %.

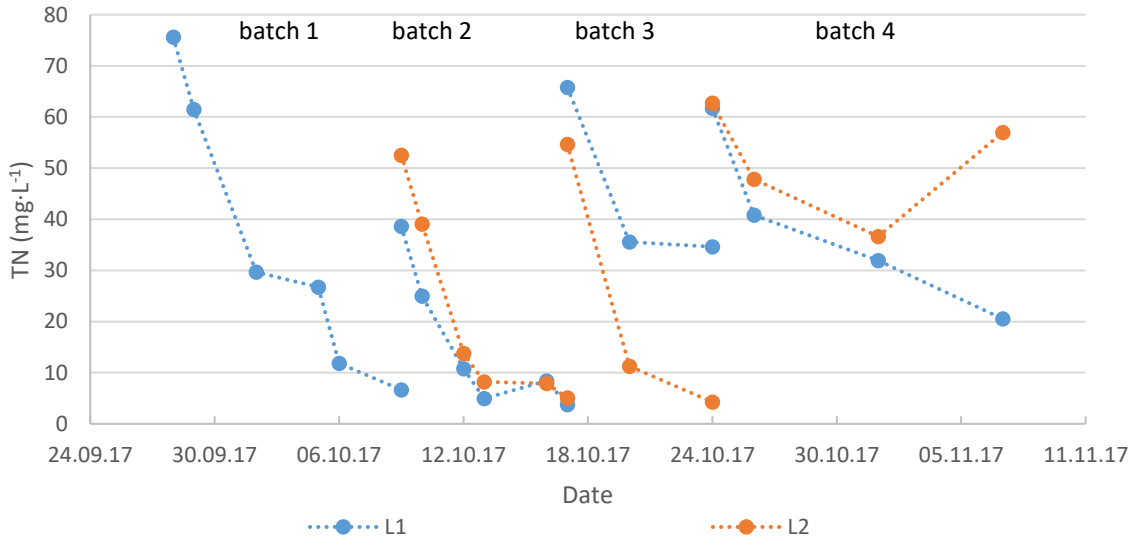

Figure 80: TN concentration in L1 and L2 during the full-scale experiment in the fall season

Table 46: Initial concentration, final concentration and RE related to TN in L1 and L2 during the full-scale experiment in the fall season

Parameter	L1				L2		
	1st Batch	2nd Batch	3rd Batch	4th Batch	2nd Batch	3rd Batch	4th Batch
Initial concentration ($mg \cdot L^{-1}$)	75.6	38.7	54.3	47.9	52.5	54.7	62.7
Final concentration ($mg \cdot L^{-1}$)	6.64	3.77	34.6	20.6	5.05	4.26	36.6
RE (%)	91	90	47	67	90	92	42

These increased NO_3-N and NO_2-N concentrations are explained by NO_x supply through flue gas. Although the analysis used for the determination of flue gas (gas chromatography) did not allow

determining the NO_x content, fluctuating NO_x concentrations could be assumed. Furthermore, the PPFD was greatly reduced during the 3rd and 4th batches and represented only 55 % to 61 % of the PPFD during the 1st batch test. Accordingly, the higher final TN, NO_3-N and NO_2-N concentrations were observed only at the end of the 3rd and 4th batch tests. At the beginning of the repeated batch tests, enough light was available, and, despite the continuous addition of NO_x, the microalgae could completely remove NO_3-N and NO_2-N. However, the microalgae were probably not able to assimilate the NO_x at too low PPFD levels. The difference between L1 and L2 during the 3rd batch test, where 47 % and 92 % of TN removal were achieved in L1 and L2 respectively, is probably due to the lower cultivation volume/illuminated area ratio of the PBR of L2.

5.2.2.2.1.3 TP

Except for the 3rd batch test in L1 (see section 5.2.2.2.1.1), more than 96 % TP was systematically removed from both cultures and final concentrations lower than 0.23 $mg \cdot L^{-1}$ were achieved (Figure 81 and Table 47). In L2, the lower cultivation volume/illuminated area ratio of the PBR resulted in higher RC_{TP}. These reached 1.7, 2.5 and 1.2 $mg \cdot L^{-1} \cdot d^{-1}$ during the 2nd, 3rd and 4th batch tests in L2 and 1.1, 1.0 and 0.7 $mg \cdot L^{-1} \cdot d^{-1}$ in L1. Overall, RC_{TP} in L2 was 35 %, 59 % and 43 % better than in L1.

Within all the batch experiments, the number of days needed to reach the final concentrations were very variable. While only four and three days were needed to reach very low concentrations during the 2nd batch in both lines and the 3rd batch in L2, this value amounted to 11 and 14 days during the 1st and the 4th batch test respectively. Except for the 1st and the 2nd batch test in L1, the differences observed were similar to the tendency of the results related to RC_{TP}. During the 1st batch test, RC_{TP} was higher than during the other batch tests but the relatively high initial NH_4-N and TP concentrations (64.9 and 9.6 $mg \cdot L^{-1}$ respectively) explain the longer time needed to reach low nutrient concentrations. On the contrary, nutrient concentration was relatively low at the beginning of the 2nd batch in L1. Therefore, despite low RC_{TP} values, only 4 days were needed to totally remove the nutrients.

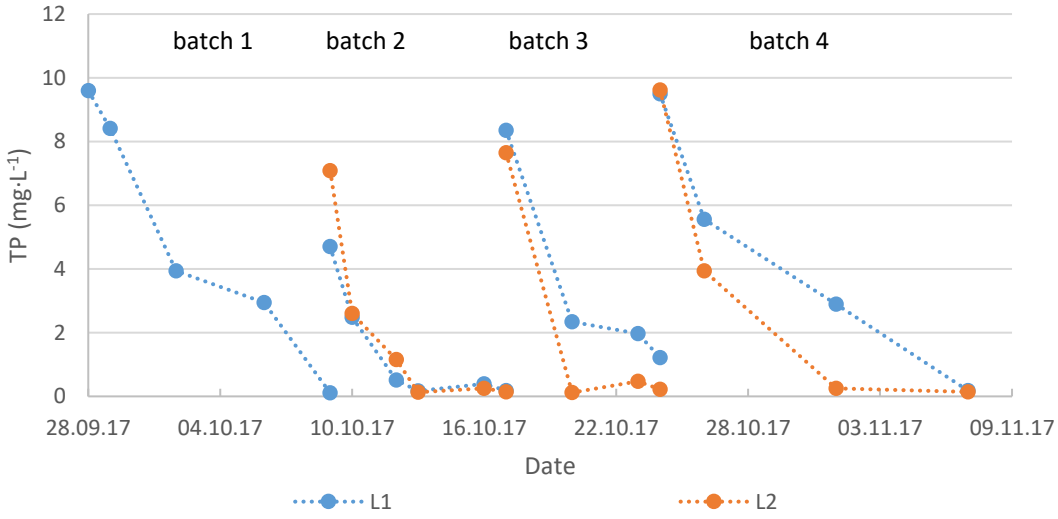

Figure 81: TP concentration in L1 and L2 during the full-scale experiment in the fall season

Table 47: Initial and final concentrations, RE and RC related to TP in L1 and L2 during the full-scale experiment in the fall season

Parameter	L1				L2		
	1st Batch	2nd Batch	3rd Batch	4th Batch	2nd Batch	3rd Batch	4th Batch
Initial concentration ($mg \cdot L^{-1}$)	9.60	4.71	8.35	9.50	7.09	7.65	9.62
Final concentration ($mg \cdot L^{-1}$)	0.12	0.18	1.22	0.18	0.14	0.23	0.15
RE (%)	99	96	85	98	98	97	98
RC ($mg \cdot L^{-1} \cdot d^{-1}$)	0.9	1.1	1.0	0.7	1.7	2.5	1.2

5.2.2.2.2 Microalgae growth

In terms of biomass production, at the end of the 1st, 2nd, 3rd and 4th batch tests, average biomass concentrations of 1.28, 1.27, 0.78 and 0.59 $g \cdot L^{-1}$ were reported (see Figure 82 and Table 48). Between the 1st and the 4th batch test, according to the decrease of light availability, the BPR decreased from 0.10 to 0.04 $g \cdot L^{-1} \cdot d^{-1}$ in L1 and from 0.13 to 0.01 $g \cdot L^{-1} \cdot d^{-1}$ in L2. Accordingly, μ_{max} decreased from 0.30 d^{-1} to 0.16 d^{-1} between the 2nd and 4th batch test in L2. In L1, μ_{max} also significantly decreased between the 2nd and 4th batch test. However, in the 1st and 4th batch, μ_{max} was similar. This was probably caused by an adaptation time needed by the microalgae in the 1st batch test.

In the 4th batch test, an almost nonexistent growth in L2 culture starting from the 2nd day was observed. As the microalgae in L1 continued their growth, light availability limitation can not explain this observation. These results are also non-coherent with $RC_{NH4\text{-}N}$ and RC_{TP}, which were higher in L2 than in L1. Several reasons could explain the absence of growth in L2. First, the average temperature only amounted to 16.8 °C, being 3 degrees less than in L1. This low temperature combined with the low light availability could have first led to a lower microalgae growth, and, then, to a premature stop of the growth. As no contamination was observed in L2, other possible explanations are more sedimentation than usual in the pipes and the fact that two biologic systems respond differently to extreme abiotic conditions.

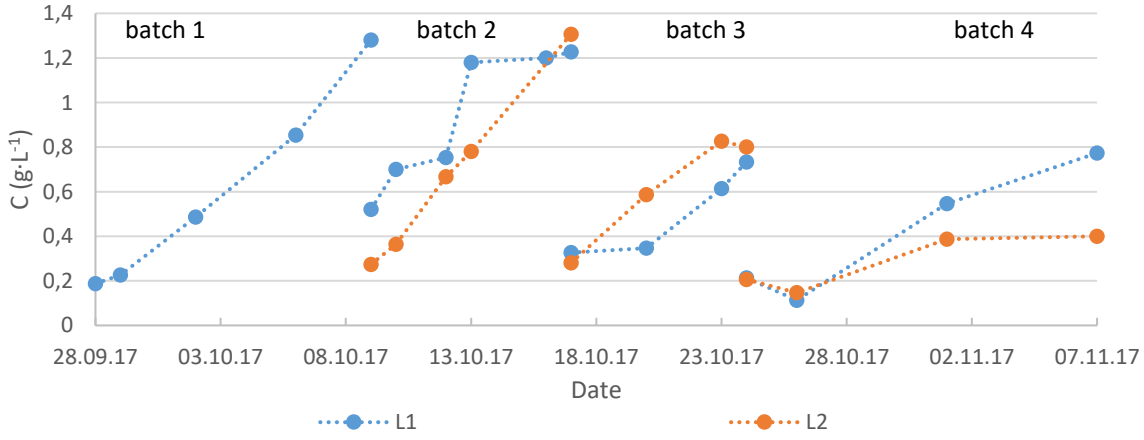

Figure 82: Biomass concentration in L1 and L2 during the full-scale experiment in the fall season

Table 48: Initial and maximal biomass concentrations, BPR, BPR_{α}, μ_{max} and PE in L1 and L2 during the full-scale experiment in the fall season

Parameter	L1				L2		
	1st Batch	2nd Batch	3rd Batch	4th Batch	2nd Batch	3rd Batch	4th Batch
Initial concentration ($g \cdot L^{-1}$)	0.19	0.52	0.33	0.21	0.27	0.28	0.21
Maximal concentration ($g \cdot L^{-1}$)	1.28	1.23	0.73	0.77	1.31	0.83	0.40
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	0.10	0.09	0.06	0.04	0.13	0.07	0.01
BPR_{α} ($g \cdot m^{-2} \cdot d^{-1}$)	5.2	4.6	3.0	2.1	3.0	1.7	0.3
μ_{max} (d^{-1})	0.25	0.44	0.19	0.26	0.30	0.25	0.16
PE (%)	1.8	2.1	1.7	1.3	1.2	0.9	1.2

BPR_{α} and PE ranged 0.3 - 5.2 $g \cdot m^{-2} \cdot d^{-1}$ and 0.9 - 2.1 % respectively. Although the BPR was higher in L2, microalgae growth related to the illuminated area was better in L1. Indeed, in the 2nd, 3rd and 4th batch tests, BPR_{α} in L1 was 53 %, 76 % and 600 % higher than in L2. As the economic costs of microalgae production can depend on the surface used for the processes, this parameter is essential. Likewise, higher PE were reached in L1. In this line, between 1.3 and 2.1 % of the light energy was used for biomass production. This parameter only ranged 0.9 - 1.2 % in L2. These differences between both lines are poorly explainable. A lower temperature in L2 could eventually partially explain these results. Nevertheless, the failure of the storage function of the control system during the 2nd and 3rd batch tests makes any comparison impossible. Moreover, as the PBRs of L1 and L2 contained a similar volume of culture and L2 has a lower cultivation volume/illuminated area ratio, a bigger proportion of the culture L2 is in the dark in the pipes. This could have influenced the parameter BPR_{α}.

5.2.2.3 2nd experiment in the summer season

5.2.2.3.1 Nutrient removal

5.2.2.3.1.1 NH_4-N

During the 2nd experiment in summer 2018, throughout the six batch tests in L1 and the five batch tests in L2, the nutrient NH_4-N was totally removed from the culture medium (Figure 83). The initial concentrations amounted to between 65.1 and 101 $mg \cdot L^{-1}$ and were in average 73 % higher than during the 1st experiment in fall 2017 (Table 49). All the final concentrations achieved values lower than 0.1 $mg \cdot L^{-1}$, and, except for the 4th and the 6th batch tests, very low NH_4-N concentrations were reached within 1 day. During the 4th batch test, this time was extended to 3 days, probably because of the cessation of flue gas supply into the PBRs for one day. Almost two days were needed during the 6th batch test to remove completely this nutrient. This was explained by the relatively low PPFD combined with the high NH_4-N initial concentration. This reached 99.5 $mg \cdot L^{-1}$ and represented together with the 5th batch test the highest initial concentration. However, while the PPFD achieved 544 $\mu mol \cdot s^{-1} \cdot m^{-2}$ during the 5th batch test, it only amounted to 287 $\mu mol \cdot s^{-1} \cdot m^{-2}$ during the 6th batch test.

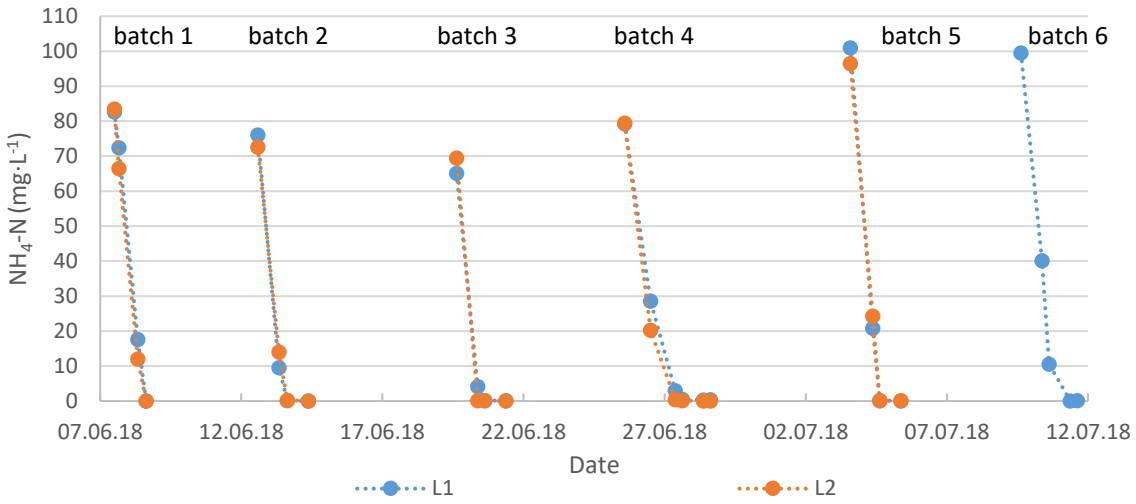

Figure 83: NH_4-N concentration in L1 and L2 during the full-scale experiment in the summer season

RC_{NH4-N} achieved values comprised between 39 and 97 $mg \cdot L^{-1} \cdot d^{-1}$. No significant difference was observed between L1 and L2. The lowest values of 39 and 44 $mg \cdot L^{-1} \cdot d^{-1}$ corresponded to the technical problem during the 4th batch test. As expected, the highest values of 97 and 93 $mg \cdot L^{-1} \cdot d^{-1}$ were reached during the 5th batch test characterized by the highest PPFD value of 544 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Although the PPFD was respectively 46 % and 75 % higher in the 1st batch than in the 2nd and the 3rd batch test, no great difference was observed in the values related to RC_{NH4-N} of the first three batch tests. This result can be explained by the adaptation time needed by the microalgae during the 1st batch to become accustomed to the permeate.

Table 49: Initial concentration, final concentration, RE and RC related to NH_4-N in L1 and L2 during the full-scale experiment in the fall season

Parameter	L1						L2				
	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch	6th Batch	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch
Initial concentration ($mg \cdot L^{-1}$)	82.7	76.1	65.1	79.3	101	99.5	83.5	72.6	69.5	79.5	96.5
Final concentration ($mg \cdot L^{-1}$)	0.070	0.075	0.095	0.277	0.084	0.079	0.041	0.076	0.082	0.036	0.106
RE (%)	100	100	100	100	100	100	100	100	100	100	100
RC ($mg \cdot L^{-1} \cdot d^{-1}$)	73	73	65	39	97	57	74	69	69	44	93

5.2.2.3.1.2 TN

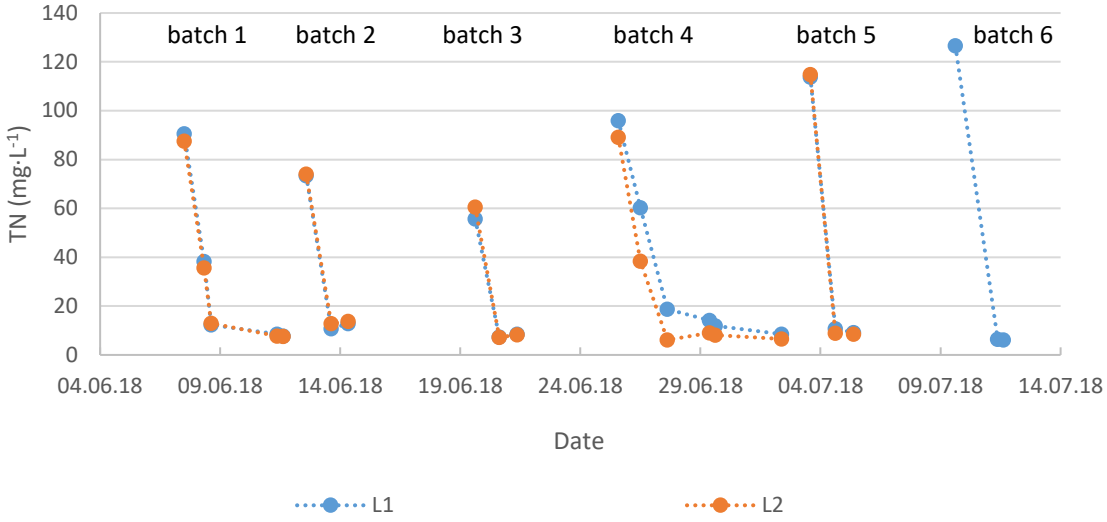

Figure 84: TN concentration in L1 and L2 during the full-scale experiment in the summer season

TN results are represented in Figure 84 and Table 50. During this experiment, TN removal reached values varying from 83 % to 95 %. As for the nutrient NH_4-N, no significant difference was observed between L1 and L2. Independently from the initial TN concentration, the final concentration always achieved similar values in the range 6.16 - 12.9 mg·L^{-1}. Except for the 2nd batch test, RE_{TN} higher than 90 % were achieved and the final TN concentrations reached values lower than 10 mg·L^{-1}. This represented the main goal of the experiments according to the European regulations related to TN discharge into the water bodies.

Table 50: Initial concentration, final concentration and RE related to TN in L1 and L2 during the full-scale experiment in the summer season

Parameter	L1						L2				
	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch	6th Batch	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch
Initial concentration (mg·L^{-1})	90.6	73.4	80.4	96.0	113.8	126.6	87.6	74.0	75.4	89.1	114.8
Final concentration (mg·L^{-1})	7.66	10.90	7.30	8.50	9.12	6.16	7.62	12.90	7.14	6.51	8.50
RE (%)	92	85	91	91	92	95	91	83	91	93	93

5.2.2.3.1.3 TP

The initial TP concentrations ranged 15.1 - 29.8 mg·L^{-1} and were in average 159 % higher than during the 1st experiment in fall 2017. Within the six batch tests, TP was totally removed from the cultures (96 - 100 %) and achieved very low final concentrations comprised between 0.047 and 0.674 mg·L^{-1} (Figure 85 and Table 51). During the 2nd, 3rd and 5th batch tests, very low TP concentrations were reached within 1 or 2 days. Relating to the 4th and 6th batch tests, 4 and 3 days were needed to reach

very low TP concentrations. For these batches, the same explanations as for the longest time needed to remove NH_4-N are valid (see section 5.2.2.3.1.1). Moreover, in the 1st batch test, very low TP concentrations were presumably reached after 2 days of cultivation. Nevertheless, the samples could only be taken after 1 and 4 days of cultivation.

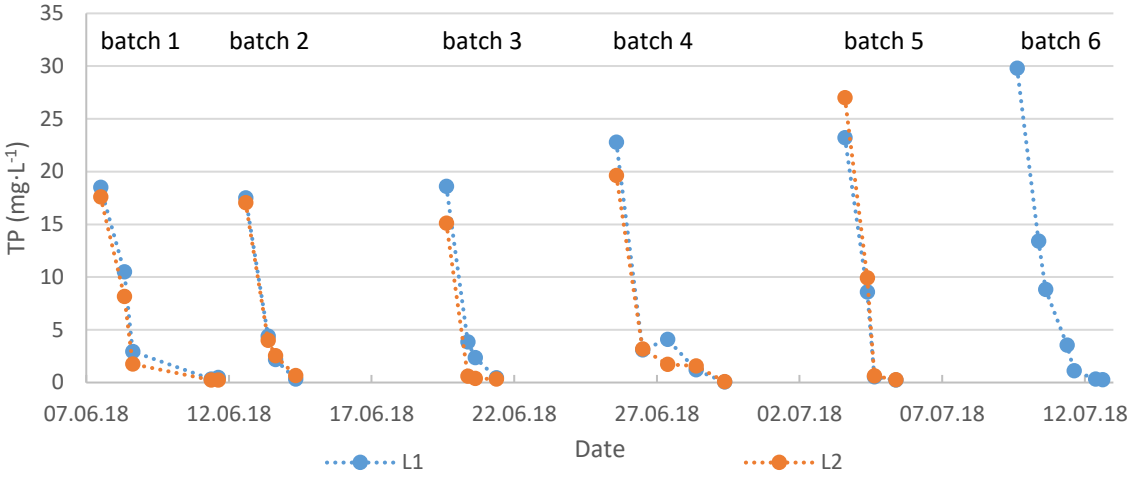

Figure 85: TP concentration in L1 and L2 during the full-scale experiment in the summer season

As for NH_4-N and TN, no significant difference was observed between L1 and L2, except the 50 % higher RC_{TP} of 15 $mg \cdot L^{-1} \cdot d^{-1}$ in L2 during the 3rd batch test. As for NH_4-N, the highest RC_{TP} of 22 and 25 $mg \cdot L^{-1} \cdot d^{-1}$ corresponded to the 5th batch test characterized by the highest PPFD of 544 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Except during the 4th batch test characterized by a technical failure, the values related to RC_{TP} during the other batch tests ranged between 10 and 15 $mg \cdot L^{-1} \cdot d^{-1}$.

Table 51: Initial concentration, final concentration, RE and RC related to TP in L1 and L2 during the full-scale experiment in the fall season

Parameter	L1						L2				
	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch	6th Batch	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch
Initial concentration ($mg \cdot L^{-1}$)	18.5	17.5	18.6	22.8	23.2	29.8	17.6	17.0	15.1	19.6	27.0
Final concentration ($mg \cdot L^{-1}$)	0.316	0.340	0.450	0.047	0.238	0.253	0.232	0.674	0.384	0.089	0.274
RE (%)	98	98	98	100	99	99	99	96	97	100	99
RC ($mg \cdot L^{-1} \cdot d^{-1}$)	14	10	10	6.0	22	11	14	9.8	15	5.2	25

5.2.2.3.2 Microalgae growth

During the 1st batch test, the microalgae grew well in both PBRs. Starting from a concentration of 0.78 and 0.68 $g \cdot L^{-1}$ in L1 and L2 respectively, this reached 3.9 and 3.4 $g \cdot L^{-1}$ after 5 days (Figure 86 and Table 52). The BPR amounted to 0.64 $g \cdot L^{-1} \cdot d^{-1}$ in L1 and 0.56 $g \cdot L^{-1} \cdot d^{-1}$ in L2 and μ_{max} reached extremely high

values of 2.96 and 2.00 d^{-1} in L1 and L2 respectively. During the 2nd batch test, despite a lower initial concentration of 0.35 and 0.28 $g \cdot L^{-1}$ in L1 and L2 respectively, a similar maximum concentration of 3.3 and 3.6 $g \cdot L^{-1}$ was achieved after 6 days. Compared to the 1st batch test, the BPR parameter achieved lower values of 0.48 $g \cdot L^{-1} \cdot d^{-1}$ in L1 and 0.55 $g \cdot L^{-1} \cdot d^{-1}$ in L2. Likewise, μ_{max} amounted to 1.43 and 1.90 d^{-1} and was then 52 % and 5 % lower than during the 1st batch test. These results were explained by the lower PPFD during the 2nd batch test.

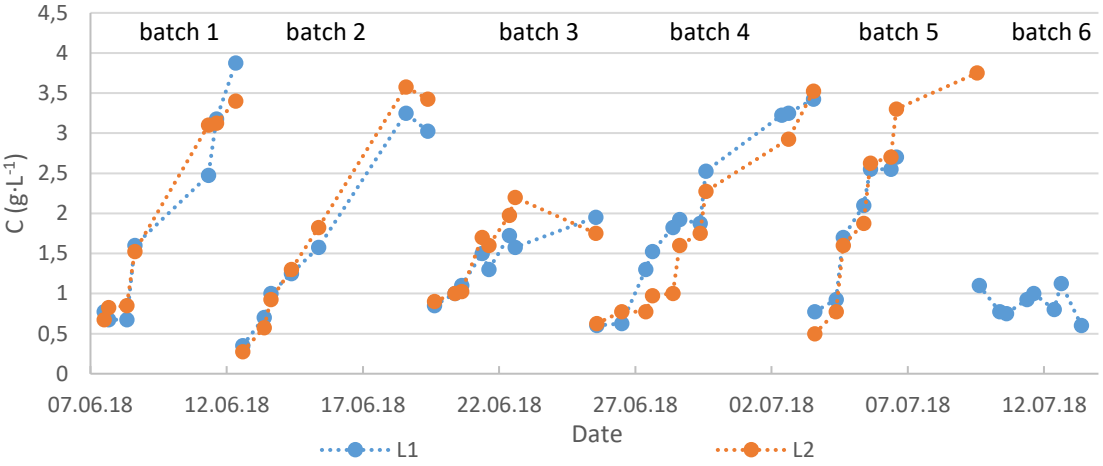

Figure 86: Biomass concentration in L1 and L2 during the full-scale experiment in the summer season

During the 3rd batch test, despite of a higher initial concentration, the cultures reached a lower maximum concentration of 2.0 $g \cdot L^{-1}$ after 6 days and 2.2 $g \cdot L^{-1}$ after 3 days in L1 and L2 respectively. This can be eventually explained by the weather conditions, which were characterized by the lowest PPFD of 263 $\mu mol \cdot s^{-1} \cdot m^{-2}$ within all the batches. Contrary to L1, in L2, the microalgae stopped to grow starting from the 3rd day of cultivation. As TP was removed faster in L2 than in L1, the microalgae had probably no nutrient intake for too long. As no contamination was reported, sedimentation in the pipes could be an additional cause. Overall, the BPR reached 0.25 and 0.44 $g \cdot L^{-1} \cdot d^{-1}$ in L1 and L2 respectively and μ_{max} achieved 0.41 d^{-1} in L1 and 0.67 d^{-1} in L2.

Compared to the 1st and 2nd batch tests, the 4th batch test was characterized by similar maximum concentrations. However, because of the technical failure leading to the cessation of flue gas supply, it lasted 8 days to reach these concentrations. As a result, the BPR was lower than in the 1st and 2nd batch tests.

The highest average BPR and μ_{max} values were achieved during the 5th batch test. The BPR amounted to 0.64 $g \cdot L^{-1} \cdot d^{-1}$ in L1 and 0.93 $g \cdot L^{-1} \cdot d^{-1}$ in L2 and μ_{max} reached 2.43 and 2.90 d^{-1} in L1 and L2 respectively. These higher values were a direct consequence of the comparatively high PPFD value of 544 $\mu mol \cdot s^{-1} \cdot m^{-2}$. However, great differences between L1 and L2 were observed. After 2 days of cultivation, the biomass concentration reached a plateau in L1 and a maximum concentration of 2.7 $g \cdot L^{-1}$. However, multiple measurements of the last sample taken on 09.07.2018 led to incoherent results. Consequently, it is not possible to confirm if the biomass concentration continued to stagnate between the 06.07.2018 and the 09.07.2018 or if the microalgae further grew. On the contrary, in L2, although the microalgae grew slower starting from the 05.07.2018, no stagnation was observed and a maximum concentration of 3.8 $g \cdot L^{-1}$ was reached.

Table 52: Initial and maximal biomass concentrations, BPR, BPR_{α}, μ_{max} and PE in L1 and L2 during the full-scale experiment in the summer season

Parameter	L1						L2				
	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch	6th Batch	1st Batch	2nd Batch	3rd Batch	4th Batch	5th Batch
Initial concentration ($g \cdot L^{-1}$)	0.78	0.35	0.85	0.60	0.78	0.78	0.68	0.28	0.90	0.63	0.50
Maximal concentration ($g \cdot L^{-1}$)	3.9	3.3	2.0	3.4	2.7	1.1	3.4	3.6	2.2	3.5	3.8
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	0.64	0.48	0.25	0.35	0.64	0.16	0.56	0.55	0.44	0.36	0.93
BPR_{α} ($g \cdot m^{-2} \cdot d^{-1}$)	10.7	8.1	5.3	5.9	10.7	2.6	10.3	10.0	8.0	6.7	17.1
μ_{max} (d^{-1})	2.96	1.43	0.41	1.43	2.43	0.31	2.00	1.90	0.67	1.88	2.90
PE (%)	1.2	1.2	0.85	0.53	0.98	0.46	1.2	1.5	1.3	0.60	1.6

Finally, microalgae growth was nonexistent over the 4 days of the 6th batch. As TP and NH_4-N were completely removed from the cultures, this result seems poorly explainable. Comparatively low light conditions characterized this last batch test. However, the PPFD was in the same order of magnitude as in the 2nd and the 3rd batch test, during which a satisfactory growth was observed. Neither contamination nor technical problems were identified during this last batch test. Mixing problems or great sedimentation in pipes could have occurred. However, no reliable explanation for the absence of microalgae growth despite nutrient assimilation could be found.

By eliminating the 4th and the 6th batch test characterized by a technical failure and an inexplicable absence of microalgae growth, PE ranged 0.88 - 1.6 %. Compared to L1, PE in L2 was systematically equal or up to 70 % higher. This could be eventually due to the slight difference in orientation, L2 being more positioned to the south direction. These results were in the same order of magnitude as the PE values reported during the 1st experiment in fall 2017. Hence, no correlation between PE and PPFD was found.

5.2.3 Performance of microalgae cultivation with permeate at laboratory scale

5.2.3.1 Performance of the permeate cultures compared to the control cultures using a synthetic culture medium

During the experimental work conducted at the University of Hamburg with *Acutodesmus obliquus*, in each batch, nutrient uptake occurred much faster with the enriched permeate (B) than with the control culture using a commercial fertilizer (C). The microalgae in (B) showed likewise higher BPR and higher final biomass concentrations. The same observations were done for the permeate (A) during the 1st batch, where the lack of micronutrients in the culture medium did not yet influence the nutrient uptake rates and the BPRs. These significant differences were attributed to the great pH fluctuations in the

control culture (C). Indeed, despite a daily adjustment of the pH close to the neutrality, very low pH values comprised between 3 and 5 were systematically achieved during the first two days of each batch. The pH value is known as one of the most critical environmental conditions (Qiu *et al.* 2017). Both instable and extreme pH values cause direct physiological effects on the microalgae cells (Chen and Durbin 1994) and a rapid acidification of the medium can even lead to cell death (Lee and Zhang 2014). On the contrary, in the present study, pH in permeate cultures was most of the time stable at a neutral level, which represents a significant advantage.

During the experiments conducted in the TU Berlin with *Chlorella vulgaris*, similar pH behaviors were observed. Throughout all the batches, the cultures with permeate stayed without regulation stable at a neutral level. On the contrary, in the synthetic culture medium, the pH reached values of 4.3 and 5.3 at the beginning of the experiments (1) and (3) and needed to be regularly corrected. However, except for the 3rd batch of (1) characterized by a lack of micronutrients leading to a reduction of TN removal in the permeate culture, the nutrient uptake rates and the BPRs were similar in these culture media. Compared to the experiments conducted at the University of Hamburg, these contradictory results are explained by the comparatively more stable pH values in the control culture in the experiments conducted with *Chlorella vulgaris*. This was probably due to a better control of CO_2 supply in the set-up used in the TU Berlin.

Finally, using *Chlorella sorokiniana*, similar TN and TP removal in the permeate culture and in the synthetic culture medium were observed. However, the TN uptake rates and the BPRs were slightly higher in the synthetic culture medium.

Throughout the experiments conducted with permeate, it was stated that this culture medium has adapted buffer capacities that lead to stabilized pH values during NH_4-N uptake by the microalgae. In full-scale plants, these remarkable buffer capacities could allow microalgae cultures without external pH regulation.

5.2.3.2 Micronutrient addition to the culture with permeate (A)

The experiment conducted with *Acutodesmus obliquus* at the University of Hamburg showed significant differences relating to TN uptake and microalgae growth in the permeate (A) compared to the enriched permeate (B) and the control culture (C). During the experiment (1) using *Acutodesmus obliquus* at the TU Berlin, the incomplete TN removal in the permeate (A) was confirmed. The decrease of TN removal first appeared during the 3rd batch (University of Hamburg) and the 2nd batch (TU Berlin). This was explained by the use of a pre-culture of microalgae as initial biomass at the beginning of the first batch. These microalgae grew in a nutrient-rich synthetic culture medium containing enough micronutrients. During the pre-culture phase, the micronutrients contained in the synthetic culture medium were assimilated and stored in the microalgae cells. Therefore, it was assumed that the stock of micronutrients in the cells was still sufficient during the first batches. The accumulated reserves gradually decreased, resulting in a total inhibition of TN removal after a few batches.

The experiments (2) and (3) conducted in the TU Berlin with *Acutodesmus obliquus* aimed at the determination of the chemical playing a role in the incomplete TN removal from the permeate (A). The addition into permeate of iron (II) sulfate in presence of EDTA as a chelating agent permitted to overcome the decrease observed in TN removal. Hence, for an efficient wastewater treatment, where the main goal is to obtain an effluent with low nutrient concentrations, iron (II) sulfate addition is sufficient. The experiments (1) and (2) conducted with the species *Chlorella vulgaris* proved that the

decrease of TN removal observed with the permeate culture (A) was not strain specific to *Acutodesmus obliquus* and would probably be observed with most microalgae species. Moreover, it clearly demonstrated that only the addition of Fe to the permeate was obligatory to achieve a complete NH_4-N removal. This confirms literature sources reporting Fe as a key element in TN assimilation and one of the most important micronutrients for microalgal biomass production (Naito *et al.* 2005; Quiggg 2016).

During the culture of *Chlorella sorokiniana*, no decrease of TN removal was observed. This was probably due to the nutrient and micronutrient concentrations in the permeate, which were much higher compared to the previous experiments conducted with *Acutodesmus obliquus* and *Chlorella vulgaris*. Hence, while Fe concentration amounted to a maximum of 0.31 $mg \cdot L^{-1}$ in the permeate used for the experiments with *Acutodesmus obliquus* and *Chlorella vulgaris* characterized by a decrease of TN removal, it amounted to 0.20 $mg \cdot L^{-1}$ in the experiment (1) and ranged 0.40 - 0.47 $mg \cdot L^{-1}$ in the experiments (3) and (4) with *Chlorella sorokiniana.*

5.2.3.3 Comparison of the performance obtained with the different microalgae species

Table 53: Comparison of the performance obtained at a laboratory scale by cultivating the species Acutodesmus obliquus, Chlorella vulgaris and Chlorella sorokiniana with permeate (A)

Species	***Acutodesmus obliquus***		***Chlorella vulgaris***		***Chlorella sorokiniana***	
Experiment	University of Hamburg, 3 batches	TU Berlin, Exp. (1), 2 batches	TU Berlin, Exp (1), 3 batches	TU Berlin, Exp (3), 2 batches	TU Berlin, Exp. (1), 2 batches	TU Berlin, Exp. (3) and Exp. (4), 4 batches
Material	350-mL tubular pipes	1-L bottles	1-L bottles		1-L bottles	
TN initial concentration ($mg \cdot L^{-1}$)	115	95	64	83	98	160
TP initial concentration ($mg \cdot L^{-1}$)	14	13	7.1	7.5	16	37
TN/TP ratio	8	7	9	11	6	4
RE_{TN} (%)	95[1]	95[2]	92[1]	95	91	92
RE_{TP} (%)	97	97	98	98	70	48
$RC_{NH4\text{-}N}$ ($mg \cdot L^{-1} \cdot d^{-1}$)	21	12	15[1]	23	15	13
RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)	7.2	2.4	2.3	3.6	1.3	1.3
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	0.26[1]	0.23	0.24[1]	0.32	0.24	0.32
μ_{max}	0.43[1]	0.56	0.58	0.94	0.50	0.48

[1]: Value corresponding to the 3rd batch not taken into account.
[2]: Value corresponding to the 2nd batch not taken into account.

The cultivation of *Acutodesmus obliquus* and *Chlorella vulgaris* in permeate (A) led to very similar results (Table 53). Except for the batches characterized by iron deficiency in the permeate, NH_4-N and TP were completely removed and TN removal amounted to between 92 % and 95 %. In the experiments conducted at the TU Berlin, the BPR, μ_{max}, NH_4-N and TP removal capacity were slightly higher with *Chlorella vulgaris* and, for this species, increased with increasing TN initial concentrations. However, this comparison is limited by the fact that the initial TP concentration was 78 % higher with *Acutodesmus obliquus*. This difference could have influenced the nutrient uptake rates.

The cultivation of *Chlorella sorokiniana* showed also a complete NH_4-N removal and a high TN removal of 91 - 92 %. However, in average, only 70 % (experiment (1)) and 48 % (experiments (3) and (4)) of TP was removed. The causes of the incomplete TP removal are discussed in the following section. In addition, $RC_{NH4\text{-}N}$, μ_{max} and the BPR were similar to the results obtained with *Acutodesmus obliquus* and *Chlorella vulgaris* in the experiments conducted at the TU Berlin. They ranged 13 - 15 $mg \cdot L^{-1} \cdot d^{-1}$, 0.48 - 0.50 d^{-1} and 0.24 - 0.32 $g \cdot L^{-1} \cdot d^{-1}$ respectively.

5.2.3.4 Influence of the TN/TP ratio and of the use of high strength domestic wastewater as substrate of the AnMBR

TN and TP concentrations in the permeate used for the experiments conducted in this work were significantly higher than those described in the literature. Whitton *et al.* (2015) reported that wastewater effluents with NH_4-N and TP concentrations in the range of 0.1 to 36 $mg \cdot L^{-1}$ and 0.04 to 12 $mg \cdot L^{-1}$ respectively are usually used in microalgae cultures. Over the whole experiments conducted at a lab-scale, permeate cultures had an initial NH_4-N concentration in the range 54 - 142 $mg \cdot L^{-1}$ and an initial TP concentration in the range 6.4 - 38 $mg \cdot L^{-1}$. Under nutrient-rich conditions, a complete nutrient removal by microalgae is difficult to achieve (Su *et al.* 2012). With increasing initial nutrient concentrations, this difficulty intensifies.

During the experiments conducted with *Acutodesmus obliquus* and *Chlorella vulgaris* (NH_4-N and TP initial concentrations ranging 54 - 93 $mg \cdot L^{-1}$ and 6.4 - 17 $mg \cdot L^{-1}$ respectively), the use of high strength domestic wastewater as substrate of the AnMBR did not represent an obstacle for nutrient removal. Under the conditions that enough light and micronutrients are available for the microalgae, this work shows that a complete nutrient removal can be achieved even with higher TN and TP initial concentrations in the permeate. The complete nutrient removal was also surely due to an adapted TN/TP ratio of 7 - 9 for *Acutodesmus obliquus* and 9 - 11 for *Chlorella vulgaris*.

Compared to these experiments, the permeate used for the batch experiments conducted with *Chlorella sorokiniana* was characterized by much higher nutrient levels. The initial NH_4-N and TP concentrations in the permeate culture (A) amounted to between 77 and 142 $mg \cdot L^{-1}$ and between 16 and 38 $mg \cdot L^{-1}$ respectively.

During the experiment (1) with this species, NH_4-N could be completely removed from the permeate. However, TP removal was incomplete. As these macronutrients were entirely removed with similar initial nutrient concentrations in the previous experiments conducted with *Acutodesmus obliquus* and *Chlorella vulgaris*, the incomplete TP removal was unexpected. Here, the TN/TP ratio amounted to 6 and was supposedly too low, as a high TP concentration was still present after the complete removal of NH_4-N.

In the experiment (2), the addition of potassium nitrate into the permeate after 8 days, which represented a supply of 52 $mg \cdot L^{-1}$ of TN, led to a complete TP removal. If this nitrogen source had been added at the beginning of the batch, the TN/TP ratio would have amounted to 10, which is in the order of magnitude of the TN/TP ratios in the previous experiments conducted with *Acutodesmus obliquus* and *Chlorella vulgaris*. Hence, the hypothesis was verified and the incomplete TP removal in the first two batches was only due to an unbalanced TN/TP ratio in the permeate used for this experiment.

Compared to the experiment (1), the permeate used for the following experiments was characterized by much higher nutrient concentrations. NH_4-N and TP initial concentrations amounted to between 127 and 142 $mg \cdot L^{-1}$ and to between 35 and 38 $mg \cdot L^{-1}$ respectively. It resulted in TN/TP ratios of 3.7 to 5.0, which were even smaller than in the previous batches. In the 1st batch of the experiment (3), the addition of 97 $mg \cdot L^{-1}$ of NH_4-N on day 0 and 71 $mg \cdot L^{-1}$ of NH_4-N on day 13 did not influence TP removal. TP behavior was very similar to the culture (A) containing only permeate. While 74 % of TP removal was achieved in the culture (A), this amounted to 77 % in the permeate enriched with ammonium sulfate. Based on the initial TN and TP concentrations in the permeate, with the quantity of ammonium sulfate added on day 0 and day 13, the TN/TP ratio would have amounted to 9. This ratio was sufficient for the culture of *Acutodesmus obliquus* and *Chlorella vulgaris* with permeate characterized by lower nutrient concentrations. Hence, it was concluded that a TN/TP ratio of 9 in the permeate is too small for the species *Chlorella sorokiniana* and for permeate characterized by these extremely high nutrient concentrations.

During the 2nd batch of (3) characterized by the supply of 67 $mg \cdot L^{-1}$ of NH_4-N on day 0 and 166 $mg \cdot L^{-1}$ of NH_4-N on day 8 (TN/TP ratios of 5.9 and 10.4), starting from day 8, TP concentration in the permeate culture enriched with nitrogen had a different developing from the permeate culture (A). On day 20, while TP concentration amounted to 23.5 $mg \cdot L^{-1}$ in the permeate (A), it only amounted to 13.5 $mg \cdot L^{-1}$ in the permeate culture enriched with ammonium sulfate. The experiment was stopped after 20 days but according to RC_{TP} between day 13 and day 20, TP would have been entirely removed starting from day 26. Hence, a TN/TP ratio of 10.4 is sufficient in the permeate.

The experiment (4) confirmed that it was not a lack of micronutrients that was responsible for the incomplete TP removal. Indeed, compared to the control culture with commercial fertilizer, TP developing was similar to the permeate (A). Indeed, RE_{TP} achieved 42 % in the permeate (A) and 51 % in the control culture.

5.2.3.5 Comparison of lab-scale results with the literature

5.2.3.5.1 Comparison of the performance of *Acutodesmus obliquus* cultivation at lab-scale with literature

Vasconcelos Fernandes *et al.* (2015) investigated the treatment of nutrient-rich blackwater in an UASB reactor and subsequent cultivation of *Acutodesmus obliquus* at lab-scale. An average BPR of 0.34 $g \cdot L^{-1} \cdot d^{-1}$ at 175 $\mu mol \cdot s^{-1} \cdot m^{-2}$ was achieved (Table 54). In the present work, during the lab-scale experiments conducted with tubular pipes at the University of Hamburg, despite the higher PPFD, a relatively lower average BPR of 0.26 $g \cdot L^{-1} \cdot d^{-1}$ was determined under similar culture conditions (material, pH and temperature). This was probably due to the differences in wastewater composition. Indeed, nutrient concentration in the wastewater effluent used in Vasconcelos Fernandes *et al.* (2015) was in average 10 times higher, which leads to higher growth rates (Xin *et al.* 2010). While 93 % of phosphate was removed from the blackwater, ammonium uptake amounted to only 27 %. According

to the authors, the difference between ammonium and phosphate removal could be explained by a unusual high TP requirement of the microalgae cells or most probably by the phenomenon of luxury uptake. RC_{TN} and RC_{TP} amounted to 16 and 5.9 $mg \cdot L^{-1} \cdot d^{-1}$ respectively. This was slightly lower than the nutrient RC reached in the lab-scale experiments at the University of Hamburg.

Table 54: Comparison of the results obtained with Acutodesmus obliquus at lab-scale with literature

Author	**Vasconcelos Fernandes *et al.* (2015)**	**Gupta *et al.* (2016)**	**Martínez (2000)**	**Ruiz-Martinez *et al.* (2012)**	**Present work: permeate (A)**	
Species	*Acutodesmus obliquus*	*Acutodesmus obliquus*	*Acutodesmus obliquus*	Polyculture of *Chlorophyceae* and Cyanobacteria	*Acutodesmus obliquus*	
Culture medium	Effluent of an UASB reactor treating blackwater	Filtrated and aerated municipal wastewater	Filtrated wastewater effluent after biological treatment in a municipal WWTP	Effluent of a submerged AnMBR treating pre-treated wastewater from a municipal WWTP	Effluent of a decentralized AnMBR treating household wastewater	
Reactor	300-mL Erlenmeyer flasks (batch experiments)	2-L bottle (batch experiments)	1-L bioreactor (batch experiments)	10-L PBR (semi-continuous process)	350-mL tubular pipes	1-L bottles
TN initial concentration ($mg \cdot L^{-1}$)	1210	53	27	43 - 81[1]	115	95
TP initial concentration ($mg \cdot L^{-1}$)	105	8.5	12	5.1 - 10.5[2]	14	13
TN/TP ratio	12	6.2	2.3	-	8.2	7.3
RE_{TN} (%)	27[1]	99	100	67[1]	95	95
RE_{TP} (%)	93[2]	98	98	98[2]	97	97
RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)	16[1]	3.5	-	20[1]	21[1]	12
RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)	5.9[2]	0.55	-	3.7[2]	7.2	2.4
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	0.34	-	0.025	0.234	0.26	0.23
μ (d^{-1})	-	-	0.768	0.66	0.43[4]	0.56[4]
PPFD ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	175	80	204[3]	143 - 209	260	270

[1]: NH_4-N, [2]: PO_4-P, [3]: 11334 Lux - Conversion in $\mu mol \cdot s^{-1} \cdot m^{-2}$ (Lang *et al.* 1981), [4]: maximum value

Regarding nutrient removal, the results of this work are in line with those of Martínez (2000). Cultivating *Acutodesmus obliquus* in wastewater that was filtered after biological purification in a WWTP, this author determined RE_{TP} and $RE_{NH4\text{-}N}$ of respectively 98 % and 100 % under similar experimental conditions (pH, temperature, PPFD and reaction times). The initial biomass concentration of 0.01 $g \cdot L^{-1}$ explains the comparatively lower BPRs in the study. On the contrary, with a

value of 0.768 d^{-1}, µ was much higher than in the present work. This was explained by the low biomass concentration in the culture, which permitted a better light availability for the microalgae cells.

Using the same species and wastewater with a similar nutrient concentration, Gupta *et al.* (2016) achieved 99 % and 98 % TN and TP removal with filtered and aerated municipal wastewater under similar pH and temperature conditions. Hence, a complete nutrient removal was also reached in this study.

Only few studies report the use of the effluent of an AnMBR for microalgal biomass production. Using the effluent of a submerged AnMBR treating wastewater from a municipal WWTP and characterized by lower nutrient concentrations than in the permeate used in this work, Ruiz-Martinez *et al.* (2012) achieved an average productivity of 0.234 $g \cdot L^{-1} \cdot d^{-1}$ under a PPFD in the range 143 - 209 $\mu mol \cdot s^{-1} \cdot m^{-2}$. This is comparable to the average BPRs of 0.23 - 0.26 $g \cdot L^{-1} \cdot d^{-1}$ achieved in the present work. As the biomass concentration in the reactor was always less than 0.6 $g \cdot L^{-1}$, the absence of self-shading effect led to a comparatively higher µ of 0.66 d^{-1}. Finally, in the study, TP was completely removed.

However, in Ruiz-Martinez *et al.* (2012), on average, only 67 % NH_4-N was removed. This lower value was attributed to the variation of NH_4-N concentration in the wastewater. At the lowest NH_4-N concentration, this nutrient was only found in very low concentrations after microalgae culture. Since the residence time of two days remained stable throughout the experiment, no optimal removal was achieved at the highest NH_4-N concentration. Hence, except for the lower $RE_{NH4\text{-}N}$, the results of Ruiz-Martinez *et al.* (2012) are in accordance with the results obtained in the present work.

The results of the present work are generally similar to existing literature. Contrary to several studies, an advantage of the results obtained in this work with *Acutodesmus obliquus* is the simultaneously entire removal of both ammonium and phosphorus. This permits to avoid a post-treatment before discharge of the effluents in the water bodies.

5.2.3.5.2 Comparison of the performance of *Chlorella vulgaris* cultivation at lab-scale with literature

Under the same experimental conditions as for *Acutodesmus obliquus* (section 5.2.3.5.1), Vasconcelos Fernandes *et al.* (2015) achieved ammonium and phosphate removal of respectively 22 % and 26 % (Table 55). Compared to the results achieved in the present work, this very low removal is explained by the extremely high TN and TP concentration contained in the anaerobically treated blackwater. Indeed, it is extremely difficult to achieve a complete nutrient removal from effluents characterized by very high nutrient levels (Su *et al.*, 2012). With values of respectively 13 and 1.4 $mg \cdot L^{-1} \cdot d^{-1}$, RC_{TN} and RC_{TP} amounted to respectively 58 - 90 % and 39 - 50 % of the values achieved in the present study. Nevertheless, in Vasconcelos Fernandes *et al.* (2015), the BPR reached 0.28 $g \cdot L^{-1} \cdot d^{-1}$, which was in line with the BPRs of 0.24 and 0.31 $g \cdot L^{-1} \cdot d^{-1}$ obtained in this work.

Table 55: Comparison of the results obtained with Chlorella vulgaris at lab-scale with literature

Author	**Cabanelas *et al.* (2013)**			**Vasconcelos Fernandes *et al.* (2015)**	**Gouveia *et al.* (2016)**	**Ren *et al.* (2017)**	**Present work: permeate (A)**	
Species	*Chlorella vulgaris*			*Chlorella vulgaris*	*Chlorella vulgaris*	*Chlorella vulgaris*	*Chlorella vulgaris*	
Culture medium	E1[3]	E2[3]	E3[3]	Effluent of an UASB reactor treating blackwater	Urban wastewater collected after primary treatment in a municipal WWTP	Centrate from a municipal WWTP	Effluent of a decentralized AnMBR treating household wastewater	
Reactor	2-L bioreactors (batch experiments)			300-mL Erlenmeyer flasks (batch experiments)	Outdoor 150-L PBR (batch experiments)	350-mL Erlenmeyer flasks (batch experiments)	1-L bottles (batch experiments)	
TN initial concentration ($mg \cdot L^{-1}$)	88	52	64	1210	119[1]	200	64	83
TP initial concentration ($mg \cdot L^{-1}$)	8.9	8.8	9.1	105	8.3[2]	82	7.1	7.5
TN/TP ratio	10	6	7	12	14	2	9	11
RE_{TN} (%)	90	92	98	26[1]	Maximum of 84[1]	77	92	95
RE_{TP} (%)	92	93	96	22[2]	Maximum of 95[2]	90	98	98
RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)	6.6	4.0	5.2	13[1]	-	77	14.5[1]	22.5[1]
RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)	0.68	0.68	0.73	1.4[2]	-	37	2.8	3.6
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	0.12	0.12	0.13	0.28	0.10	0.42	0.24	0.31
μ (d^{-1})	0.22	0.18	0.21	-	-	-	0.58[4]	0.94[4]
PPFD ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	150			175	-	50	270	

[1]: NH_4-N, [2]: PO_4-P, [3]: E1: Pre-treated urban wastewater 1, E2: Pre-treated urban wastewater 2, E3: Anaerobically treated wastewater, [4]: maximum value

Cabanelas *et al.* (2013) investigated nutrient uptake and *Chlorella vulgaris* growth using several wastewater streams. Compared to the present work, three streams had similar initial TN and TP concentrations (52 - 88 $mg \cdot L^{-1}$ and 9 $mg \cdot L^{-1}$ respectively). While two of these streams were pre-treated urban wastewater from two different WWTPs, the last stream was anaerobically treated wastewater. The present results related to nutrient removal are in line with Cabanelas *et al.* (2013) results. Indeed, the authors reported at least 92 % TP removal and 90 % TN removal. For comparison, up to 96 % TP removal and 98 % TN removal was achieved in the present work. Moreover, while the highest nutrient uptake was obtained with a TN/TP ratio of 11 in the present study, this was achieved with a TN/TP ratio of 7 in Cabanelas *et al.* (2013). Due to the much lower PPFD and temperature used by these authors, an exhaustive comparison of the BPR, μ, RC_{TN} and RC_{TP} is not possible.

Using urban wastewater collected after primary treatment in a municipal WWTP, Gouveia *et al.* (2016) conducted batch experiments with *Chlorella vulgaris* in an outdoor 150-L PBR. Compared to the permeate composition of this work, the wastewater was characterized by a similar TP concentration but a higher initial TN concentration, which led to a relatively high TN/TP ratio of 14. RE_{TN} and RE_{TP} amounted to 84 % and 95 % respectively. In the present work, while RE_{TP} was similar, RE_{TN} was much higher, reaching values comprised between 92 % and 95 %. This significant difference could be due to the 43 % higher initial TN concentration in the wastewater used in Gouveia *et al.* (2016). As the author conducted outdoor experiments (PPFD not given), the maximum BPR of 0.1 $g \cdot L^{-1} \cdot d^{-1}$ was hardly comparable with the present work.

Finally, using the nutrient-rich centrate from a WWTP (200 $mg \cdot L^{-1}$ TN and 82 $mg \cdot L^{-1}$ TP), Ren *et al.* (2017) achieved 77 % TN removal and 90 % TP removal. Hence, both macronutrients were not completely removed, which could be caused by the extremely high nutrient concentration in the centrate. On the contrary, because of the very high initial nutrient concentration and the initial biomass concentration of 1.1 $g \cdot L^{-1}$, the BPR, RC_{TN} and RC_{TP} were much higher than in the present work and achieved 0.42 $g \cdot L^{-1} \cdot d^{-1}$, 77 $mg \cdot L^{-1} \cdot d^{-1}$ and 37 $mg \cdot L^{-1} \cdot d^{-1}$ respectively.

5.2.3.5.3 Comparison of the performance of *Chlorella sorokiniana* cultivation at lab-scale with literature

The literature related to the culture of *Chlorella sorokiniana* with wastewater streams or commercial fertilizers reports different results. Vasconcelos Fernandes *et al.* (2015), who investigated microalgae culture using the effluent of an UASB reactor treating blackwater, observed very low ammonium and phosphate removal of 30 % and 39 % respectively with a temperature of 25 °C (Table 56). However, with a temperature of 37 °C, which is reported as the optimum temperature for this species, ammonium and phosphate were totally removed. Hence, Vasconcelos Fernandes *et al.* (2015) demonstrated that the temperature is a key element for nutrient assimilation by *Chlorella sorokiniana*. In the experiment conducted at 25 °C, $RC_{NH4\text{-}N}$, RC_{TP} and the BPR amounted to 17 $mg \cdot L^{-1} \cdot d^{-1}$, 2.1 $mg \cdot L^{-1} \cdot d^{-1}$ and 0.33 $g \cdot L^{-1} \cdot d^{-1}$ respectively and were slightly higher than in the present work. This was probably due to a more favorable TN/TP ratio of 12, as well as the slightly higher temperature.

Gupta *et al*. (2016) worked with filtrated and aerated municipal wastewater characterized by much lower TN and TP concentrations of 53 and 8.5 $mg \cdot L^{-1}$ respectively. With a TN and TP removal of 87 % and 68 % respectively, nutrient uptake was very similar to the experiment (1) with *Chlorella sorokiniana* of the present work. This was surely due to the similar TN/TP ratio of 6.2. However, the nutrient uptake rates were much lower than in the present work. This was presumably due to the low initial nutrient concentrations.

With similar initial nutrient concentrations and TN/TP ratios to the experiments (3) and (4) conducted with *Chlorella sorokiniana* in the present work, Kim *et al.* (2013) obtained TN and TP removal of only 28 % and 25 % respectively. However, RC_{TN} was similar to the present work and RC_{TP} was even higher. While it only achieved 1.3 $mg \cdot L^{-1} \cdot d^{-1}$ in this study, it amounted to 3.4 $mg \cdot L^{-1} \cdot d^{-1}$ in Kim *et al.* (2013). This lower nutrient removal could be due to the shorter investigation time of 6 days combined with the low PPFD of 80 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

Table 56: Comparison of the results obtained with Chlorella sorokiniana at lab-scale with literature

Author	Vasconcelos Fernandes *et al.* (2015)	Vasconcelos Fernandes *et al.* (2015)	Gupta *et al.* (2015)	Kim *et al.* (2013)	Shriwastav *et al.* (2014)	Present work: permeate (A)	
Species	*Chlorella sorokiniana*	*Chlorella sorokiniana*	*Chlorella sorokiniana*	*Chlorella sorokiniana*	*Chlorella sorokiniana*	*Chlorella sorokiniana*	
Culture medium	Effluent of an UASB reactor treating blackwater	Effluent of an UASB reactor treating blackwater	Filtrated and aerated municipal wastewater	Synthetic culture medium	Synthetic culture medium	Effluent of a decentralized AnMBR treating household wastewater	
Reactor	300-mL Erlenmeyer flasks (batch experiments)	Two 380-mL PBR	2-L bottle (batch experiments)	1-L bottle	2-L bottle	1-L bottles	
TN initial concentration ($mg \cdot L^{-1}$)	1210	1210	53	200	1.95 - 25	98	160
TP initial concentration ($mg \cdot L^{-1}$)	105	105	8.5	50	0.37 - 8.42	16	37
TN/TP ratio	12	12	6.2	4	0.5 - 146	6	4
RE_{TN} (%)	30[1]	98[1]	87	28	73 - 97	91	92
RE_{TP} (%)	39[2]	100[2]	68	25	21 - 100	70	48
RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)	17[1]	82[1]	3.1	13.1	-	15	13
RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)	2.1[2]	8.9[2]	0.39	3.4	-	1.3	1.3
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	0.33	-	-	-	-	0.24	0.32
PPFD ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	175	100 - 150	80	60	80	270	
Temperature (°C)	25	37	22	25	22	Ambient air	

[1]: NH_4-N, [2]: PO_4-P

Finally, using a synthetic culture medium, Shriwastav *et al.* (2014) investigated the influence of the TN/TP ratio on nutrient removal. The initial TN and TP concentrations ranged 1.95 - 25 $mg \cdot L^{-1}$ and 0.37 - 8.42 $mg \cdot L^{-1}$ respectively, meaning TN/TP ratios amounting from 0.5 to 146. TN removal was always relatively high and achieved values comprised between 73 and 97 %. However, the TN/TP ratio had an important influence on TP removal, which varied from 21 to 100 %. With a TN/TP ratio of 0.52, TP removal only amounted to 21 %. With increased ratios of 1.96 and 6.12, TP removal achieved an average of 60 %. Finally, with TN/TP ratios of 14.6 and 24.43, more than 90 % of TP was assimilated. Hence, the results of this study concurred with the results obtained in the present work with *Chlorella sorokiniana*, since TP removal significantly increased with increasing TN/TP ratios.

5.2.4 Performance of *Acutodesmus obliquus* cultivation with permeate at the pilot-plant Hamburg-Reitbrook

5.2.4.1 Comparison of the performance obtained in the fall and in the summer season at full-scale

The two experiments conducted in the pilot-plant Hamburg-Reitbrook were characterized by very different abiotic conditions. First, the average temperature in the PBRs amounted to 18.9 °C in fall 2017 and 23.6 °C in summer 2018. In both experiments, significant temperature differences were observed in the PBRs within a day. During the fall and summer experiments, these reached up to 15 °C and 20 °C respectively within only 12 hours. If the heat exchanger would not have been present, these differences would have been much higher. However, the installation of a heat exchanger characterized by a higher capacity could assure PBR temperatures in the range 20 - 30 °C.

Throughout the batches, pH regulation led to average pH values in the neutral level. Nevertheless, punctual technical problems with flue gas supply were observed and led to pH increase up to 11. As sudden changes in pH have direct physiological effects on the microalgae cells that can even lead to their death (Chen and Durbin 1994; Lee and Zhang 2014), this situation should be avoided. Moreover, high pH values favorite indirect nutrient removal throughout the production of ammonia gas caused by the displacement of the equilibrium ammonia/ammonium as well as the precipitation of phosphate with metal ions. A solution would consist of the installation of a circuit connecting a CO_2 bottle to the PBRs and enabling the supply of this gas in such cases.

The most significant difference in the abiotic conditions between the fall and the summer season experiments was the PPFD. While the average PPFD in fall amounted to 111 $\mu mol \cdot s^{-1} \cdot m^{-2}$, this amounted to 406 $\mu mol \cdot s^{-1} \cdot m^{-2}$ in summer.

Table 57 compares the results obtained during the two full-scale experiments. Concerning the experiments conducted in the fall season, only the results obtained with L2 are represented. Indeed, contrary to L1, it corresponds to the PBR model used in the summer season.

Despite the significant differences in the PPFD values, TP and NH_4-N were completely removed in both seasons. Nevertheless, with a value reaching 91 %, TN removal was 21 % higher in the summer season. In order to avoid the high nitrate and nitrite concentrations leading to lower TN uptake in the culture medium in the fall season, a lower flue gas flow should be set and adapted in accordance to the nitrate and nitrite concentrations in the system.

The nutrient removal capacity was logically higher with a higher PPFD. While the PPFD was 310 % higher in summer, $RC_{NH4\text{-}N}$ and RC_{TP} were respectively 518 % and 656 % higher. Consequently, higher HRTs were needed in fall. They amounted between 3 days and 14 days in L2. In comparison, only two days were generally needed to obtain a complete NH_4-N, TN and TP uptake in summer. The HRT is a significant parameter for wastewater treatment, as lower HRTs lead to lower reactor volumes or higher flow rates. In addition to the incomplete TN removal, the relatively high HRT would represent the main disadvantage of this process during fall or spring in Germany. At the contrary, in the summer season in countries with a temperate climate or throughout the year in countries with a lot of sunshine, this process represents an appealing technology, which combines the advantages of flue gas recycling, nutrient reuse and microalgal biomass production. However, more experimental data should be used

for an exhaustive comparison. To that purpose, a several-months experiment in a semi-continuous mode should be performed, for example from spring until fall.

Table 57: Comparison of the main results obtained during Acutodesmus obliquus cultivation at full-scale in the fall and in the summer season

Parameter	Fall 2017 (L2)		Summer 2018		Increase between fall 2017 and summer 2018 (%)
	Range	Mean value	Range	Mean value	
PPFD ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	81 - 126	99	263 - 544	406	310
RE_{NH4-N} (%)	98 - 100	99	100 - 100	100	1.0
RC_{NH4-N} ($mg \cdot L^{-1} \cdot d^{-1}$)	3 - 16	11	39 - 97	68	518
RE_{TN} (%)	42 - 92	75	83 - 95	91	20
RE_{TP} (%)	97 - 98	98	96 - 100	99	1.0
RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)	1.2 - 2.5	1.8	5.2 - 25	13.6	656
Maximum biomass concentration ($g \cdot L^{-1}$)	0.40 - 1.31	0.85	1.1 - 3.9	3.0	253
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	0.01 - 0.13	0.07	0.16 - 0.93	0.49	600
BPR_{α} ($g \cdot m^{-2} \cdot d^{-1}$)	0.3 - 3.0	1.7	2.6 - 17	8.7	412
μ_{max}	0.16 - 0.44	0.26	0.31 - 2.96	1.67	530
PE (%)	0.9 - 2.1	1.1	0.46 - 1.6	1.1	0

In summer, the average maximum microalgae concentration, μ_{max} and BPR_{α} amounted to respectively 3.0 $g \cdot L^{-1}$, 1.67 d^{-1} and 8.7 $g \cdot m^{-2} \cdot d^{-1}$. Compared to fall 2017, this represents an increase of 253 %, 530 % and 412 % respectively. Moreover, PE was not dependent to the season and amounted to 1.1 % in both seasons. This shows that the same ratio of sunlight was used for microalgae growth in the fall and summer season. Hence, the significant differences in the results were only caused by the differences of abiotic conditions.

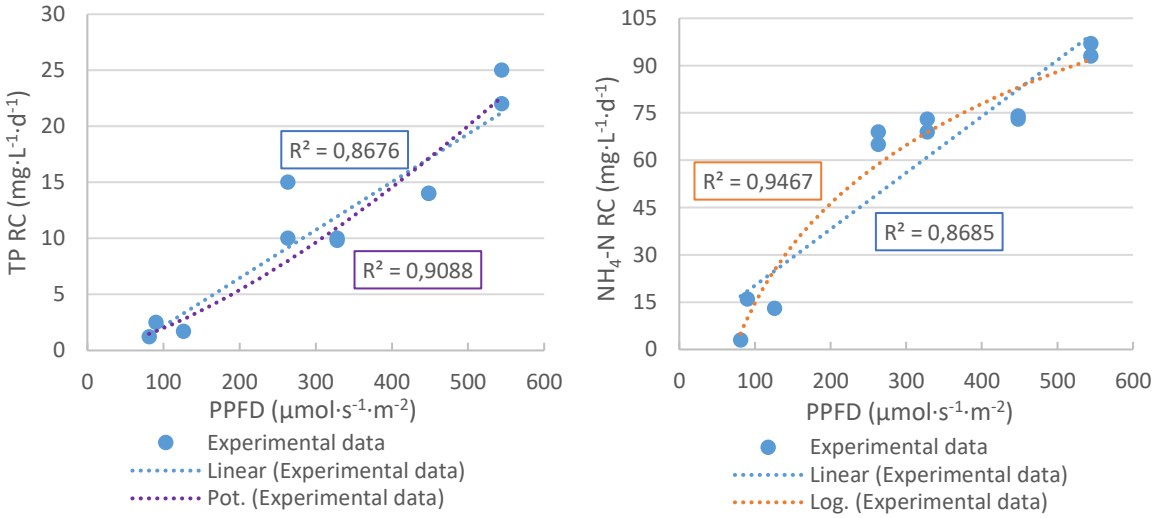

Figure 87: Nutrient removal capacity according to the PPFD and related correlations during the full-scale experiments with Acutodesmus obliquus in the fall and in the summer season - left: TP - right: NH_4-N

The relation between the nutrient removal capacity and both the PPFD and the temperature was deeper investigated throughout graphical representation and mathematical functions permitting to predict the relation between these parameters (Figure 87 and Figure 89). The same work was conducted for the BPR and μ_{max} as a function of the PPFD and the temperature (Figure 88 and Figure 90). To this purpose, the values related to the 4th batch and the 6th batch of the experiment conducted during the summer were not taken into account. Indeed, during the 4th batch, the nutrient RC and the BPR were strongly influenced by the technical problem affecting the flue gas supply during one whole day and the 6th batch was characterized by an absence of microalgae growth. For each parameter represented as a function of the PPFD, a linear trend line could be established with a coefficient of determination ranging between 0.86 and 0.90. While a logarithmic trend line was more accurate for the parameter $RC_{NH4\text{-}N}$, the most fitting trend lines were power and exponential functions for the parameters RC_{TP} and μ_{max} respectively. Nonetheless, these three different functions could not fit with the other parameters, so that a linear approach was preferred. Concerning the parameters represented as a function of the temperature, linear trend lines were established with a comparatively lower coefficient of determination ranging between 0.41 and 0.79. Hence, RC_{TP}, $RC_{NH4\text{-}N}$, BPR and μ_{max} are more dependent on the PPFD than on the temperature. In addition, Figure 91 and Figure 92 represent RC_{TP}, $RC_{NH4\text{-}N}$, BPR and μ_{max} as a function of both the temperature and the PPFD. In future experiments, these graphics will be useful to predict the evolution of these parameters according to the weather conditions.

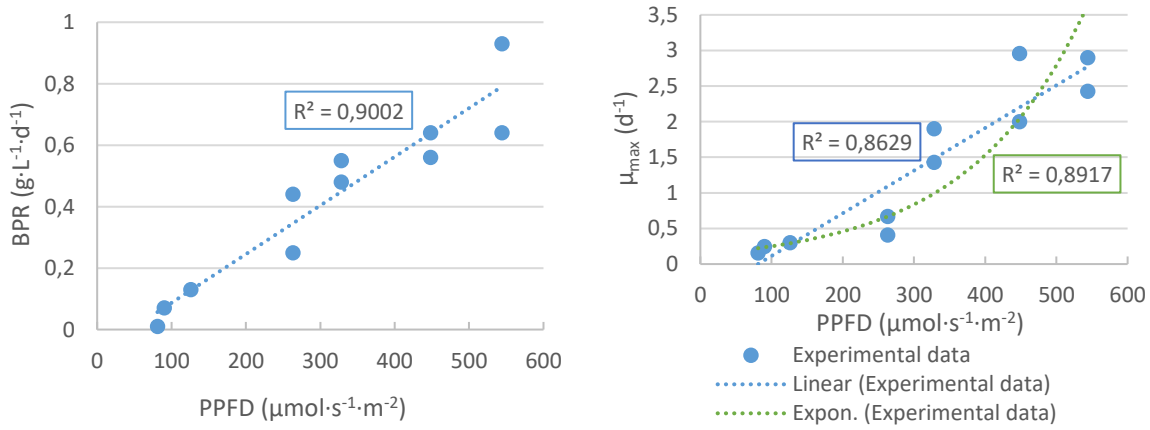

Figure 88: BPR (left) and μ_{max} (right) according to the PPFD and related correlations during the full-scale experiments with Acutodesmus obliquus in the fall and in the summer season

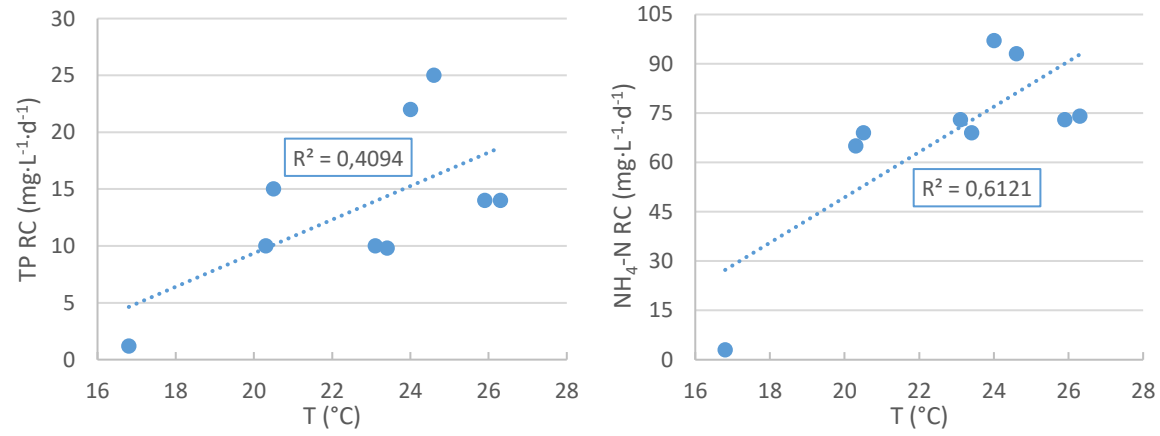

Figure 89: Nutrient removal capacity according to the temperature and related correlations during the full-scale experiments with Acutodesmus obliquus in the fall and in the summer season - left: TP - right: NH_4-N

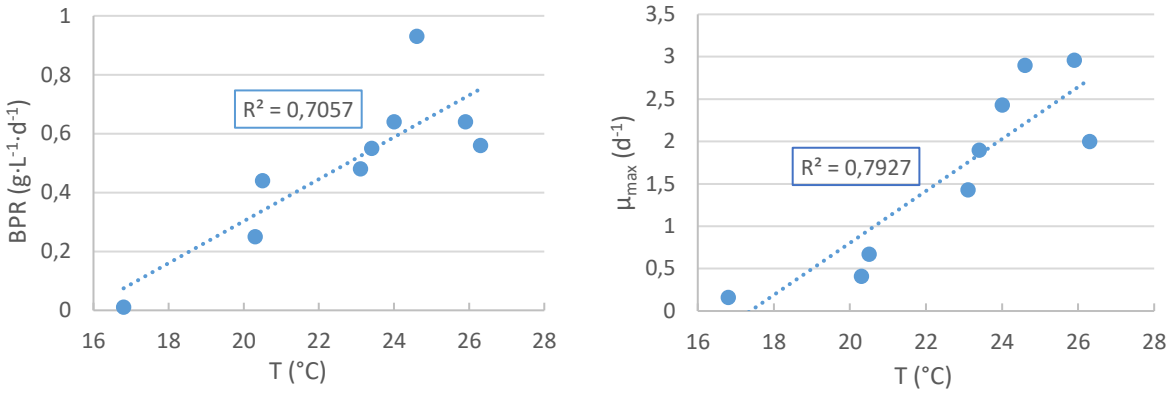

Figure 90: BPR (left) and μ_{max} (right) according to the temperature and related correlations during the full-scale experiments with Acutodesmus obliquus in the fall and in the summer season

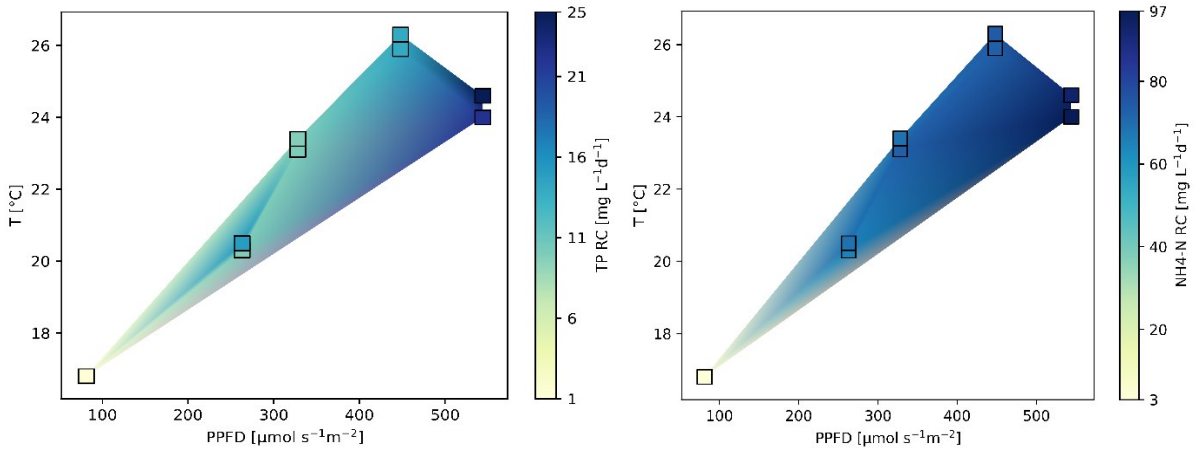

Figure 91: Nutrient removal capacity according to the temperature and the PPFD during the full-scale experiments with Acutodesmus obliquus in the fall and in the summer season - left: TP - right: NH_4-N

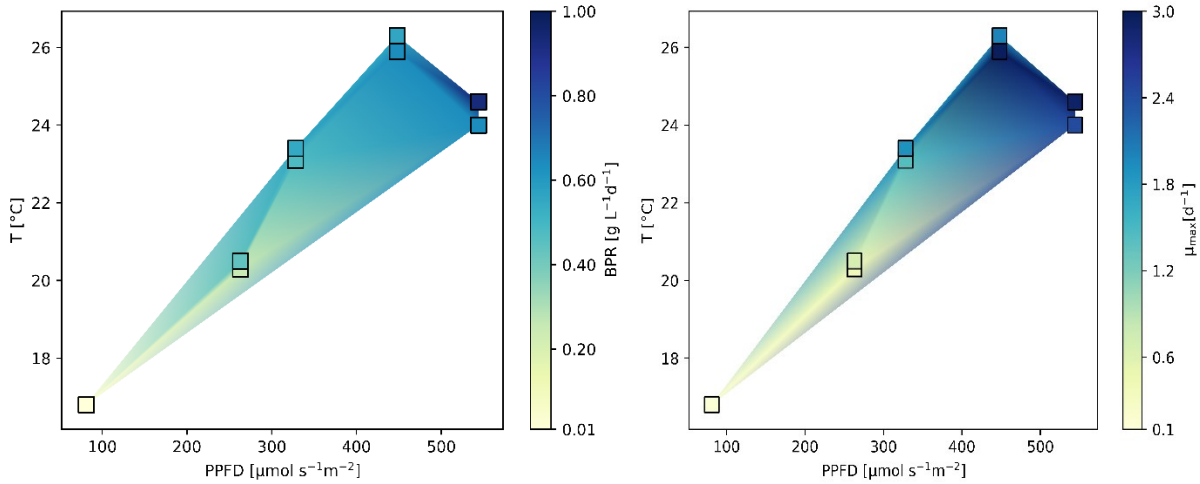

Figure 92: BPR (left) and μ_{max} (right) according to the temperature and the PPFD during the full-scale experiments with Acutodesmus obliquus in the fall and in the summer season

5.2.4.2 Comparison of the performance obtained with *Acutodesmus obliquus* at full-scale and at lab-scale

The lab-scale and full-scale experiments did not show any difference in nutrient removal. However, differences in nutrient uptake rates and in the BPRs were observed. The values related to PPFD ranged between 260 and 270 µmol·s^{-1}·m^{-2} during the lab-scale experiments. With mean PPFDs of 328, 263 and 287 µmol·s^{-1}·m^{-2}, similar light conditions were measured during the 2nd, 3rd and 6th batch tests of the experiment conducted in the summer season in the outdoor pilot-plant. During this experiment, without taking into account the 6th batch test characterized by an inexplicable absence of growth, the BPR amounted to between 0.25 and 0.55 g·L^{-1}·d^{-1}. This is comparatively better than the BPR reached during the laboratory tests (0.23 - 0.26 g·L^{-1}·d^{-1}). In addition, during these batches in the outdoor pilot-plant, RC_{NH4-N} and RC_{TP} ranged 57 - 73 mg·L^{-1}·d^{-1} and 10 - 15 mg·L^{-1}·d^{-1} respectively. These values were up to 6 times higher than in the lab-scale experiments with bottles (TU Berlin) and 3 times higher than in the lab-scale experiments with tubular pipes (University of Hamburg).

The great differences with the lab-scale experiments conducted in the TU Berlin are easily explained by the geometry of the reactor. Indeed, in the TU Berlin, because of the use of bottles, the ratio volume/ illuminated area was four times higher than in the full-scale plant and the cells situated in the middle of the bottles received less light. Consequently, the amount of light available for each microalgae cell was lower.

The significant differences between the results of the lab-scale experiment conducted in the University of Hamburg and the full-scale experiment first seem surprising, since these experiments were characterized by similar PPFD values and volume/illuminated area ratios. Nevertheless, some differences remained. First, during the full-scale experiments, the mixing of the PBR was enhanced throughout constant circulation of the culture medium in addition to the turbulences created by flue gas supply. In the University of Hamburg, the mixing was only caused by flue gas supply and a slight sedimentation of the microalgae was occasionally observed in the pipes. Moreover, during the experiments conducted in the University of Hamburg and the pilot-plant Hamburg-Reitbrook, the COD concentration in the permeate ranged between 800 and 1,000 mg·L^{-1} (troubles of the anaerobic process) and between 200 and 300 mg·L^{-1} respectively. The higher COD concentrations in the permeate were accompanied by higher turbidities that probably affected the transmission of light in the culture.

5.2.4.3 Comparison of the performance of *Acutodesmus obliquus* cultivation at full-scale with literature

Viruela *et al.* (2016) reported the performance of an outdoor full-scale plant using as culture medium the effluent of an AnMBR fed with pre-treated wastewater from a municipal WWTP (Table 58). Three flat PBRs with a working volume of each 550 L were used to conduct semi-continuous experiments. The system was characterized by the vast predominance of the species *Scenedesmus sp.*, which is one of the most frequently observed species in the wastewater/microalgae systems. Over the four months experiments divided into five periods, the PPFD ranged between 124 and 402 µmol·s^{-1}·m^{-2} and the temperature between 15.4 and 24.6 °C. During the full-scale experiments, in the first two batches in fall 2017 and in the 1st, 2nd, 3rd and 6th batch tests in summer 2018, the PPFD amounted to between 126 and 448 µmol·s^{-1}·m^{-2} and the temperature to between 20.3 and 26.3 °C. As the light conditions were similar and the temperature was only slightly higher than in Viruela *et al.* (2016), the results are comparable. In Viruela *et al.* (2016), NH_4-N and PO_4-P removal of respectively 36 - 75 % and 36 - 78 %

were described. In the present work, RE_{NH4-N} and RE_{TP} removal reached an average of 100 % and 98 % respectively. Hence, nutrient removal was much higher in the present work. While the RC_{NH4-N} and RC_{PO4-P} ranged respectively 0.81 - 4.8 $mg \cdot L^{-1} \cdot d^{-1}$ and 0.17 - 0.61 $mg \cdot L^{-1} \cdot d^{-1}$ in Viruela *et al.* (2016), in the present study, they achieved 5.9 - 74 $mg \cdot L^{-1} \cdot d^{-1}$ and 0.9 - 14 $mg \cdot L^{-1} \cdot d^{-1}$ respectively. The BPRs ranged between 14 and 41 $mg \cdot L^{-1} \cdot d^{-1}$ in Viruela *et al.* (2016). This was likewise lower than in the full-scale plant Hamburg-Reitbrook, where the BPRs amounted to between 90 and 640 $mg \cdot L^{-1} \cdot d^{-1}$.

Table 58: Summary of microalgae cultivation with wastewater effluents at full-scale in the literature

Author	**Arbib *et al.* (2013)**		**Novoveská *et al.* (2016)**	**Romero Villegas (2017)**	**Viruela *et al.* (2016)**	**Viruela *et al.* (2018)**
Species	*Acutodesmus obliquus*		Evolving polycultures of microalgae	*Nannochloropsis gaditana*	*Scenedesmus sp.*	*Scenedesmus sp.*
Culture medium	Secondarily pre-treated wastewater from a municipal WWTP		Filtrated and disinfected raw municipal wastewater	Centrate from a municipal WWTP diluted with seawater	Effluent of an AnMBR treating pre-treated wastewater from a municipal WWTP	Effluent of an AnMBR treating pre-treated wastewater from a municipal WWTP
Reactor	530-L HRAP	380-L airlift tubular PBR	12 to 48 floating offshore PBRs (4 to 15 m^3 per PBR)	Three outdoor 340-L tubular PBRs	Three outdoor 550-L flat PBRs	Two lines of each two outdoor 550-L flat PBRs connected with a 500-L ultrafiltration membrane unit
TN initial concentration ($mg \cdot L^{-1}$)	25 - 26		40	146[1]	55 ± 16[1]	52 ± 10[1]
TP initial concentration ($mg \cdot L^{-1}$)	1.8 - 2.2		4.2	44	6.8 ± 1.7[2]	6.8 ± 1.6[2]
RE_{TN} (%)	65	90	75	100[1]	36 - 75[1]	-
RE_{TP} (%)	59	87	93	> 98	36 - 78[2]	-
RC_{TN} ($mg \cdot L^{-1} \cdot d^{-1}$)	-	-	-	30 - 40[1]	0.81 - 4.75[1]	4.4 - 7.6[1]
RC_{TP} ($mg \cdot L^{-1} \cdot d^{-1}$)	-	-	-	4 - 6	0.17 - 0.61[2]	0.40 - 0.97[2]
BPR ($g \cdot L^{-1} \cdot d^{-1}$)	5 - 8[3]	13 - 22[3]	6.8 ± 1.7[3]	0.4 - 0.6	0.014 - 0.041[6]	0.011 - 0.031[6]
PPFD ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	100 - 560		250 - 600[5]	1200 ± 170	124 - 402	169 - 362
Duration (months)	4		12	5	4	4
Temperature in PBR (°C)	5 - 15[4]		10 - 30[5]	25 ± 5	15.4 - 24.6[4]	26 - 29
HRT (d)	10	5	2 - 7	-	8 - 14	8 - 9
PE (%)	-	-	-	1.4 - 2.7	-	1.4 - 6.3

[1]: NH_4-N, [2]: PO_4-P, [3]: $g \cdot m^{-2} \cdot d^{-1}$, [4]: ambient temperature, [5]: monthly average, [6]: $g\ VS \cdot L^{-1} \cdot d^{-1}$

In Viruela *et al.* (2018), two membrane photobioreactors (MPBR) composed of each two 550-L PBRs and a 500-L ultrafiltration membrane unit were investigated in order to avoid the washout of the biomass. While RC_{NH4-N} ranged between 4.39 and 7.61 $mg \cdot L^{-1} \cdot d^{-1}$, RC_{TP} amounted to between 0.40 and 0.97 $mg \cdot L^{-1} \cdot d^{-1}$ and the BPR between 11 and 30.6 $mg \cdot L^{-1} \cdot d^{-1}$. These results were in the low range of the results obtained in the full-scale plant Hamburg-Reitbrook. Compared to the previous experiments without membrane unit, the higher temperatures and the complete microalgal biomass retention explained the higher removal rates.

Hence, the nutrient RE and RC were systematically better in the present work. The reasons explaining these differences are not clear but can be presumed. According to Viruela *et al.* (2016; 2018), the fluctuation of NH_4-N and PO_4-P concentrations in the effluent of the AnMBR (40 - 100 $mg \cdot L^{-1}$ and 6 - 11 $mg \cdot L^{-1}$ respectively) may explain the low nutrient removal. In addition, the authors observed that the highest nutrient removal results were established with the lowest nutrient concentration in the effluent and vice versa. Compared to the process used in the present work, the system ran semi-continuously, which means that a part of the fluid phase of the microalgae culture was replaced by AnMBR effluent every day. The HRT could have not been totally adapted to the system, which would explain the relatively low nutrient removal.

Besides, because of the daily washout of the biomass, biomass concentration in the culture always remained under 0.5 $g \cdot L^{-1}$. It is well known that, with a lower biomass concentration, the nutrients are assimilated slower. Moreover, the reactors consisted of 550-L PBRs, so the difference observed could be a usual scale-up problem. This is characterized by the difficulty to construct large volume reactors with a short light path enhancing light availability for the microalgae, to control and optimize the mixing conditions and to prevent contamination in the reactors. One of the deciding factors explaining the differences with the present study could be the volume/area ratio of the PBRs used in Viruela *et al.* (2016). This amounted to 250 $L \cdot m^{-2}$, which is between 5 and 11 times bigger than the ratio in Hamburg- Reitbrook. With a 25 cm light path for each PBR, it is to presume that the light availability for the microalgae was not optimal. This is confirmed by the fact that the authors stated that solar irradiance was the key factor affecting the nutrient removal capacity in their work. The BPRs achieved in Viruela *et al.* (2016; 2018) were in the low range of those obtained in the present work. Thus, compared to the nutrient RE and RC, the differences related to the BPR were not so pronounced.

Novoveská *et al.* (2016) investigated during one year the performance of 12 to 48 floating offshore PBRs with a volume of each 4 to 15 m^3. The initial culture was mostly composed of *Scenedesmus dimorphus* and naturally evolved to a polyculture dominated by the species *Chlorella, Cryptomonas* and *Scenedesmus*. The microalgae were supplied with raw wastewater that was previously filtrated and disinfected. In addition, the process worked in repeated batches that lasted between 2 and 7 days. TN and TP removal reached an average of 75 % and 93 % respectively. With a TN removal of 74 % and a TP removal of 96 %, the results obtained in the full-scale plant Hamburg-Reitbrook in the fall season were very similar. However, with a TN removal of 91 %, nutrient removal in the summer season in Reitbrook was better. As the values given in Novoveská *et al.* (2016) are yearly averages and the monthly results as a function of the PPFD and the temperature are not given, exhaustive comparisons relating to nutrient removal are not possible.

In Novoveská *et al.* (2016), for a PPFD ranging between 263 and 567 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and a temperature ranging between 20 and 26 °C, which corresponds to the abiotic conditions in the summer season in the pilot-plant Hamburg-Reitbrook, BPR_{α} amounting to between approximately 5 and 15 $g \cdot m^{-2} \cdot d^{-1}$ were

achieved (Figure 93). In the present work, in the summer season, BPR_{α} ranged 2.6 - 17 $g \cdot m^{-2} \cdot d^{-1}$ and was in the same order of magnitude. As the minimum PPFD in Novoveská *et al.* (2016) reached 200 $\mu mol \cdot s^{-1} \cdot m^{-2}$, no comparison with the full-scale experiments in the fall season (maximum PPFD of 150 $\mu mol \cdot s^{-1} \cdot m^{-2}$) was allowed.

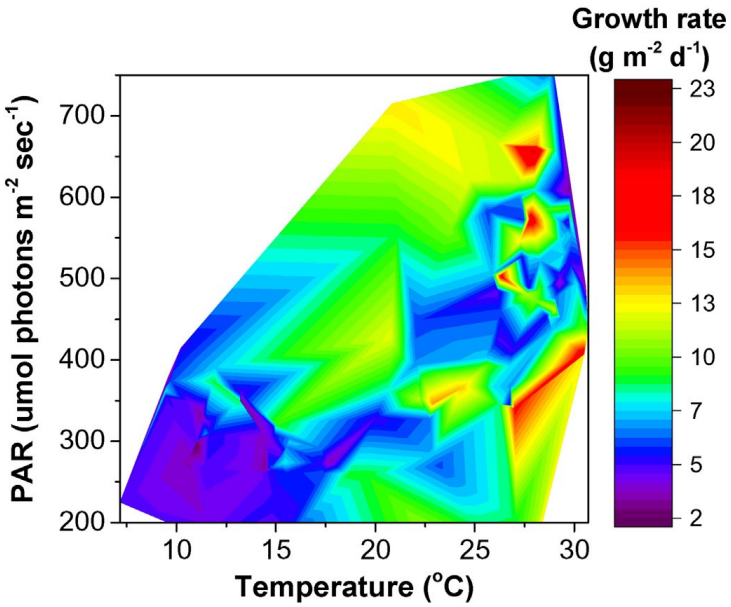

Figure 93: BPR_{α} (growth rate) according to the PAR (PPFD) and the temperature in floating offshore photobioreactors (Novoveská et al. 2016)

Romero Villegas *et al.* (2017) investigated the performance of three outdoor 340-L tubular PBRs cultivating *Nannochloropsis gaditana* with the centrate of a WWTP diluted to 30 % with seawater. The average PPFD of 1200 $\mu mol \cdot s^{-1} \cdot m^{-2}$ was two times higher than the maximum PPFD of 567 and 544 $\mu mol \cdot s^{-1} \cdot m^{-2}$ reached during the 4th and 5th batch tests in the summer experiments at full-scale. Contrary to the other literature sources, with NH_4-N and TP initial concentrations amounting to 146 and 44 $mg \cdot L^{-1}$ respectively, nutrient concentration was slightly higher than in the full-scale experiments conducted in the summer season. In Romero Villegas *et al.* (2017), NH_4-N and TP uptake was likewise complete. However, in the 5th batch test in the summer season characterized by the best abiotic conditions and the absence of technical failure, $RC_{NH4\text{-}N}$ and RC_{TP} were at least 133 % and 267 % higher than in Romero Villegas *et al.* (2017). Compared to the BPR values comprised between 0.4 $g \cdot L^{-1} \cdot d^{-1}$ and 0.6 $g \cdot L^{-1} \cdot d^{-1}$ in Romero Villegas *et al.* (2017), this parameter was also slightly better in the present work, where it amounted to 0.64 and 0.93 $g \cdot L^{-1} \cdot d^{-1}$ in L1 and L2 respectively in the 5th batch test.

As similar BPRs were found under the same experimental conditions with a synthetic fertilizer as culture medium in Romero Villegas *et al.* (2017), the lower BPRs obtained were not due to the presence of pollutants in the centrate inhibiting microalgae growth. Moreover, the microalgae species used in this experiment is known to lead to high BPRs, so the differences in the results were presumably not caused by the use of this species. Consequently, we can suppose that the relatively slow nutrient assimilation and BPRs were due to the use of tubular PBRs. While they have the advantage to be simple reactors with a large illumination surface, they face photolimitation and mass transfer problems, which usually lead to CO_2 depletion and O_2 accumulation in the system (Huang *et al.* 2017).

With the same microalgae species as in this work, Arbib *et al.* (2013) investigated a four-month continuous process consisting of a 530-L HRAP and a 380-L airlift tubular PBR. With initial TN and TP concentrations ranging respectively 25 - 26 $mg \cdot L^{-1}$ and 1.8 - 2.2 $mg \cdot L^{-1}$, the wastewater effluent was

much less concentrated than in the present work. However, the PPFD values recorded in Arbib *et al.* (2013) (100 - 560 $\mu mol \cdot s^{-1} \cdot m^{-2}$) permit a comparison with the results obtained in the summer season as well as in the fall season during the 1st and 2nd batch tests. In the tubular PBR, TN removal was the same as in this study. Nevertheless, in the present work, with 99 % RE_{TP} in the summer season and 98 % RE_{TP} in the first two batches in the fall season, TP uptake was higher than in Arbib *et al.* (2013) (87 %). The authors reported TP removal between 78 and 94 % over the four periods studied and a strong dependence of RE_{TP} on the ambient temperature, especially on the temperature drops down to 5 °C during the night. Hence, the weather conditions explain the differences observed between this work and Arbib *et al.* (2013).

Despite a twice higher HRT, TN and TP removal in the HRAP (open system) only reached 65 % and 59 %. These low results were explained by the light limitation in the HRAP caused by the 30 cm depth of the system, the poor mixing and CO_2 depletion in the process. Likewise, while BPR_α only ranged between 5 and 8 $g \cdot m^{-2} \cdot d^{-1}$ in the HRAP, this amounted to between 13 and 20 $g \cdot m^{-2} \cdot d^{-1}$ in the tubular PBR. In the first two batches in the fall season as well as in the batches in the summer season, BPR_α reached values comprised between 2.6 and 17 $g \cdot m^{-2} \cdot d^{-1}$. This was significantly better than the results obtained in the HRAP. However, the BPR_α obtained in this work were lower than the BPR_α achieved in Arbib *et al.* (2013) (13 - 22 $g \cdot m^{-2} \cdot d^{-1}$), which shows that the process of the full-scale plant Hamburg-Reitbrook could be optimized. The explanations for these differences could be the shear stress in the flat panel PBRs caused by the continuous circulation of the culture medium. Moreover, the dark zone in the tubular PBR in Arbib *et al.* (2013) represented less than 12 % of the total volume. On the contrary, in the present study, the dark volume represented up to 46 % of the total volume of the line. Furthermore, the wastewater effluent in Arbib *et al.* (2013) had presumably a lower turbidity due to the much lower nutrient concentrations. Coupled with the lower biomass concentration in the culture, the solar irradiance effectively attaining the microalgae was higher in Arbib *et al.* (2013).

Overall, only few authors reported data about the photosynthetic efficiency (PE), which is an essential parameter to evaluate the performance of the outdoor full-scale plants. While the theoretical maximum PE for microalgae amounts to approximately 10 % (Richmond and Hu 2013), this parameter is rarely higher than 6.5 % in full-scale plants (Carvalho *et al.* 2006). In the Netherlands, PE usually amounts to 1.5 % for HRAPs, 3 % for tubular PBRs and 5 % for flat panel PBRs (Norsker *et al.* 2011). In the present work, PE ranged 0.9 - 2.1 % in the fall season and, except for the 4th and 6th batch tests characterized by technical problems and an absence of growth, it ranged 0.85 - 1.6 % in the summer season. While no significant difference was observed between L1 and L2 in the summer season, PE was much higher in L1 than in L2 in the fall season. This was probably due to the lower illuminated area for a similar volume of culture irradiated. Compared to the reference PE value of 5 % given by Norsker *et al.* (2011), optimization of the process is needed. As the dark zone represented 40 % to 57 % of the total volume of the lines, PE will easily increase with a smaller dark zone.

Compared to the authors presented in this section, the present results are in the lower range of Romero Villegas *et al.* (2017), who reached PE ranging 1.4 - 2.7 % with tubular PBRs. Viruela *et al.* (2018) achieved PE from 1.4 % to 6.3 %. The better results were caused by the low dark zone and the long-term optimization of the process. Furthermore, at lab-scale, Soletto *et al.* (2008) showed that the decrease of CO_2 supply into the system leads to an increase of PE. Indeed, due to biochemical mechanisms that still need to be explained, the excess of inorganic carbon in the culture medium leads to lower growth of the microalgae. In the full-scale plant Hamburg-Reitbrook, it is not feasible to decrease the dark volume of the process. Nevertheless, the observations based on the long-term

operation of this process in a semi-continuous mode would lead to the optimization of the process. During this long-term operation, the variation of the flow rate of flue gas could enable to find the optimal flow rate leading to the highest PE value.

5.2.5 TP and TN fractions in the microalgae cells

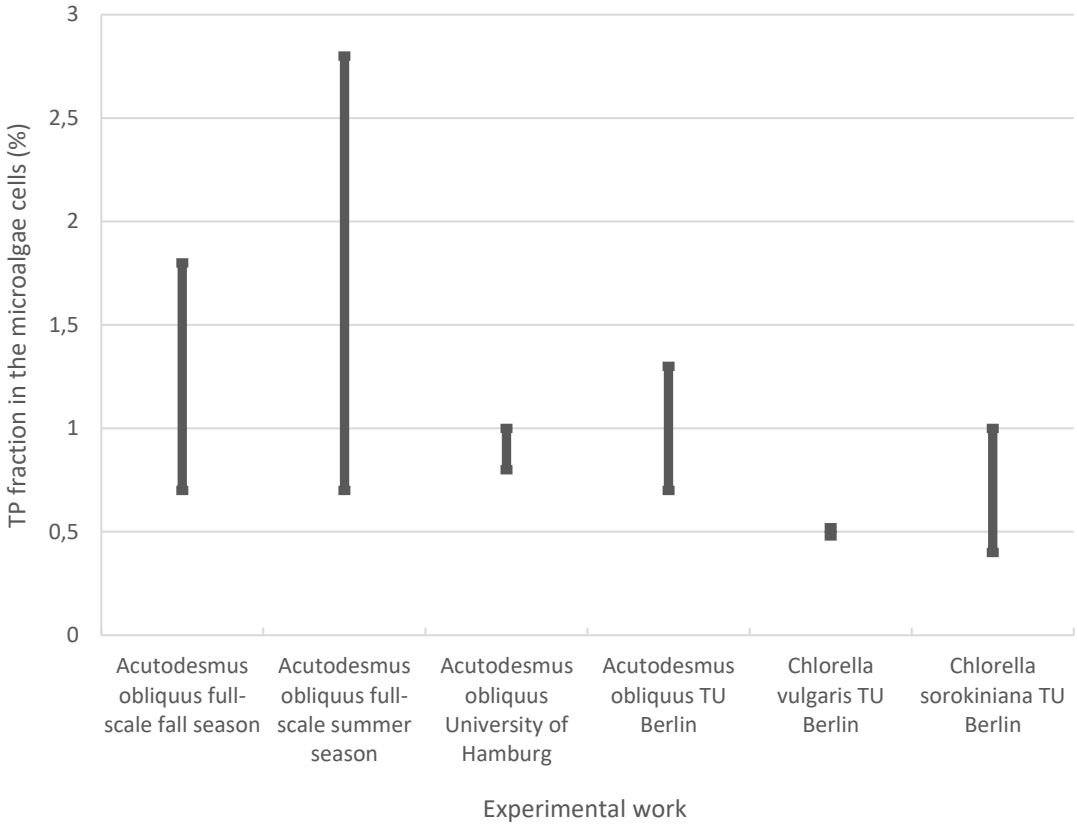

Figure 94: TP fraction in the microalgae cells during the experimental work conducted with the species Acutodesmus obliquus, Chlorella vulgaris and Chlorella sorokiniana

In the present section, the fractions of TN and TP in the biomass produced in permeate cultures are shown (Figure 94 and Figure 95). To this purpose, the hypothesis of an entire assimilation by the microalgae of the TN and TP removed from the culture medium is taken into account. Indeed, it is assumed that no indirect removal took place, as pH stayed neutral in the permeate cultures. In order to enable an accurate comparison between the species, TP and TN fractions in the microalgae biomass were calculated the day from which TP and TN have been completely removed or have remained at constant concentrations. In most of the cases, the experiments lasted longer, meaning an increase of biomass concentration in absence of TP and TN supply. This logically led to a decrease of TN and TP concentrations in the microalgae cells and, therefore, to an increase of lipids and carbohydrates contents.

The results related to the cultivation of *Acutodesmus obliquus* at lab-scale were similar. For the experiment conducted at the University of Hamburg, the 1st batch of the permeate (A) and the three batches of the permeate enriched with micronutrients (B) were taken into account. TP fraction ranged between 0.8 % and 1.0 % and TN fraction ranged between 5 % and 8 %. This is in accordance with Beuckels *et al.* (2015) reporting values between 0.5 % and 1.7 % and between 3 and 8 % for TP and TN respectively, and with Reynolds (2006) reporting values between 0.03 % and 3 % and between 3 % and

12 % for TP and TN respectively. The fraction of TN increased as the batches progressed. Hence, the microalgae cells probably adjusted TN and TP concentrations in their biomass depending on TP and TN supply. This phenomenon is well documented in the literature (Whitton *et al.* 2015). During the experiment (1) conducted with *Acutodesmus obliquus* at the TU Berlin, TP fraction in the biomass amounted to between 0.7 % and 1.3 % and TN fraction amounted to between 5 % and 8 %. Between both batches, TP fraction in the cells increased by 73 %. This was presumably due to the decrease of TN/TP ratio from 9 to 6. Hence, more TP was proportionally present in the culture medium and the cells consequently adapted the nutrient concentrations in their cells.

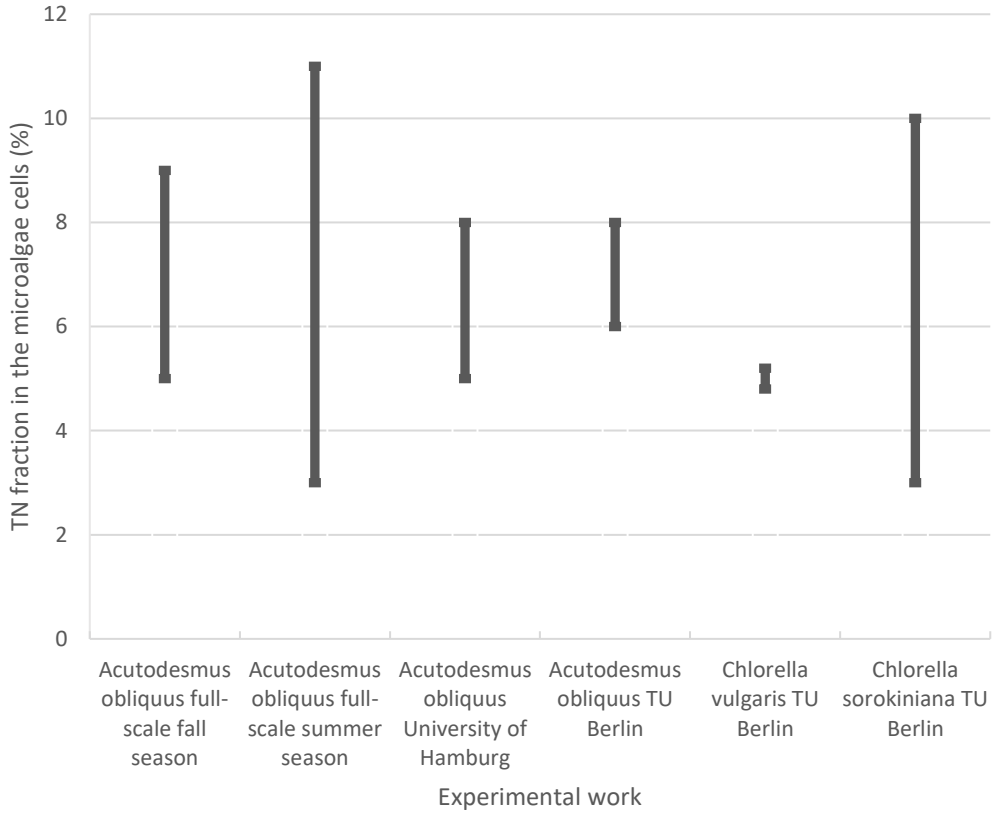

Figure 95: TN fraction in the microalgae cells during the experimental work conducted with the species Acutodesmus obliquus, Chlorella vulgaris and Chlorella sorokiniana

Concerning the full-scale experiment conducted in the fall season in the pilot-plant Hamburg-Reitbrook, the 4th batch in L2 was not taken into account as no growth took place. TP and TN fractions in the biomass ranged between 0.7 % and 1.8 % and between 5 % and 9 % respectively. Between each batch test, the fraction of TP in the biomass increased. This could be due to the decrease of TN/TP ratio from 7.8 to 6.5 between the 1st and the 3rd batch or to the decrease of PPFD leading to stress conditions for the microalgae and consequently to storage of TP in the vacuole of their cells. In addition, great differences related to TN fraction in the biomass were observed between L1 and L2 during the 3rd batch. Indeed, TN was not entirely assimilated in L1 but microalgae growth was similar in L1 and L2. Overall, the results related to TN fraction were in the same order of magnitude as at lab-scale. However, as NO_x was supplied into the system in the form of flue gas, it is possible that the fraction of TN in the biomass was higher.

In the summer season at full-scale, TP and TN fractions in the biomass showed great variations and amounted to between 0.7 % and 2.8 % and to between 3 % and 11 % respectively. Except during the 1st and the 4th batch tests, TP fraction in the biomass was systematically higher than 1.6 %. This can be explained by the comparatively low TN/TP ratio amounting to between 4 and 5 or by the phenomenon of luxury uptake. Since the microalgae cells were deprived of TP supply during 3 - 6 days in each batch test, at the beginning of the following batch, they could have assimilated more TP than needed in a purpose of storage for future times of depravation. This did not happen during the 1st batch, as the microalgae had a constant supply before the beginning of this batch throughout culture in a synthetic culture medium, and during the 4th batch that was characterized by a technical failure of flue gas supply. Except during the 1st and the 4th batch tests, TN fraction reached 6 - 11 % and was in the highest range of the other experiments conducted with *Acutodesmus obliquus*. During these experiments at full-scale, no relation between the nutrient fraction in the biomass and the PPFD was showed.

During *Chlorella vulgaris* cultivation (experiment (3)), TP and TN fractions in permeate amounted to 0.5 % and 5 % respectively. For the species *Chlorella*, Beuckels *et al.* (2015) reported values ranging between 0.5 % and 1.3 % and between 5 % and 10 % for TP and TN respectively. Hence, both values are in the low range of the literature. This is also in the low range of the results obtained in this work with the species *Acutodesmus obliquus* and *Chlorella sorokiniana*. In addition to the species used, the only difference to the other experiments is the comparatively high TN/TP ratio amounting to 11. Nevertheless, a correlation between TN/TP ratio and both TP and TN fractions in the biomass has not been demonstrated before.

In *Chlorella sorokiniana* culture (experiment (1)), TP and TN fractions amounted to between 0.4 % and 1.0 % and to between 3 % and 10 % respectively. Compared to the other experiments of this work, TP and TN fractions were in the same order of magnitude. In comparison to the other species studies, *Chlorella sorokiniana* is able to assimilate comparatively more TN. Consequently, wastewaters streams defined by a high TN/TP ratio should be used for the cultivation of this species.

5.2.6 Effluent quality after microalgae cultivation

5.2.6.1 Nutrient concentration after microalgae cultivation

One of the main goals of the combination of the AnMBR technology with microalgae cultivation is to obtain a high-quality effluent with regard to the final macronutrient concentrations. Concerning the discharge of wastewater streams into sensitive areas, countries belonging to the European Union (EU) must apply the European directive 91/271/EEC. In this directive, sensitive areas are defined as *"natural waters which are found to be or may become eutrophic in the near future if protective action is not taken, or those which need more advance treatment to reach compliance with other EU directives"*. The directive allows maximum TP and TN concentrations of 1 $mg \cdot L^{-1}$ and 10 $mg \cdot L^{-1}$ respectively in the effluents of large WWTPs (>100,000 PE) and maximum TP and TN concentrations of 2 $mg \cdot L^{-1}$ and 15 $mg \cdot L^{-1}$ respectively in the effluents of smaller WWTPs. Independently of the size of the WWTPs, TP and TN concentrations need to be reduced by 80 % before discharge.

In Germany, it is the so-called "Abwasserverordnung" (Wastewater regulation) dating back to 1997 that regulates the requirements for domestic wastewater discharge into water bodies. For the WWTPs of the size category 1 and 2 (< 300 kg $BOD_5 \cdot d^{-1}$ - see Table 60), no NH_4-N, TN or TP limit is set. A maximum NH_4-N concentration of 10 $mg \cdot L^{-1}$ is permitted in the effluents of the plants belonging to the

size category 3. In the size category 4, maximum NH_4-N, TN and TP concentrations of 10 $mg \cdot L^{-1}$, 18 $mg \cdot L^{-1}$ and 2 $mg \cdot L^{-1}$ respectively are allowed. For the larger WWTPs (size category 5), the maximum NH_4-N, TN and TP concentrations amount to 10 $mg \cdot L^{-1}$, 13 $mg \cdot L^{-1}$ and 1 $mg \cdot L^{-1}$ respectively.

Table 59: Comparison of the results of the present study with German and European regulations for discharge of wastewater effluents into the water bodies - green: the related regulation is systematically respected for all parameters; yellow: one parameter rarely does not respect the related regulation; orange: at least one parameter often does not respect the related regulation; red: at least one parameter systematically does not respect the related regulation

Law	Requirement	***Acutodesmus obliquus*** **lab-scale**	***Chlorella vulgaris*** **lab-scale**	***Chlorella sorokiniana*** **lab-scale**	***Acutodesmus obliquus*** **Full-scale Fall 2017**	***Acutodesmus obliquus*** **Full-scale Summer 2018**
German wastewater regulation, category 3	NH_4-N < 10 $mg \cdot L^{-1}$					
German wastewater regulation, category 4	NH_4-N < 10 mg $\cdot L^{-1}$ TN < 18 $mg \cdot L^{-1}$ TP < 2 $mg \cdot L^{-1}$					
German wastewater regulation, category 5	NH_4-N < 10 $mg \cdot L^{-1}$ TN < 13 $mg \cdot L^{-1}$ TP < 1 $mg \cdot L^{-1}$					
European directive 91/271/EEC: sensitive water bodies, between 10,000 and 100,000 PE	TN < 15 $mg \cdot L^{-1}$ TP < 2 $mg \cdot L^{-1}$					
European directive 91/271/EEC: sensitive water bodies, > 100,000 PE	TN < 10 $mg \cdot L^{-1}$ TP < 1 $mg \cdot L^{-1}$					

Table 60: Categorization of the WWTPs in Germany

Wastewater treatment plant size category	**1**	**2**	**3**	**4**	**5**
Daily organic load (kg $BOD_5 \cdot d^{-1}$)	< 60	60 - 300	300 - 600	600 - 6,000	> 6,000

Apart from the lab-scale experiments where NH_4-N assimilation was not complete because of a lack of micronutrients in the permeate, NH_4-N systematically remained at very low concentrations at the end of the lab-scale and full-scale experiments. Thus, the NH_4-N requirements for discharge into the water bodies were easily respected for all the regulations.

In each lab-scale experiment conducted with the species *Acutodesmus obliquus and Chlorella vulgaris*, with TP final concentrations ranging between 0.12 and 0.88 $mg \cdot L^{-1}$, the most stringent TP requirement of 1 $mg \cdot L^{-1}$ was systematically achieved (Table 59). This requirement was also met six times in seven during the four successive batches at the outdoor full-scale plant in fall 2017. In the batch that failed to meet this requirement, the residence time in the related PBR was presumably too low and the final TP concentration amounted to 1.22 $mg \cdot L^{-1}$. If the batch test in this PBR had lasted a few days more, a final concentration lower than 1 $mg \cdot L^{-1}$ would have been assuredly achieved. In summer 2018, during the 6 and 5 successive batch tests conducted in L1 and L2, the most stringent legal TP requirement was systematically met.

Except for the batch tests where a micronutrient deficiency in the permeate cultures avoided a complete TN assimilation, final TN concentrations ranging between 3.9 and 6.8 $mg \cdot L^{-1}$ were achieved during the lab-scale experiments conducted with permeate and the species *Acutodesmus obliquus and Chlorella vulgaris*. This also meets the strictest legal requirement of 10 $mg \cdot L^{-1}$.During the outdoor full-scale experiments in fall 2017, final TN concentrations amounting to between 3.8 $mg \cdot L^{-1}$ and 37 $mg \cdot L^{-1}$ were reached and, four times in seven, a lower concentration than 10 $mg \cdot L^{-1}$ was achieved. Nevertheless, in the summer 2018, except for the 2nd batch test, TN concentration was systematically lower than 9.1 $mg \cdot L^{-1}$. Hence, the strictest TN regulations were respected. During the 2nd batch test, final TN concentrations amounting to 10.9 and 12.9 $mg \cdot L^{-1}$ in L1 and L2 respectively were reached. Hence, except for the European directive 91/271/EEC related to sensitive water bodies and larger WWTPs, these final concentrations respect all the European and German regulations.

With the species *Chlorella sorokiniana*, as TP was not efficiently removed from the permeate culture (A), the effluent only respected the requested quality for the German WWTPs of the size categories 1 to 3. Hence, in small decentralized WWTPs, after microalgae cultivation, the permeate effluent could be discharged as it in the water bodies. However, as TP final concentrations reached up to 24 $mg \cdot L^{-1}$, it is not recommended to discharge this effluent in the water bodies. This would be against the aims of this project, which are the protection of the water bodies against the phenomenon of eutrophication, as well as the recovery of TN and TP throughout biomass production. Therefore, solutions must be chosen for future AnMBR operations with such extremely high nutrient concentrations and unfavorable TN/TP ratios.

During the culture of *Chlorella sorokiniana*, due to the increase of nutrient concentration in the permeate, the batches lasted up to 25 days. This represents for future full-scale applications an extremely high HRT. Moreover, this high HRT leads to an extremely high biomass concentration in the reactor. This is often accompanied by the development of bacteria and contamination in the reactors or the release of dissolved organic matter (DOM) leading to extremely high COD and BOD_5 concentrations in the fluid phase. Hence, the solution proposed here is to dilute the permeate with rainwater, greywater, seawater or underground water.

The experiments with *Acutodesmus obliquus* and *Chlorella vulgaris* showed that a TN/TP ratio of 7 was enough to completely remove both NH_4-N and TP from the permeate defined with lower initial nutrient concentrations. Using these species, it should be investigated if a smaller initial TN/TP ratio also enables the total uptake of these two macronutrients.

In case where a dilution process is too complex and costly to install or no water is available for dilution, a utilization of the permeate without dilution is also an acceptable solution. However, a post-treatment related to TP must be set. The proposed post-treatments or alternatives are:

- The use of a microalgae species defined by a lower optimum TN/TP ratio. For instance, Choi and Lee (2015) demonstrated that a low TN/TP ratio could lead to a total TP removal with *Chlorella vulgaris*.
- A significant pH increase throughout the stop of CO_2 supply leading to phosphate precipitation with the microalgae biomass.
- Chemical precipitation using iron or aluminum salts. This solution is effective and used in most WWTPs but does not enable TP recovery.
- TP adsorption by use of iron hydroxide, hydroxide mineral materials, activated carbon or agricultural waste. This process enables the recovery of TP under the form of struvite or calcium phosphate. However, it is not as effective as chemical precipitation.

5.2.6.2 Comparison of nutrient removal achieved in this work with German WWTPs

The performance of the German WWTPs is summarized in the DWA (2010), in which 5,949 WWTPs representing 92 % of the German WWTPs participated. In average, TP and TN removal amounts to 90 % and 81 % respectively (Table 61). In the effluents, TP, TN and NH_4-N are present at an average concentration of 0.71, 7.3 and 1.27 $mg \cdot L^{-1}$ respectively. However, the study shows that nutrient removal in a WWTP is strongly influenced by its size category. While the highest TN removal of 82.7 % is reached in the WWTPs of the size category 3, TN removal only amounts to 71.3 % and 76.7 % in the WWTPs of the size categories 1 and 2. The discrepancy related to the TP results is much higher. Despite an average removal of 90 %, only 70 % of TP is removed in the WWTPs belonging to the categories 1 and 2. The difference of the processes performed explains the dependence of the results on the size category (Table 62). The WWTPs of the size category 5 only use activated sludge processes with anaerobic sludge stabilization (ASAN). The WWTPs of the size categories 3 and 4 perform to 77 % and 95 % respectively activated sludge processes with aerobic sludge stabilization (ASA) and ASAN. On the contrary, 65 % and 32 % of the plants belonging to the size categories 1 and 2 are characterized by trickling filters, waste stabilization ponds or constructed wetlands, which do not show a high nutrient removal efficiency. The ponds and the constructed wetlands are only to find in the WWTPs of these size categories.

With *Acutodesmus obliquus* and *Chlorella vulgaris*, TP removal ranged in the present work between 96 % and 99 %. This removal is at least 6 % better than the average TP removal in German WWTPs. Most notably, TP removal with microalgae was much more efficient than in the smaller WWTPs of the categories 1 and 2, which only reach an average TP removal of 70 %. In lab-scale experiments as well as in full-scale experiments in the summer season, with these species, TN removal amounted to between 90 % and 94 %. Compared to the average of all the WWTPs and the WWTPs of the category 1, TN removal was respectively 10 % and 21 % higher. Hence, using microalgae, a significant amelioration of nutrient uptake is observed.

TN removal in the full-scale plant in the fall season only reached an average of 74 %. This was slightly better than the category 1 of the WWTPs (71 %) but 7 % less than the average TN removal in German WWTPs. As a reminder, this was due to both the low PPFD and the supply of flue gas, which led to abnormally high concentrations of nitrate and nitrite in the PBRs. In the seasons characterized by low PPFD, a decrease of flue gas supply will automatically lead to a better TN removal.

Table 61: Comparison of nutrient removal and final concentrations of the present study with the German WWTPs

Wastewater treatment	TP removal (%)	TN removal (%)	TP final concentration ($mg \cdot L^{-1}$)	TN final concentration ($mg \cdot L^{-1}$)	NH_4-N final concentration ($mg \cdot L^{-1}$)
WWTPs category 1	70	71	-	-	-
WWTPs category 2	70	77	-	-	-
WWTPs category 3	80	83	-	-	-
WWTPs category 4	91	81	-	-	-
WWTPs category 5	94	80	-	-	-
Average WWTPs	90	81	0.71	9.3	1.27
Lab-scale *Acutodesmus obliquus*	97	94	0.37	6.2	0.13
Lab-scale *Chlorella vulgaris*	97	94	0.20	4.9	0.14
Full-scale *Acutodesmus obliquus* Fall 2017	96	74	0.32	15.9	0.57
Full-scale *Acutodesmus obliquus* Summer 2018	99	91	0.30	8.4	0.093

Table 62: Percentage of each wastewater treatment technology according to the WWTP size category in Germany

WWTPs size category	Number of WWTPs	Percentage of the municipal wastewater streams in Germany	Activated sludge with anaerobic sludge stabilization (ASAN)	Activated sludge with aerobic sludge stabilization (ASA)	Sequencing batch reactor	Trickling filter	Waste stabilization pond	Constructed wetland
1	997	0.78	0	28	7	11	46	8
2	1,602	4.1	0	61	7	15	17	0
3	744	4.8	9	77	5	8	0	0
4	1,645	41	55	40	3	2	0	0
5	207	50	100	0	0	0	0	0

During the present experiments, while TP final concentrations were 48 % to 72 % lower than the average of the German WWTPs, this amounted to between 55 % and 93 % for final NH_4-N concentrations. Hence, nutrient removal using microalgae and the effluent of the AnMBR does not only represent a free and eco-friendly culture medium for microalgal biomass production, but also an accurate alternative to processes commonly performed in WWTPs for nutrient removal. This combination of an AnMBR process and microalgae production could especially be implanted as a

decentralized small WWTP and would represent a solution to the only partial nutrient elimination in the small WWTPs in Germany.

Because of the unfavorable TN/TP ratio in the permeate used for the experiments conducted with *Chlorella sorokiniana*, the results related to this species are not compared with conventional German WWTPs in this section.

5.2.6.3 COD and BOD_5 concentration after microalgae cultivation

The AnMBR process aims to use the largest possible amount of organic matter contained in the wastewater to both produce a large volume of biogas and guaranty a low COD concentration in the permeate. COD concentration in the permeate after microalgae cultivation must respect the legal requirements related to this parameter. Hence, besides the understanding of the variation of COD concentration in the permeate according to the wastewater characteristics and the AnMBR operation parameters, the analysis of the variation of COD concentration during microalgae cultivation is essential.

On the one hand, in the full-scale experiment conducted in the fall season with *Acutodesmus obliquus* as well as in the lab-scale experiments with *Chlorella vulgaris* and *Chlorella sorokiniana*, a first correlation between the COD variation and the BPR was established (Appendix 6). While a low BPR usually leads to a decrease or a slight increase of COD concentration, high BPRs or high biomass concentrations lead to increase of this parameter up to 486 %. This is presumably due to the release of dissolved organic matter (DOM) by the microalgae into the culture medium. According to Hulatt and Thomas (2010), the release of DOM by microalgae achieves typical values of 0 - 30 % of the carbon fixed during photosynthesis and can even reach 80 %. This release is normal during a healthy microalgae growth and is caused by both passive leakage across the cell membrane and active exudation to the culture medium (Thornton 2014). Nevertheless, the exact causes, mechanisms, physiological conditions and environmental conditions stay unclear. Consequently, during continuous microalgae growth in a full-scale plant aiming at the discharge of the culture medium into the water bodies, regular COD measurements as well as low biomass concentrations should be considered.

Table 63: COD variation according to the initial COD concentration by cultivation of microalgae with permeate

COD concentration in the permeate ($mg \cdot L^{-1}$)	**100 - 150**	**150 - 200**	**200 - 250**	**250 - 350**	**> 350**
Variation of COD concentration	increase	Constant or slight variations	Constant or low decrease	Decrease	Significant decrease

On the other hand, a correlation between the variation of COD concentration during normal growth of *Acutodesmus obliquus* and *Chlorella vulgaris* and the initial COD concentration in the permeate was established, too. This correlation is resumed in Table 63 and similar observations are reported in the literature. For instance, Wang *et al.* (2010) showed that, during microalgae cultivation, the reduction of the concentration of dissolved COD in the culture medium increases with increasing initial concentrations of dissolved COD. The significant decrease of COD concentration starting from initial COD concentration of 350 $mg \cdot L^{-1}$ is explained by the fact that microalgae are able to uptake the dissolved organic carbon remaining in the permeate. In this case, the uptake of this carbon source

leads despite assumed DOM release to higher decrease of COD concentration in the culture medium. By initial COD concentrations amounting to approximately 200 $mg \cdot L^{-1}$, compared to the inorganic carbon source, little organic carbon is available for the microalgae. Hence, the uptake of dissolved organic carbon remaining in the permeate approximates DOM release from microalgae, leading to slight variations of the COD concentration in the culture medium. For initial COD concentrations lower than 150 $mg \cdot L^{-1}$, the phenomenon of DOM release is more important than dissolved organic carbon uptake from the permeate and leads to an increase of COD concentration in the culture medium. The measuring ranges given in this section are approximating values and the variations of COD concentration are simultaneously dependent of the BPR and the biomass concentration in the culture.

Although a significant COD decrease is possible during microalgae culture, the goal of the AnMBR process is to reach the lowest possible COD concentration in the permeate. This means that the COD contained in the wastewater is mostly transformed to biogas and represents an indication that the AnMBR process is running well. However, in punctual periods characterized by a process disruption leading to higher COD concentrations in the permeate, it might be still possible to respect the legal requirements thanks to the capacity of microalgae to assimilate organic carbon. However, this situation should be avoided. According to the present work and the legal requirements, it should be considered to reach a COD concentration slightly lower than 150 $mg \cdot L^{-1}$ in the permeate and adapt the HRT and the biomass concentration of a continuous microalgae cultivation process in a way that leads to very low variations of COD concentration in the culture medium.

Because of the harder samples conservation, the complex measuring method, the high standard deviation as well as the lack of reproducibility of the measurements, BOD_5 developing during microalgae culture was not investigated as often as COD. Nevertheless, the observed trends were always similar to COD, so that the observations related to COD in this section can be applied to this parameter.

5.3 Energy balance of the combination of an AnMBR process with microalgae cultivation

In the previous sections, the technical feasibility of the combination of an AnMBR process with microalgae cultivation for both domestic wastewater treatment and nutrient recovery has been proved. However, for an expansion of this technology, the combined process needs to be energetically and economically profitable, particularly compared to a conventional WWTP. In this section, the actual energy costs of conventional domestic wastewater treatment and their dependence on parameters such as the size of the plant are first presented. Secondly, energy demand and energy recovery of actual AnMBR processes at lab-scale and at full-scale are reported. Thereafter, a similar work is carried out for the microalgae cultivation step.

5.3.1 Energy consumption of conventional wastewater treatment plants

Although wastewater contains two to four times the energy needed for its treatment (Gandiglio *et al.* 2017), the energy cost of WWTPs is comparatively high, meaning that the energy efficiency for wastewater treatment can still be optimized and WWTPs as net energy producers is an achievable aim. However, today, WWTPs are still frequently the biggest energy consumers for municipalities (Gu *et al.* 2017). Wastewater treatment has clearly direct environmental benefits but the emission of greenhouse gases related to wastewater treatment must be significantly reduced.

Table 64: Average energy consumption in conventional WWTPs

Author	DWA 2017	Gandiglio *et al.* (2017)	Guerrini *et al.* (2017)		Panepinto *et al.* (2016)	Seib *et al.* (2016)
Energy consumption (kWh·m^{-3})	0.39[1]	0.41 - 0.87	0.26 - 0.84	0.67[1]	0.3 - 2.1	0.3 - 0.6

[1]: in Germany

In a conventional WWTP, the cost of energy amounts to between 25 % and 40 % of the total operating costs. The highest consumption of energy is required for the active sludge treatment due to the aeration needed for the process (55 - 70 %) (Panepinto *et al.* 2016). Overall, different energy costs are reported in the literature for WWTPs using activated sludge processes. While Seib *et al.* (2016) report values between 0.3 and 0.6 kWh·m^{-3}, according to Panepinto *et al.* (2016), Guerrini *et al.* (2017) and Gandiglio *et al.* (2017), energy consumptions for conventional WWTPs range 0.3 - 2.1, 0.26 - 0.84 and 0.41 - 0.27 kWh·m^{-3} respectively (Table 64). In Germany, the average energy consumption (all the size categories taken into account) amounts to 0.67 kWh·m^{-3} according to Guerrini *et al.* (2017) and 0.39 kWh·m^{-3} according to DWA (2017).

Table 65: Average energy consumption in conventional WWTPs according to the size of the plant

Author	Gandiglio *et al.* (2017)	Guerrini *et al.* (2017)
Energy consumption in small WWTPs (kWh·m^{-3})	5.5	3.21[1]
Energy consumption in large WWTPs (kWh·m^{-3})	0.13	0.25[1]

[1]: in kWh·kg^{-1} COD

Furthermore, according to Guerrini *et al.* (2017), the COD concentration in wastewater, the plant capacity combined with the rate of used capacity positively influence the energy efficiency of a WWTP. Here, the influence of the plant capacity is particularly relevant, as the thesis concentrates on small WWTPs. Hence, Guerrini *et al.* (2017) report an energy consumption of 3.21 kWh·kg^{-1} COD and 0.25 kWh·kg^{-1} COD for plants with a capacity lower than 2,000 PE and higher than 100,000 PE respectively (Table 65). Taking into consideration an average COD concentration of 0.5 g·L^{-1}, this corresponds to 1.61 and 0.13 kWh·m^{-3} respectively. According to Gandiglio *et al.* (2017), an energy cost of 5.5 kWh·m^{-3} is reached in small WWTPs (<10,000 PE), whereas it only amounts to 0.13 kWh·m^{-3} in large WWTPs (>100,000 PE).

5.3.2 Energy consumption of AnMBR processes

At the beginning of the implementation of the AnMBR technology, one of the main disadvantages reported was the high energy demand of the membranes. Meanwhile, today, in addition to the search of an excellent effluent quality coupled with a low HRT, the emphasis is largely put on the energy efficiency of AnMBR processes. The main aim is to achieve a lower energy demand than conventional WWTPs using activate sludge processes, and even to be energy neutral or a net energy producer. In the following, literature values for the energy cost reported at lab-scale and full-scale are presented. Thereafter, the energy demand and the energy gain of the present AnMBR are calculated.

5.3.2.1 Energy consumption of AnMBR processes at a lab-scale

In the publications of the last years, different energy costs are reported for crossflow and submerged membranes. Martin-Garcia *et al.* (2011) report an energy cost of 0.3 $kWh \cdot m^{-3}$ and 3.7 $kWh \cdot m^{-3}$ for submerged and crossflow membranes respectively (Table 66). Martin *et al.* (2011) simulations estimate for submerged and crossflow membranes energy demands in the range 0.03 - 5.7 $kWh \cdot m^{-3}$ and 0.23 - 16.52 $kWh \cdot m^{-3}$ respectively. The significant variations are due to the fluctuations of biogas sparging requirements according to the different membranes used (submerged membranes) and the fluctuations of crossflow velocity and viscosity of the sludge (crossflow membranes). As the electrical energy produced is estimated to amount to 0.3 $kWh \cdot m^{-3}$ for a COD concentration in wastewater of 1.14 $g \cdot L^{-1}$, the AnMBR process could be a net energy producer. If high-strength domestic wastewater is used, due to the high amount of organic matter for a similar wastewater volume, the energy gain is even higher.

Using both theoretical values and their own experiments at a lab-scale, Mei *et al.* (2018) report that energy recovery by means of an AnMBR process is possible at temperatures of 25 °C and 35 °C. Energy recovery throughout biogas production of 0.42, 0.34 and 0.17 $kWh \cdot m^{-3}$ at 35, 25 and 15 °C respectively can be reached. Overall, taking into consideration theoretical values for biogas sparging, pumping, heating and mechanical stirring and according to both the flux applied to the membrane and the SRT applied to the 31-L AnMBR, an energy cost of -0.31 - 0.33 $kWh \cdot m^{-3}$, -0.19 - 0.58 $kWh \cdot m^{-3}$ and 0.04 - 0.97 $kWh \cdot m^{-3}$ at 35, 25 and 15 °C respectively can be achieved. Nevertheless, these values do not take into account the efficiency of the conversion of the biogas into electrical energy, which is usually estimated to amount to 30 - 35 %. Taking into account an efficiency of 33 %, the energy cost of an AnMBR at lab-scale would reach -0.03 - 0.61 $kWh \cdot m^{-3}$, 0.03 - 0.80 $kWh \cdot m^{-3}$ and 0.16 - 1.09 $kWh \cdot m^{-3}$ at 35, 25 and 15 °C respectively. For optimization of the efficiency of AnMBR processes, the authors recommend the use of high-strength wastewater, as it is the case in the present study and generally in small decentralized WWTPs.

Table 66: Energy consumption in AnMBR processes at a lab-scale in the literature

Author	Martin *et al.* (2011)	Martin-Garcia *et al.* (2011)	Mei *et al.* (2018)	Seib *et al.* (2016)
Energy consumption in AnMBR processes with submerged membranes ($kWh \cdot m^{-3}$)	-0.27 - 5.4	0.3	-0.31 - 0.97	-
Energy consumption in AnMBR processes with crossflow membranes ($kWh \cdot m^{-3}$)	-0.07 - 16.22	3.7	-	0.00 - 0.08

In lab-scale experiments with four 3.3-L AnMBR operated at 10 and 25 °C with a crossflow membrane, Seib *et al*. (2016) report an energy demand of 0.02 - 0.05 $kWh \cdot m^{-3}$ for the membrane module. Combined with an energy cost of 0.02 - 0.08 $kWh \cdot m^{-3}$ for the reactor, 0.05 $kWh \cdot m^{-3}$ for dissolved CH_4 removal and 0.12 $kWh \cdot m^{-3}$ for nutrient removal by means of ion exchange, a total energy demand of 0.22 - 0.30 $kWh \cdot m^{-3}$ is reached. Considering an energy gain of 0.22 $kWh \cdot m^{-3}$, the AnMBR processes could be energy neutral. Nevertheless, this study also does not take into consideration the efficiency of 30 - 35 % for electrical conversion of biogas into electricity. With an efficiency of 33 %, the total energy cost of the AnMBR process would range between 0.15 - 0.23 $kWh \cdot m^{-3}$.

Overall, the energy consumption of such processes at lab-scale is generally better compared to conventional WWTPs using activated sludge processes.

5.3.2.2 Energy consumption in AnMBR processes at full-scale

The only publication related to the energy balance of pilot-scale AnMBRs is the review of Shin and Bae (2018), which overall includes eleven full-scale AnMBR processes and nine of them for an energy study. The AnMBRs are defined by HRTs comparable to those of conventional aerobic WWTPs (2.2 - 33 h) (Table 67). While the COD concentration in wastewater amounts to between 198 and 1462 $mg \cdot L^{-1}$, only three plants and one plant respectively are characterized by COD concentrations higher than 800 and 1000 $mg \cdot L^{-1}$ respectively. Hence, the average COD concentration in the wastewater is comparatively lower than in the present study. The temperature of the processes amounts to between 9 and 35 °C, with most of the values comprised between 18 and 30 °C. The AnMBR full-scale plants are operated with commercial membrane modules as well as real domestic wastewater. The vast majority of them uses two-stage systems combining an anaerobic reactor using biogas sparging or mechanical stirring for mixing with an external submerged membrane tank using biogas sparging to provide shear stress as well as sludge recirculation in the membrane tank for more turbulence.

Overall, the energy consumption for fouling control amounts to between 0.04 and 1.35 $kWh \cdot m^{-3}$ with an average of 0.41 $kWh \cdot m^{-3}$. When no data is available for the other functions that are energy consuming, as mechanical stirring, heating or pumping, a theoretical value of 30 % of the total energy consumption is considered. With an energy demand ranging between 0.01 and 0.38 $kWh \cdot m^{-3}$ for these other functions, the energy demand for the whole process achieves values ranging between 0.10 and 1.66 $kWh \cdot m^{-3}$.

The electrical energy recovery considers an electrical conversion efficiency of 33 %. Hence, the energy gain ranges 0.08 - 0.92 $kWh \cdot m^{-3}$. Consequently, the energy consumption of the whole process ranges overall -0.62 - 1.16 $kWh \cdot m^{-3}$ with an average of 0.14 $kWh \cdot m^{-3}$.

Here, a significant difference of the total energy consumption is observed according to the influent COD concentration. While the average energy consumption amounts to 0.29 $kWh \cdot m^{-3}$ for COD concentration in wastewater less than 0.8 $g \cdot L^{-1}$, this reaches -0.25 $kWh \cdot m^{-3}$ for the three pilot-scale plants defined by higher COD concentrations in wastewater. The energy consumption reported is in the lower range of the lab-scale results reported in the previous section (-0.31 - 16.22 $kWh \cdot m^{-3}$). This shows that the low-energy costs achieved in lab-scale experiments can also be reached in full-scale plants. This represents a real advantage for the future expansion of the technology.

Table 67: Energy demand, energy recovery and total energy consumption in AnMBR processes at full-scale (Shin and Bae 2018)

Parameter	COD concentration in wastewater ($g \cdot L^{-1}$)	HRT (h)	Temperature (°C)	Energy demand for fouling control ($kWh \cdot m^{-3}$)	AnMBR total energy demand ($kWh \cdot m^{-3}$)	Electrical recovery of methane ($kWh \cdot m^{-3}$)	AnMBR total energy consumption ($kWh \cdot m^{-3}$)
Range	0.198 - 1.462	2.2 - 33	9 - 35	0.04 - 1.35	0.10 - 1.66	0.08 - 0.92	**-0.62 - 1.16**
Average	0.653	13	24	0.41	0.46	0.32	**0.14**

5.3.2.3 Energy consumption of the AnMBR process in the present work

5.3.2.3.1 Methodology

This section aims to estimate the energy consumption of a decentralized AnMBR treating domestic wastewater and having a similar temperature and wastewater composition to the HAWANA AnMBR pilot-plant. For this purpose, both the energy demand and the electrical potential of the AnMBR are calculated. Concerning the energy demand of the AnMBR, this category is divided into three parameters: fouling control of the membrane, heating and others. This last category includes pumping and mixing by means of biogas sparging or mechanical stirring. In this study, the AnMBR is not energetically optimized, the main goal being to test the coupling between the effluent of the AnMBR and microalgae cultivation. Consequently, the model of a two-stage AnMBR with a submerged membrane, as the vast majority of the AnMBR models in Shin and Bae (2018), is privileged in this energy study.

The energy demand for fouling control using biogas sparging and for the parameter "others" including pumping and stirring is calculated as the average of the values given in the review. For the heating of the anaerobic reactor, calculations are done with a heating capacity of 1.16 $kWh \cdot m^{-3} \cdot K^{-1}$ (Mei *et al.* 2018). As the HRT is higher in the present study, the energy demand for the parameter "others" should be accordingly increased. However, the case of an AnMBR characterized by an optimal microorganisms population in the sludge leading to similar HRTs to the literature is considered. As a reminder, because of the too low TS concentration in sludge, comparatively low OLRs and high HRTs were reached during this experimental work (see 5.1.8).

Finally, for the electrical energy recovery of the AnMBR, a similar procedure to Shin and Bae (2018) is carried out. For this purpose, the results of the four experimental phases characterized by a COD concentration in the permeate less than 150 $mg \cdot L^{-1}$ (respect of the legal requirements relating to discharge of wastewater from small WWTPs) are taken into consideration. This concerns the phases 1, 2 and 3 of the first experimental period (PI 1, PI 2 and PI 3) and the phases 1 and 5 of the second experimental period (PII a and PII 5). However, because of the absence of data related to methane production, the phase PII 1 is not taken into account. The methane production at the experimental temperatures as well as an energy potential related to methane of 11 $kWh \cdot m^{-3}$ (Mei *et al.* 2018) and an electrical conversion efficiency of 33 % (Shin and Bae 2018) are considered. The values obtained are also compared with theoretical values (0.35 $m^3 CH_4 \cdot kg^{-1}$ COD under standard conditions adapted to the experimental temperature and pressure as well as 25 % of energy lost during anaerobic conversion of organic matter into methane). Consequently, the energy cost of a decentralized AnMBR process defined by high-strength wastewater with a COD concentration amounting to between 0.97 and 1.93 $g \cdot L^{-1}$ is calculated and conclusions about the energy balance of such plants are done.

5.3.2.3.2 Results

Under the experimental temperature and pressure conditions of the four experimental phases, between 0.57 and 1.15 $kWh \cdot m^{-3}$ would have been recovered throughout electrical conversion of methane (Table 68). The lowest energy recovery is reached with the lowest COD concentration in wastewater of 0.97 $g \cdot L^{-1}$ and, at the contrary, the highest value is achieved with the highest COD concentration of 1.93 $g \cdot L^{-1}$. The theoretical values of electrical recovery range 1.06 - 2.15 $kWh \cdot m^{-3}$, which is up to 87 % higher than the experimental values. Hence, with an optimized anaerobic process, enhanced energy recovery could be reached.

Simultaneously, the energy demand for fouling control and for pumping and reactor mixing reaches 0.35 kWh·m^{-3} and 0.11 kWh·m^{-3} respectively. Hence, without the heating parameter, the present AnMBR process would be energy positive with an energy consumption amounting to between -0.69 and -0.11 kWh·m^{-3}. However, taking into consideration wastewater heating from a temperature of 20 °C to the related experimental temperatures, an energy demand of 14.85 - 19.95 kWh·m^{-3} is obtained for this function, which totally stops the good energy efficiency of the AnMBR process.

Here, two solutions can be envisaged. The first solution is to further heat the anaerobic reactor at mesophilic conditions. Simultaneously, the energy lost during the electrical conversion of methane in the form of heat and the heat contained in the permeate should be recovered using heat exchangers. If 90 % of both the heat contained in the permeate and the heat lost during the electrical conversion of methane are recovered, the energy demand for the heating function will range between 0.09 and 0.63 kWh·m^{-3}. This will correspond to a total energy consumption of the AnMBR process of -0.60 to 0.47 kWh·m^{-3}. Hence, also with reactor heating, the AnMBR process could be a net energy producer. In addition, the highest value of 0.47 kWh·m^{-3} is in the range of the average energy costs for wastewater treatment in Germany and is much lower than the energy cost of wastewater treatment for small WWTPs.

The second solution would be to operate the AnMBR process at ambient temperature. If the process was optimized, between 0.17 and 0.24 m^3 CH_4·kg^{-1} COD would be produced. This is similar to the values obtained in the present study, which means that the energy consumption of such processes could also approximately range between -0.69 and -0.11 kWh·m^{-3}.

Table 68: Energy recovery, energy demand and total energy consumption of the HAWANA AnMBR process during the experimental phases PI 1, PI 2, PI 3 and PII 5

Experimental phase	PI 1	PI 2	PI 3	PII 5
COD concentration in wastewater (g·L^{-1})	1.03	1.74	0.97	1.93
Electrical methane recovery (kWh·m^{-3})	0.88	1.03	0.57	1.15
Theoretical electrical methane recovery (kWh·m^{-3})	1.16	1.94	1.06	2.15
Energy demand for pumping and mixing (kWh·m^{-3})	0.11	0.11	0.11	0.11
Energy demand for fouling control (kWh·m^{-3})	0.35	0.35	0.35	0.35
Energy demand for heating without heat recovery from the permeate and methane conversion (kWh·m^{-3})[1]	19.95	18.44	14.85	18.79
Energy demand for heating with 90 % heat recovery from the permeate and methane conversion (kWh·m^{-3})[1]	0.63	0.23	0.58	0.09
Total energy consumption without heating (kWh·m^{-3})	**-0.42**	**-0.58**	**-0.11**	**-0.69**
Total energy consumption with heating (kWh·m^{-3})[1]	**19.53**	**17.87**	**14.74**	**18.10**
Total energy consumption with heating throughout 90 % heat recovery from the permeate and methane conversion (kWh·m^{-3})	**0.21**	**-0.35**	**0.47**	**-0.60**

[1]: based on an influent temperature of 20 °C

Hence, considering only the AnMBR process without nutrient removal or nutrient recovery, the system is definitively profitable and can even be a net energy producer. As the energy recovery throughout methane production increases with increasing COD concentrations in wastewater, the use of a high-

strength domestic wastewater, which mostly characterizes small decentralized WWTPs, positively influences the energy consumption of the plant.

If the effluent of the AnMBR process is used as it for fertigation, that is the use of the permeate and the nutrients contained in the permeate for vegetable cultivation purposes, the energy cost of the AnMBR further stays very low. However, this solution is only applicable in rural areas. According to Seib *et al.* (2016), nutrient removal using ion exchange before effluent discharge would lead to an additional energy cost of 0.12 $kWh \cdot m^{-3}$ and, after further steps, enable nutrient recovery. Hence, in any case, the process would be still profitable in comparison to a conventional aerobic WWTP, particularly for a small decentralized WWTP. Nevertheless, the use of ion exchange has rarely been studied at a full-scale.

A large number of publications describes the use of wastewater effluents for microalgae cultivation as extremely promising. However, conclusions related to the energy balance of the combination AnMBR/microalgae cultivation can not be drawn so easily. This is the purpose of the next section, where the energy costs of microalgae cultivation using the effluent of the AnMBR are discussed.

5.3.3 Energy consumption of microalgae cultivation at full-scale in flat photobioreactors and high rate algal ponds

5.3.3.1 Methodology

The energy consumption of the microalgae cultivation step is calculated based on both literature values for energy demand during this process and the experimental results obtained with the outdoor full-scale experiments in the pilot-plant Hamburg-Reitbrook. For the simulation of the energy costs, two different models of reactors are chosen: a flat PBR (closed system) and a HRAP (opened system) (Table 69). Calculations relating to the flat PBR are done considering a power demand of 50 $W \cdot m^{-2}$ (PBR where the energy efficiency is optimized) and 500 $W \cdot m^{-2}$ (PBR not energetically optimized) as well as a volume/surface ratio of 0.07 m (Slade and Bauen 2013). For the HRAP, a power demand of 1 $W \cdot m^{-2}$ is taken into account and the volume/surface ratio is set to 0.25 m (Norsker *et al.* 2011; Slade and Bauen 2013).

Table 69: Presentation of the three different reactor models used for the energy balance of microalgae cultivation

Parameter	Optimized flat PBR	Non-optimized flat PBR	HRAP
Power demand ($W \cdot m^{-2}$)	50	500	1
Volume/surface ratio (m)	0.07	0.07	0.25
Energy demand for harvesting ($kWh \cdot m^{-3}$)		1 - 4	

However, these values for energy demand only consider the cultivation step, meaning the energy required for pumping and mixing the culture in the reactor. The energy demand for harvesting, which is usually done using centrifugation, and the drying and dewatering of the biomass must be also taken into consideration. The related energy demand amounts to between 1 and 4 $kWh \cdot m^{-3}$ (Schlagermann *et al.* 2012; Slade and Bauen 2013).

Here, three cases are considered according to the different seasons defined by the different PPFD. These cases are presented in Table 70 and are defined by PPFD values of 401, 98 and 245 $\mu mol \cdot s^{-1} \cdot m^{-2}$, which represent the summer season, the fall season and the average sunlight

conditions in Germany (Christian Münch GmbH). The HRT values in the summer and fall season are the times that were needed to reach the maximum nutrient removal. For the other case, the HRT is calculated according to a linear correlation between the PPFD and the HRT in the summer and fall season. A final biomass concentration ranging between 0.8 and 2.5 $g \cdot L^{-1}$ is considered, which corresponds to the maximum biomass concentration that can be produced according to the nutrient concentration in the permeate.

Table 70: Presentation of the three cases used for the energy balance of microalgae cultivation

Parameter	Summer season	Fall season	Average in Germany
PPFD ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	401	98	245
HRT (d)	1	7	4.1
Biomass concentration ($g \cdot L^{-1}$)	0.8 - 2.5	0.8 - 2.5	0.8 - 2.5

Finally, the reuse of the microalgal biomass produced includes five scenarios: use of the biomass as fertilizer, methane production throughout anaerobic digestion in the AnMBR with microalgae as co-substrate of wastewater, energy recovery throughout combustion of the microalgae, conversion of microalgae to biodiesel and conversion of microalgae to biodiesel followed by anaerobic digestion of the remaining microalgae, as only the lipids contained in the microalgae can be converted into biodiesel. For the anaerobic digestion, methane production of 178 L $CH_4 \cdot kg^{-1}$ and 330 L $CH_4 \cdot kg^{-1}$ for *Acutodesmus obliquus* and *Chlorella vulgaris* respectively are considered (Murphy *et al.* 2015). As in the previous section, an energy recovery of 11 $kWh \cdot m^3$ CH_4 with an electrical conversion efficiency of 33 % is considered. For the combustion of the microalgae, a low heating value (LHV) amounting to between 15 and 20 $MJ \cdot kg^{-1}$ is taken into account, as most of the publications report values in this range. Finally, the parameters taken into account for the calculations relating to lipid extraction and conversion to biodiesel are presented in Table 71. Moreover, an efficiency of 42 % in the diesel engine is considered. The values of electrical or energy recovery relating to these four scenarios are resumed in Table 72.

Table 71: Summary of the parameters taken into consideration for conversion of microalgae into biodiesel (Schlagermann et al. 2012)

Parameter	Value
Lipid content of microalgae (%)	40
Energy demand for oil extraction ($kWh \cdot kg^{-1}$)	0.6
Energy demand for oil conversion to biodiesel ($kWh \cdot kg^{-1}$)	0.2
Efficiency of oil conversion to biodiesel (%)	90
LHV of biodiesel ($kWh \cdot kg^{-1}$)	10

Table 72: Electrical and energy recovery of microalgae throughout combustion, anaerobic digestion, biodiesel production and biodiesel production combined with anaerobic digestion

Parameter	Electrical energy recovery throughout combustion ($kWh \cdot m^{-3}$)	Electrical energy recovery throughout anaerobic digestion ($kWh \cdot m^{-3}$)	Energy recovery throughout biodiesel production ($kWh \cdot m^{-3}$)	Energy recovery throughout biodiesel production + anaerobic digestion ($kWh \cdot m^{-3}$)
Value	1.1 - 4.6	0.5 - 3.0	0.8 - 2.3	1.1 - 4.1

5.3.3.2 Results

Table 73: Total energy demand in optimized PBR, non-optimized PBR and HRAP

Parameter	Summer season	Fall season	Average in Germany
Total energy demand for microalgae cultivation and harvesting in optimized PBR (kWh·m^{-3})	1.08 - 4.08	1.59 - 4.59	1.34 - 4.34
Total energy demand for microalgae cultivation and harvesting in non-optimized PBR (kWh·m^{-3})	1.84 - 4.84	6.88 - 9.88	4.44 - 7.44
Total energy demand for microalgae cultivation and harvesting in HRAP (kWh·m^{-3})	1.01 - 4.01	1.08 - 4.08	1.05 - 4.05

In the optimized PBR and in the HRAP, independently from the average PPFD, harvesting and drying of the biomass is the main energy consumer (Table 73). Therefore, in these two types of reactors, the priority must be put on the optimization of the energy efficiency of this step. Solutions could be the optimization of centrifugation or the use of a solar dryer for biomass drying. Overall, for the optimized PBR and the HRAP, the energy demand for microalgae cultivation and harvesting ranges between 1.01 and 4.59 kWh·m^{-3}. In the non-optimized PBR defined by a higher energy demand for mixing and pumping, for the average PPFD in Germany, the energy demand is much higher than for the optimized PBR and the HRAP and extremely dependent from the HRT in the PBR. Hence, in Germany, the related average energy demand reaches 4.44 - 7.44 kWh·m^{-3}.

For the use of the microalgae biomass as a fertilizer, the energy demand for microalgae cultivation and harvesting is considered to be the total energy consumption of the process. Hence, depending on the reactors used and the energy efficiency of harvesting, between 1.01 and 9.88 kWh·m^{-3} are needed for microalgae production. Therefore, the use of the biomass as a fertilizer does not seem to be viable. In this case, the use of the permeate directly after the AnMBR process for fertigation should be privileged. However, the production of a fertilizer based on ammonium nitrate leads to an energy consumption of 3.9 kWh·kg^{-1} (Fertilizers Europe 2011), which is to compare with the energy consumption amounting to between 1.3 and 4.0 kWh·kg^{-1} for fertilizer based on microalgae. Hence, compared to synthetic fertilizers, an energy gain will be anyhow obtained. In the case where fertigation can not be implemented, the use of the biomass as a fertilizer could be viable compared to the actual processes to produce fertilizers.

The use of anaerobic degradation for electrical energy recovery from the microalgae is mostly energetically viable in the optimized PBR and the HRAP, on the condition that harvesting is optimized. A net energy recovery could be even obtained if the permeate is defined by the same nutrient level as in the full-scale experiments in the summer season. Hence, for the average light conditions in Germany, up to 2 kWh·m^{-3} could be recovered during this process (Table 74). For a PBR that is energetically non-optimized, it is more difficult to reach a low energy consumption. In this case, the process would be energetically viable only with an optimized harvesting, with the use of the permeate defined by the highest nutrient concentrations and in a climate characterized by yearly PPFD average of approximately 401 µmol·s^{-1}·m^{-2}. In Germany, the process would not be energetically viable, except if a wastewater defined by even more nutrient levels is used.

Furthermore, the anaerobic digestion of wastewater with microalgae as co-substrate is a relatively new topic and has not been yet studied at a full-scale. The use of nutrient-rich microalgae could eventually lead to a higher nutrient concentration in the permeate but also to a higher COD

concentration in the permeate. This must be avoided, as a low COD concentration in wastewater must be reached for discharge in wastewater bodies. Nevertheless, this process would be meaningful, as a reactor defined by anaerobic conditions is already present in the system.

Table 74: Total energy consumption in optimized PBR, non-optimized PBR and HRAP in case of electrical recovery of microalgal biomass throughout anaerobic digestion

Parameter	Summer season	Fall season	Average in Germany
Total energy consumption for optimized PBR ($kWh \cdot m^{-3}$)	-1.9 – 3.6	-1.4 – 4.1	-1.7 – 3.8
Total energy consumption for non-optimized PBR ($kWh \cdot m^{-3}$)	-1.2 – 4.3	3.9 – 9.4	1.4 – 6.9
Total energy consumption for HRAP ($kWh \cdot m^{-3}$)	-2.0 – 3.5	-1.9 – 3.6	-2.0 – 3.5

Related to electrical recovery throughout combustion of the biomass, with an optimized harvesting of 1 $kWh \cdot m^{-3}$, the system is most of the time a net energy producer in the optimized PBR and the HRAP (up to 3.6 $kWh \cdot m^{-3}$) (Table 75). In average in Germany, the total energy consumption ranges -3.3 - 3.2, 0.4 - 6.9 and -3.5 - 3.0 $kWh \cdot m^{-3}$ for optimized PBR, non-optimized PBR and HRAP. Hence, this system can be also a net energy producer in Germany but harvesting needs to be optimized and the permeate must have a nutrient concentration similar to the level reached during the full-scale experiment in the summer season. The energy consumption for this process is comparatively lower than for the anaerobic digestion and seems promising. Nevertheless, microalgae combustion has been scarcely researched until now and the combustion of microalgae biomass leads to greenhouse gases emissions as high as fossil energy.

Table 75: Total energy consumption in optimized PBR, non-optimized PBR and HRAP in case of electrical recovery of microalgal biomass throughout combustion

Parameter	Summer season	Fall season	Average in Germany
Total energy consumption for optimized PBR ($kWh \cdot m^{-3}$)	-3.5 – 3.0	-3.0 – 3.5	-3.3 – 3.2
Total energy consumption for non-optimized PBR ($kWh \cdot m^{-3}$)	-2.8 – 3.7	2.3 – 8.8	-0.2– 6.3
Total energy consumption for HRAP ($kWh \cdot m^{-3}$)	-3.6 – 2.9	-3.5 – 3.0	-3.6 – 3.0

Biodiesel production from microalgae leads to similar results than anaerobic digestion and comparatively lower results than combustion. In Germany, values ranging -1.0 - 3.5, 2.1 - 6.6 and -1.3 - 3.2 $kWh \cdot m^{-3}$ would be obtained in optimized PBR, non-optimized PBR and HRAP respectively (Table 76). In countries defined by high sunlight conditions all year round, this process would always be energy positive with optimized harvesting or use of very high strength wastewater. In Germany, in optimized PBR and in HRAP, all the best conditions relating to wastewater composition and energy efficiency of the process must be met to lead to energy recovery.

Table 76: Total energy consumption in optimized PBR, non-optimized PBR and HRAP in case of recovery of microalgal biomass throughout biodiesel production

Parameter	Summer season	Fall season	Average in Germany
Total energy consumption for optimized PBR ($kWh \cdot m^{-3}$)	-1.2 – 3.3	-0.7 – 3.8	-1.0 – 3.5
Total energy consumption for non-optimized PBR ($kWh \cdot m^{-3}$)	-0.5 – 4.0	4.6 – 9.1	2.1 – 6.6
Total energy consumption for HRAP ($kWh \cdot m^{-3}$)	-1.3 – 3.2	-1.2 – 3.3	-1.3 – 3.2

Table 77: Total energy consumption in optimized PBR, non-optimized PBR and HRAP in case of recovery of microalgal biomass throughout biodiesel production combined with anaerobic digestion

Parameter	Summer season	Fall season	Average in Germany
Total energy consumption for optimized PBR ($kWh \cdot m^{-3}$)	-3.0 – 3.0	-2.5 – 3.5	-1.8 – 3.2
Total energy consumption for non-optimized PBR ($kWh \cdot m^{-3}$)	-2.3 – 3.7	-2.8 – 8.8	0.3 – 6.3
Total energy consumption for HRAP ($kWh \cdot m^{-3}$)	-3.1 – 2.9	-3.0 – 3.0	-3.1 – 2.9

Furthermore, this process needs to focus on optimization of process parameters to obtain the highest lipids concentration as possible. This combined with the need of several processes for oil extraction and conversion to biodiesel is challenging for small decentralized WWTPs and seems complicated to set up. As an anaerobic reactor is already present in the process, the anaerobic degradation of the remaining microalgae will be meaningful and enable more energy recovery under the form of electricity. In this case, the average energy recovery in Germany would reach -1.8 - 3.2, -0.3 - 5.7 and -3.1 - 2.9 $kWh \cdot m^{-3}$ in optimized PBR, non-optimized PBR and HRAP (Table 77).

In conclusion, as for the AnMBR process, energy can be recovered from the microalgae cultivation step in Germany, under the conditions that an optimized PBR with a low energy consumption or a HRAP is used, and energy consumption for harvesting and biomass drying is optimized. Nevertheless, the present results are based on full-scale experiments with flat panel PBRs and the use of HRAP is here only simulated (HRT, biomass production). Furthermore, if the choice of a HRAP is considered, full-scale experiments should first be conducted to determine if nutrient removal is as high as in flat panel PBRs, as it is the main aim of the microalgae step process. Among all the solutions presented above, for a small decentralized WWTP, the energy recovery of microalgae biomass throughout anaerobic digestion or as a fertilizer seems the most plausible solution, as an anaerobic reactor is already present and the use of the biomass as a fertilizer does not need an additional process. However, in the case of the use of the biomass as a fertilizer, the energy balance of the microalgae cultivation process would be negative in Germany but the energy consumption still lower than the energy consumption for synthetic fertilizers production.

However, the direct use of permeate for fertigation would be to privilege if the use of fertilizers is considered. The use of the microalgae biomass for combustion or biodiesel production would also permit under favorable conditions a net energy recovery. However, these both processes are also accompanied by several disadvantages and seem very difficult to set up in small decentralized WWTPs, particularly biodiesel production. In countries defined by high sunlight conditions all year round, all the four possibilities for energy recovery would lead most of the time to a net energy production. Overall, this process is very attractive for climates characterized by regular and high sunlight conditions.

The present study is only a general energy balance. The topic of energy recovery or fertilizer production throughout microalgae cultivation is much debated and different opinions are drawn. However, it is always reported that the energy consumption during the microalgae cultivation step and the energy demand for harvesting are the main deciding parameters in the energy viability of microalgae production. For instance, Slade and Bauen (2013) showed in their study that a net energy recovery was reached in 6 out 8 HRAP and 0 out 3 PBRs studied. Hence, energy recovery is possible but the energy efficiency of the technology must still be optimized. Furthermore, to know if microalgae production

could be profitable depends on other parameters, as for example the cost of diesel or synthetic fertilizer production. As synthetic fertilizers need phosphorus and this resource is announced to be depleted during the present century, microalgae production from wastewater for fertilizer use could be a very interesting and promising alternative, as phosphorus is naturally present in wastewater streams. Since crude oil resources are also finite and present only in few countries, the production of biodiesel from microalgae could become profitable in Germany in the event of a sharp price increase and promote the country's energy independence. Hence, independently from the energy balance, microalgae production presents several advantages that could be shortly determining factors because of the announced depletion of crude oil and phosphorus resources.

6 Summary and Outlook

The present thesis aims at the investigation of the feasibility of the combination of an AnMBR process with microalgae cultivation for decentralized wastewater treatment. Related to the AnMBR process, the work is based on the 327-days operation of an 850-L AnMBR pilot-plant installed in the residential building "BIQ-The Algae House" in Hamburg-Wilhelmsburg and treating domestic wastewater produced there. The results show that the application of the AnMBR process is suitable for the treatment of the organic matter contained in the domestic wastewater, as well as for nutrient recovery for microalgae cultivation.

The wastewater was defined by a COD concentration comprised between 0.97 and 2.49 $g \cdot L^{-1}$ and was, therefore, defined as high-strength wastewater, which is characteristic of small decentralized WWTPs. Over the experimental period, a very high COD removal amounting to from 86 % to 94 % was achieved. Besides, BOD_5 removal reached 99 %. Consequently, the results related to COD and BOD_5 removal are situated in the upper range of the literature values for AnMBR plants and small decentralized WWTPs and compete with German conventional WWTPs.

However, the OLR and methane production are in the lower range of the literature values. These low values were mainly due to the microbial biocenosis contained in the reactor, which was significantly lower in this work compared to other studies. This led to higher HRTs, which were comprised between 4 and 9 days. This is particularly high but will be significantly reduced by optimization of the sludge concentration in the reactor. Hence, literature shows that AnMBR processes enable similar HRTs to conventional WWTPs (1-2 d).

One of the main goals of the study was obtaining a permeate quality enabling its discharge in the water bodies (COD concentration < 150 $mg \cdot L^{-1}$). Despite the very good COD removal in each experimental phase, due to the high-strength domestic wastewater, an excellent permeate quality was only reached in the phases 1, 2 and 3 during PI and in the phases 1 and 5 during PII. This corresponded to HRTs ranging 4.8 - 9.2 days (PI) and 7.0 - 7.5 days (PII). The related OLR amounted to between 0.11 and 0.34 kg $COD \cdot m^{-3} \cdot d^{-1}$ (PI) and to between 0.26 and 0.28 kg $COD \cdot m^{-3} \cdot d^{-1}$ (PII).

With higher OLRs comprised between 0.35 and 0.45 kg $COD \cdot m^{-3} \cdot d^{-1}$, an optimum methane production was achieved (0.048 - 0.067 m^3 $CH_4 \cdot m^{-3} \cdot d^{-1}$). However, in this OLR range, the permeate quality deteriorated and COD values up to 253 $mg \cdot L^{-1}$ were reached. Hence, for future operations of decentralized AnMBRs treating domestic wastewater, two solutions are possible. The first one is to stay at a low OLR enabling permeate discharge into the water bodies, which is the simplest and the most practical solution for small WWTPs. The second one is to run the plant at an OLR leading to the optimum biogas production and to implement a post-treatment step. This solution may be considered only if it is energetically and economically favorable.

Moreover, because of the constant fluctuations of wastewater composition, one of the main difficulties of the project was the establishment of correlations between the different parameters defining the AnMBR process. Due to the random sampling of wastewater once to three times a week, the correlations found in the present thesis are not accurate. Longer operation time of the AnMBR combined with automatic sampling devices is needed to obtain more precise results. In addition to a better knowledge of the exact wastewater composition and an increase of the sludge concentration in the reactor, the energy performance of the AnMBR pilot-plant must be enhanced. Furthermore, solutions leading to the recovery of the biogas dissolved in the permeate must be implemented.

However, as it is the first time that the use of an AnMBR for decentralized domestic wastewater treatment is reported in the literature, these first results are fundamental for the development of the AnMBR technology in decentralized applications.

Related to microalgae cultivation using the permeate of the AnMBR and aiming at both nutrient recovery and biomass production, the present study demonstrated that the three microalgae species *Acutodesmus obliquus*, *Chlorella vulgaris* and *Chlorella sorokiniana* can grow in this effluent. Compared to a synthetic culture medium, similar performance was reached. Moreover, the use of the permeate has a significant advantage, as its adequate buffer capacity leads to a stable pH level without regulation. However, in some experiments, a decrease of TN removal and biomass production was observed. This was due to a lack of iron in the permeate. Hence, for cultures at full-scale, an iron salt combined with a chelating agent may be supplied at a very low concentration.

During the laboratory experiments conducted with *Acutodesmus obliquus* and *Chlorella vulgaris,* TN and TP initial concentrations ranged 64 - 115 $mg \cdot L^{-1}$ and 7.1 - 14 $mg \cdot L^{-1}$ respectively. The related TN/TP ratios amounted to between 7 and 11. While NH_4-N and TP were systematically completely removed from the permeate (in case of no iron deficiency), TN removal amounted to between 92 % and 95 %.

For the experiments conducted with *Chlorella sorokiniana*, nutrient concentration in the permeate significantly increased. Hence, TN and TP concentrations in the permeate amounted to between 98 and 160 $mg \cdot L^{-1}$ and to between 16 and 37 $mg \cdot L^{-1}$ respectively. The relating TN/TP ratios were much lower than in the previous experiments and ranged 4 - 6. Because of these unfavorable TN/TP ratios, TP could not be efficiently removed from the culture medium. To avoid the eutrophication of the water bodies by the discharge of this effluent, an adequate solution needs to be found. This solution could be the use of another microalgae species that can adapt to low TN/TP ratios, pH increase leading to TP precipitation or TP precipitation with iron or aluminum salts. Moreover, to avoid long HRTs in the reactors leading to very high biomass concentrations with the risk of bacterial contamination and dissolved organic matter release, the permeate could be diluted with rainwater, greywater, seawater or phreatic water. If no dilution is considered, COD and BOD_5 levels should be regularly measured and a post-treatment set in case of too high concentrations of these parameters.

During the two experiments conducted in the fall and the summer season at full-scale with two flat panel photobioreactors, the species *Acutodesmus obliquus* was successfully cultivated. While NH_4-N and TP were completely removed in both seasons, TN final concentration respected the requirements for wastewater discharge in the water bodies only in the summer season. This was due to the continuous supply of flue gas as CO_2 source for the microalgae into the photobioreactors. This gas contains NO_x, which led to very high concentrations of nitrates and nitrites in the system. In the summer season, this did not represent a problem, as enough light was available to enable fast assimilation of these nutrients by the microalgae. In the fall season, throughout continuous monitoring of nitrate and nitrite concentration in the liquid phase, flue gas supply should be correctly adapted.

Overall, high influence of the weather conditions (PPFD) on the nutrient uptake rates and the BPRs was observed. Hence, the experiments in the summer season gave very satisfactory results, with $RC_{NH4\text{-}N}$, RC_{TP} and the BPR averaging 68 $mg \cdot L^{-1} \cdot d^{-1}$, 14 $mg \cdot L^{-1} \cdot d^{-1}$ and 0.49 $g \cdot L^{-1} \cdot d^{-1}$ respectively. Compared to the literature for similar PPFD, nutrient uptake was higher and BPR similar. However, comparatively lower PE were reached. These were explained by the dark volume, which represented up to 46 % of the total volume of the culture.

The results related to nutrient removal during microalgae cultivation permit to compete with conventional WWTPs. However, further steps are required to improve the process. Indeed, only repeated batch tests were investigated in this study. To plan the performance of a full-scale application, the pilot-plant must be operated at least over one year in a semi-continuous mode. Moreover, in the present study, at full-scale, only microalgae culture in a flat panel photobioreactor was investigated. Since this process is expensive, energy-intensive and complex to install, an open system like a HRAP may be considered, too. Hence, microalgae cultivation using permeate should be also investigated in an open system in a semi-continuous mode over for at least one year.

If the combination of the AnMBR with microalgae cultivation in a semi-continuous process at full-scale also permits to obtain an effluent with adequate quality, this new decentralized wastewater treatment system could be expanded, especially in remote areas without sewerage. For the expansion of this new wastewater treatment technology, as energy is the main parameter accounting for the costs of a WWTP, the total energy consumption of this system is a key parameter.

In the present AnMBR process, if an optimized energy efficiency for pumping, mixing and filtration is considered and with 90 % heat recovery from methane conversion and permeate, the total energy consumption during the experimental phases defined by a COD concentration in permeate less than 150 $mg \cdot L^{-1}$ would have amounted to between -0.60 and 0.40 $kWh \cdot m^{-3}$. This is similar to other AnMBR processes at full-scale and could even be enhanced, as the theoretical methane production was not achieved in this study. Compared to the average energy consumption ranging 0.26 - 2.1 $kWh \cdot m^{-3}$ for conventional WWTPs and even reaching up to 5.5 $kWh \cdot m^{-3}$ in small WWTPs, the use of an AnMBR for high-strength domestic wastewater is viable. With permeate and nutrient reuse using fertigation, which is the process that combines irrigation and fertilization, the technology is energetically extremely appealing.

The energy balance results relating to nutrient reuse and biomass production throughout microalgae cultivation are disparate and indicate that the viability of this process is extremely dependent on the energy demand of the reactor used as well as the energy demand for the harvesting and drying of the biomass. In a small decentralized WWTP in Germany, energy recovery throughout anaerobic digestion seems one of the most appealing possibilities for microalgae biomass reuse but still needs to be intensively researched. This solution could lead to net energy production of up to 2.0 $kWh \cdot m^{-3}$ if harvesting and cultivation are energetically optimized and if a high nutrient concentration characterizes the permeate. Hence, shortly, the combination of the AnMBR process with microalgae cultivation could be energetically and economically viable, under the condition that the emphasis is put on energy optimization of microalgae cultivation and harvesting. In countries defined by high sunlight levels all year round, the combination of these two technologies is particularly appealing.

In this thesis, essential research towards the performance of decentralized wastewater treatment applicable in remote or poor areas is conducted. The characteristically high nutrient concentrations that define these wastewater streams differ strongly from the composition of municipal wastewater reported in the literature. In the present work, it is the first time that wastewater treatment using an AnMBR process is carried out at house-scale with domestic wastewater streams defined by such high nutrient concentrations. Consequently, the effluent of the AnMBR is also characterized by high nutrient concentrations. The coupling of such an effluent with microalgae cultivation has never been investigated before. Moreover, literature about the coupling of wastewater streams characterized by high nutrient concentrations and microalgae cultivation is scarce. As nutrient assimilation from the microalgae becomes more difficult with increasing nutrient concentrations in the culture medium, this

work is essential for a better understanding of the coupling of a decentralized AnMBR treating domestic wastewater with microalgae cultivation. Since the energy balance of both processes increases with increasing nutrient concentrations, the data provided in this work is a key element for the future development of this technology. Furthermore, only one author reports the use of the effluent of an AnMBR as a culture medium for microalgae cultivation in an outdoor full-scale process facing weather dependency and using flue gas as a carbon source. Hence, the data related to microalgae cultivation using two outdoor full-scale PBRs presented in this work is also essential for the further development of the coupling of both processes. Finally, the role of micronutrients for the coupling of wastewater treatment with microalgae cultivation has not been investigated before.

Considering the urgency of solutions for small decentralized wastewater treatment, particularly in remote areas and in developing countries, as well as the need for nutrient reuse from wastewater streams due to the depletion of the resource phosphorus, the use of this new technology should significantly increase during the present century. To this purpose, the present work brings key elements.

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10 Appendix

Appendix 1: Steps for membrane cleaning

Due to fouling, membrane cleaning is regularly required. For this purpose, the membrane is dismantled from the membrane circuit and set up in a parallel and closed circuit consisting of a 20 L tank, a circulating pump and the membrane. Following the membrane manufacturer recommendations (Table 78), the membrane is first flushed with tap water at a temperature in the range 35-40 °C. Subsequently, the membrane is alkaline cleaned with sodium hydroxide diluted with tap water for 2 hours at pH 10-11 and 35-40 °C. This step aims the removal of organic, colloidal and biofouling. After a further rinse with tap water at 35-40 °C, the membrane is neutral rinsed for about 30 min at a similar temperature with citric acid at pH 6-8. This enables the removal of inorganic fouling and metal scaling. Before the membrane is rebuilt in the membrane circuit, the membrane is flushed again with warm tap water. This protocol was applied until December 2017.

Due to an inefficient flux recovery after cleaning, contact with the membrane manufacturer was initiated in January 2018. Since this time, the following new protocol is applied. First, the membrane is flushed briefly with tap water to remove residual sludge. Thereafter, it is neutral cleaned with tap water enriched with 1 % P3-Ultrasil 53 (Ecolab) in the temperature range 35-40 °C for a period of 2 hours. After this step, the membrane is flushed briefly with tap water. Then, the membrane is alkaline cleaned with tap water enriched with 1 % P3-Ultrasil 141 and 0.1 % P3-Hypochloran (Ecolab) in the temperature range 35-40 °C for a period of 30 minutes. After the alkaline cleaning, the membrane is flushed briefly with tap water and rebuilt in the membrane circuit.

Table 78: Procedure applied for membrane cleaning

Step	Process	Medium (April - December 2017)	pH (April - December 2017)	Medium (starting from January 2018)	pH (starting from January 2018)	temperature (°C)	Time (min)
1	Flushing	tap water	neutral	tap water	neutral	35-40	15
2	Rinsing	NaOH	10-11	P3-Ultrasil 53 (1 %)		35-40	120
3	Flushing	tap water	neutral	tap water	neutral	35-40	15
4	Rinsing	Citric acid	6-8	P3-Ultrasil 141 (1 %) and P3-Hypochloran (0.1 %)		35-40	30
5	Flushing	tap water	neutral	tap water	neutral	35-40	15

Appendix 2: Standard cuvette tests procedures

NH_4-N concentration is determined by the indophenol blue method (ISO 7150-1, DIN 38406 E5-1) (Table 79). In alkaline conditions, the NH_4-N ions react with hypochlorite and salicylate ions in the presence of sodium nitroprusside as a catalyst to form a blue color indophenol dye. The Koroleff method (EN ISO 11905-1) is performed for TN determination. Nitrogen is digested in presence of peroxodisulphate and oxidized to nitrate. Then, nitrate reacts with 2.6-dimethylphenol in a solution composed of sulfuric and phosphoric acid to form 4-nitro-2,6-dimethylphenol. The cuvettes are then measured at a wavelength of 370 nm.

Table 79: Summary of the chemical parameters measured by means of standard cuvette tests

Parameter	Method	Cuvette designation	Measuring range ($mg \cdot L^{-1}$)
TP and PO_4-P	phosphorus-molybdenum-blue method	LCK 350	2.0 - 20.00
		LCK 348	0.5 - 5.0
		LCK 349	0.05 - 1.5
TN	2.6 - Dimethylphenol method	LCK 338	20 - 100
		LCK 238	5 - 40
		LCK 138	1 - 16
NH_4-N	indophenol blue method	LCK 302	47 - 130
		LCK 303	2 - 47
		LCK 305	1 - 12
		LCK 304	0.015 - 2.0
COD	dichromate method	LCK 014	1000 - 10000
		LCK 514	100 - 2000
		LCK 114	150 - 1000
		LCK 614	50 - 300
		LCK 314	15 - 150
BOD_5*	dilution method	LCK 555	4 - 1650
		LCK 554	0.5 - 12
NO_3-N	2.6 - Dimethylphenol method	LCK 340	5 - 35
		LCK 339	0.23 - 13.5
NO_2-N	diazotization method	LCK 342	0.6 - 6
		LCK 341	0.015 - 0.6
Fe	1.10 - Phenantrolin method	LCK 321 + Crack-set LCW902	0.2 - 6.0
Mg	metalphthalein method	LCK 326	0.5 - 50
Mn	reaction with oxims	00816 (firm WTW)	0.1 – 5.0
SO_4-S	barium sulfate method	LCK 153	40 - 150

* also with the respirometry method

The phosphorus-molybdenum-blue method is used to determine TP and PO_4-P concentrations (ISO 6878-1-1986, DIN 38405 D11-4). Phosphorus components are first acidified by sulfuric acid to give phosphoric acid. Then, phosphoric acid reacts with molybdate acid, forming a phosphormolybdate complex. Using ascorbic acid as a reducer and antimony as a catalyzer, the complex becomes phosphomolybdenum blue.

COD concentration is determined by the dichromate method (ISO 6060-1989, DIN 38409-H41-H44). Under acidic conditions (addition of sulfuric acid), potassium dichromate is a strong oxidizing agent and reacts with the organic compounds. The green coloration of the remaining chromium (III) ions permits to know the amount of oxygen consumed during the reaction.

BOD_5 determinations of the permeate were carried out by the dilution method (EN 1899-1). BOD_5 in wastewater was also measured by the dilution method until September 2017. From October 2017 until March 2018, instead of using cuvette tests, BOD_5 in wastewater was determined by respirometry (Oxytop IS6). Starting from April 2018, the dilution method was used again to measure BOD_5 concentration in wastewater. With the dilution method, the sample is pipetted into the cuvette in presence of allythourea that inhibits ammonia oxidation. After 5 days of storage at 20 °C in a place deprived of light, an alkaline solution with a pyrocatechol derivative in the presence of ions Fe^{2+} is added to the cuvette. Depending on oxygen concentration, a red dye is formed. By respirometry, the sample is placed during 5 days at 20 °C in a bottle that is hermetically sealed. The microorganisms present in the bottle require oxygen to oxidize the biodegradable compounds. The oxygen consumption causes a pressure decrease, from which the BOD_5 concentration can be calculated. Since the microorganisms present in the bottle produce CO_2, this gas is absorbed using NaOH.

The parameters nitrate (NO_3-N), nitrite (NO_2-N), iron (Fe), magnesium (Mg), manganese (Mn) and sulfate (SO_4-S) were only measured occasionally. NO_3-N measurement uses the 2.6 - Dimethylphenol method (ISO 7890-1-2-1986, DIN 38405 D9-2), NO_2-N the diazotization method (EN ISO 26777, DIN 38405 D10), Fe the 1.10-Phenantrolin method (ISO 6332-1988, DIN 38406 E1-1), Mg the metalphthalein method and SO_4-S the barium sulphate method. Before Fe measurements, the samples were prepared using a crack-set. In the presence of an oxidizing agent, undissolved and complexly bound iron was dissolved by boiling under acidic conditions. Mn was measured using cuvette tests of the firm WTW (DIN 38406-2).

Before NH_4-N, PO_4-P, NO_3-N, NO_2-N, Fe, Mg, Mn and SO_4-S measurements of wastewater, sludge and microalgae cultures were performed, the samples were centrifuged and the supernatant was filtered using a syringe filter (cellulose acetate or PVDF, 0.45 µm, Labsolute, Germany).

Appendix 3: Performances of the AnMBR process during the phases and subphases of the experimental periods PI and PII

Table 80: AnMBR performance during the phase 1 and 2 of PI - red: main phases - italic: average value - bold: median value

Parameter	Phase 1 28.06. - 18.07.		Phase 2a 30.08. - 28.09.		Phase 2b 28.09. - 17.10.		Phase 2c 25.10. - 23.11.		Phase 2 30.08. - 23.11.	
Duration (d)	21		29		19		29		86	
HRT (d)	9.2		5.1		4.2		5.4		5.1	
Temperature (°C)	37.2		36.7		35.6		35.2		35.9	
pH	6.8		6.6		6.1		6.2		6.3	
COD in wastewater ($g \cdot L^{-1}$)	*1.17*	**1.03**	*2.28*	**1.37**	*4.46*	**5.48**	*1.21*	**0.82**	*2.09*	**1.74**
VS in sludge ($g \cdot kg^{-1}$)	*0.77*	**0.76**	*1.30*	**1.38**	*1.61*	**1.55**	*1.81*	**1.81**	*1.61*	**1.62**
COD in permeate ($mg \cdot L^{-1}$)	*191*	**144**	*153*	**156**	*137*	**135**	*141*	**97**	*145*	**135**
COD removal (%)	*85*	**88**	*93*	**89**	*97*	**97**	*88*	**88**	*93*	**93**
COD biological removal (%)	*82*	**83**	*85*	**74**	*95*	**96**	*82*	**78**	*87*	**86**
CH_4 content (%)	80		80		71		61		69	
OLR ($kg\ COD \cdot m^{-3} \cdot d^{-1}$)	*0.13*	**0.11**	*0.45*	**0.27**	*1.05*	**1.30**	*0.22*	**0.15**	*0.41*	**0.34**
F/M ratio ($kg\ COD \cdot kg^{-1}\ VS \cdot d^{-1}$)	*0.16*	**0.15**	*0.34*	**0.19**	*0.65*	**0.84**	*0.12*	**0.08**	*0.25*	**0.21**
SBP ($m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}$)	0.023		0.042		0.068		0.037		0.048	
methane yield ($m^3\ CH_4 \cdot kg^{-1}$ COD)	*0.18*	**0.20**	*0.09*	**0.16**	*0.07*	**0.05**	*0.17*	**0.24**	*0.12*	**0.14**
Methane production ($m^3\ CH_4 \cdot kg^{-1}\ COD_{rem}$)	*0.21*	**0.23**	*0.10*	**0.18**	*0.07*	**0.05**	*0.19*	**0.24**	*0.13*	**0.14**
BSP ($kg\ VS \cdot kg^{-1}\ COD_{rem}$)	*0.10*	**0.11**	*0.04*	**0.07**	*0.01*	**0.01**	*0.00*	**0.00**	*0.02*	**0.03**

Table 81: AnMBR performance during the phase 3 of PI - red: main phase - italic: average value - bold: median value

Parameter	Phase 3a 19.12 - 24.01		Phase 3b 24.01 - 19.02		Phase 3 19.12 - 19.02	
Duration (d)	36		26		62	
HRT (d)	4.3		5.6		4.8	
Temperature (°C)	33.9		31.1		32.8	
pH	6.1		6.1		6.1	
COD in wastewater ($g \cdot L^{-1}$)	*1.52*	**1.12**	*0.87*	**0.87**	*1.31*	**0.97**
VS in sludge ($g \cdot kg^{-1}$)	*2.41*	**2.19**	*2.62*	**2.64**	*2.48*	**2.40**
COD in permeate ($mg \cdot L^{-1}$)	*175*	**130**	*88*	**84**	*146*	**110**
COD removal (%)	*88*	**88**	*90*	**91**	*88*	**89**
COD biological removal (%)	*76*	**72**	*33*	**34**	*66*	**60**
CH_4 content (%)	71		72		71	
OLR ($kg\ COD \cdot m^{-3} \cdot d^{-1}$)	*0.35*	**0.26**	*0.15*	**0.16**	*0.27*	**0.21**
F/M ratio ($kg\ COD \cdot kg^{-1}\ VS \cdot d^{-1}$)	*0.15*	**0.12**	*0.06*	**0.06**	*0.11*	**0.09**
SBP ($m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}$)	0.039		0.017		0.029	
methane yield ($m^3\ CH_4 \cdot kg^{-1}\ COD$)	*0.11*	**0.15**	*0.11*	**0.11**	*0.11*	**0.14**
Methane production ($m^3\ CH_4 \cdot kg^{-1}\ COD_{rem}$)	*0.12*	**0.17**	*0.12*	**0.12**	*0.12*	**0.15**
BSP ($kg\ VS \cdot kg^{-1}\ COD_{rem}$)	*0.06*	**0.08**	*0.26*	**0.26**	*0.11*	**0.13**

Table 82: AnMBR performance during the phases 1 and 2 of PII - red: main phase - italic: average value - bold: median value

Parameter	Phase 1 10.05. - 22.05.		Phase 2a 23.05. - 05.06.		Phase 2b 06.06. - 21.06.		Phase 2 23.05. - 21.06.	
Duration (d)	13		14		16		30	
HRT (d)	7.5		4.9		3.7		4.2	
Temperature (°C)	41.0		38.6		36.7		37.6	
pH	6.4		6.4		6.6		6.5	
COD in wastewater ($g \cdot L^{-1}$)	*1.71*	**1.97**	*1.61*	**1.59**	*1.37*	**1.26**	*1.48*	**1.42**
VS in sludge ($g \cdot kg^{-1}$)	*2.69*	**2.69**	*2.53*	**2.53**	*2.24*	**2.24**	*2.38*	**2.38**
COD in permeate ($mg \cdot L^{-1}$)	*124*	**120**	*188*	**188**	*218*	**197**	*206*	**195**
COD removal (%)	*93*	**94**	*88*	**87**	*84*	**85**	*86*	**86**
COD biological removal (%)	*40*	**48**	*63*	**62**	*84*	**85**	*75*	**74**
CH_4 content (%)	65		72		72		72	
OLR ($kg\ COD \cdot m^{-3} \cdot d^{-1}$)	*0.23*	**0.26**	*0.33*	**0.32**	*0.37*	**0.34**	*0.36*	**0.34**
F/M ratio ($kg\ COD \cdot kg^{-1}\ VS \cdot d^{-1}$)	*0.09*	**0.10**	*0.13*	**0.13**	*0.16*	**0.15**	*0.15*	**0.14**
SBP ($m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}$)	-		-		-		-	
methane yield ($m^3\ CH_4 \cdot kg^{-1}\ COD$)	-	-	-	-	-	-	-	-
Methane production ($m^3\ CH_4 \cdot kg^{-1}\ COD_{rem}$)	-	-	-	-	-	-	-	-
BSP ($kg\ VS \cdot kg^{-1}\ COD_{rem}$)	*1.16*	**0.84**	*0.00*	**0.00**	*0.00*	**0.00**	*0.00*	**0.00**

Table 83: AnMBR performance during the phases 3 and 4 of PII - red: main phase - italic: average value - bold: median value

Parameter	Phase 3a 22.06. – 23.07.		Phase 3b 24.07. – 13.08.		Phase 3 22.06. – 13.08.		Phase 4a 14.08. – 30.08.		Phase 4b 31.08. – 11.09.	
Duration (d)	32		21		53		17		12	
HRT (d)	5.6		5.8		5.7		4.8		7.5	
Temperature (°C)	36.9		37.3		37.0		36.7		36.7	
pH	6.7		6.8		6.7		6.6		6.7	
COD in wastewater ($g \cdot L^{-1}$)	*2.07*	**2.05**	*2.09*	**2.01**	*2.07*	**2.02**	*2.78*	**3.12**	*2.59*	**2.50**
VS in sludge ($g \cdot kg^{-1}$)	*2.52*	**2.61**	*3.18*	**3.13**	*2.81*	**2.68**	*3.41*	**3.36**	*3.30*	**3.30**
COD in permeate ($mg \cdot L^{-1}$)	*204*	**201**	*198*	**209**	*202*	**209**	*260*	**251**	*245*	**260**
COD removal (%)	*90*	**90**	*90*	**89**	*90*	**89**	*90*	**92**	*90*	**89**
COD biological removal (%)	*92*	**92**	*77*	**76**	*87*	**86**	*83*	**85**	*93*	**92**
CH_4 content (%)	77		73		76		74		74	
OLR ($kg\ COD \cdot m^{-3} \cdot d^{-1}$)	*0.37*	**0.36**	*0.36*	**0.35**	*0.37*	**0.36**	*0.58*	**0.65**	*0.34*	**0.33**
F/M ratio ($kg\ COD \cdot kg^{-1}\ VS \cdot d^{-1}$)	*0.15*	**0.14**	*0.11*	**0.11**	*0.13*	**0.13**	*0.17*	**0.19**	*0.10*	**0.10**
SBP ($m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}$)	0.056		0.060		0.057		0.052		0.067	
methane yield ($m^3\ CH_4 \cdot kg^{-1}$ COD)	*0.15*	**0.15**	*0.17*	**0.17**	*0.16*	**0.16**	*0.09*	**0.08**	*0.20*	**0.20**
Methane production ($m^3\ CH_4 \cdot kg^{-1}\ COD_{rem}$)	*0.16*	**0.16**	*0.21*	**0.23**	*0.18*	**0.19**	*0.11*	**0.09**	*0.21*	**0.22**
BSP ($kg\ VS \cdot kg^{-1}\ COD_{rem}$)	*0.00*	**0.00**	*0.07*	**0.08**	*0.03*	**0.03**	*0.08*	**0.07**	*-0.19*	**-0.20**

Table 84: AnMBR performance during the phases 4 and 5 of PII - red: main phase - italic: average value - bold: median value

Parameter	Phase 4 14.08. – 11.09.		Phase 5a 12.09. – 28.09.		Phase 5b 29.09. – 14.10.		Phase 5 12.09. – 14.10.	
Duration (d)	29		17		16		33	
HRT (d)	5.6		6.8		7.1		7.0	
Temperature (°C)	36.7		36.1		36.3		36.2	
pH	6.64		6.6		6.5		6.5	
COD in wastewater ($g \cdot L^{-1}$)	*2.55*	**2.49**	*2.03*	**1.88**	*1.87*	**1.94**	*1.95*	**1.93**
VS in sludge ($g \cdot kg^{-1}$)	*3.37*	**3.36**	*3.16*	**3.07**	*2.68*	**2.63**	*2.86*	**2.81**
COD in permeate ($mg \cdot L^{-1}$)	*254*	**253**	*159*	**151**	*104*	**98**	*133*	**139**
COD removal (%)	*90*	**89**	*92*	**92**	*94*	**95**	*93*	**92**
COD biological removal (%)	*87*	**86**	*95*	**95**	*114*	**114**	*102*	**102**
CH_4 content (%)	74		73		74		73	
OLR ($kg\ COD \cdot m^{-3} \cdot d^{-1}$)	*0.46*	**0.45**	*0.30*	**0.27**	*0.26*	**0.27**	*0.28*	**0.28**
F/M ratio ($kg\ COD \cdot kg^{-1}\ VS \cdot d^{-1}$)	*0.14*	**0.13**	*0.09*	**0.09**	*0.10*	**0.10**	*0.10*	**0.10**
SBP ($m^3\ CH_4 \cdot m^{-3} \cdot d^{-1}$)	0.058		0.036		0.041		0.038	
methane yield ($m^3\ CH_4 \cdot kg^{-1}\ COD$)	*0.13*	**0.13**	*0.12*	**0.13**	*0.16*	**0.15**	*0.14*	**0.14**
Methane production ($m^3\ CH_4 \cdot kg^{-1}\ COD_{rem}$)	*0.15*	**0.15**	*0.13*	**0.14**	*0.14*	**0.13**	*0.13*	**0.14**
BSP ($kg\ VS \cdot kg^{-1}\ COD_{rem}$)	*0.00*	**0.00**	*-0.05*	**-0.05**	*-0.06*	**-0.06**	*-0.05*	**-0.05**

Appendix 4: Acutodesmus obliquus cultivation at the University of Hamburg

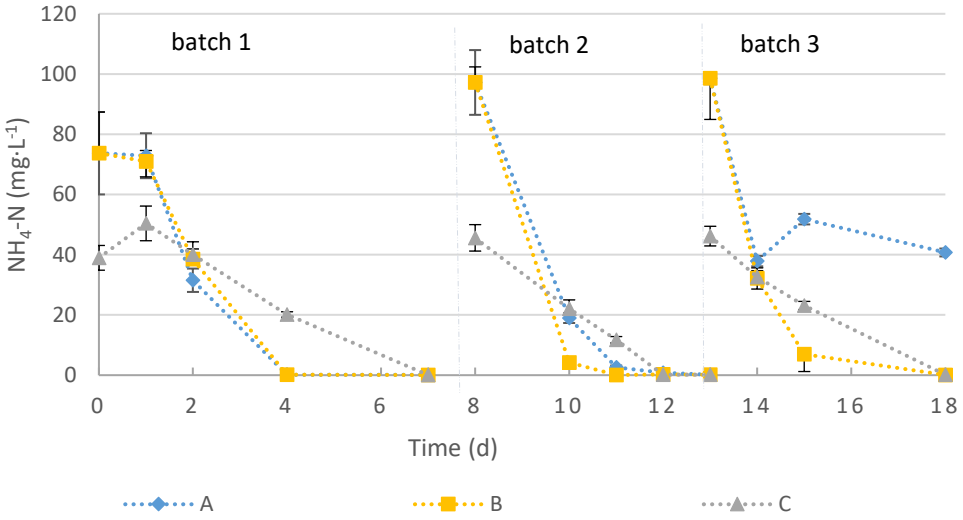

Figure 96: NH_4-N concentration in the permeate (A), the permeate enriched with micronutrients (B) and the commercial fertilizer (C) during the experiment conducted with Acutodesmus obliquus at the University of Hamburg

Appendix 5: pH during the experiments conducted with *Chlorella vulgaris*

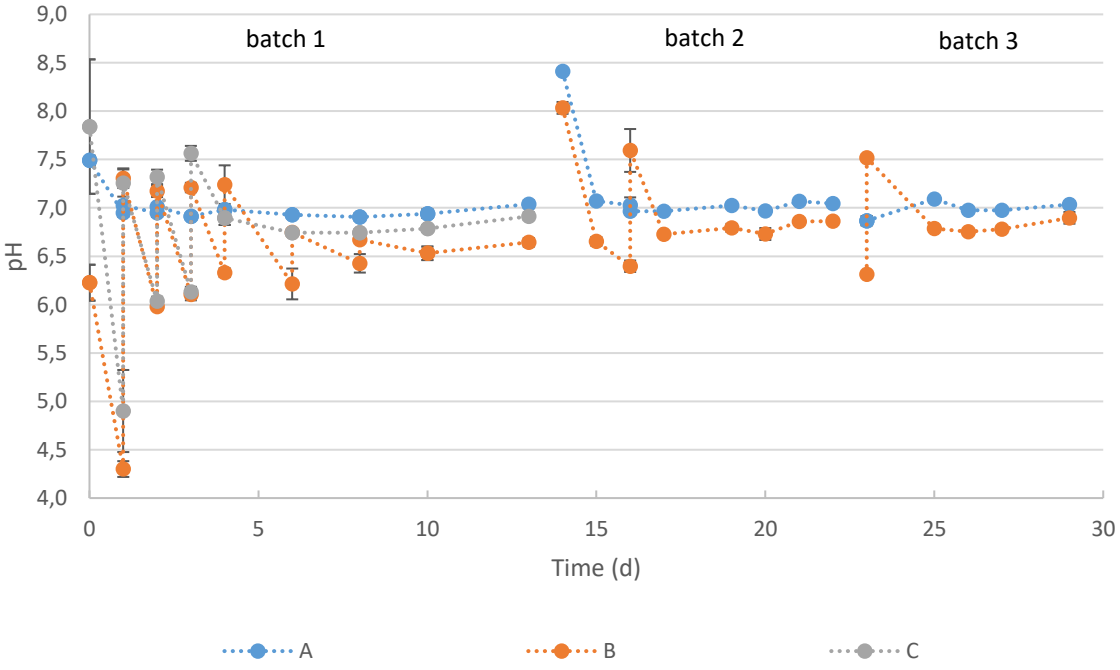

Figure 97: pH in the permeate (A), the synthetic culture medium enriched with ammonium sulfate (B) and the synthetic culture medium enriched with ammonium nitrate (C) during the experiment (1) of Chlorella vulgaris cultivation

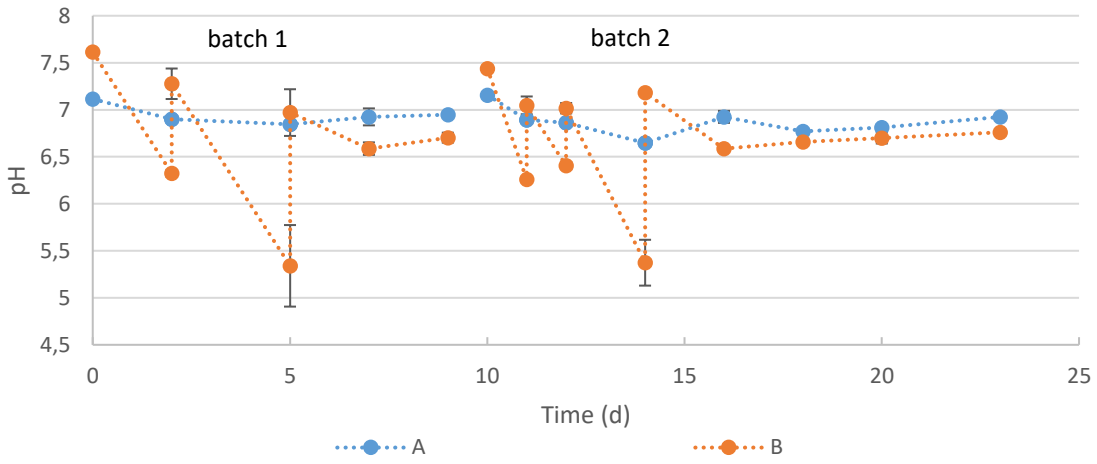

Figure 98: pH in the permeate (A) and the synthetic culture medium enriched with ammonium sulfate (B) during the experiment (3) during the experiment (1) of Chlorella vulgaris cultivation

Appendix 6: COD variation in the culture medium during microalgae cultivation

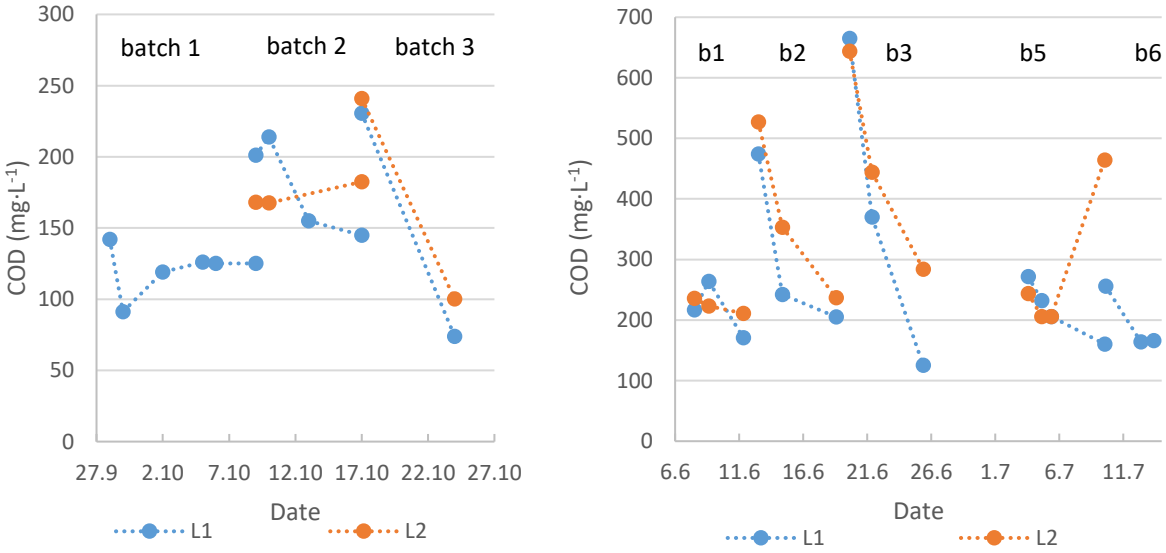

Figure 99: COD concentration during the full-scale experiments in L1 and L2 in the fall season (left) and in the summer season (right) - b: batch

Table 85: COD development, BPR and final biomass concentration during the cultivation of Chlorella vulgaris with permeate at a lab-scale

Experiment	Initial COD concentration ($mg \cdot L^{-1}$)	Final COD concentration ($mg \cdot L^{-1}$)	COD variation (%)	BPR ($g \cdot L^{-1} \cdot d^{-1}$)	Final biomass concentration ($g \cdot L^{-1}$)
3rd batch of the experiment (1)	111 ± 5	74 ± 2	-33	0.18	1.20 ± 0.08
1st batch of the experiment (3)	117 ± 3	133 ± 1	+14	0.28	2.67 ± 0.09
2nd batch of the experiment (3)	73 ± 1	111 ± 7	+51	0.35	4.67 ± 0.09

Table 86: COD development, BPR and final biomass concentration during the cultivation of Chlorella sorokiniana with permeate at a lab-scale

Experiment	Initial COD concentration ($mg \cdot L^{-1}$)	Final COD concentration ($mg \cdot L^{-1}$)	COD variation (%)	BPR ($g \cdot L^{-1} \cdot d^{-1}$)	Final biomass concentration ($g \cdot L^{-1}$)
1st batch of (1)	108 ± 18	269 ± 31	+60	0.25	1.17 ± 0.17
2nd batch of (1)	123 ± 3	116 ± 14	-6	0.22	2.12 ± 0.09
1st batch of (2)	188 ± 8	513 ± 68	+173	0.54	3.55 ± 0.14
2nd batch of (2)	183 ± 5	231 ± 21	+26	0.21	4.36 ± 0.28
1st batch of (3)	223 ± 2	208 ± 9	-7	0.24	4.07 ± 0.09
2nd batch of (3)	160 ± 3	157 ± 18	-2	0.26	2.22 ± 0.11