Improving the nitrogen removal in algal wastewater stabilization ponds

vorgelegt von
M.Sc. Ta Hoa Binh
aus Hanoi, Vietnam

von der Fakultät VI
der Technischen Universität Berlin
zur Erlangung des akademischen Grades

Doktor der Ingenieurwissenschaften
-Dr.-Ing.-
genehmigte Dissertation

Promotionsausschuss:

Vorsitzender	: Prof. Dr. Uwe Tröger
Gutachter	: Prof. Dr.-Ing. Matthias Barjenbruch
Gutachterin	: Prof. Dr.-Ing. Heidrun Steinmetz

Tag der wissenschaftlichen Aussprache: 22.10.2013

Berlin 2013
D83

Danksagung

Zunächst möchte ich meinen Betreuern, Prof. Dr.-Ing. Matthias Barjenbruch und Prof. Dr.-Ing. Heidrun Steinmetz, Anerkennung und Dankbarkeit aussprechen. Es war mir eine große Freude, mit 2 Experten in deren Bereich zusammen zu arbeiten und von ihnen zu lernen. Ich schätze besonders ihr Vertrauen in mich, mir ein großes Maß an Freiheit zu lassen, meine Forschungsarbeit nach eigenen Ideen durchzuführen und zu definieren, außerdem die vielen Anregungen, die sie mir gaben; die Geduld, die sie aufbrachten; die kontinuierliche Unterstützung; die Möglichkeit, fremde Länder zu besuchen, um mich mit Forschern aus dem Ausland bekannt zu machen und die Kultur in Deutschland zu erleben.

Außerdem möchte ich den unzähligen Kollegen von der TU Berlin, dem FG Siedlungswasserwirtschaft, dem Labor der SIWAWI, dem FG Baustoffe und Bauchemie, dem Labor der Biotechnologen, dem Labor des Leibniz Centre for Agricultural Landscape Research (ZALF), dem Institut für Landscape Hydrology (LWH) für ihre Unterstützung meiner Recherche und das Ausüben von Tätigkeiten Dankbarkeit und Anerkennung aussprechen.

Namentlich: Kathrin Gantner, Dagmar Balla, Stefan Rettig, Carsten Riechelmann, Christian Berbig, Sabine Rühmland, Alexander Wriege-Bechtold, Paul Kober, Cathrin Hinz, Oscar Aimé Yemba Sassy, Tosca Piotrowski, Rosemarie Rehausen-Scherer, Elke Dalmann, Miroslav Brkovic, Daniel Venghaus, etc., vielen Dank für die Übernahme so vieler meiner Aufgaben und für die Geduld mit meiner chaotischen Arbeitsweise. Es war eine Freude mit Ihnen zusammenzuarbeiten. Ohne Ihre Hilfe, hätte ich meine Arbeit niemals rechtzeitig zum Abschluss gebracht.

Ich möchte dem MOET-Vietnam und dem DAAD meine Wertschätzung und Dankbarkeit für die Organisation der finanziellen Unterstützung meiner Forschungsarbeit und für ihre Großzügigkeit im Austausch von Daten und Know-how aussprechen.

Zu guter Letzt, besondere Dankesworte an meine Eltern, meine Familie für ihre Liebe und Unterstützung.

Dieses Abenteuer endet hier, aber neue können beginnen.

Berlin, December 2013 Ta Hoa Binh

ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge both my supervisors, Prof. Dr.-Ing. Matthias Barjenbruch and Prof. Dr.-Ing. Heidrun Steinmetz. It was a very pleasure to learn from and cooperate with two experts in their respective fields. I especially appreciate their trust in me by giving me a large degree of freedom to define and carry out my research, giving me many suggestions, keeping patients, continuous supporting during this thesis work, giving me the opportunities to visit foreign countries, to introduce me with another researcher from abroad and to experience the culture of Germany.

I would further like to acknowledge the support I got from the countless colleagues of TU Berlin, FG Siedlungswasserwirtschaft, the lab of SIWAWI, FG Baustoffe und Bauchemie-Lab Bioengineering Faculty, the lab of Leibniz Centre for Agricultural Landscape Research (ZALF), Institute of Landscape Hydrology (LWH) for both my research and practicing activities.

The persons in particular: Kathrin Gantner, Dagmar Balla, Stefan Rettig, Carsten Riechelmann, Christian Berbig, Sabine Rühmland, Alexander Wriege-Bechtold, Paul Kober, Cathrin Hinz, Oscar Aimé Yemba Sassy, Tosca Piotrowski, Rosemarie Rehausen-Scherer, Elke Dalmann, Miroslav Brkovic, Daniel Venghaus, etc., thank you for taking over so many of my duties and for your patience in my chaotic way of working. It was a pleasure to work together with you. Without your help, I never would have finished this study in time.

I would like to express my appreciation to MOET-Vietnam, DAAD organization for financially supporting part of this research and for their generosity in sharing data and know-how.

And last but not least, a special word of thanks to my parents, my family for their love and support.

This adventure ends here, but new adventures can begin.

Berlin, December 2013 Ta Hoa Binh

KURZFASSUNG

Abwasserteiche können in gewissen Grenzen Kohlenstoff und Stickstoff aus dem Abwasser entfernen. Algen kommen in der Natur vor, deshalb geht nur ein überschaubarer Umweltschaden von ihnen aus. Wie jede Pflanze können Algen Ammonium als Nährstoff direkt für den Zellaufbau verwenden. Vorhergehende Untersuchungen konnten zeigen, dass für jede produzierte Tonne Algenbiomasse (Trockenmasse), 1,3 bis 1,8 Tonnen Kohlenstoffdioxid assimiliert oder verbraucht werden. Die im Wasser unter günstigen Lichtbedingungen wachsenden Algen treten mit aeroben Bakterien in eine für beide Seiten vorteilhafte Beziehung. Die Algen nutzen Kohlenstoff aus der Luft und absorbieren die Nährstoffe, die durch aeroben bakteriellen Abbau organischen Materials entstehen. Gleichzeitig geben die Algen Sauerstoff durch Photosynthese frei. Der von den Algen produzierte Sauerstoff ist dabei der wichtigste Faktor für das autotrophe aerobe Bakterienwachstum, für die Oxidation der Abwasserinhaltsstoffe und für den Abbau des organischen Materials. Auf diese Weise können geeignete Bedingungen für das Wachstum von autotrophen nitrifizierenden Bakterien erzeugt werden. Mit diesem Wissen entstand die Idee, das Wachstum von Algen in Abwasserteichen zu erhöhen, um einen gesteigerten Ammoniumabbau zu erzielen.

Diese Studie enthält zwei Untersuchungen im Labormaßstab. Die erste Studie beinhaltet Algen- und Wasserlinsenexperimente und die zweite Studie umfasst den Algenreaktor mit Strömungsleitplatten. Diese Experimente wurden durchgeführt, um die Wirkung verschiedener Konfigurationen auf die Ammoniumentfernungsleistung zu bestimmen und zu vergleichen. Im Ergebnis produzierten die Algenreaktoren mit Strömungsleitplatten sehr hohe Sauerstoffkonzentrationen von etwa 6 mg O_2/l. In dem Algenreaktor mit der Strömungsleitplatte wurden ca. 90% der NH_4^+-N-Konzentration (von den 67 mg NH_4^+-N/l im Zulauf), 81% des CSB's und 86-89% des BSB_5's eliminiert. Dafür wurde kein Belüftungssystem verwendet und es wurde kein CO_2 zugeführt. Weiterhin zeigte die Untersuchung, dass der Schlamm aus dem System rechtzeitig (mindestens alle drei Wochen) entfernt werden muss, damit die Effizienz der Ammonium-Stickstoff-Entfernung nicht reduziert wird. Dieser Effekt kann durch den Abbau der Biomasse erklärt werden, der die Nährstoffe wieder in die Wasserphase überführt. Die Ergebnisse der verschiedenen Versuchsdesigns weisen darauf hin, dass Licht- und Temperaturbedingungen sowie das Rückführverhältnis die wichtigsten Faktoren sind, um die Reinigungsleistung zu erhöhen. Die Nitrifikations-, Denitrifikations- und Assimilationsprozesse sind die wichtigsten Mechanismen zur Stickstoffentfernung in den Algen/Wasserlinsen-Experimenten und dem Algenreaktor mit den Strömungsleitplatten. Diese Promotion verdeutlicht die Eignung von Algen für die Abwasserreinigung. Auf diese Weise können die Algenreaktoren zum Schutz von Gewässern und Süßwasserressourcen beitragen. Außerdem ermöglicht dieses technisch einfache System die Reduktion der Abwasserbehandlungskosten. Die erzeugte Algenbiomasse kann verwendet werden, um beispielsweise Bioenergie sowie Dünger für die Landwirtschaft zu produzieren.

Supervisor:

Prof.-Dr-Ing. Matthias Barjenbruch

Abstract

Algae have a minimum environmental impact. Like a plant, algae can directly use ammonia as a nutrient for their growth. It is proved that for every ton of algae biomass (drying weight) produced, 1.3 to 1.8 tons of carbon dioxide has been either biologically fixed, or consumed.

The algae grow on wastewater under adequate light conditions establish a mutually beneficial relationship with aerobic bacteria. The algae utilize carbon and nutrient produced through aerobic bacterial by degradation of organic matter. The algae subsequently release oxygen by photosynthesis. Thus, suitable conditions for autotrophic bacteria growth and its assimilation of ammonia nitrogen can be created. Knowing this, the idea came up to use algae to reduce ammonia concentration in wastewater.

This study includes two investigations on a laboratory scale. The first setup was established for algal and duckweed experiments; the second are two differently baffled algal reactors. These experiments have been implemented to determine and compare the effect of different setups on ammonia removal efficiencies. As a result, the baffled algal reactors produced very high oxygen concentration of approximately 6 mg O_2/l. The oxygen produced by the algae is the most important factor for autotrophic, aerobic bacterial growth, substances oxidation and decomposition of detritus. In the baffled algal reactors approximately 90% of 67 mg NH_4^+-N/l in the influent, 81% of COD and 86-89% of BOD_5 removal efficiencies were observed without any aeration systems or CO_2 addition.

Furthermore the research revealed that, if the sludge is not removed from the system on time (every three weeks at least), the efficiency of ammonia nitrogen elimination will reduce. This effect could be explained by an increased decay rate of organisms and the recycling of organic matters into the water body. From the investigations with different experimental conditions and different designs, it could be indicated that light regimes, temperature conditions and effluent recycling are the important factors to increase substances removal efficiency from municipal wastewater. The nitrification, denitrification and assimilation processes were the major mechanisms for ammonia nitrogen removal in both algal/duckweed experiments and baffled algal reactors.

This study emphasized the feasibility of algae for wastewater treatment. The algal treatment ponds can contribute to the protection of natural water bodies and fresh water resources. In addition, these technically simple systems can reduce the wastewater treatment costs. The produced algal biomass can be used to produce e.g. bio energy or fertilizer for agriculture.

Supervisor

Prof.-Dr-Ing. Matthias Barjenbruch

Table of content

List of figures

Page

List of table

Abbreviation

APHA	: American public health association
AQUASIM	: computer program for the identification and simulation of aquatic systems
DIN EN	: German Institute Standardization in English version
DWA	: German Water Association
EU-15	: European Union with 15 countries
F.A.O	: Food and agriculture organization of the United Nations
ISO	: International standard organization
O.E.C.D	: Organization for economic co-operation and development
USEPA	: U.S environmental protect agency
$\mu_{avg.}$	: average growth rate
$\mu E/s.m^2$	: micro Einstein per second and square meters
μ_{max}	: maximum growth rate
$\mu_{spec.}$	: specific growth rate
ASP	: acid-soluble polyphosphate
ATP	: adenosine Triphosphate
BOD_5	: biological oxygen demand by measuring 5 days
C/N	: the ratio of carbon per nitrogen
Cd	: cadimi
C_e	: concentration effluent
CH_2O	: methanal
Chl-α; a_{chl}	: the short form of several types of chlorophyll content
$C_{in/out}$	: Concentration of specific substrate in or out
cm^3	: cubic centimeters
C_o	: concentration in influent
CO_2	: dioxide carbon
COD	: chemical oxygen demand
COD/N	: the ration of chemical oxygen demand per nitrogen
Conc.	: concentration
D	: diameter (cm)

DO	: dissolved oxygen
DS/g	: dry solid per gram
Exp	: mathematical function
$g/m^{3.}d$	: gram per cubic meters and day
H	: height (cm)
H^+	: hydronium ions
ha	: hectare
HCl	: acid clohydric
Hg^+	: mercury ions
hr or h	: hour
HRAP	: high rate algal ponds
I_k	: co-efficiency of irradiance
I_{max}	: co-efficiency of maximum irradiance
I_o	: co-efficiency of irradiance at beginning of time = o
I_t	: co-efficiency of irradiance at the time is different to zero
Kj/mol	: kilojoules per molecular
km/h	: kilometers per hour
K_s	: saturation co-efficiency of substrates
L	: length (cm)
l/d	: litter per day
log_{10};LOG10	: nature logarithm
Lux	: light intensity unit
m/d	: meter per day
m^2	: square meters
m^3	: cubic meters
$mg.Cg^{-1}.h^{-1}$	: milligram carbon utilized per hour
min/max	: minimum or maximum
MLSS	: mixed liquor suspended solid
MLVSS	: mixed liquor volatile suspended solid
n	: number of samples
N or N_2	: nitrogen
NADPH2	: methylenetetrahydrophosphate reductase
NH_4^+/NH_3	: ammonia species
NH_4^+-N	: ammonia nitrogen
nm	: nano meters
NO_3^--N	: nitrates nitrogen
N-org.	: organic nitrogen
NPOC	: non-purgeable organic carbon
O_2	: oxygen

OH^-	: hydroxide
Pb	: lead
PBR	: photo bioreactors
pH	: water acidity
photon/m^2s	: illumination photon per square meters and second (time)
P_{max}	: maximum photosynthesis conversion efficiency
$Q_{in/out}$	: flow of water in or out
Q_{max}	: maximum of specific substrate utilization
R^2 or r^2	: correlation factor
RBC	: rotating biological contactors
TAN	: Total ammonia nitrogen
TC	: total carbon
TKN or Kjeldahl-N	: total Kjeldahl nitrogen
TN_{in}	: mass of total nitrogen inflow
TN_{out}	: mass of total nitrogen out flow
TOC	: total organic carbon
T^{oC} ; Temp.	: temperature degree
Tss	: total suspended solid
Vss	: volatile suspended solid
W	: power unit (Watts)
w	: wind
W.	: width
WEF	: water environment federation (US)
ΔN	: mass balance of total nitrogen
Θ	: (theta) temperature is defined as that at which the excess chemical potential is zero
λNH_3	: percentage of un-ionized ammonia
σ	: standard deviation
(g)	: gas phase
ANAMMOX	: anaerobic ammonium oxidation
AOB	: ammonia oxidizing bacteria
aq.	: liquid phase (Aqueous)
ASM	: activated sludge model
ASP	: acid-soluble polyphosphate
CANON	: completely autotrophic nitrogen removal over nitrite
CWs	: constructed wetlands
Eff.	: effluent
hab.year	: habitant and year

HQ40D	: name of handout electronic equipment
HRT	: hydraulic retention time
LCK	: Hach Lange cuvette kit
Linpor-CN	: Linpor carbon nitrogen process
MBR	: membrane biological reactor
OXiTop	: name of handout electronic equipment to measure BOD_5
S	: substrates
SHARON	: Single reactor high activity ammonia removal over nitrite
sp.	: species
UASB	: upon anaerobic sludge blanket
WWTP	: wastewater treatment plant
ALg.T	: algal experiment
DWd.T	: duckweed experiment
Ref.T	: reference experiment
BAR(s)	: baffled algal reactor(s)
T1	: baffled algal reactor with downward and upward flow
T2	: baffled algal reactor with sideward flow
T3 Ref.	: algal reactor without baffles (to compare)

Chapter I.
INTRODUCTION

1. INTRODUCTION AND PROBLEM STATEMENT

The United Nations (2012) has reported that in the year 2011, more than the half of the world's population (52%) was living in the urban areas. The rapid urban population growth and industrialization, especially in developing countries, leads to depletion of natural resources and environmental problems. With increasing population in urban areas, the rate of wastewater production has been increasing over time. Hence, wastewater treatment methods, selection of appropriate technologies and operation costs of treatment plants have remained the primary cause of concern for all countries. Appropriate technologies need to be not only economically suitable but also environmentally and culturally. As suggested by Mara *et al.* (1992), USEPA (1998) and Kayombo *et al.* (2005), even though a combination of different technologies can be used to improve wastewater treatment performance, algal ponds, waste stabilization ponds and constructed wetlands are effective options due to their low energy consumption and minimal operational requirements. However, the applied technical systems had some shortcomings, such as requiring large space, long time of treatment.

Nitrogen is an abundant element on earth, making up nearly 80% of the earth's atmosphere. The major sources of nitrogen in the environment and waterways are wastewater and application of fertilizers. The use of nitrogen fertilizers in agriculture is increasing in tandem with the rise in the world's population and the demand for food. As a result, countries such as China, India, Malaysia, etc., are rapidly expanding the use of nitrogen fertilizers for increased production of dietary protein through nitrogen inputs into crop, livestock production systems, and water (F.A.O 2011). Therefore, a large volume of nitrogen compounds is being contributed to the natural environment. At present, these large volumes of nitrogen-containing wastewater of domestic and industrial activities are discharged directly into the environment

without proper treatment, which leads to pollution of the air, soil, water and bio-ecological systems. For example, in Rajasthan (India) the raw sewage received at the activated sludge plant had a biological oxygen demand (BOD) of 600–800 mg/l and an ammonia nitrogen (NH_4^+-N) concentration of 80–110 mg/l. In domestic wastewater from one septic tank in China, it was 54-74 mg NH_4^+-N/l (Zeng *et al.* 2009). In the north of Germany, the raw wastewater contributed a Chemical Oxygen Demand (COD) of 860 mg/l and a concentration of over 80 mg NH_4^+-N/l according to the statistics of DWA (Germany Water Association, 2011). In Vietnam, some statistics have shown more than 84 mg NH_4^+-N/l in wastewater. In Tanzania, pollution of rivers such as Karanga, Njoro and Rao in Moshi; Mirongo in Mwanza and Themi in Arusha is the cause of frequent disease outbreaks in downstream communities (Senzia *et al.* 2003).

Techniques to eliminate the majority of pollutants from these effluents are essential in developed countries and are becoming increasingly important from an environmental and human health point of view in the developing countries. The impact of increased nitrogen deposition on biological, ecological systems and human beings is diverse, but the most important effects are:

- Water pollution caused by nitrogen compounds causes eutrophication and acidification in fresh waters (Krause-Jensen *et al.* 2008; Grizzetti *et al.* 2011 as cited by Sutton *et al.* 2011). Biodiversity loss, toxic algal blooms and dead zones (fish kills) are all examples of its effects (Grizzetti *et al.* 2008 as cited in Sutton *et al.* 2011).

- High nitrate nitrogen (NO_3^--N) concentrations in drinking water are considered dangerous for human health, as they may lead to cancer and infant methaemoglobinaemia. Nitrate levels in freshwater across most of Europe greatly exceed the threshold. About 3% of the population in EU-15 is potentially exposed to levels exceeding the standard for drinking water of 50 mg NO_3^--N per litre (van Grinsven *et al.* 2006; Erisman *et al.* 2011).

- In addition, increasing nitrates in ground water threatens the long-term quality of the resource, due to its long-term persistence in aquifers (Alley *et al.* 2002*;* Jackson *et al.* 2007; Schlesinger 2009).

- Ammonia is highly toxic to fish even at low concentrations. The presence of unionized ammonia species increases at higher pH and temperatures. Nitrogen, together with phosphorus, stimulates the overgrowth of algae and other plants. Under certain

situations, eutrophication can occur due to the death of algae, leading to a high ammonia contribution to the water due to recycling of organic materials resulting in an increased demand for oxygen.

- Nitrogen in the atmosphere comes from the emission of ammonia due to human activities and from combustion sources (Jackson *et al.* 2000). This N contributes, in the form of nitric acid to acid rain, which despoils lakes, rivers, and forests (Keeney and Muller 2000).

- In land ecosystems, excess atmospheric N may enhance the growth of exotic species or accelerate tree growth, causing disruption of ecosystem functions (Vitousek *et al.* 1997; Jordan and Weller 1996 as cited in Follett and Hatfield 2001; Reid *et al.* 2005), and changes in (competitive) relationships between species, resulting in a loss of biodiversity.

Domestic wastewater contains a number of nitrogen compounds, and ammonia nitrogen is the one of the most challenging compounds to remove from wastewater. Therefore, removal of ammonia nitrogen is a very important step before returning wastewater to water bodies.

Besides the conventional nitrogen removal process such as trickling filters, conventional nitrification/denitrification processes, pond systems, rotating biological contactors, sequencing batch reactors, many processes and cost-effective biological nitrogen elimination processes have been developed, including simultaneous nitrification and denitrification, anaerobic ammonium oxidation – ANAMMOX (Ahn 2006), and completely autotrophic nitrogen removal over nitrite – CANON (Breisha 2010).

However, these processes require high operation/maintenance/investment costs and large energy consumption, and are difficult to apply in low income countries. The combination of biological treatment methods to remove nitrogen concentrations from wastewater with the activated sludge system by applying nitrification and denitrification are getting more beneficial, less expensive and more effective. Thus, it has been used as a standard method worldwide to achieve low nitrogen emissions (Khin 2004a,b).

Traditional and novel biological nitrogen elimination technologies are being reviewed. Recent studies dealing with temperature, dissolved oxygen, salinity, pH or free ammonia concentration as factors affecting the nitrogen removal efficiency have also been incorporated with biological treatment processes (Breisha 2010).

The approaches that this study follows are the use of natural materials such as algae and/or duckweed to remove nitrogen compounds from wastewater. It is proved that algae and duckweed are highly efficient in removing these substances in wastewater, for example:

- 80% - >90 % of NH_4^+-N removed by algal wastewater stabilization pond (Middlebrooks *et al.* 1982; Silva 1982; Gijzen 2001; Karin 2006).

- 75% of COD removal in algal & duckweed pond with hydraulic retention time 21 days (Zimmo 2003).

- 80-85% of BOD_5 could be removed (Zirchky and Reed 1988; Zimmo 2003)

- >10 mg O_2/l could be produced (Gutzeit 2006).

Other advantages of using algae or duckweed are: low energy consumption, minimal operational requirements, easy, environmental friendly implementation, low operation/investment costs, ease of operation and maintenance even for low skilled labour and high efficiency when hydraulic retention time (HRT) is sufficient. Therefore, it could be considered as an effective option to improve wastewater treatment performance (Mara *et al.* 1992; USEPA 1998; Kayombo *et al.* 2005).

2. RESEARCH OBJECTIVES AND APPROACHES

2.1 General objective

The research will focus on discussing the behaviour of algal, duckweed experiments and baffled algal treatment reactors. The results obtained from baffled algal reactors could contribute to the development of a model describing the biological processes in the reactor for ammonia nitrogen removal from wastewater on a lab-scale for both experiments.

- Description and interpretation of the performances of the algal and duckweed experiments and the baffled algal reactors.

- Description of the hydraulic conditions of the reactor based on tracer studies.

- Quantification of the importance of baffled algal reactors during the conversion of nitrogen.

The aim of this research is to utilize algal and duckweed materials for wastewater treatment in reactors with different setups and to examine the influence of different baffle patterns on

hydraulic flows. The experiments are intended to obtain the highest ammonia nitrogen removal rate and to improve ammonia nitrogen removal efficiency. It is expected that by applying upward and downward flows, algal and duckweed experiments show a high ammonia removal efficiency.

The baffles designed in algal reactors will prevent the wastewater for moving directly from the inlet to the outlet and increase the space for algal growth, so that more ammonia nitrogen and nutrients and other substances can be eliminated. This research also intends to solve the problem of limited surface area for the attachment of nitrifiers in the baffled algae reactors through the allocation of suitable sticking surface area by the baffles.

2.2 Specific objectives

- To examine an upflow/downflow pattern in algal and duckweed experiments. It is expected that the process of nitrification will improve by increasing the aerobic zone, resulting from the delivery of more oxygen to the deeper parts of the treatments. Hence, it increases the denitrification process.

- To introduce vertical baffles in the different designs for upward and downward flows or for sideways flows in algal reactor with a view to improve the hydraulic performance of the reactors by creating continuous flow patterns, minimize problems associated with short-circuiting and stratification. Vertical baffles will increase the oxygen delivery to deeper part of reactor and results in obtaining more oxidized substances. In contracst, horizontal baffles will reduce the death zone and crease the well mix substances in water. Moreover, on examining the baffles in algal reactors, an increase in the attached surface is expected.

- To study if there is an improvement in the overall performance of the baffled algae reactors in terms of all parameters associated with ammonia nitrogen removal from wastewater.

- To study if there overall performance of the baffled algae reactors will improve in terms of BOD_5, COD removal. An increase in oxygen concentration and attachment surface area for bacteria will result in increased BOD_5 and COD degradation and more ammonia nitrogen uptake by algae.

The main part of the research is to understand and determine how to obtain the most effective removal of NH_4^+-N, BOD_5, COD, NO_3^--N and to optimize oxygen production. It also

considers all factors that can affect nitrogen removal and natural processes in the reactor, such as the hydraulic flow pattern, oxygen concentration, pH, organic loading, DO, COD/N ratio, nitrification and denitrification rate, temperature and retention time. The second part of the research will introduce the conceptual model framework for interpreting ammonia nitrogen removal via algal reactor within the principles of activated sludge model ASM1, ASM3.

The study was carried out at the Department of Urban Water Management, Institute of Civil Engineering, Faculty of Planen Bauen Umwelt, Technische Universität Berlin, Germany beginning from October 2009.

Chapter II.

LITERATURE REVIEW OF NITROGEN REMOVAL FROM WASTEWATER

1. REASONS FOR REDUCING NITROGEN COMPOUNDS IN WASTEWATER

1.1 Nitrogen cycle

1.1.1 In the air and on earth

On earth, there are two pools of nitrogen, with a relatively little exchange between them: the gaseous dinitrogen (N_2) of the atmosphere, and the N that is chemically bound to other elements such as carbon, hydrogen or oxygen, which has been described as 'reactive nitrogen' for its tendency to react with other elements (Galloway *et al.* 2004). Reactive N includes inorganic reduced forms (e.g. ammonia NH_3, and ammonium NH_4^+), inorganic oxidized forms (e.g. nitrogen oxides NO_x, nitric acid HNO_3, nitrous oxide N_2O, nitrate NO_3^- and nitrite NO_2^-) and organic compounds (e.g. urea, amines, proteins and nucleic acids).

1.1.2 In the water

In water, nitrogen exists in the form of NH_4^+, NH_3, NO^-_3 and NO^-_2 (as can see in the Fig. 1). The term ammonia refers to two chemical species which are in equilibrium in water: NH_3 (un-ionized) and NH_4^+ (ionized). Ammonia's toxicity is primarily attributable to the un-ionized form (NH_3), as opposed to the ionized form (NH_4^+). In general, the higher the pH, the more the NH_3 and the greater the toxicity. When dissolved in water, normal ammonia (NH_3) reacts to form an ionized species called ammonium (NH_4^+).

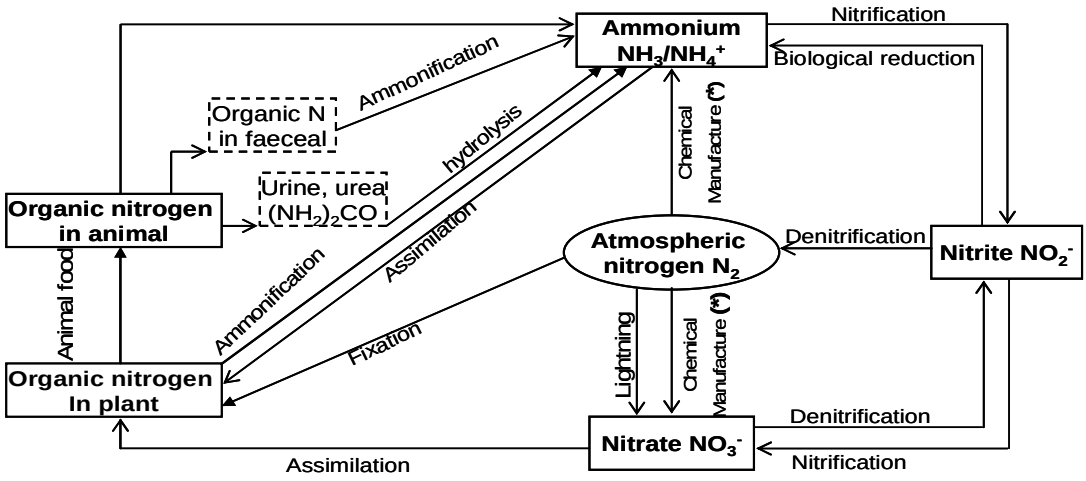
$$\text{org. N (e.g. } H_2N\text{-CO-}NH_2) + H_2O \rightarrow NH_4^+ + OH^-$$

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

A nitrite in water is mostly produced by bacteria of the genus *Nitrosomonas*. Nitrite is less toxic than ammonia, but is still toxic (McCaffert 1981; Cole 1983). High levels of nitrite can kill many aquatic organisms. Fortunately, a further nitrification reaction can occur:

$$2NO_2^- + O_2 \leftrightarrow 2NO_3^-$$

According to McCaffert (1981), Cole (1983), the end product here, nitrates (NO_3^-), is even less toxic than nitrite, and can be used by many plants as a nitrogen source. This reaction is carried out by bacteria of the genus *Nitrobacter* in aquatic and terrestrial systems. In a typical marine aquarium, nitrate may approach toxic levels, but this process takes a months. In reality, there are a number of denitrification reactions take place which reduce nitrate levels. As nitrite levels build and peak, *Nitrobacter* populations will thrive and convert the nitrite to nitrate, reducing nitrite concentrations to near zero. It is usually wise to monitor this process through daily tests of ammonia and nitrite levels.



(*): Haber-Bosch synthesis

$$N_2 + 3H_2 \rightarrow 2NH_2$$

$$NH_3 + 2O_2 \rightarrow HNO_3 + H_2O$$

$$HNO_3 + NaOH \rightarrow NaNO_3 + H_2O$$

Figure 1. The aqueous nitrogen cycle in wastewater treatment
(Adapted and modified from Barnes and Bliss 1983)

1.2 Ammonia nitrogen impact on water bodies

Domestic wastewater effluents contain high concentrations of inorganic and organic nitrogen that may lead to eutrophication of the bodies of water receiving them (Mallick 2002; de-Bashan *et al.* 2004*;* Foley *et al.* 2005) and the raw water sources should no longer be used as

sources of potable water without treatment (Asadi *et al.* 2002; Jalali 2005; Barton *et al.* 2006). According to Constantine (2008), there are several important reasons for removing ammonia nitrogen from wastewater:

- Ammonia nitrogen is a nutrient, so it can support algae growth.
- It can limit the oxygen demand in the bodies of water receiving them.
- Free or un-ionized fraction of ammonia nitrogen is toxic to aquatic life.

The nitrogen cycle is normally in balance, when it becomes unbalanced, it usually results in an ecological problem (Kuenen and Robertson 1985). Nitrogen is becoming increasingly significant in water and wastewater management because the discharge of nutrients such as ammonium nitrogen into rivers and lakes can influence on our environment and life adversely. For example, it has been shown that algal bloom in ponds produces certain toxins which can poison livestock and even people.

Furthermore, nitrogen compounds were also implicated in the acid rain problem (Codd 1984). Physical-chemical systems have frequently been used to remove the amount of ammonia nitrogen in wastewater treatment plants (Hurse and Connor 1999). The utilization of biological nutrient removal processes for the treatment of wastewater has environmental, economical and operational benefits.

1.3 Typical compositions of untreated domestic wastewater

Untreated municipal wastewater generally contains high levels of organic materials, numerous pathogenic microorganisms, as well as nutrients and toxic compounds (Turka *et al.* 2011). The majority of wastewater treatment plants (WWTP) constructed today aim to reduce the concentration of pollutants such as suspended solids, organic matters or pathogens to an acceptable level before discharging the effluent into the watercourse. Moreover, the removal nitrogen compounds are obligatory in the EU.

The ultimate goal of wastewater treatment and management is the protection of the environment in a manner commensurate with public health and socio-economic concerns. The typical composition of untreated wastewater of predominantly domestic sewage in Brazil, Egypt, US, Denmark and in several developing countries can be summarised in the table 1 (von Sperling *et al.* 2005; Henze *et al.* 2008; DWA 2011 and Vietnam Water resource).

Table 1. Typical untreated municipal wastewater (von Sperling *et al.* 2005; Henze *et al.* 2008; DWA 2011 and Vietnam reported by Liqa Raschid-Sally *et al.* 2011)

Contaminant	Range of concentration (mg/l)		
	Worldwide	**Germany**	**Vietnam**
Total suspended solids (Tss)	700-1350		400
Volatile suspended solid	480		180
BOD_5, 20ºC	250-560	280-410	140
TOC	-	-	290
COD	400-1200	438-932	269
Total nitrogen	30-100	41-81	-
Ammonia-N	20-75	75->100	90
Organic-N	15-25		8.3
Total phosphorus	6-25	6-13	-
pH	6.7-8		6-8.5

2. PHYSICAL, CHEMICAL AND BIOLOGICAL EFFECTS ON NITROGEN REMOVAL

This section will discuss the effects of physical, chemical and biological processes (nitrification, denitrification, ammonification, ammonia volatilization, assimilation or fixation) and another supporting process for removing ammonia from water.

2.1 Physical and chemical processes

2.1.1 The oxygen dissolve and transfer

Dissolved oxygen is an important parameter to understand the nitrite route. If the oxygen concentration inside the reactor is less than 1 mg O_2/l (as demonstrated in the Fig. 2), there is no nitrate created, because the ammonia oxidation rate is favoured in front of the nitrite oxidation kinetics (Picioreanu *et al.* 1997; Pollice *et al.* 2002). Furthermore, the nitritation at low dissolved oxygen will only be stable if it is properly coupled with the denitrification (Hanaki *et al.* 1990a,b; Salem *et al.* 2004).

There are two mechanisms that can supply oxygen to the treatment reactor. Firstly, oxygen produced by phytoplankton and secondly oxygen diffusion from the inlet. According to

Shilton (2005), passive or naturally aerated ponds rely on oxygen produced by phytoplankton during photosynthesis, and the oxygen transfer due to surface aeration is generally limited to the windy periods of the day. The equation of oxygen exchange can be calculated as follows:

$$\text{Oxygen exchange (mg/l·d)} = \frac{K_{LO} \times (C_S - C)}{d}$$

Where:

K_{LO}: reareration mass transfer coefficient (hr^{-1}).
C_s: oxygen saturation concentration (mg/l).
C: dissolved oxygen concentration (mg/l).
d: day

The reareration mass transfer coefficient by wind (w: km/h) can be calculated (Grau *et al.* 1996):

$$K_{LO}\,(\text{m/d}) = (0.384 \times w^{0.5} - 0.088 \times w + 0.029 \times w^2) \times \theta_{k,20}^{T-20}$$

When θ is the temperature correction factor. A calculation to estimate the oxygen saturation in mg/l as a function of the temperature T in ^{o}C is possible:

$$C_s\,(\text{mg/l}) = 14.652 - (0.4622 \times T) - (0.007991 \times T^2) - (7.7774 \times 10^{-5})$$

Based on studies by many researchers in the past, the dissolved oxygen level acts as the main diffusion control parameter regulating the extent of simultaneous nitrification and denitrification in membrane biological reactor (MBR) operation with different mixed liquor suspended solid (MLSS) levels.

Ali Zafarzadeh *et al.* (2011) found that highest nitrite could be accumulated (50%- 52%) at the dissolved oxygen concentration of 1-1.5 mg/l in moving bed biofilm reactors (MBBRs). This study also showed that the average nitrification rate is about 0.96 gN/m^2 per day while the maximum nitrification rate is about 2 gN/m^2 per day.

Ahmed *et al.* (2007) indicated that for each 5% changes in dissolved oxygen in aerobic reactors, 10% removal of nitrogen should be achieved. Clearly, dissolved oxygen in water is the one of the important factors that affects the nitrogen removal efficiency. Previous studies have shown that the nitrification rate increases on increasing the dissolved oxygen to the range of 1-3 mg/l and decreased for a dissolved oxygen range of 0.3-0.5 mg/l (investigated by

Qasim 1999 as cited in Ahmed *et al.* 2007). Hsu and Chiang (1997) as cited in Ahmed *et al.* (2007) showed that to obtain NH_4^+-N removal efficiency of more than 60%, the dissolved oxygen concentration in the aerobic system should be maintained above 1 mg/l.

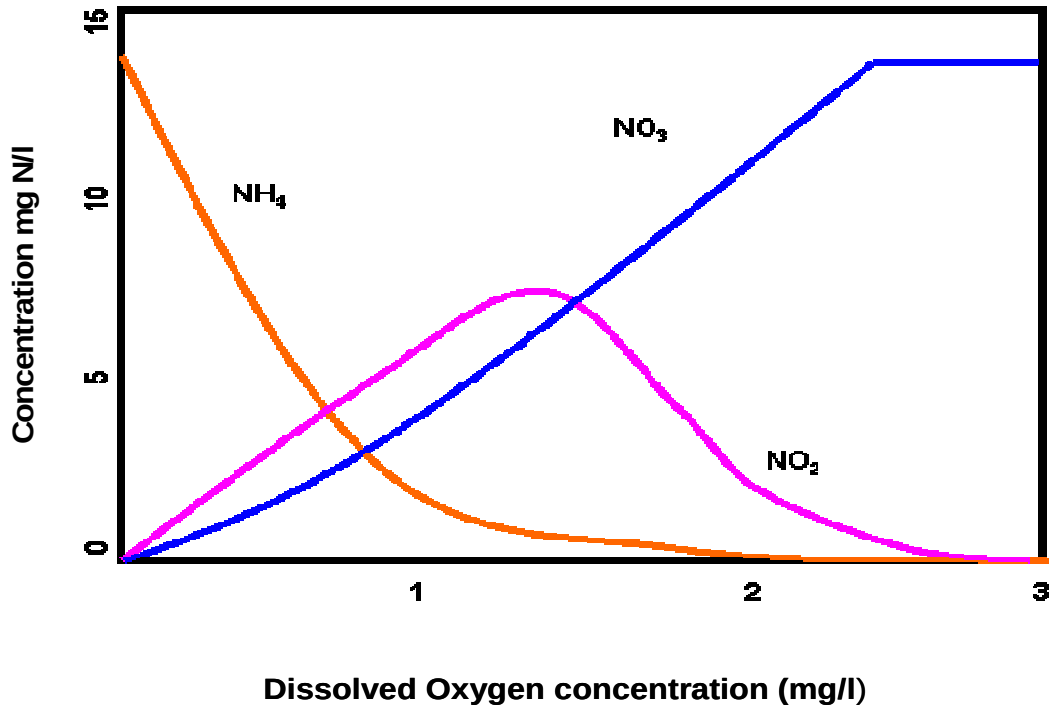


Figure 2. Stationary states of nitrogen for different dissolved oxygen (Salem *et al.* 2004).

The more the dissolved oxygen concentration in water, the higher the NH_4^+-N removal could be achieved. On the other hand, dissolved oxygen in water is an essential factor for the nitrification process because it acts as an electron acceptor in the biochemical reaction. To prevent the possibility of oxygen shortage, the dissolved oxygen concentration in water must be higher than 2 mg/l (Ahmed et al. 2007). According Gutzeit (2006), high ammonia removal efficiency of 67% NH_4^+-N could be obtained from algal photobioreactor while produced oxygen in system was more than 10 mg O_2/l.

2.1.2 Temperature effects

Temperature affects wastewater treatment in many ways, such as affecting the biomass, increasing certain biochemical processes, increasing the efficiency of enzymes involved, etc. There is a wide array of factors that are influenced by temperature: the electron donor or acceptor availability, the chemical forms of the substrate, ammonia volatilization, water vaporization, pH, etc. For example:

- The temperature has a strong effect on the growth of nitrifiers (Metcalf and Eddy 1991). The growth rate increases as temperature increases up to approximately 35^{o}C (Churchwell *et al.* 1980). The acceptable temperature has an upper limit of 45 to 50^{o}C. The co-efficiency of temperature influence to nitrification is 1.103, to *Nitrosomonas* is 1.10 and *Nitrobacter* is 1.06 (Bever and Teichmann 1990).

- The optimal temperature for nitrifiers activity has been reported to be as low as 15^{o}C (Charley *et al.* 1980), but more typically appears to increase with increasing temperature for up to approximately 30^{o}C, slowing down as the temperature increases beyond that (Groeneweg *et al.* 1994).

- For growth on denitrification, optimum reaction rates occur at 35–50^{o}C. The higher threshold makes it apparent that a high temperature is a greater concern for the nitrification process than for the denitrification process because at a high temperature, the nitrification process becomes faster than the denitrification process (Barnes and Bliss 1983) as can see on the Fig. 3.

- Several researches used the parameter Q_{10} to determine temperature effects on algal growth. It is indicated that for batch-cultured algae with optimal growth temperatures in the range 5-40^{o}C is 1.88 (Raven and Geider 1988). The influent temperature on heterotrophic metabolism is 1.072 (Wang et al. 2009).

The maximum nitrification rate (*k*) varies from a low of 0.0085 at 4^{o}C and pH 7, to a high of 0.175 mg/l NH_3^--N/l MLVSS/d at 33^{o}C and pH 8.3 (Shammas 1982). Wild *et al.* (1970) found that the rate varied from a maximum of 0.185 at pH of 8.4 to a minimum of 0.020 mg N/mg MLVSS/d at a pH of 6. Bishop *et al.* (1976) reported the rate of 0.11 mg N/mg MLVSS/d at 27^{o}C that decreased down to 0.032/d at 15^{o}C. Sutton *et al.* (1981) as cited in Wang *et al.* (2009a) showed that at the MLVSS concentration of 1,700 mg/l, pH 7 to 8, and a temperature of 21^{o}C, the rate *k* was 0.0216 mg N/mg MLVSS/day.

They also reported that at 10^{o}C the sludge retention time had to be doubled from 30 up to 60 days to attain the same extent of nitrification. The effects of temperature and pH on *k* at different MLVSS concentrations indicates that the optimum operating temperature is just above 25^{o}C and a pH value of 8.0 (Sedlak 1991; WEF 2000; Debabrata 2004; 2010; Liu *et al.* 2005; Wang *et al.* 2005).

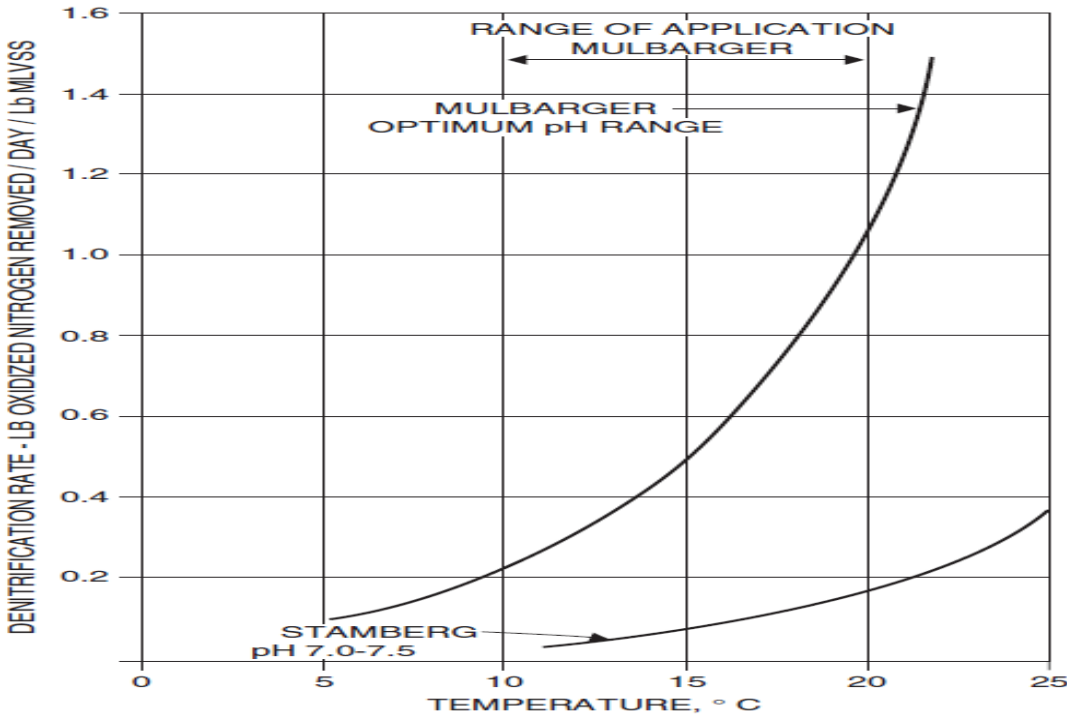


Figure 3. Effect of temperature on rate of denitrification (USEPA 1973, Barnes and Bliss 1983).

2.1.3 Diffusion

Diffusion is the spontaneous net movement of particles from an area of high concentration to an area of low concentration in a given volume of fluid down the concentration gradient. There are two types of diffusion existing in a system such as molecular diffusion and turbulent diffusion. Several studies indicate that the wind is one of the sources of the diffusion process in waste stabilization ponds. The coefficients of the diffusion process are shown in annex 5.

2.1.4 Ammonia volatilization

Ammonia volatilization is an important process of nitrogen reduction in water (by vaporization) and is also a major source of atmospheric ammonia. Ammonia in an aqueous solution can be lost through diffusion to the atmosphere at high temperatures and pH. In water, ammonia exists as NH_3 ion and NH_4^+ forms.

At low pH value the ammonia fraction dominates over ammonium species (NH_4^+). If pH and temperature can be kept low, little potential exists for NH_3 to volatilize. As the Fig. 4 shows below, at pH 7.5, less than 1% of the ammonium N is actually in the form of NH_3-N.

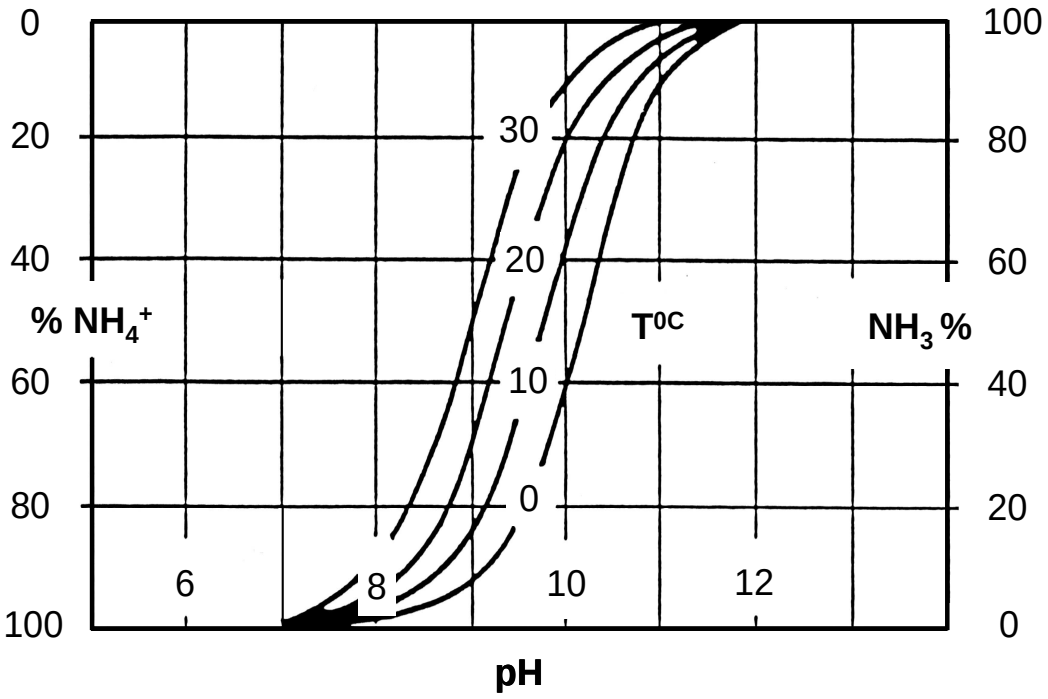


Figure 4. Distribution of ammonia between liquid and gas phase with pH (Koppe and Stozek 1999)

According to Senzia *et al.* (2002), The rate of NH_3-N volatilisation depends on the concentration of ammonia gas in the liquid, depth of the system and the mass transfer coefficient (K_L). It can be determined by using the proposed equation:

$$r_v = \frac{NH_3 - N(g) \times K_L}{d}$$

Where:

r_v: rate of NH_3-N volatilisation

K_L: the mass transfer coefficient in the liquid phase

d: depth of the pond or reactor (m)

The free concentration of NH_3 (mg/l) is pH and temperature dependent and modelled as follows (NH_3-N mg/l):

$$NH_3 - N = \frac{NH_3 - N}{1 + 10^{(10.5 - 0.03T - pH)}}$$

Mass transfer coefficient K_L was modelled as proposed by Stratton (1968) and Stratton (1969) as cited in Senzia *et al.* (2002) in accordance to:

$$K_L = 0.0566 \times \text{Exp}(0.13(T - 20)); \text{ where } T \text{ is the water temperature in °C}$$

It should be noted that the removal of ammonia through ammonia volatilization is important but only at a high pH ≥ 9 - 11.5.

2.1.5 Hydraulic flow pattern effects on nitrogen removal

The reduction of pollutants is directly proportional to the continuity of reactions and the retention times of wastewater in the system (Shilton and Harrison 2003). Therefore, it is clearly visible that the treatment efficiency is linked to the degree of short-circuiting. This can seriously hinder attempts to achieve high conversion of a substance from wastewater (Finney and Middlebrooks 1980; Levenspiel 1999).

A horizontal flow pattern in a pond as well as in a treatment system normally fails to move substrate from aerobic to anaerobic zones to accomplish the denitrification processes. Several studies have shown a rapid movement of wastewater from the inlet to the outlet due to wind effects. Also, water can move rapidly over the upper thermal layers. Both these factors prevent the movement of water from the aerobic to the anaerobic zones (Shilton *et al.* 2000). It is assumed that the transport of nitrates by diffusion may not be sufficient, resulting in limited denitrification rate.

Modifying the hydraulic flow patterns into upward and downward flows should be a solution that will improve the efficiency of ammonia nitrogen removal. This is being applied through the mechanical components of wastewater treatment plants that put water in motion.

At the surface layer, wastewater is in contact with oxygen in the atmosphere and so can have a high oxygen concentration. This is also helpful for the growth of the autotrophic bacteria and nitrifiers. However, this aerobic zone is limited to only about 40 cm from the water surface of algae and duckweed waste stabilization ponds (van der Steen 2000). Two flow patterns are important in the system considering the following arguments:

- The downward flow pattern will take the high oxygen concentration from the top layer into the deeper layer of the pond or biological treatment reactor. Hence, it is increasing the oxygen concentration for nitrification, while this flow pattern of water can effectively transport substances from the aerobic to the anaerobic zones at the bottom.

- In contrast, at the bottom of the ponds or the reactors, oxygen cannot diffuse fast enough to reach the bottom. Because of the mass transfer's kinetic limitations, the conditions at the bottom are suitable for denitrification. Furthermore, the un-oxidized ammonia that was taken to the bottom by the downward flow, it cannot undergo nitrification. There is a need to shift this water to the upper aerobic zones. This can be achieved by the upward flow pattern to the next cell.

In several studies it is assumed that the flows in the waste stabilisation ponds are almost completely mixed (Zimmo *et al.* 2003). But in reality, the flow in the treatment plants are neither completely mixed nor plug flow but instead consist of dispersed flow conditions.

In scientific studies, Lithium chloride (LiCl) is used to assess the hydraulic performance in the reactors. Because, Lithium does not absorb or react with the substances/particles in the wastewater, it shows low molecular diffusivity, has no effect on the main flow and is also not present in the inlet. This it makes easy to analyse flow behaviour in a system (Metcalf and Eddy 2003).

According to Sheppard (1962), the tracer test method is one of the techniques that provides information about the system or some parts of it through the observation of the behaviour of a specific substance. The tracer test method usually presupposes the use of a trace to label, or to make a specific phase of the system easily identifiable.

According to Shilton and Harrison (2003), the most effective hydraulic design will always be "plug flow". The authors also suggest that the stub baffles appears to have made the performance of the vertical inlet more effective and reliable, the stub baffle can work extremely well in some cases. Another advantage of stub baffles is that since the stub baffles are sensitive to changes to the pond configuration, they provide similar treatment improvements as longer baffles.

In this research, the theoretical models of short horizontal and vertical baffles developed by Watter *et al.* (1973) as cited in Shilton and Harrison (2003) and Shilton (2005) were used to determine the construction of system (shown in the Fig. 5, 6, 7). Shilton's model provides a visualization to monitor and construct different reactor models for plug-flow and various hydraulic flow patterns caused by the baffles in the reactors.

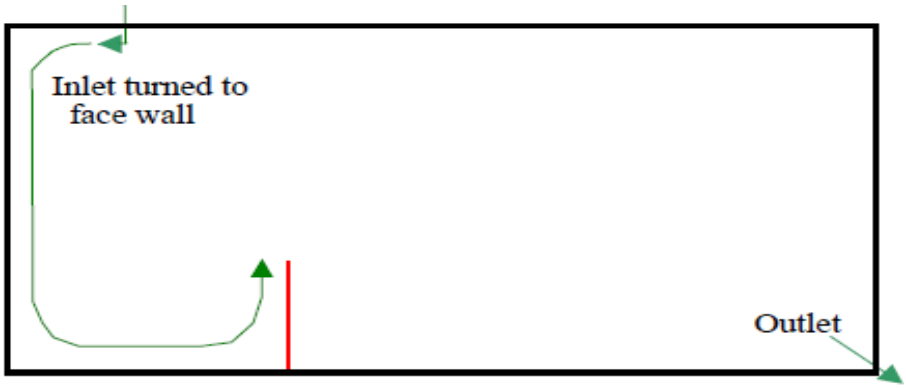


Figure 5. Pond with modified inlet and stub baffle

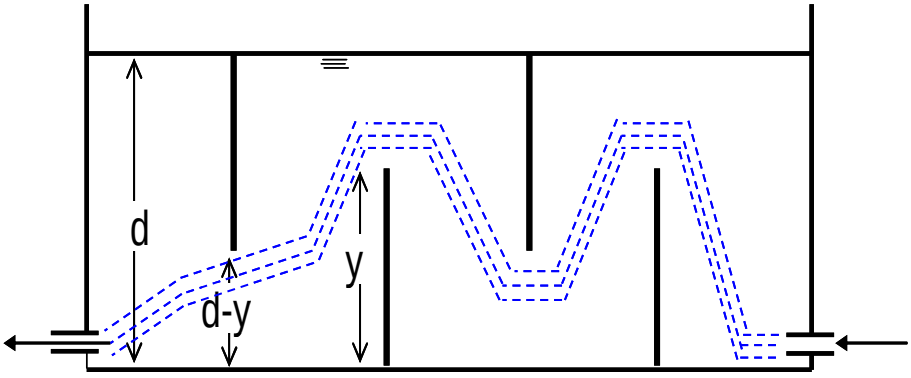


Figure 6. Experimental set-up for vertical baffle

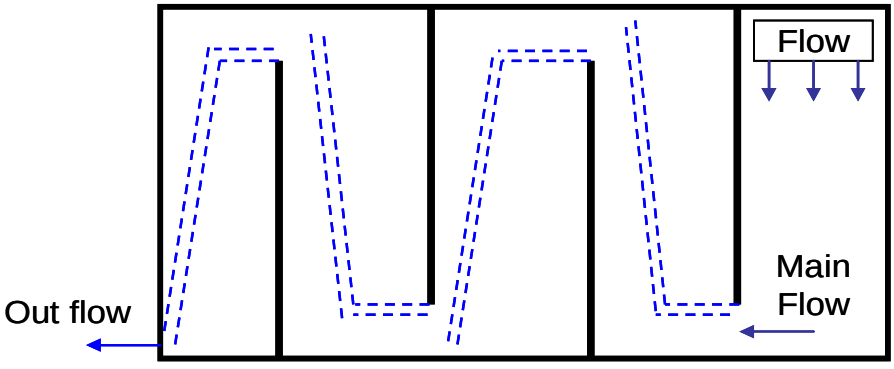


Figure 7. Channelling due to width baffles applied

2.2 Ammonia nitrogen removal from wastewater through biological processes

Biological processes generally provide the most economic means for controlling nitrogen in wastewater (Barnes and Bliss 1983). Hence, its examination has been done while providing information of the primarily biological processes that are involved in the ammonia nitrogen removal.

One of the most effective biological processes for the removal of nitrogenous chemicals from wastewater treatment plants involves nitrification and denitrification (Am Jang and Kim 2004). The transformation of nitrogen and the relationships between these various nitrogen forms that may occur in biological treatment systems are often conveniently expressed as in the Fig. 8 below.

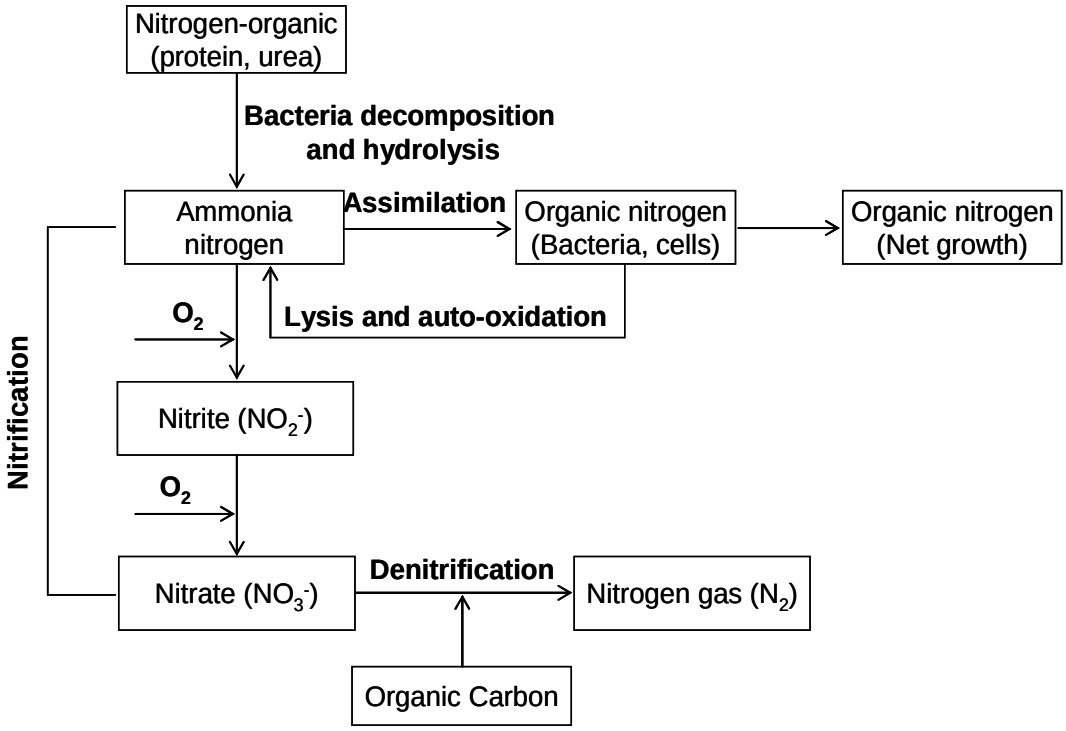


Figure 8. Nitrogen transformation in biological treatment processes (Sedlak 1991)

Organic nitrogen present in raw wastewater may be transformed to ammonia through decomposition of protein matter and hydrolysis of urea by bacteria living in water. Therefore, ammonia nitrogen is assimilated in newly formed cells of organisms (Udo Wiesmann *et al.* 2007). Removal of nitrogen is obtained with different efficiency by several processes such as assimilation, plant-uptake, and by conversion to nitrogen gas through the nitrification and denitrification processes (Sedlak 1991).

2.2.1 Nitrification and denitrification processes

- Nitrification process

Nitrogen is commonly removed from wastewater through conventional aerobic autotrophic nitrification and anoxic heterotrophic denitrification. Biological nitrification is the conversion or oxidisation of ammonium ions to nitrite and later nitrate ions. Nitrification of ammonium (NH_4^+) with molecular oxygen as electron acceptor yields nitrite (NO_2^-) and nitrites (NO_3^-) through the action of aerobic ammonia-oxidizing and nitrite-oxidizing bacteria (Grady *et al.*

1999). Nitrification includes two reaction steps: ammonium is first oxidized to nitrite by ammonia-oxidizing *Nitrosomonas*. Then the nitrite is further converted to nitrate by nitrite-oxidizing *Nitrobacter*. The reaction equations are the following:

$$2NH_4^+ + 3O_2 \xrightarrow{\textit{Nitrosomonas + Nitrosopira}} 2NO_2^- + 4H^+ + 2H_2O + 352 \text{ kJ/mol } NH_4^+\text{-N}$$

$$2NO_2^- + O_2 \xrightarrow{\textit{Nitrosobacter + Nitrosopira}} 2NO_3^- + 73 \text{ kJ/mol } NH_4^+\text{-N}$$

Overall reaction:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ + 425 \text{ kJ/mol } NH_4^+\text{-N}$$

The ammonia-oxidizing bacteria responsible for the first step of nitrification are chemolithoautotroph organisms. This process is actually a net reaction of two reactions that occur for nitrite-oxidizing.

The first reaction involves the enzyme ammonium monooxygenase, which catalyses the oxidation of NH_4^+ to hydroxylamine (NH_2OH). The enzyme ammonium monooxygenase is a membrane-bound protein that is inhibited by acetylene. The second reaction is catalysed by hydroxylamine oxidoreductase with water as the source of oxygen, this reaction generates energy (Hyman *et al.* 1988; Juliette *et al.* 1995).

Nitrate ions play a central role in wastewater nitrogen removal by using biological processes because these ions are the initial product of the denitrification process and become the source of nutrition when ammonium ions are not available. Arciero and Hooper (1993) showed that the electrons are presumed in the process above to branch into two pathways. The two are passed to the terminal electron cytochrome oxidize to be utilized for ATP and NAD(P)H generation (Arciero and Hooper 1993; Arciero *et al.* 1993). The reactions are as follows (Kuai and Verstraete 1998):

$$NH_4^+ + 0.5O_2 + 2e^- = NH_4OH + 2H^+$$

$$NH_4OH + H_2O = NO_3^- + 5H^+$$

According to Rittmann and McCarty (2001), the ammonia-oxidizing bacteria yield is higher than the yield of nitrite-oxidizing bacteria (shown in the table 2). The growth and reproduction of the nitrifiers are strongly influenced by many physical and chemical factors, such as temperature, oxygen concentration, pH, nutrients, concentration of toxic and inhibitory substances (Barnes and Bliss 1983).

Table 2. Basic parameters for aerobic ammonia-oxidizers at 20°C and 25°C (Rittmann and McCarty 2001)

Parameter	Aerobic ammonium oxidizers	
	T = 20°C	**T = 25°C**
Y, mg Vss/mg NH_4^+-N	0.33	0.33
Y_N, mg Vss/mg NO_2^--N	-	-
μ_{max}, d^{-1}	0.76	1.02
K_N, mg Vss/mg NH_4^+-N/l	1.00	1.50
K_N, mg Vss/mg NO_2^--N/l	-	-
K_O, mg O_2/l	0.50	0.50
b, d^{-1}	0.11	0.15

Knowles *et al.* (1965) as cited in Henze *et al.* (1996), Focht and Chang (1975) suggested that when there is low dissolved oxygen, the concentration of aerobic ammonium oxidizers does not sufficiently support the nitrite oxidizers with their substrate, not even at increased temperatures. The growth of nitrifying bacteria accelerates up to the optimal temperature of 30°C. A low pH in wastewater has a primary effect on nitrifying bacteria by inhibiting enzymatic activity. Therefore, the optimal pH range where the nitrification processes begin is 7.2–8.0 (Gerardi 2002).

Nitrification in the aeration reactor can occur in the form of either incomplete or complete nitrification. When the ammonium ions and the nitrite ion concentration in the mixed liquor effluent are less than 1mg/l each and the nitrate concentration is as big as possible, the nitrification has occurred and is considered to be complete (Gerardi 2002).

Incomplete nitrification is effected by several parameters such as operating conditions, temperature or temporary low oxygen concentration. Although there are many organisms that are capable of oxidising ammonium and nitrite ions, the rate of nitrification obtained from *Nitrosomonas, Nitrobacteria* is usually greater than the rate achieved by other organisms under the same conditions (Gerardi 2002). Following Barnes and Wilson (1983), several factors affecting nitrification in wastewater treatment plants are:

- The significantly increased oxygen demand 4.2 g O_2/g NH_4^+-N removed and decreased DO level.

- The very low cell yield per unit of ammonium nitrogen oxidized; and

- The requirement for alkalinity to buffer the system against hydrogen ions produced during nitrification, amounting to approximately 7 g alkalinity for each g NH_4^+-N oxidized.

In biological treatment plants, nitrification may be determined by the presence of several biological, chemical, physical indicators (Gerardi 2002):

- The growth of algae or duckweed in clarifier
- Decreased mixed liquor dissolved oxygen level
- Increase alkalinity/pH in secondary clarifiers
- Molecular nitrogen in secondary clarifier

- Denitrification process

Denitrification is the anoxic process done by heterotrophic bacteria, which involves the conversion of nitrite and nitrate to nitrogen in the gaseous form (N_2). This is a dissimilatory nitrate reduction process. In 2001, Rittmann and McCarty summarized the denitrification proceeds in a stepwise process as follows:

$$NO_3^- + 2H^+ = NO_2^- + H_2O$$
$$NO_2^- + 2H^+ = NO\,(g) + H_2O$$
$$2NO + 2H^+ = N_2O\,(g) + H_2O$$
$$N_2O + 2H^+ = N_2\,(g) + H_2O$$

Obviously, nitrogen products in the gaseous form can be released to the atmosphere. Madigan *et al.* (1949) found that denitrification is essential for wastewater treatment, since it effectively decreases the available nitrogen that can support excessive algal growth. For the simulation of heterotrophic denitrification, there are a wide variety of electron donors that can support the process (Davies 1973; Tam *et al.* 1992*;* Akunna *et al.* 1993; Thalasso *et al.* 1997; Costa *et al.* 2000).

Anoxic growth of heterotrophs is the growth in the absence of oxygen. Organisms use nitrate as an electron acceptor. The bacteria that perform denitrification belong mainly to the genera

Bacillus, Pseudomonas and *Alcaligenes*, and are facultative anaerobic. This process leads to a production of heterotrophic biomass (0.6–0.7 g DS/g BOD_5) and nitrogen gas, and is inhibited by the presence of dissolved oxygen. The reduction steps that are involved:

$$NO^-_3 \rightarrow NO^-_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

In contrast, nitrification does not remove nitrogen from wastewater. The process simply transforms the amount of ammonia/ammonium available in wastewater into nitrate ions (Kuenen and Robertson 1994). Afterwards, the denitrification process takes place to remove nitrogen from wastewater by converting it to the gas form that escapes to the atmosphere (Gerardi 2002).

The denitrification process is affected by nitrate, carbon source, temperature and dissolved oxygen concentrations in accordance with separate Monod-type expressions. Under unsuitable conditions of pH and temperature, the processing rate decreases. This leads to a reduction of the ammonia nitrogen removal rate. There are several indicators and in the same time limiting-factors for denitrification (McGraw-Hill 2005):

- The increase in alkalinity, pH
- $BOD_5/N > 4$ (required)
- The reduction of redox potential
- The concentration of dissolved oxygen (< 0.5 mg O_2/l)

- Factors effect on the nitrification and denitrification processes

* Oxygen effects

The optimal efficiency of denitrification process can be obtained in a DO range of 0.15-0.35 mg/l (Nelson 1978; Nielsen *et al.* 1990; Grady *et al.* 1999; Gómeza *et al.* 2002; Sattayatewa *et al.* 2010). In this situation, nitrate becomes the primary oxygen source for microorganisms. When bacteria break apart nitrate (NO_3^-) to gain oxygen, the nitrate concentration is reduced to nitrous oxide (N_2O), and in turn, nitrogen gas (N_2) escapes into the atmosphere as gas bubbles. Free nitrogen is released to the air without any negative effect to the environment.

Several studies on nitrate production have shown that the concentration of the nitrite and/or nitrate in wastewater treatment plants depends on oxygen-limited conditions (Bernet *et al.* 2001; Han *et al.* 2001; Pollice *et al.* 2002; Ruiz *et al.* 2003). Therefore, at low dissolved

oxygen levels in water, together with high NH_3^--N concentrations, nitrite oxidizers reduce their activity and cause unstable conditions for nitrite creation (Wyffels *et al.* 2003; Wyffels 2004).

It has been commonly found that the nitrification process dependents significantly on the dissolved oxygen in the wastewater. Dochain *et al.* (2003) said that the process increases up to 30% due to an increase in the oxygen concentration in water. The oxygen uptake rates in a suspended culture is optimal at DO concentrations of 1 - < 11 mg/l. An oxygen concentration of 12 mg/l or higher leads to a reduction in the uptake rate because the exceeded DO levels becomes toxic to the nitrifying micro-organisms (Am Jang and Kim 2004).

* pH effects

A wide variety of biological processes depend on the pH, which in turn affects the growth and activity of organisms. In brief, the pH is known to affect enzymes of interest, affinity for the substrate, as well as substrate or product inhibition (Groeneweg *et al.* 1994).

The pH is a significant parameter influencing to the nitrification and denitrification processes. The optimum pH range for nitrification is generally accepted to be 6.5 to 8.5. The optimum pH for denitrification process is between 7.0 and 8.0 (Shammas 1971, 1982, 1983; Henze *et al.* 1987a; Fleit *et al.* 2008; Wang *et al.* 2009a). The effects of pH on the growth of microorganisms can be summarised as:

- The optimum pH for both *Nitrosomonas* and *Nitrobacter* lies between 7.5 and 8.0 and will grow within a range of approximately 2 pH units (Szwerinski *et al.* 1986; Prosser 1989; Lessard and Bihan 2003; Henze *et al.* 2008).

- *Nitrosomonas* prefers an optimum pH range of 6.0 to 9.0 at temperature 20-30^{o}C for growth. Nitrification stops at pH < 6.0 (Metcalf and Eddy 1991; 2003; Gerardi 2002; Gray 2004).

In this regard, it has been shown that the efficiency of nitrogen removal is strictly correlated with the value of pH. In a treatment reactor, if the reactor runs under too acidic conditions, the process nitrification would be interrupted (Caicedo *et al.* 2000; Zimmo *et al.* 2000).

2.2.2 Nitrogen fixation, assimilation and ammonification

Nitrogen fixation: nitrogen in the atmosphere is reduced to ammonia nitrogen by bacteria and cyanobacteria. This process is related to several biological processes for the reduction of N_2 to

NH_3 that occur in the synthesis of organic compounds. Since nitrogen rarely appears in nitrogen-rich wastewater, the transformation can be neglected in wastewater treatment systems (Kadlec and Wallace 2009). In ammonification: the organic nitrogen is mineralized to ammonium, nitrite and nitrate. Finally, the assimilation is the process where nitrogen compounds such as NH_4^+ are incorporated as nutrient into the microorganisms' cell for its growth (Metcalf and Eddy 1991).

3. BIOLOGICAL PROCESSES FOR REMOVING AMMONIUM NITROGEN FROM WASTEWATER

3.1 Conventional biological processes or technologies

Wastewater treatment starts with a pre-treatment process depending on the type of wastewater and subsequent treatment systems. Primary treatment in settling tanks allows for settling of the organic suspended solids to form raw sludge. The secondary treatment of wastewater employs various technologies to reduce up to 85% of the nitrogen, BOD, COD and suspended solids.

The technological processes to treat wastewater can be grouped into four general process categories: source separation, physical/chemical processes, biological nitrification/denitrification, and natural systems (Hazen and Sawyer 2009). Hence, according to the selected process category, the design of the system for nitrogen removal must follow different general concepts (Mulder 2003; Carrera *et al.* 2003; Murat *et al.* 2003).

Biological treatment systems that can remove ammonia nitrogen from wastewater include: activated sludge, wastewater lagoons, trickling filter process, submerged fixed bed reactor, biological filters, biological contactor process, moving-bed process, suspended biomass/biofilm, Linpor-CN, constructed wetlands etc., (Davis and Masten 2004: Valigore 2011). Nitrogen exists in different forms because of the different states of oxidation (from -3 to +5). In the environment, changes from one oxidation state to another can be accomplished biologically by living organisms (Sedlak 1991, Madigan *et al.* 1949; Reed 1984).

Biological nitrification is the process of converting ammonia in wastewater to nitrate using aerobic autotrophic bacteria in the treatment process. Nitrification is actually a two-step process for removing ammonia from wastewater using two different types of autotrophic bacteria that oxidize ammonia to nitrite (N*itrosomonas*) and then oxidize nitrite to nitrate (*Nitrobacter*).

The incorporation of such autotrophic and heterotrophic nitrogen removal processes has been studied by different researchers over the past. They have analysed the biochemistry, microbiology and physiology of the autotrophic and heterotrophic nitrogen removal processes. A number of laboratories and pilot-scale reactors have been operated. However, there were still several questions remaining towards an implementation for obtaining a high efficiency of nitrogen removal and the approach for upgrading the processes to full scale:

- Analysing/optimising the process operating conditions, such as the influence of concentrations, pH, temperature, wind speed or the conditions considering the light regime.

- Defining the ideal residence time in the operating system.

- Listing the necessary methods and suitable conditions to control the process.

- Describing and predicting the nitrogen removal by a certain modelling development.

In the absence of sufficient organic carbon supply, the denitrification process will not completely convert nitrate into nitrogen gas (Gerardi 2002). To avoid this issue in the denitrification zone, most WWTP first send the wastewater to a mixing-basin where it is stirred without air supply before flowing to the aeration tank (pre-denitrification). During this process, nitrification may occur.

3.1.1 Activated sludge process

The activated sludge process is a wastewater treatment method that is widely used around the world. To date, the largest application of the activated sludge process systems has been to treat domestic or industrial wastewater. The highest quality of effluent could be achieved from this process. Both aerobic and anaerobic bacteria may exist in this process, but the predominant species is facultative bacteria.

However, the activated sludge process system is more mechanised in comparison with other wastewater treatment systems. It requires a large energy consumption for aeration and is difficult to apply in low income countries.

The following units in activated sludge process are integral and essential to any continuous-flow (describe in the Fig. 9):

- Aeration tank (main reactor)
- Settling tank (secondary sedimentation tank)

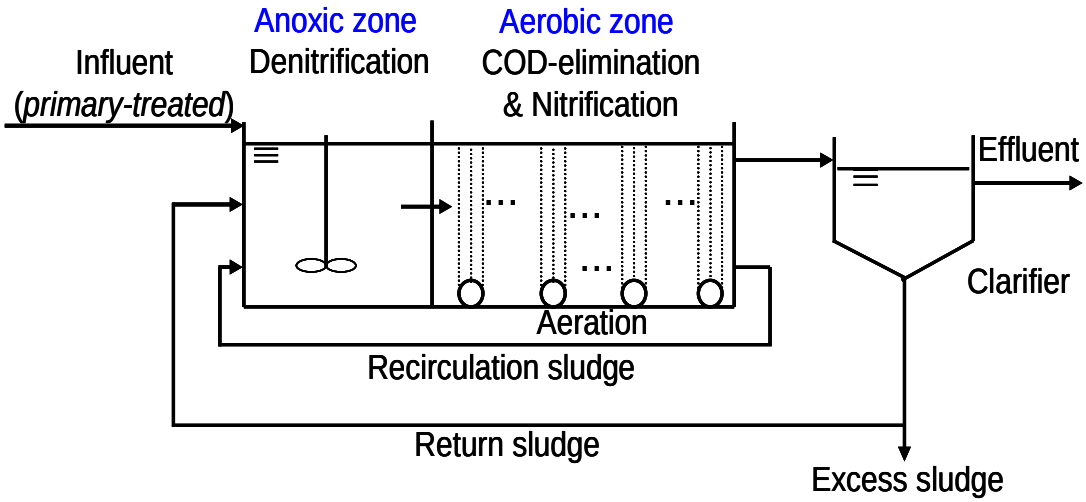


Figure 9. Standard activated sludge process (DWA 2011)

3.1.2 Trickling filter

Trickling filter consists of a fixed biological bed of coarse contactor media such as crushed traprock, granite, limestone, clinkers, wood slats, plastic tubes, corrugated plastic sections, hard coal, or other material over which wastewater is distributed or contacted. Biological slimes form on the media which assimilate and oxidize substances in the wastewater. Various contact beds and trickling filters have been developed for the transfer of dissolved organic matter and fine suspended solids from settled wastewater to contact surfaces (Wang *et al*. 2009a).

However, an uncovered trickling filter is vulnerable to below freezing weather and it is less effective in the treatment of wastewater containing high concentrations of soluble organics. It has only limited flexibility and control, and needs long recovery time with upsets. The process creates odor problems if improperly operated (Wang *et al*. 2009a).

3.1.3 Rotating biological contactor

According to Wang *et al*. (2009a), rotating biological contactor (RBC) is an attached-growth biological process, which consists of a series of rotating plastic media all coated with a layer of biofilm. The biofilm or slime on the media aerobically reacts with substances in a waste stream for bio-oxidation and nitrification, or anaerobically reacts with the substances for denitrification.

The general concept of rotating biological contactors is to let wastewater flow through the tank, and to rotate the medium in the wastewater to be treated, alternatively exposing the

medium (and the attached biological growth) to air and the wastewater. The rotated treatment units are about 40% immersed in the wastewater for aerobic removal of organic waste by the biological film developing on the media. Wastewater treatment efficiency in terms of carbonaceous oxidation and nitrification can be significantly increased by the multiple staging of rotating biological contactors. RBC systems can also be used for biological denitrification.

The most important factor affecting performance of the rotating biological contactors is the biological slime of those microorganisms that grow on a series of thin media, such as discs, mounted side by side on a shaft. The treatment efficiency decreases with decreasing wastewater temperature below 13^{o}C. Other limiting-factors reducing the efficiency of the process are: high organic and hydraulic overload, pH too high or too low, toxic materials in influent. The performance of a typical four stage RBC system with primary and secondary clarifiers is (Wang *et al.* 2009a**)**:

- BOD_5 removal 80% to 90%
- SS removal 80% to 90%
- Phosphorus removal 0% to 30%
- NH_4^+-N removal up to 95%

3.2 New technologies for removing nitrogen from wastewater

Nitrate containing wastewater from the aeration basin is re-circulated and mixed with the carbon-rich fresh wastewater entering the mixing basin. With such a combination, more than 70% of nitrogen reaching the wastewater can be eliminated. Hellinga *et al.* (1998) pointed out that some wastewaters have a relatively high ammonium concentration but low organic carbon content (COD/N (mg/l) was COD 810/N_{TKN}1050), making it difficult to remove the ammonium by nitrification and denitrification without adding any organic carbon source. In order to treat this wastewater, a process called the SHARON (single-reactor high activity ammonia removal over nitrite) process was developed. The process distinguishes itself from other biological wastewater treatment processes by a complete absence of sludge retention (van Kempen *et al.* 2001). The main advantages of this process are nitrification/denitrification with nitrite as intermediate under stable process conditions. It could be reduced 85% ammonium from influent while saves 25% on aeration energy and 40% on BOD addition in comparison with conventional systems (Hellinga *et al.* 1998).

Another process configuration for ammonium removal consists of a combination of nitrification and anaerobic ammonium oxidation. Since these two reactions occur fully autotrophically, the process is called completely autotrophic nitrogen removal over nitrite

(CANON). This process configuration eliminates the need for adding organic carbon to achieve denitrification, since all involved bacteria are autotrophs (Strous 2000; Egli *et al.* 2001; Third *et al.* 2001). In the first step, ammonium is converted anaerobically to nitrite, and in the second step, ammonium is oxidized aerobically with nitrite to form dinitrogen. These processes have been more or less applied in The Netherlands, United Kingdom, Sweden, France and USA, etc.

To get more benefits in obtaining sustainable biological media through nitrification and denitrification processes, essential environmental conditions must be ensured (Lowe *et al.* 2006). For each and every process, a distinctive group of bacteria is required. Each group of microorganisms requires separate reactors as electron donors used by the denitrification organisms.

The most important factors to affect these groups are: temperature, aeration condition, pH and the C/N ratio. Various treatment plants and reactor configurations have been implemented with the goal of optimizing the efficiency of nitrification and denitrification processes by using these organisms, the contact time of the microorganisms with substances and to reduce the required energy as well as the implementation costs.

3.3 Natural system for removing ammonia nitrogen from wastewater

3.3.1 Algal ponds

The discharge of high concentrations of nutrients, nitrogen and phosphorus into the receiving water causes growth of algae and cyanobacteria in lakes, river and the sea, which can end up undergoing eutrophication. This has a significant effect on the water quality and aquatic ecology of the water body. Therefore, a large quantity of substances and sources of nitrogen in the wastewater need to be removed (Lowe *et al.* 2006). A variety of simple and cost effective nitrogen reduction technologies are available for wastewater treatment.

Using natural algal material to remove nutrients such as ammonia and ammonium nitrogen from wastewater is not a new idea and has been applied in warmer climates (algae involved in nutrient removal from wastewater are further discussed in section **4** of this chapter). Several projects have improved and developed the techniques for producing algae in terms of nutrient removal capacity. The basis of this method are the algae as photosynthetic, autotrophic organisms that can assimilate nutrients from the water to use it for their biomass growth and oxygen production (N/biomass average 8%, and 0.16-5% for phosphorus according to Hemens and Mason (1968) and Shilton (2005).

Some reports proved that algal growth could remove up to 90% of the phosphorus or nitrogen from wastewater (Doran and Boyle 1979). In biological treatment reactors, algae may provide heterotrophic organisms in secondary treatment with oxygen. They can also be used to absorb metals from wastewater (Monteiro and Castro 2012) and can be used to produce bio-energy (de la Noüe *et al.* 1992). Moreover, to obtain a high effect on ammonia nitrogen removal from wastewater, many algal systems were also investigated (Siegrist *et al.* 1998; Helmer *et al.* 2002). The development of an algal habitat in the reactor on carrier materials enables the development of slow growing microorganisms like nitrifiers and provides suitable living conditions for them.

Siegrist *et al.* (1998), Kwangyoung & Lee (2002) and Helmer *et al.* (2002) discovered that algae effectively removed ammonium from wastewaters without the need of an extra organic carbon source or only a small organic carbon consumption. The process releases mainly dinitrogen gas with a small amount of nitrite/nitrate. This can be explained as follows: when microalgae cells are cultured under photoautrophic conditions, these cells can utilize molecular CO_2 from air or bicarbonate ions. Thus, microalgae cultural could remove nitrogen together with CO_2 while minimizing the reduction in COD and BOD_5. One of the advantages of the algal system is that it can maintain the suitable conditions for a long term establishment of both aerobic and anaerobic ammonia oxidizers. This is mainly due to the growth of algae and their ability to maintain the system's stability (Mata *et al.* 2010; 2012). However, in an algal system, the physical parameters such as pH, oxygen and temperature supply are more difficult to control in comparison with other systems (Helmer *et al.* 2002).

3.3.2 Duckweed ponds

The duckweed genera *lemna sp.*, *Spirodela sp*. and *Wolffia sp*. have all been used in biological wastewater treatment systems. There are many full-scale systems in operation, which use floating plants. Duckweed genera are free-floating, freshwater plants with a leaf-like form, small size and a very fast reproduction rate (ISO 2001).

Duckweed can be used to treat wastewater containing very high total ammonia concentrations as long as certain pH levels are not exceeded. The degradation of organic material is enhanced by duckweed through two additional processes: more oxygen supply and additional surface for bacterial growth. The duckweed could be responsible for three-quarters of the total nitrogen (N) and phosphorus (P) loss in very shallow systems (Körner *et al.* 2003). A number of studies of duckweed growth in relation to nutrient uptake and utilization in polluted waters have been reported (Oron 1994; Al-Nozaily *et al.* 2000; Al-Nozaily 2001; Caicedo 2005). It

was estimated that as much as 15 to 20% of the nitrogen can be fixed by duckweed growth biomass (Underwood and Baker 1991).

Moreover, many studies in the past for different type of wastewater at laboratory scales, as well as in outdoor experiments proved that duckweeds are highly efficient for nitrogen uptake, especially for removing nutrients. Körner and Vermaat (1998) reported 73 to 97% removal of the initial Kjeldahl-nitrogen within 3 days in laboratory scale duckweed-covered systems (18.5 cm diameter and 4.5 cm depth).

Ozengin and Elmaci (2007) via a laboratory scale of *Lemna minor L.* showed, the removal efficiencies of about 73-84% of COD, 83-87% of total nitrogen and 70-85% of total photphorus could be achieved under laboratory condition (the Fig. 10). Al-Khier *et al.* (2007) reported a removal efficiency of 82% of NH_4^+, 89% of COD, 91% of BOD_5 and 96% of TSS in outdoor natural conditions for eight days at temperatures of 20-29^oC.

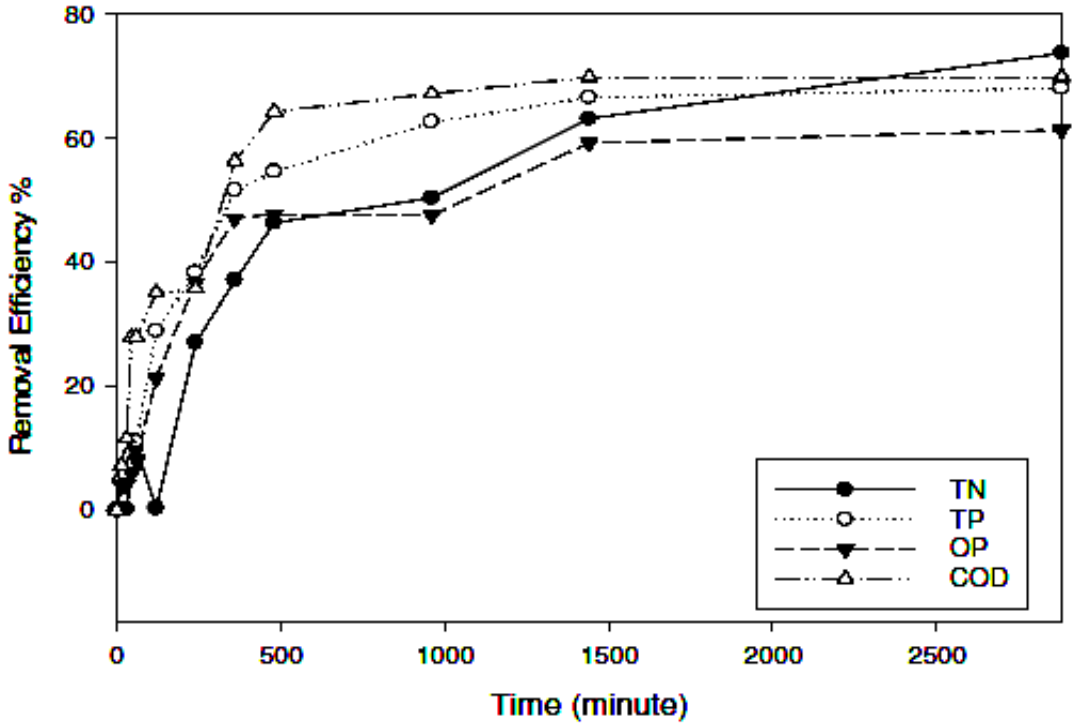


Figure 10. Removal of COD, total nitrogen (TN), total photporus (TP), ortho phosphorus (OP) throughout monitoring of municipal wastewater
(Ozengin and Elmaci 2007)

3.4 Comparison of removal efficiency of differents processes and technologies

The table 2 shows the comparison of wastewater treatment technologies. The data in this table was collected from different sources, regions and technologies. For example, in the research

of von Sperling *et al.* (2006), the summarized data were collected from the warm climate regions with a mean yearly temperature range of 20–26^{o}C. The author focused on several parameters, such as solids, organic matter, nitrogen, phosphorus, faecal contamination, COD and BOD removal efficiencies. Another parameter in the research was provided about the sludge age (in completely mixed aerated lagoons, the detention time varies in the range of 2 to 4 day; in UASB reactors, it usually exceed 30 days), the depth of the pond (2.5 to 4.0 m), etc.

Table 3. Comparison of wastewater treatment technologies
(Nurdogan and Oswald 1995; Zimmo 2003; Garcıa *et al.* 2004; Grönung 2004; von Sperling *et al.* 2006; German Water Association - DWA 2011)

System	Average removal efficiency %				Land m^2 /habitant	Installed power W/habitat
	BOD_5	COD	NH_4^+-N	Total N		
Primary treatment (septic tank)	30-35	23-35	10 - < 30	10 - < 30	0.03 - 0.05	-
Facultative pond	75-85	65-80	< 50	< 60	2 - 4	-
Anaerobic-Facultative pond+Algea removal	85-90	75-83	< 50	< 60	1.7 - 3.2	-
Constructed wetland	80-90	75-85	< 50	< 60	3.0 - 5.0	-
UASB reactor	60-75	55-70	< 50	< 60	0.03 - 0.10	
Conventional activated sludge	85-93	80-90	> 80	< 60	0.12 - 0.25	2.5 - 4.5
Activated sludge (Germany)	99	95	1.2 mg effluent	82		34 Kwh/ hab.year*(1)*
Low rate trickling filter	85-93	80-90	65-85	< 60	0.15 - 0.3	-
Rotating bio.contactor	88-95	83-90	65-85	< 60	0.1 - 0.2	-
Algal and duckweed pond	85-92	75-90	55-85			-
High rate algal ponds	90	90-95	90	57-73		-

(1): Energy consumption

4. ALGAL-BACTERIA INVOLVED IN NUTRIENT REMOVAL FROM DOMESTIC WASTEWATER

4.1 Potentials of application of algal for wastewater treatment

Oswald (1988) pointed out that by using auto-phototrophic organisms are not new for removing nutrient from wastewater. The cultivation of algae offers the combined advantages

of treating the wastewaters and simultaneously producing algal biomass, which can further be exploited for producing bio-energy. In comparison with heterotrophic systems like activated sludge plants, algal ponds are using low-grade technology while saving of energy. The photosynthetic organisms can produce oxygen, and hence it could replace mechanical aeration.

Numerous efforts have been devoted to create a suitable condition and technology for culturing microalgae (Richmond & Becker 1986; Oswald 1988). In this context, use of algal material for wastewater treatment has been proposed for obtaining the high substances removal efficicency (de la Noüe *et al.* 1992). Application of technology to algal wastewater treatment provides more flexibility in the reactor design when compared with conventional treatment systems.

One of the major and practical limitations by using algal material for treating wastewater is that algae requires high temperature and light intensity for its growth biomass. Therefore, solar radiation is an important factor for algal photosynthesis. Several research have been revealed that in different solar radiance conditions in the world different growth rates and removal efficiencies of algal must be examined. From this point, the environmental scientists who are engaged in wastewater management with algal ponds could consider this condition to get high potential of algae photosynthesis.

The Fig. 11 shows the global irradiant for the period 1981-2000. In this figure, the high irradiance regions from 40^{o} North to 40^{o} South, and the average radiation intensity is around 1.500-2.500 kWh/m^2.

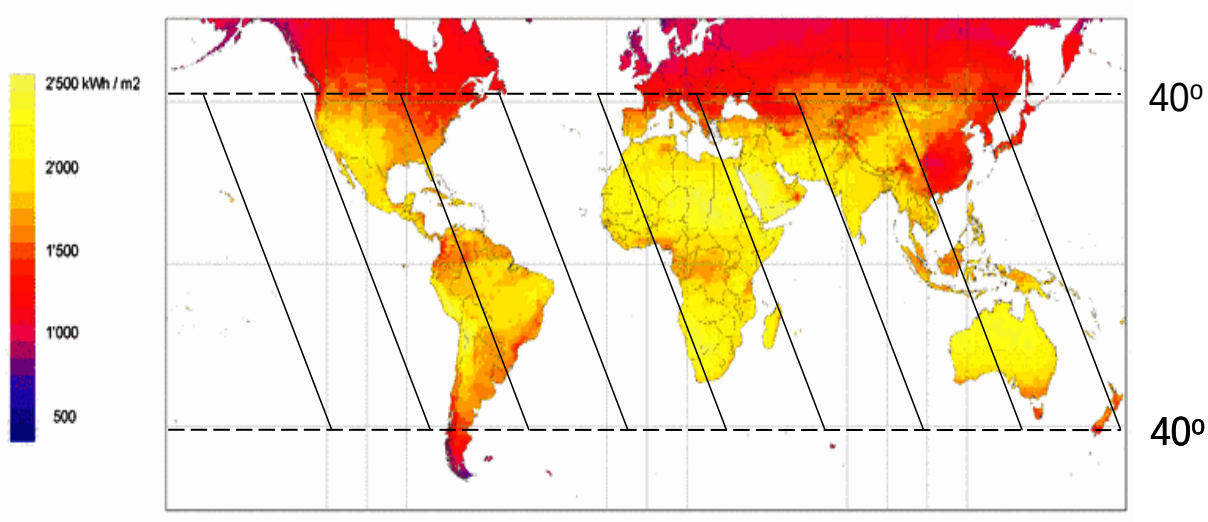


Figure 11. Global irradiance (www.meteronorm.com)

4.2 Algal biological and ecological

Algae are identified by their primary pigmentation characteristics. They belong to the heterogeneous group of eukaryotic, photosynthetic, unicellular, and multicellular organisms lacking true tissue differentiation. In a treatment system, algae biomass grows and produces oxygen to the bacterial decomposition of organic matter (shown in the Fig. 12).

In the Bergey's Manual of Determinative Bacteriology edited by Buchanan and Gibbons (9^{th} edition-1999), cyanobacteria are classified as blue-green organisms. The blue-green species have a mixture of photosynthetic pigments that produce the shades of colour. Some blue-green algae have the ability to fix atmospheric nitrogen. A chemical formula for the algal cell is $C_{106}H_{181}O_{45}N_{16}P$ (Oswald 1988). Green algae belong to the algae species most closely related to plants. They have the same pigments (chlorophyll a and b, and carotenoids), the same chemicals in their cell walls (cellulose), and the same storage product (starch) as plants.

Green algae may be unicellular or form filaments, nets, sheets, spheres, or complex moss like structures. They exist as both freshwater and marine species. Green algae can be subdivided in two bigger groups: *Chlorophycophyta* and *Euglenophycophyta* as motile organisms that are differentiated by their characteristic physiology. Both groups use chlorophyll as their photosynthetic pigment and metabolize inorganic nutrients in water. Blue-green algae, technically known as cyanobacteria, are microscopic organisms that are naturally present in lakes and streams in low numbers. Blue-green algae can become very abundant in warm, shallow, undisturbed surface waters that receive a lot of sunlight (NYS DOH, 2008). When this occurs, they can form blooms that discolour the water or produce floating rafts or scum on the surface of the water.

Algae and cyanobacteria play a positive role in wastewater treatment by recycling nutrients, taking up carbon, nitrogen or phosphorus. Therefore, they could reduce the nutrient content in the effluent and the pollutant load of a reactor or treatment system.

A number of techniques have been developed, implemented and improved with the aim of producing algal biomass for wastewater treatment. These include using high algal oxidation ponds, packaged bag of algae, rotating algal disks, photo bioreactors, etc., for removing ammonia as well as other toxic substances from wastewater. However, algae can be a problem when “blooming” where excessive algal growth in the receiving water can deplete the available oxygen. The colour range of algae changes from green to yellow-green and from brown to red. It depends on the species and their specific environment.

A study by Ammann and Fraser-Smith (1968) has indicated that there is about 3.0 milligram oxygen produced per milligram of carbon utilized from carbon dioxide. Another study by Hannan and Patouillet (1963) on *Chlorella* species pointed out that the species can produce about 2.8 milligram oxygen for each milligram carbon used. The photosynthesis done by algae is very important for the biosphere. It reduces the amount of carbon dioxide and increases the amount of oxygen in the atmosphere (as described in the Fig. 12). Some types of algae have been found in sewage lagoons or in stabilization ponds (William and Sauter 2005).

In a treatment system utilizing algae material, the dissolved oxygen concentration in reactors is usually high (10 mg O_2/l) due to algal photosynthesis activity under a temperature of 20^{o}C (Gutzeit 2006). Hence, the reactors employing algae can overcome the above-mentioned problem by feeding a higher biomass population attached to natural or synthetic materials. The removal rate of the ammonia nitrogen from wastewater could reach up to 95% via an algal system because of an increased oxygen concentration due to photosynthesis activities. But this influence is relatively difficult to compare with other reports in the literature for waste stabilization ponds, either due to different system configurations and different operational conditions (Middlebrooks *et al.* 1982*;* Silva *et al.* 1995; Lee *et al.* 2009).

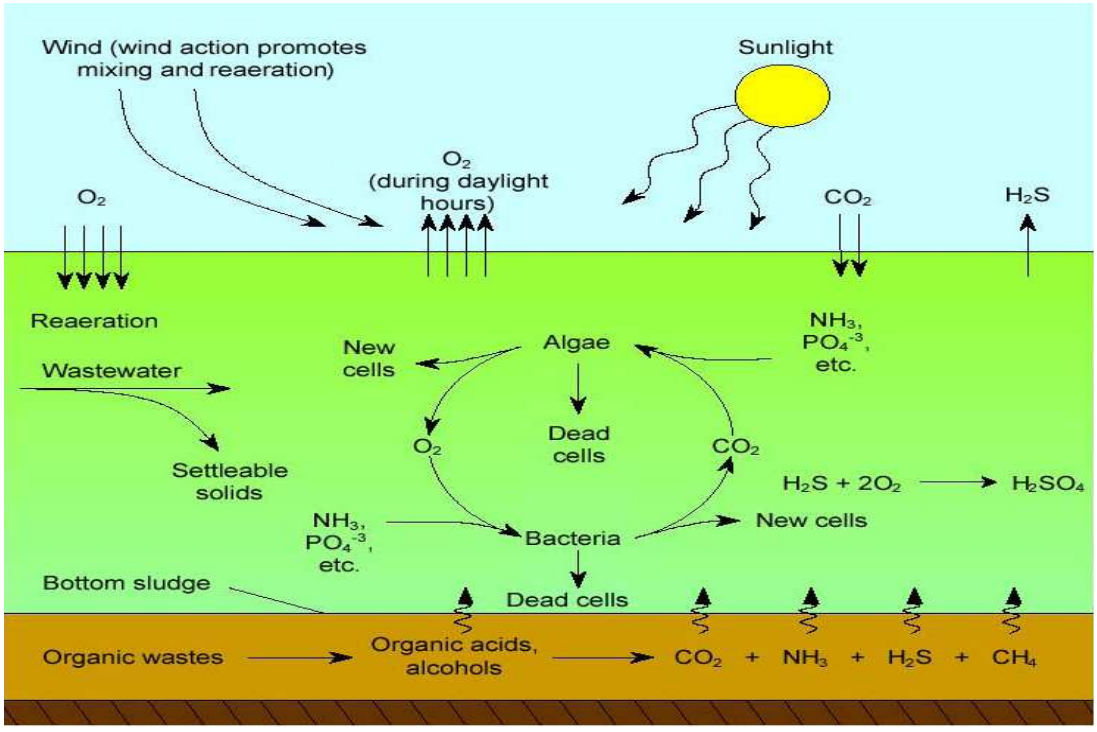


Figure 12. Schematic of algae and bacteria interplay in wastewater pond (Adapted and modified from Metcalf and Eddy 1991)

Microalgae can be used for tertiary treatment of wastewater due to their capacity to assimilate nutrients. Domestic wastewater contains high concentrations of necessary nutrients for algae growth. The growth of algae depends on limiting factors like light, temperature, pH, substrate, water depth, turbulence and hydraulic retention time. For example, pH becomes toxic to algae at high values, overdose of intense light will damage the photo system, and the growth will cease if the temperature is higher or lower than optimum levels. Thereby, it may become one of the technologies suitable for removing high concentrations of ammonia nitrogen or nutrients from wastewater, while saving space and obtain a higher removal efficiency than suspended growth methods.

4.3 Assimilation into algal and bacterial biomass

Wastewater treatment systems with algae are particularly attractive, because of their high effectiveness in nutrient removal, low operational costs and easy implementation. The biggest benefit of using algae for wastewater treatment is their capacity to do photosynthesis to transform sunlight into biomass while taking up ammonia nitrogen from wastewater. In doing so, they use the degraded carbon dioxide and the nutrients assimilated by bacteria. The main mechanisms of algal ammonia nitrogen removal are the uptake of the substance into the cell and the facilitation of stripping the ammonia through the elevated pH (Aslan and Kapdan 2006).

Studies in the past have shown that, algae tend to prefer consuming ammonium over nitrate, and the nitrate consumption process does not occur until the ammonium is almost completely consumed (Maestrini *et al.* 1986). Therefore, wastewaters with high ammonium concentrations could be effectively used to support algae grow biomass. Algae have a great potential for the removal of many contaminant substances such as nitrogen, phosphorus or to absorb metal from wastewater (Mehta and Gaur 2005; Monteiro and Castro 2012).

According to Gray (2005), algal and bacterial cells are able to assimilate nutrients and coexist in a symbiotic relationship. Through their cooperation, algae and bacteria can make substances soluble and absorb organic matters from wastewater into their biomass. This can subsequently be removed by settling and sludge removal.

The nutrient composition of algae grown on domestic wastewater varies between 0.6-16% (average 8%) for nitrogen and 0.16-5% for phosphorus (Hemens and Mason 1968, Shilton 2005). According to Becker (1994), Oliver and Ganf (2000), nitrogen may comprise more than 10% of algal biomass. The nitrogen uptake rate was dependent on different algal species.

For example, the nitrogen uptake rate of algae species *C. vulgaris* was 0.512 while *S. obliquus* was 0.621 for (Ruiz-Martin *et al.* 2010).

4.4 Physical and chemical effects on algae growth

4.4.1 pH effects on algal growth and its effect on ammonia nitrogen removal

The major mechanism of N-removal through the algal system is the biological uptake of nitrogen for biomass production. The algal mass is partly settled or attached to the walls of the reactor. An increased rate of algae growth can be observed in higher pH ranges of about 7-9, which obviously represent the favourable conditions for algae development (Matusiak 1977; Wang *et al.* 2009a). Kim and Lee (2001) indicated that *Chlorella vulgaris* develops well under the pH condition around 5-7 as described in the Fig. 13 below.

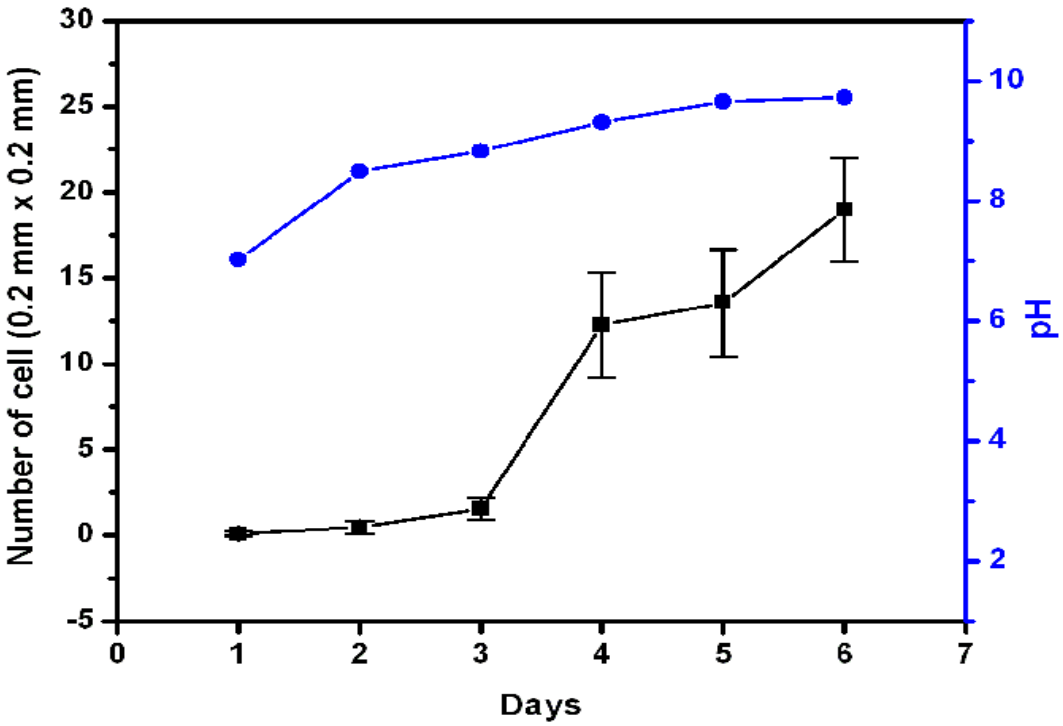


Figure 13. Growth kinetic and pH change of *Chlorella vulgaris* dependent upon initial pH variance in different cultural media (Kim and Lee 2001)

Fontes *et al.* (1987) found that optimal productivity of the cyanobacterium *Anabaena variabilis* was obtained at pH 8.2-8.4, slowing down at pH 7.4-7.8. The growth rate of algae decreased dramatically at pH 9 or above. However, some species can accept pH values higher than that (marine algal species).

When the water environment has a high concentration of free ammonia accompanied with high pH level, this becomes toxic to algal growth by affecting the photosynthesis inhibition.

E.g when the ammonia nitrogen concentration in an environment was ≥ 54 mg/l together with pH=9.5, these combined factors have led to a 90% reduction in the photosynthesis activity of algae (Azov and Goldman 1982; Veenstra *et al.* 1995 in Shilton 2005).

The variation of pH due to algal growth has also been recognized in the studies of Harremöes (1978) as cited in Henze *et al.* (1996), Arvin and Kristensen (1982) and Szwerinski *et al.* (1986). Kuenen *et al.* (1986) indicated that an increased pH will support the photosynthetic activity of algae. The pH value of the water can also affect algal growth, and the pH of the medium has a strong influence on algal growth. It directly affects the algal photosynthesis.

Therefore in changing the pH value further changes the growth rate (Danilov *et al.* 2001). In high rate algal ponds, this pH increase can be compensated by respiration deeper in the ponds, and the pH can then be regulated by letting in more organic material, thereby enhancing the respiration (Borowitzka, 1998 as cited in Becker 1988).

4.4.2 Temperature effects

Eppley (1972), Goldman and Carpenter (1974) have reviewed the effect of temperature on algal growth. They concluded that at low temperature levels, the assimilation rate of organic carbon is reduced or only maintained at a base level. As a result, only a small fraction of the total carbon can be used by the algae (Cohen and Parnas 1976). The direct influence of temperature on phytoplankton division rates is clear: within defined temperature limits division rates increase with increasing temperature.

Table 4 shows the different growth rates of algae under different temperature conditions. Obviously, algae accept a wide range of temperature from 19 to 40^{o}C. For example *Chlorella pyrenoidosa* develops with growth rate at a temperature of 25^{o}C, meanwhile the growth rate of *Chlorella sp.* is 1.8/day under the same temperature.

The growth rates of algae are often proportional to the temperature. The optimal temperature is mostly from 28^{o}C to 35^{o}C (sufficient nutrient and light provided). But it varies between algal species. e.g the acceptable range of temperature for green algae is 30-35°C and for blue-green algae *Chlorella pyrenoidosa,* it is above 35°C (Guillard and Ryther 1962; Eppley 1972; Soeder *et al.* 1985*;* Soeder 1986; Devos *et al.* 1998). It is indicated that the growth rate for batch-cultured algae with optimal growth temperatures in the range 5-40^{o}C is 1.88 (as can see in the section 2.1.2 in this chapter).

Table 4. Maximum growth rate versus temperature data from continuous culture experiments on freshwater (as cited by Goldman and Carpenter 1974)

Algal Species	Temp (^{0}C)	Maximum growth rate μ_e/day	Reference
Chlorella pyrenoidosa	19	1.36	Shelef et al. (1970)
(Emerson strain)	19	1.45	Shelef (1968)
	25	1.95	Zabat (1970)
	25	2.14	Zabat (1970)
	28.5	2.22	Shelef (1968)
Chlorella pyrenoidosa	35	3.94	Shelef et al. (1970)
	35	4.32	Shelef (1968)
	39.2	4.26	Shelef et al. (1970)
	39.2	5.65	Shelef (1968)
Chlorella sp.	25	1.88	Williams (1965)
Selenastrum capricornutum	24	1.85	Toerien et al. (1971
	27	2.45	Goldman et al. (1974)
Scenedesmus quadricauda	27	2.29	Goldman et al. (1974)

In contrast, Hulburt and Guillard (1968), found that some species of algae like *Skeletonema tropicum* had a stabilized growth rate at temperatures between 25 and 35°C, but it could cease at the temperatures below 13°C or above 35°C. The studies on *Scenedesmus* by Fogg (1975a,b) indicated a μ_{max} of 2.83/d at 25°C. Gons (1977) found a μ_{max} of 1.14/d for *Scenedesmus*. Goldman and Graham (1981) reported a μ_{max} of 1.59/d at 20°C.

Also, Ahlgren, G. (1987), Zargar *et al.,* (2006) found that the biomass production rate in an algal system is affected by the temperature conditions. The research also indicated that chlorophyll-*a* and biomass were increased after 17 days of exposure at 31^{o}C and 33^{o}C respectively, and decreased at 36^{o}C after being exposed for same test-period.

The Fig. 14 below shows the effect of different temperature levels on chlorophyll-*a* content of the alga *Scenedesmus quadricauda*. It supports that the suitable temperature condition for algal growth is in a range of 20-26^{o}C.

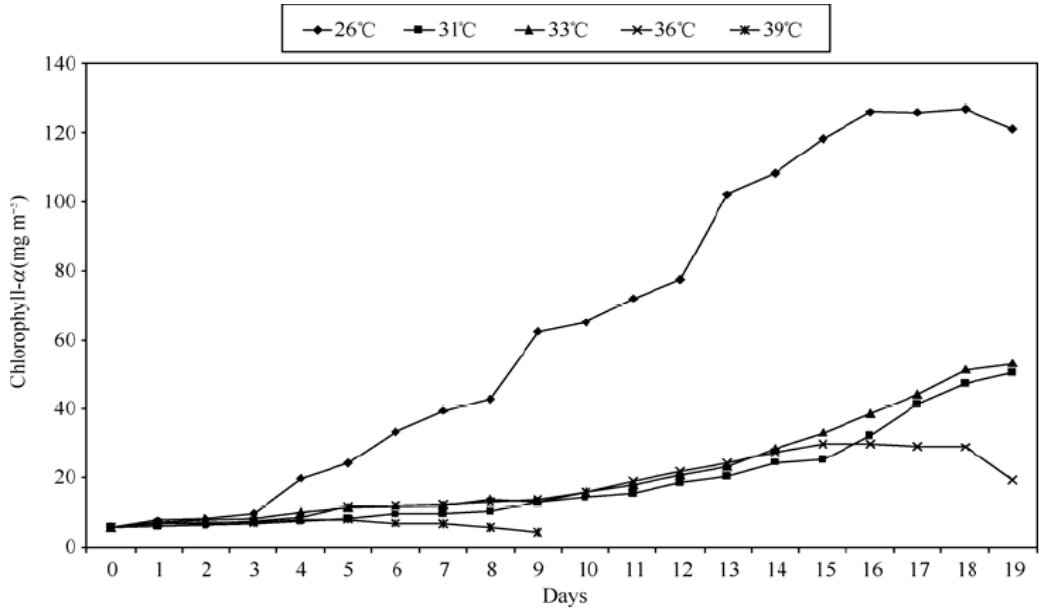


Figure 14. Effect of different temperature levels on chlorophyll-α content of the algae *Scenedesmus quadricauda*.

4.4.3 Light conditions effects on algal growth

Many tests in the past uncovered that an increased duration of irradiance could directly enhance the number of cells, as well as increase their function for nutrient removal from wastewater. Khoeyi *et al.* (2012) indicated that there was not only a different growth of chlorophyll-content, but also a different rate of biomass growth while comparing different algae species. Carvalho *et al.* (2006; 2009, 2011), Posten (2009), Carvalho *et al.* (2011) and Ifeanyi *et al.* (2011) also suggested that to obtain a high performance of the algae, they should be provided with the appropriate duration and the quantity of light (intensity and wavelength).

A number of studies observed the effect of light on algal biomass growth. The studies revealed that the algae could grow with the maximal rate only if sufficient light was provided. Then, a high chlorophyll accumulation in the algal reactor could be obtained (Jacob-lopes *et al.* 2009). However, higher light intensities over a certain threshold normally damage the photo-system (Goldman 1979*;* Grobbelaar 1982, 1991; Milligan and Cosper 1997*;* Richmond 2000, 2004; Richmond *et al.* 2003).

Nevertheless, some algae species are able to grow in the dark and use simple organic compounds as their energy and carbon source. The light energy is converted to chemical energy in the photosynthesis. The work of Duarte (1984), as shown in the Fig. 15, showed that the gross primary productivity of algae reaches steady state growth at a photon flux of

150μE/m^2s. However, higher light intensities over a certain threshold normally damage the photo-system (Goldman 1979; Grobbelaar 1982, 1991; Milligan and Cosper 1997; Richmond 2000, 2004; Richmond *et al.* 2003).

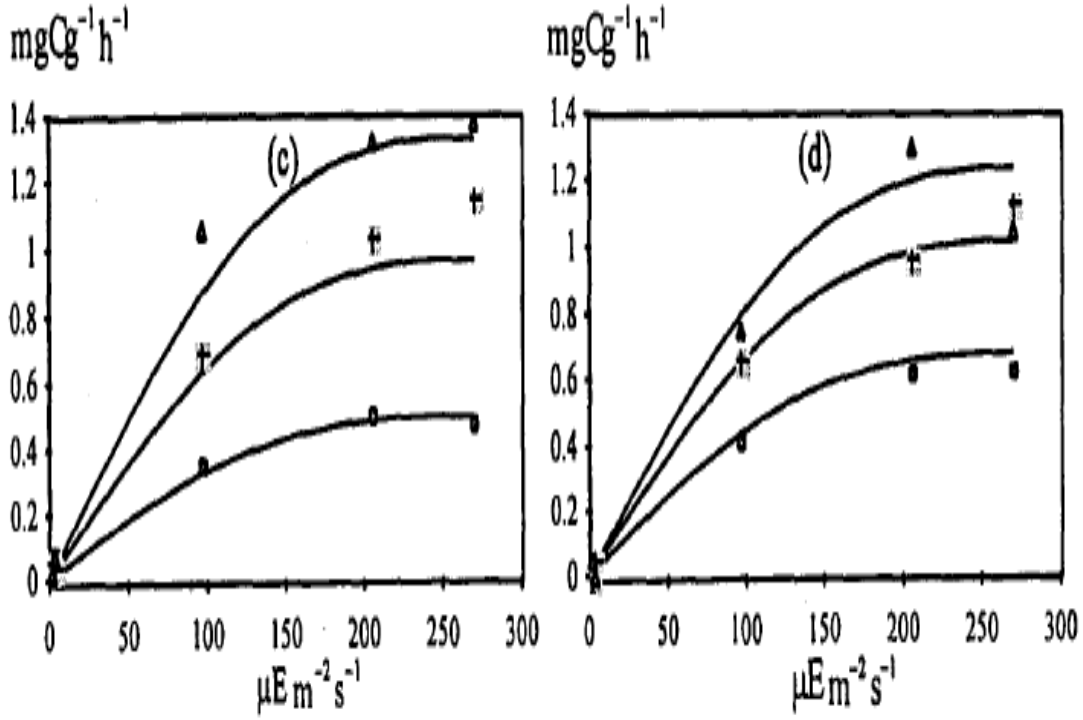


Figure 15. Gross primary productivity measured at different light intensity conditions correspondent (Duarte, 1984)

Oswald (1988) reports that in outdoor ponds, more than 90% of the total incident solar energy can be converted into heat and less than 10% into chemical energy. Fontes (1987) reported a conversion efficiency of sunlight energy into chemical energy of only 2%.

Light regime is the name for the continuous variation of irradiance over time ($\boldsymbol{I}_t$). But it has been defined by simplified forms such as dark/light residence time. It has a strong influence on the algal growth in the reactors (Molina Grima *et al.* 1997; 1999; 2001; Wu and Merchuk 2002; Janssen *et al.* 2000, 2002 and 2007; Janssen 2002; Posten 2009; Brindley *et al.* 2010).

Several authors concluded that there is no general agreement on the selection of a specific light regime. Some authors choose values such as the semi-saturation irradiance ($\boldsymbol{I}_k$), which is calculated as: $\boldsymbol{I}_k = I_{max}/2$, to describe to the irradiance approach to the light saturation level for the algal growth biomass (Acien Fernandez *et al.* 1998, 2001).

There were many theoretical approaches to determine the maximum photosynthetic capacity of organisms to increase their solar-energy conversion efficiency (Weissman and Goebel

1987; Weissman *et al.* 1988; Tillett 1988*;* Melis 2009). Walker (2002; 2009) tried to describe the "*Z-scheme*" for algae photosynthesis and development of biomass (CH_2O) via two proposed photo systems:

Photo-System I : $2H_2O = O_2 + 4H^+$
Photo-System II : $CO_2 + 4H^+ = CH_2O + H_2O$

The light energy absorbed by algae is first stored as intermediate bio-chemical reductants (NADPH2 and ATP). These are afterwards used by the algal cells to produce new biomass: CH_2O. The algal biomass and growth rate could be predicted or calculated by the maximum algal photosynthetic conversion efficiency $\boldsymbol{P}_{max}$ and energy supply $\boldsymbol{I}_o$ (Tillett 1988).

In the absence of nutrient limitation, the photosynthesis increases with increasing light intensity until the maximum growth rate is attained. If more light is provided beyond this point, it can lead to photo-oxidation, also known as photoinhibition (Bouterfas *et al.* 2002; Macedo *et al.* 2002; Torzillo *et al.* 2003; Richmond 2004).

Accordingly, if the light-limitation is the condition for the efficiency of the algal photosynthesis, it must be a result of the reduction of chlorophyll-a content. Several studies in the past have shown that the achievement of the chlorophyll-a content in algal cells corresponds to the temperature and the duration of the provided light. The variations in chlorophyll-a represent changes in the 'self-shading' package effect, and can be detected via measurements of *in vivo* absorbance combined with cell pigment analyses (Osborne and Raven 1986a,b; Raven and Geider 1988).

Regarding the magnitude of these effects on chlorophyll-a, Osborne and Raven (1986b) pointed out that it is mostly the temperature and the light regime that has an effect on the important functions of the a_{chl} synthesis of the algae. In any case, the a_{chl} concentration would be expected to increase in cells grown in suitable temperature conditions and while an optimal duration of light is provided.

Algae that photosynthetically produce oxygen can relieve biological oxygen demand (BOD) in wastewater. The dissolved oxygen levels in water could saturate up to 200% (García *et al.* 2000; Molina *et al.* 2001; Park and Craggs 2010). Moreover, algal growth in the water can absorb heavy metals. As an example, *P. typicum* and *S. quadricauda* showed that the highest percentage of metal bioremoval occurred in the first 30 min of contact, recording 97% (Hg^{2+}), 86% (Cd^{2+}) and 70% (Pb^{2+}) (Shanab et al. 2012).

Posten (2009), Ifeanyi *et al.* (2011) and Janssen (2002) suggested increasing light distribution along with sufficient agitation, aeration and energy demand for higher performance of algal growth in nutrient wastewater elimination. Rochet *et al.* (1986) and Mata *et al.* (2012) observed the response of algae and chlorophyll content to different changes in the light intensity and quality. They reported that the highest biomass production and the most suitable conditions for cultivating algae in those conditions are in an aerated culture and exposing the growth to a 12-hour period of daylight intensity of 12.000 Lux. The high chlorophyll-a content is related to the growing energy requirement (light) for higher production of biochemical compounds.

4.4.4 Effect of substances on algal growth

The growth of algae and bacteria in a treatment system consists of four phases: lag phase, log-growth phase, stationary phase, and log-death phase. The calculation of algal growth on substances of ammonia and nitrates was based on the Michaelis-Menten kinetic model as shows in the Fig. 16 (Berg *et al.* 1958).

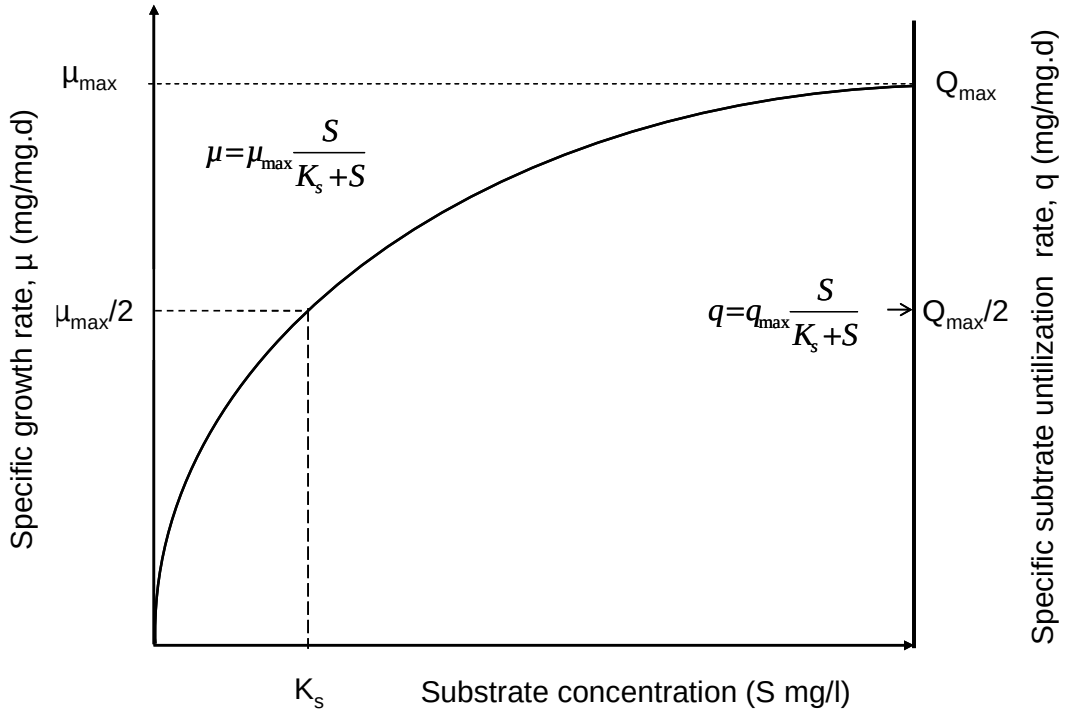


Figure 16. Monod relationship between substrate concentration (S) on growth rate of algae and bacteria (Petre 2013)

Throughout the lag-phase, microorganisms acclimatize to their new conditions and begin to reproduce. In the log growth phase, algae multiply at a rate determined by their generation

time and ability to process the substrate. When the microorganisms enter the stationary phase, they have exhausted the substrate necessary for growth, and the population is at a standstill. If no new substrate is supplemented the microorganisms begin to decrease the growth rate. Hence, in the log-death phase, the death rate exceeds the production of new cells (Liu *et al.* 1999).

The death rate is usually a function of the viable population and environmental characteristics. In some cases, the log-death phase is the inverse of the log-growth phase (Metcalf and Eddy 1991). If the concentration of available substrate is at a minimum level, the microorganisms are forced to metabolize their own protoplasm without replacement. This process, known as lysis occurs when dead cells break and the remaining nutrients diffuse out to supply the remaining cells as an extra nutrient source. This type of cell growth is sometimes referred to as cryptic growth and occurs in the endogenous phase (Liu *et al.* 1999).

Photosynthesis supplies the energy necessary for algae to fix gaseous nitrogen dissolved in the water. The solubility of nitrogen (depending on the specific nitrogen compound that is present) in water at atmospheric pressure and a temperature of roughly 20°C is close to 12-20 mg/l (Greenwood and Earnshaw 1997).

Previous studies have pointed out that using algae could reduce ammonium nitrogen from wastewater by around 67%-81%, and the nutrients in wastewater are significantly removed by some algal species (García *et al.* 2006; Khan and Yoshida 2008*;* Powell *et al.* 2009; Park and Craggs 2010; Park *et al.* 2011). This is because the algae could use the photosynthetic mechanism to convert nutrient from water into cells (Weissman and Goebel 1987; Weissman *et al.* 1988; Tillett 1988; Melis 2009; Walker 2002, 2009).

Carbon dioxide is the primary carbon source for algae. Carbon is the most important element found in algal biomass and constitutes over 50% of the typical algal biomass (Becker 1994). Some algae species like *Chlorella sp.* can grow both autotrophically and heterotrophically (Oh-Hama and Miyachi 1988). Algae grow better in water containing a high concentration of bicarbonate alkalinity than in water with low bicarbonate alkalinity.

Ammonia nitrogen is the most important source of nitrogen for algae while nitrates are the secondary source. The blue-green bacteria, still considered as algae by some phycologists, have the ability to use atmospheric nitrogen as their nitrogen source when nitrate or ammonia nitrogen is not available.

On the other hand, algae usually tend to absorb nitrogen as ammonia rather than as nitrate because ammonia is easier to be synthesized into amino acids as compare with nitrate (Fitzgerald and Rohlich 1964; Santos and Oliveira 1987; Mostert and Grobbelaar 1987; Graham and Wilcox 2000).

Phosphate is a crucial source of phosphorus for the algae. Since phosphates are limited in the natural environment, phosphorus availability is often the limiting factor in the growth of algae. Algae also need sulphates and trace metals including calcium, potassium, zinc, copper, manganese and molybdenum. The lack of sufficient trace metals will limit the magnitude of algae growth (McKinney 2004). Iron is needed for electron transfer processes. Magnesium is required for the formation of chlorophyll-a. (Etienne Paul and Liu 2012).

4.4.5 Hydraulic retention time

Based on the theory of algal growth, the hydraulic retention time (HRT) to obtain good treatment performance, as well as to remove nutrients should be between two and seven days (Tang 1995; Benemann 1989).

4.4.6 Turbulence

Richmond and Becker (1986) stated that when the nutritional requirements of mass cultured algae are satisfied and the environmental conditions are not growth-limiting, mixing for the creation of a turbulent flow constitutes the most important requisite for high yields of algal mass. It can affect algal growth by preventing the cells from sinking to the bottom of the pond, preventing the formation of nutritional and gaseous gradients, and by moving the cells through an optical gradient (Grobbelaar 1991).

In the research about the effect of light limited algal growth in homogenous turbulence, Marshall and Huang (2010) showed the effect of turbulent mixing on algae growth rate under light-limiting conditions. The authors found that for small values of the ratio of illuminated layer depth to total fluid depth, significant enhancement of algae production rate is observed with mixing. A further increase in mixing frequency does not produce additional growth rate enhancement (shows in the Fig. 17).

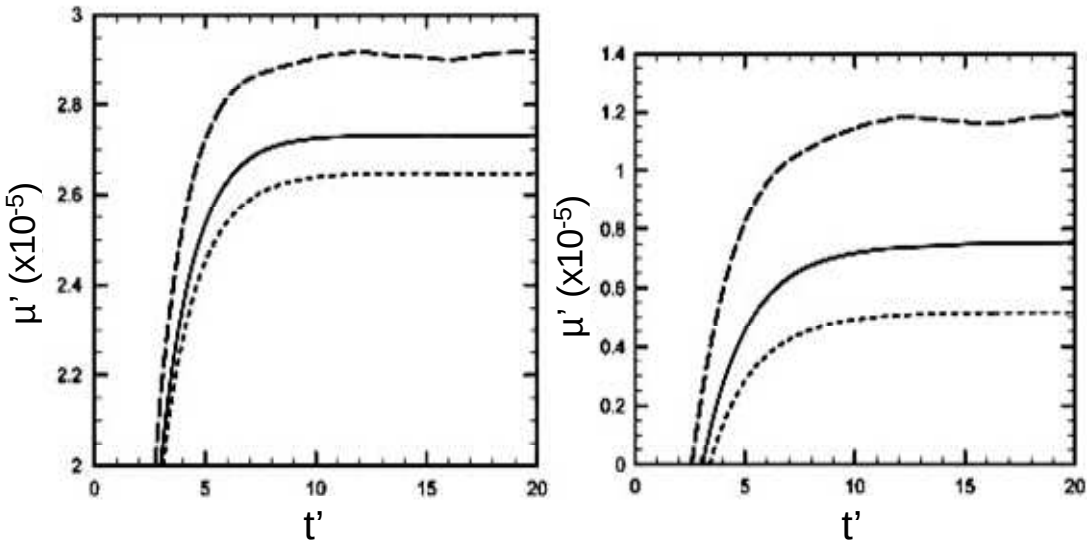


Figure 17. Dimensionless algae growth rate values with turbulent mixing for (a) $\sigma'\varphi = 2$ and (b) $\sigma'\varphi = 4$. No flow (short-dashed line), mixing with $f_T = 0.36$ (solid line), and mixing with $f_T = 1.8$ (long-dashed line). (Marshall and Huang 2010)

Where:

μ': average algae growth rate, s^{-1}

σ': algae absorption coefficient, mlm^{-1} $(10^6$ cells$)^{-1}$

φ: algae concentration, 10^6 cells/ml

f_T: dimensionless turbulence frequency

Chapter III.
RESEARCH MATERIALS, ANALYTICAL METHODS
AND EXPERIMENTAL IMPLIMENTATIONS

1. RESEARCH MATERIALS

In this study, the practical experiences during start-up and operation of algal-duckweed experiments and baffled algal reactors are discussed along with the construction of the reactors. Special attention is given to observe the possible effects of algae on ammonia nitrogen, the gradients of nitrate concentration during treatment operation as well as the removal rates of BOD_5 and COD removal from domestic wastewater, as can see in the table 5 below.

Table 5. Pre-settled wastewater concentration (*average values*)
(wastewater source: Schönerlinder wastewater treatment plant in Berlin)

Parameter	**Concentration (mg/l)**	
	Algal and duckweed experiments	**Baffled algal reactors**
BOD_5	284	268
COD	594	651
TKN	83.8	83.8
NH_4^+-N	67	63
NO_3^--N	0.3	0.4
O_2	0.7	0.6
pH	7.3	7.4

Grabbed samples from all experiments were analysed weekly in order to observe the concentration trend throughout the week. All samples were collected during the day at the effluent of tests. No samples were collected during the night. The flow rates (in/out) and recycling flow are controlled by automatic dossier pumps. Table 6 gives the information of this study on the measurement methods, sample taking points and analysing frequency.

Table 6. Overview of sample taking points and measuring methods

(**σ**: standard deviation)

Parameter	**Measuring**	**Sample taking point**	**σ**
BOD_5	5 day by Oxi-Top equipment	- Influent: each 10 days - Effluent: weekly	1÷5%
COD	Hach Lange Cuvette test	- Influent: each 10 days - Effluent: weekly	1000mg/l ± 40 200mg/l ± 40
NO_3^--N	Hach Lange Cuvette test	- Influent: each 10 days - Effluent: weekly	± 0.4-0.5 mg/l
NH_4^+-N	Hach Lange cuvette test kit	- Influent: each 10 days - Effluent: weekly	± 1.5-2 mg/l
O_2	HQ 40D equipment	- Influent: each 10 days - Effluent: weekly - At different parts of setup: . Algal- duckweed experiments: *n=4* . Baffled algal reactors: *n=10*	± 1-10%
pH	HQ 40D equipment	- Influent: each 10 days - Effluent: weekly	± 1-10%
TN	Cuvette test, Shimadzu TOC/V (for sludge)	- Influent: each 10 days - Effluent: weekly - Algal sludge: every 3 weeks	20mg/l ± 2
TOC	Shimadzu TOC/V (for sludge)	- Algal sludge: every 3 weeks	By factory and technical
TKN	Hach Lange cuvette test kit Kjeldahl	- Influent: each 10 days - Effluent: weekly - Algal sludge: every 3 weeks	± 5.5-6mg/l By technical

2. ANALYTICAL MEHTODS

2.1 Oxygen concentration, pH media and temperature in wastewater

The oxygen concentration, dissolved oxygen, pH and temperature in the reactors and samples were measured by electronic equipment HQ40D with equivalence sensors.

2.2 Chemical Oxygen Demand (COD)

According to ISO 15705, COD is the volume of oxygen equivalent to the mass of potassium dichromate that reacts with the oxidisable substances in the water under the working conditions of the method. COD is measured by adding 2ml of fresh sample into the COD cuvette. The reaction time is about 15 minutes of cooking time at 170°C by Dr. LANGE HT200S (in reality, it needed two hours from heating up to cooling down the cuvette).

The sample must be homogenized before the analysis is performed. The digestion solution of COD cuvette was prepared by adding $K_2Cr_2O_7$ and concentrated H_2SO_4. Colorimetric measurements of COD were made using Photometer DR5000 with different wavelengths (348, 448 and 605nm) for the different ranges of COD from 15 to 1.000 mg/l. The filtrated COD with membrane filter with size 0.45µm (Millipore) was used to calculate the fractions of COD.

2.3 Ammonium nitrogen (NH_4^+-N), nitrate- nitrogen (NO_3^--N)

Ammonium-nitrogen (NH_4^+-N) and Nitrate-nitrogen (NO_3^--N) concentration were determined by using the Hach Lange DR5000 Spectrophotometer and adequate cuvette test kits for NH_4^+-N: LCK 302, 302, 305, 304, and LCK 339 (Hach-Lange 1989; DIN 38 406 E5; DIN 38 405 D9) for NO_3^--N.

2.3.1 Ammonium nitrogen (NH_4^+-N)

According to DIN 38406 E5, the principle to determining ammonium nitrogen in wastewater by using Hach Lange Cuvette test is dependent on the rate of reaction of Ammonium ions at the pH value of 12.6 with hypochlorite ions (created in an alkaline medium by hydrolysis of dichloroisocyanuric acid ions) and salicylate ions in the presence of sodium prusside-sodium as a catalyst for the blue dye indophenol blue.

A filtered sample about 0.2–5 ml in volume, filtered via a membrane filter (0.45µm), is added to the cuvette and allowed to react for 15 minutes. The test result is measuring by DR5000

Spectrophotometer Hach-Lange at wavelengths 550-694nm to obtain the ammonium-nitrogen concentration in the wastewater.

2.3.2 Nitrate- nitrogen (NO_3^--N)

The analysis principle is based on the reductant of nitrate to nitrite by added hydrazine. The nitrite then undergoes diazotization with sulphanilamide and azo coupling with N-naphtyl-ethylendiamindihydrochlorid (NED) which is measured photometrically at 370-546 nm.

2.4 Total nitrogen (TN) and total organic carbon (TOC)

2.4.1 Total nitrogen

The content of total nitrogen (TN) was determined by using the automatic analysis machine (Shimadzu TOC/V). Carrier gas (purified air) is passed at a controlled flow rate of 150 ml/min through a combustion tube that is filled with thermal decomposition catalyst and heated to 720°C. When the sample pre-treatment/injection system injects the sample into the combustion tube, the TN in the sample thermally decomposes to create nitrogen monoxide.

The carrier gas carrying the nitrogen monoxide from the combustion tube is cooled and dehumidified in the dehumidifier before passing into a chemiluminescence detector, where the nitrogen monoxide is detected. The chemiluminescence detector utilizes the gas-phase chemiluminescence of ozone and nitrogen monoxide, such that the detected nitrogen monoxide analogue signal forms a peak. To measure the TN concentration of the sample, the relationship between the TN concentration and peak area (calibration curve) is predetermined using a TN standard solution, to express the peak area as a ratio of the TN concentration.

2.4.2 Total organic carbon

Total organic carbon (TOC) is the sum of organically bound carbon present in the water sample, associated with dissolved or suspended matter (European Committee for Standardization 1997; DIN EN 1484). The content of TOC was determined by machine Shimadzu TOC/V. Carrier gas (purified air) is passed at a controlled flow rate of 150 ml/min through an oxidation-catalyst-filled total carbon combustion tube, heated to 680°C.

When the sample pre-treatment/injection system injects the sample into the combustion tube, the total carbon in the sample is oxidized or decomposed to create carbon dioxide. The carrier gas carrying the combustion products from the combustion tube is cooled and dehumidified in the dehumidifier before passing via the halogen scrubber into the sample cell of the non-dispersive infrared detector (NDIR), where the carbon dioxide is detected.

The NDIR analogue signal forms a peak, and the data processor calculates the peak area. To measure the TC concentration of the sample, the relationship between the total carbon concentration and peak area (calibration curve) is predetermined using a total carbon standard solution, to express the peak area as a ratio of the TC concentration.

2.5 Biological oxygen demands measurement (BOD_5)

BOD_5 is the biochemical oxygen demand over 5 days. It was determined by the OXiTop equipment (DIN 38 409 H52). The sample was incubated in an amber flask, with the addition of sodium hydroxide (NaOH) for 5 days at 20°C. Oxygen is converted to CO_2 by the microorganisms, and this is removed from the gas phase through reaction with NaOH, which results in a pressure drop that is proportional to the amount of oxygen consumed**.** The resulting BOD_5 is obtained from the BOD_5 determination by OxiTop® (WTW) remote control.

2.6 Total Kjeldahl nitrogen (TKN)

In the presence of H_2SO_4, potassium sulphate (K_2SO_4), and cupric sulphate ($CuSO_4$) catalyst, amino nitrogen of many organic materials is converted to ammonium. Free ammonia also is converted to ammonium. After addition of a base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulphuric acid. The ammonia may be determined colorimetrically, by an ammonia-selective electrode, or by titration with a standard mineral acid. The samples were analysed using traditional Kjeldahl distillation apparatus in the SiWAWI laboratory using Kjeldahl flasks of volume 50-250 ml.

2.7 Algal chlorophyll-α content determination

Chlorophyll-α content is bound within the living cells of algae and other phytoplankton that are found in surface water. Chlorophyll-α content is a key biochemical component. Adapted the APHA (2005), the photosynthetic pigments enable algae to harvest light energy. Chlorophyll-a content (Chl-α or a_{chl}) is the preferred indicator for algal biomass quantification since it comprises 1 to 2% of the dry-weight of all algae species. The other types of chlorophyll like Chl-b, c, d, and e are the accessory pigments that can augment light adsorption. In general, several sources of bias surrounded Chl-α analysis. Firstly, Chl-α content varies depending on specie's physiology and abiotic factors (e.g., climate), which can result in perceived algal biomass differences among different microbial communities.

Secondly, chlorophylls degrade into phaeopigments as communities age and cells die. Chl-α content measurements must be corrected for these phaeopigments since they have the same absorption peak. Thirdly, sample preparation and the extraction solvent used impact the results significantly (Biggs and Kilroy 2000; Ritchie 2006; Schagerl and Künzl 2007).

Chlorophylls degrade into phaeopigments as communities age and cells die. Chl-α content measurements must be corrected for these phaeopigments since they have the same absorption peak. Thirdly, sample preparation and extraction solvent used significantly impact results (Biggs and Kilroy 2000; Ritchie 2006; Schagerl and Künzl 2007). Several methods can be used to determine algal growth biomass, such as the measurement of biomass by manual cell counting by microscope, an electronic particle counter or by calculating the fresh/drying weight of filtered algal mixed culture.

Alternative techniques, such as the *in vitro* Chlorophyll-α content fluorescence can be used to provide a satisfactory correlation with biomass (O.E.C.D 1984; DIN 38 412 Part 16, Dec. 1985; ISO/WD20079 DIN AK "Biotese"; Wentworth *et al.* 2003; Kruskopf and Flynn 2005) and therefore, the growth rate of algae identified. In this study, the photometric analysis method is used to determine chlorophyll-a content. The process is based on the appropriate standard DIN (38 412 L16):

$$\text{Chlorophyll-}\alpha\ (\mu g/l) = 29.6 \times (E_v - E_n) \times \frac{v}{V \times d}$$

Where:

E_v: specphotometer wavelength 665nm (before acidification with HCl 2 mol/l)
E_n: specphotometer wavelength 665nm (after acidification with HCl 2 mol/l)
v : volume of sample extraction (ml)
V : volume of measuring sample (ml)
d : light path of cuvette (cm)

The growth population rate of algae is defined as:

$$\mu = t^{-1} \times \ln(N_t / N_0) \quad \text{(unit: day}^{-1}\text{)}$$

Where:

N_0: biomass existing at the beginning of the test
N_t: Biomass after the incubation time
t = 1 day

2.8 Validation of cuvette tests for drinking water analysis

The cuvette tests are used for the determination of ammonium, nitrate and nitrite, COD, TOC, Total nitrogen satisfy the requirements of the 2001 Drinking Water Ordinance with regard to the performance characteristics trueness, precision and limit of detection. The performance characteristics are all below 10%. (DIN EN ISO/IEC 17025: 2005-08; ISO/IEC 17025:2005).

3. EXPERIMENTAL IMPLEMENTATIONS.

Short description of Schönerlinde wastewater treatment plant

Pre-settled wastewater from the Schönerlinde wastewater treatment plant is used to feed the experiments in this research. Therefore, a brief description of the plant is provided to better understanding of the idea behind this research. The WWTP has mechanical and biological treatments mechanisms together with biological phosphate removal in combination with nitrification and denitrification. (sources: Berliner Wasserbetriebe - BWB).

- Mechanical: cleaning capacity in dry weather conditions is 105.000 m^3/day.

- Biological treatment: eight pools are anaerobic zones; fourteen basins are anoxic and aerobic zones, total useful capacities are 11.900 and 119.750 m^3.

- Five bubble aeration are the membrane diffusers. Twelve tanks have a total capacity of 42.660 m^3 and two circular tanks with total useful volume of 10.370 m^3.

- Sludge treatment: four digesters with a total capacity are 32.000 m^3. Three centrifuges for dewatering of sludge and, three drying lines.

- Biogas utilization: two bells gas tank with a storage capacity of 5.000. The biogas is used for heating the sludge, electric power generation (CHP), heating the building and supplying hot water to the wastewater treatment plant.

3.1 Algal and duckweed experiments

Several authors have proved that algal or duckweed wastewater stabilization ponds can effectively remove nitrogen and other toxic substances. Algal/duckweed wastewater stabilization ponds have many benefits such as low construction, operational and maintenance costs. The most important role of algal/duckweed wastewater stabilization ponds is the potential for improving nitrogen removal by increasing biochemical processes. This can be achieved through the nitrification and denitrification processes. Moreover, hydraulic patterns

in ponds with aquatic plants, algae and/or duckweed can significantly impact the rate of biochemical processes.

In the past, many studies on nitrogen removal by algae and duckweed have suggested that nitrification and denitrification are important processes in total nitrogen removal (Caicedo 2005). The author has also suggested that introducing algal and duckweed ponds can improve nitrogen removal. This is due to the fact that algae and duckweed can produce oxygen via photosynthetic mechanisms, which favours the nitrification process. It is also known that algae can take up ammonium directly (Mostert and Grobbelaar 1987; Graham and Wilcox 2000). Thus it makes the ideal method to treat wastewater in several countries because it is relatively cheap, simple to use and stable.

This study will provide knowledge on improving ammonia nitrogen removal by understanding upwards and downwards flow hydraulic patterns in algae and duckweed systems. It is considered as a sustainable concept for developing another experimental module for improving the efficiency of ammonia nitrogen removal from wastewater by using algal material.

Experiments will be performed in order to understand the processes involved and to establish nitrification and dentrifications rate constants of modified wastewater stabilization ponds. The aim of this research is to investigate the process of nitrification and denitrification in algal experiments with the purpose of improving these processes. The Fig.18 and 19 describe the algal and duckweed experiments implementations.

The research will also investigate the efficiency of removal of ammonia under the temperature conditions between 11-16^{o}C. The research had been done with municipal wastewater without settle-able particles taken from the effluent of primary treatment unit of Schönerlinder wastewater treatment plant of Berlin.

In the laboratory's condition, intermittent light simulations were set up to provide light to the algal and duckweed experiments. The total volume of each experiment is 135 litres. Flow rate discharge was around 15 l/d per setup; HRT was 10-11 days. Intermittent artificial light for day/night was 12:12 hours. The three wastewater experiments had no aeration system, therefore the oxygen concentration was completely generated by the algal and duckweed via photosynthesis and it is consumed by respiration process. Carbon dioxide (CO_2) was not added.

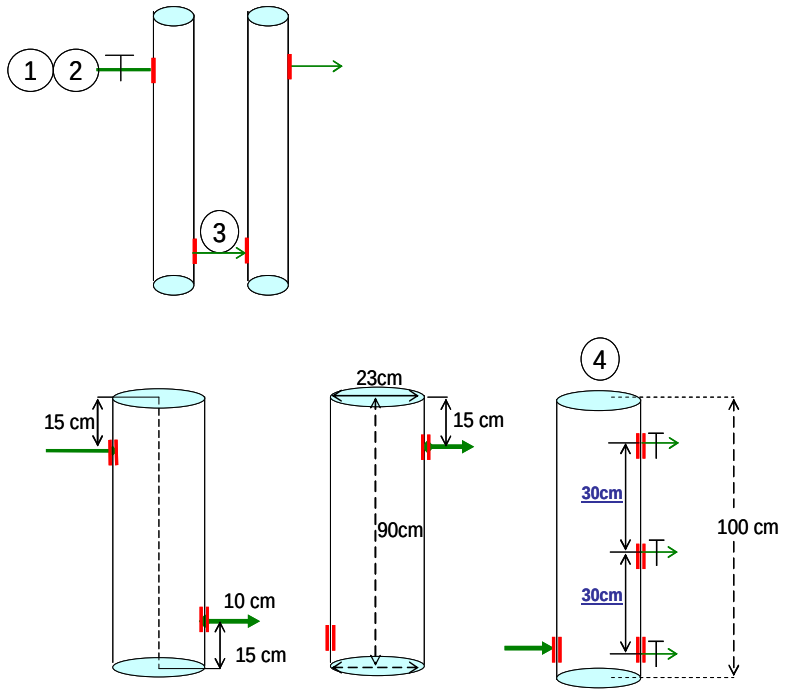
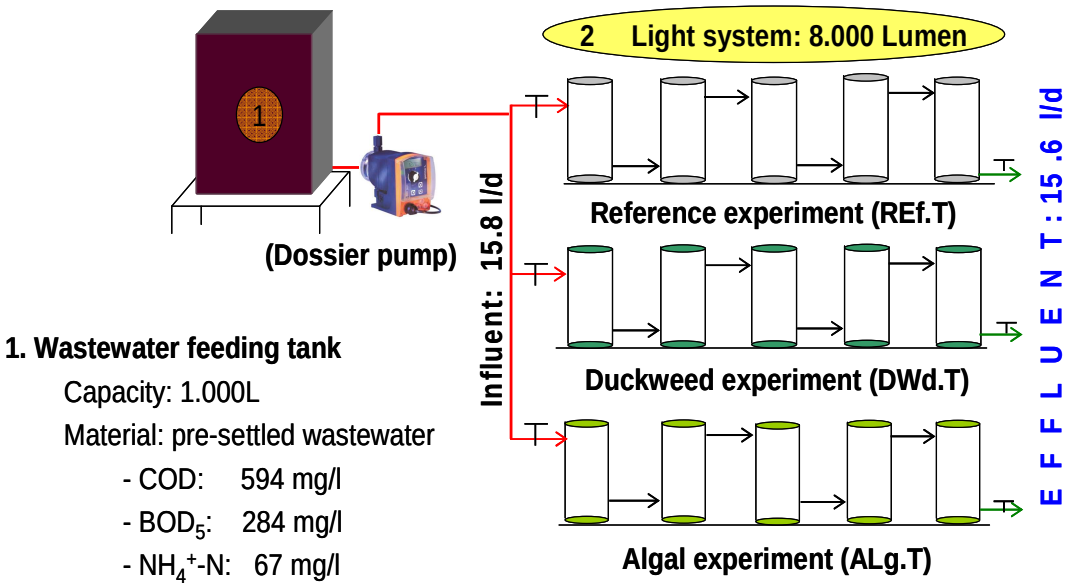


Figure 18. The detail of processing unit and dimension implementation of the Algal and Duckweed experiments



1. Wastewater feeding tank

Capacity: 1.000L

Material: pre-settled wastewater

- COD: 594 mg/l
- BOD_5: 284 mg/l
- NH_4^+-N: 67 mg/l

2. Algal, Duckweed and Reference experiments:

100 cm height; 5 units; total volume: 135 l; HRT: 10-11 days. Light provide: 8.000 lumen.

Figure 19. The implementation of the Algal and Duckweed experiments

3.2 Baffled algal reactors

The baffled algal reactors setup had been implemented. The modified setup for the study was based on the design of the process model developed by Wuhrmann, Ludzack-Ettinger, and Modified Ludzack-Ettinger process-MLE as cited by WEF (1998) for single sludge nitrogen removal, carbon oxidation and nitrification system.

The expected result of this research is that the combined-modified system shows interesting advantages of the system in comparison with the conventional process such as air supplies, which enhanced residual substance treatment by returning treated water one more time to the main reactors. The system can achieve high removal efficiency of ammonia nitrogen in order of 86-90%.

Moreover, the baffled reactor provides a significant reduction of COD and BOD_5 from influent. It also shows an interesting advantage in comparison with other systems, such as the increasing residual substance treatment. The system can practically operate under any condition (plug-flow or contact-stabilization). The advantages of the method are:

- Ease and flexible construction for wastewater reactor.
- Providing more uniform oxygen supply in the treatment reactor while lowering the peak demand to obtain a high rate of oxidation of substances.
- Allowing operational control of hydraulic residence time and increasing internal flow velocity.
- Considerably reducing the size of treatment reactor, increasing hydraulic retention time and treatment reactor’s capacity.

The experiment was established in three algal treatment reactors: two setups for the baffle's contributions in different design (upward and downward flow: **T1**; sideward flow: **T2**), while the last setup was a Reference treatment reactor (without baffles: **T3**). All designs were combined with continuous flow.

The system was operated for a period of 180-200 days. The research had been done with municipal wastewater without settle-able particles taken from the effluent of a primary treatment unit of a wastewater treatment plant in Berlin. The principle of this study was applied on an existing small–scale laboratory system, using raw wastewater without settle-able particles discharging directly into the reactors. With the purpose of increasing the

efficiency of substance removal from wastewater, a recycling line was used in order to obtain an optimal efficiency of ammonia nitrogen, COD, BOD removal, which won't be possible to obtain by using the traditional methods due to the fact that conventional nitrogen removal process require a large-scale renovation of existing facilities.

Therefore, in the laboratory conditions, artificial lights (intermittent light simulations 12:12 hours and 6:18 hours for simulating day/night) were set up to provide light to the setups for 12 hours and 6 hours per day at the different time-periods of the study.

The total volume of one reactor (plus collector) is 250 litres with usable volume of 220 litres. The flow rate discharge per each treatment reactor is 22 l/d; HRT varied between 10 and 11 days. Every three weeks, the algal sludge was removed from the reactors to keep the efficiency of ammonia nitrogen removal rate constant.

Weekly grabbed samples collected from effluent for analyses of COD, BOD_5, nitrate, ammonia nitrogen, TKN were taken for both setups (algal/duckweed experiments, baffled algal reactors) to evaluate the trend of removal substance in the experiments.

Total nitrogen (TN) and total organic carbon (TOC/NPOC) in the algal sludge and fresh algal have been analysed monthly (every 3 weeks) for each of the baffled algal reactors during the period of study using Shimadzu TOC/V. As for ammonia concentration, there were a few reductions of concentration before and after filling the feeding tank. The set-up of algal baffled reactors is given in the Fig. 20-21 below:

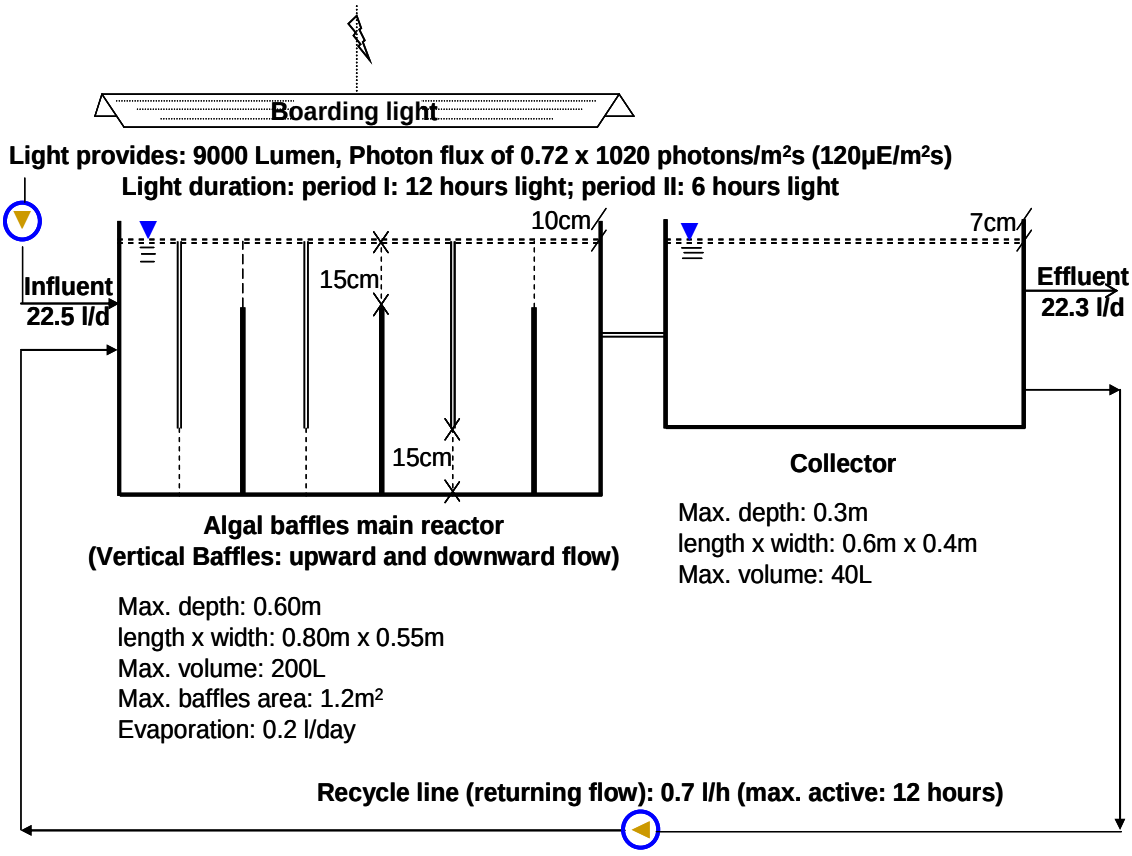


Figure 20. The schematic of BARs model with recycle line

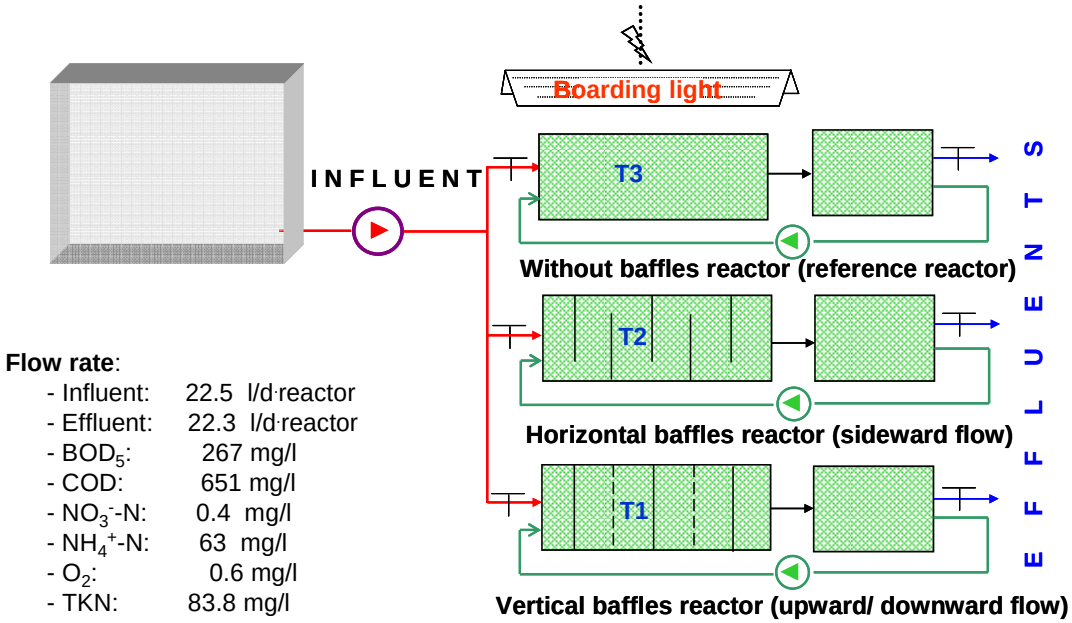


Figure 21. The implementations of the BARs with recycle line

Chapter IV.
EXPERIMENTAL RESULTS AND DICUSSIONS

The results are presented in two sections. The first section deals with the description and discussion of Algal and Duckweed experimental results from laboratory. The second section describes the Baffled Algal Reactors (**BARs**) to improve the efficiency of ammonia nitrogen removal from wastewater and to obtain high rates of biological processes in the reactors. This would provide a deeper understanding of the role that an algal treatment reactor plays in the nutrient removal.

Firstly, the Algal and Duckweed experiments were implemented. This part provides the information for the comparison of the performance of ammonia removal treatment between algae and duckweed experiments. Importantly, these experiments can give an overview about the function of algae and duckweed for treating municipal wastewater, and to answer the question of the occurrence of the nitrification and denitrification processes in these setups.

Secondly, the **BARs** were constructed. The principles of the experimental implementation are based on the results of the first experiment. This investigation evaluated ammonia nitrogen removal in **BARs** comprising laboratory-scale reactors on adding recycling lines and different designs of baffles in the reactors. The aims of the research were to promote the algal reactor's practice for wastewater treatment by increasing ammonia nitrogen and organic carbon removal efficiency through the use of baffles which increase the biofilm biomass concentrations.

The designs started with Shilton's models tracer test to determine the characteristics of each reactor. Based on the considerations, the algal-duckweed experiments and the baffled algal reactors were opened for ammonia nitrogen removal from municipal raw wastewater without settle-able particles. Weekly grabbed samples were collected at the effluent of the

experiments and analysed in order to observe the concentration trend throughout the week of treatment. All samples were collected during the day meaning that no samples were collected at night. The flowing rates (in/out) and recycling flow of baffled algal reactors are controlled by automatic dossier pumps.

About 41 samples (included influents and effluents) of the algal and duckweed experiments were collected from June 2010 to April 2011. About 52 grab samples were collected from effluent of baffled algal reactors from December 2011 to April 2013 (included influents and effluents of the reactors). The extra samples were collected at 0, 5, and 10 days to measure the changes in the concentrations in the feeding tank over time. The oxygen concentration and pH measurements were taken at 20, 40 and 60 cm from the inlet of the reactors for making the oxygen and pH profiles.

1. ALGAL AND DUCKWEED EXPERIMENTS

1.1 Calculation methods

The method used to calculate the loading rate and removal efficiency were based on simple equations shown below:

$$\text{Loading rate} = \frac{Q_{in} \; x \; C_{in}}{V} \; (g/m^3 \cdot d)$$

Where:

- Q_{in}: inflow (m^3/d)
- C_{in}: concentration of substance influent (g/l)
- V: volume of experiment (or reactor): m^3

$$\text{Removal rate} = \frac{(Q_{in} \times C_{in}) - (Q_{out} \times C_{out})}{V} \; (g/m^3 \cdot d)$$

Where:

- Q_{out}: outflow (m^3/d)
- C_{out}: concentration of substance at the effluent (g/l)
- V: volume of experiment (or reactor): m^3

$$\text{Removal efficiency (\%)} = \frac{(Q_{in} \times C_{in}) - (Q_{out} \times C_{out})}{(Q_{in} \times C_{in})} \times 100$$

Note: Q_{in} is different from Q_{out} due to a small evaporation volume (as described in the table 7).

1.2 Operating conditions

Table 7 below shows the operating conditions to carry out ammonia nitrogen removal by using algal and duckweed setups. The characteristics of each experiment included: 5 processing units with a capacity of 135 litres. Artificial light at an intensity of 8.000 lumen was provided. The intermitted light simulation was dark/light phase: 12-hour:12-hour. The observation of the water evaporation was done by monitoring the water levels in both the main reactor and the collector via metric rulers.

Table 7. Operating conditions for algal and duckweed experiments.

	Quantification
Flowing rate: - Influent - Effluent	15.80 l/d 15.60 l/d
Water evaporation from experiment	0.20 l/d
Experiment:	3 experiments
- Dimension	H: 100cm, D: 23cm; Unit: 5 columns
- Capacity (each experiment)	135 litres
- Effective capacity	132 litres
Hydraulic retention time	10 -11 days
Artificial intermitted light simulation: 12:12 light/dark	Photon flux of 0.72 x 1020 photons/m^2s (120μE/m^2s) yielding 8.000 lumen Spectral range 400-700 nm
Wastewater discharge type	Pre-settled wastewater
Temperature on surface of the experiments	22^{o}C (on average)
Testing period	180 days
Starting up of system	1 week

1.3 Experimental results

Table 8 describes the average values of wastewater influent and water quality outflow of each experiment. In total 41 samples were collected from the experiments.

As can be seen from the tables showing the obtained results, the concentration of NH_4^+-N, COD and BOD_5 influent to the treatment reactors were very high. Meanwhile, the oxygen concentration had a significantly low value of 0.7mg/l. The highest removal rates of BOD_5 of the algal and duckweed experiments achieved were around 93% of total BOD_5 influent. In contrast, the reference experiment removed approximately 76% total COD from the inflow.

Table 8. Wastewater influent and effluent characteristics (*n= 41*)

	Influent Concentration (mg/l)	Load g/(m^3·d)	Effluent concentration (mg/l)			Removal g/(m^3·d) Removal efficiency (%)		
			ALg.T	DWd.T	REf.T	ALg.T	DWd.T	REf.T
BOD_5	**284**	**33**	**20**	**21**	**87**	**31** (93%)	**31** (93%)	**23** (70%)
Min	*235*		*6*	*9*	*15*	*84 %*	*82%*	*36%*
Max	*434*		*62*	*70*	*198*	*97 %*	*97%*	*94%*
COD	**594**	**70**	**82**	**57**	**142**	**60** (86%)	**63** (91%)	**53** (76%)
Min	*372*		*44*	*33*	*69*	*74%*	*72%*	*39%*
Max	*780*		*143*	*124*	*228*	*93%*	*96%*	*89%*
O_2	**0.7**		**5**	**5**	**1**	**+ 0.5**	**+ 0.5**	**+ 0.01**
Min	*0.2*		*4*	*3*	*0.1*			
Max	*2*		*6*	*6*	*2*			
TKN	83.80							
NH_4^+-N	**67**	**8**	**18**	**22**	**58**	**6** (73%)	**5** (69%)	**1** (14%)
Min	*38*		*7*	*9*	*30*	*39%*	*33%*	*0.1%*
Max	*86*		*37*	*38*	*83*	*91%*	*86 %*	*24 %*
NO_3^--N	**0.32**		**2**	**5**	**0.4**	**+ 0.2**	**+ 0.6**	**+ 0.01**
Min	*0.1*		*0.2*	*0.2*	*0.2*			
Max	*0.5*		*7*	*18*	*1.4*			
pH	**7.3**		**7.9**	**7.7**	**7.6**			

Note:

- **REf.T***: Reference experiment*
- **ALg.T***: Algal experiment*
- **Dwd.T***: Duckweed experiment*
- +: *increase*

1.3.1 COD

The measurements of COD at the influent and the effluent of the three experiments at the different measurement times are shown in the Fig. 22. The average COD measured at the influent was 594 mg/l. The average value of COD was 82 mg/l (min: 44 mg/l, max: 143 mg/l) at the effluence of the algal and 57 mg COD/l at the effluence of the duckweed experiment (min: 33 mg/l, max: 124 mg/l). The COD decreased about 86% in the algal and 91% in the duckweed treatment, whereas the COD load was approximately 70 g COD/(m^3·d).

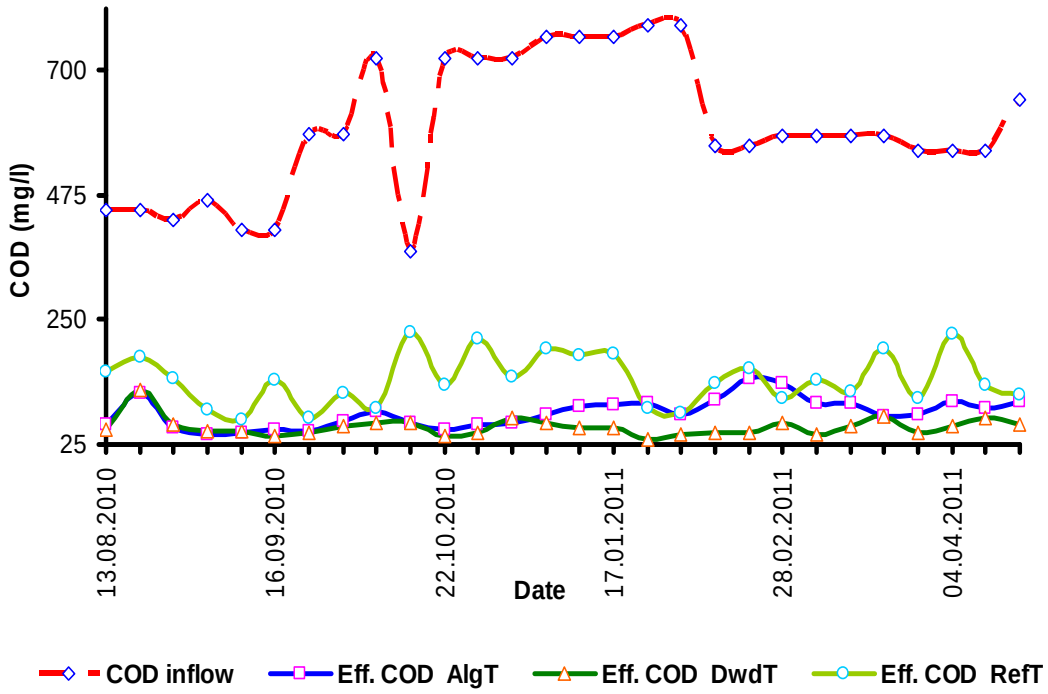


Figure 22. COD measured in the influent and at the effluent of the experiments

- COD removal efficiency

The COD decreased sharply at the effluence of the algal and duckweed experiments, as shown by the R^2 values of 0.96 and 0.98 for algal and duckweed experiments respectively in the Fig. 23. The trend of $\log_{10}$ was used to provide a better comparison of the evaluated results and theoretical result as opposed to the linear or exponential trend. When the COD - load was around 85->95 g/(m^3·d), the highest removal efficiency of the treatments were achieved (approx. 86-91% of total COD from influent). The result was in agreement with Zimmo (2003), Zimmo *et al.* (2002; 2004) and Moez Bouali *et al.* (2012), who reported over 75-90% COD removal by algal and duckweed reactors. Using algae and duckweed for treating wastewater had been reported in the literature. Mandi (1994) demonstrated that some species of duckweed (*Lemna gibba)* could be used in primary treatment of wastewater with COD

from 305-530 mg/l, but can not grow on undiluted domestic wastewater or industrial wastewater with COD of 1400-1700 mg/l. Also, Copelli *et al.* (1982) found that the maximum COD in water tolerated by duckweed ranges from 300 to 500 mg/l. Al-Nozaily *et al.* (2001) used a batch experiment with algae and supposed that with surface loading more than 800 kg filtered COD/ha, oxygen supply becomes the limiting factor for COD removal.

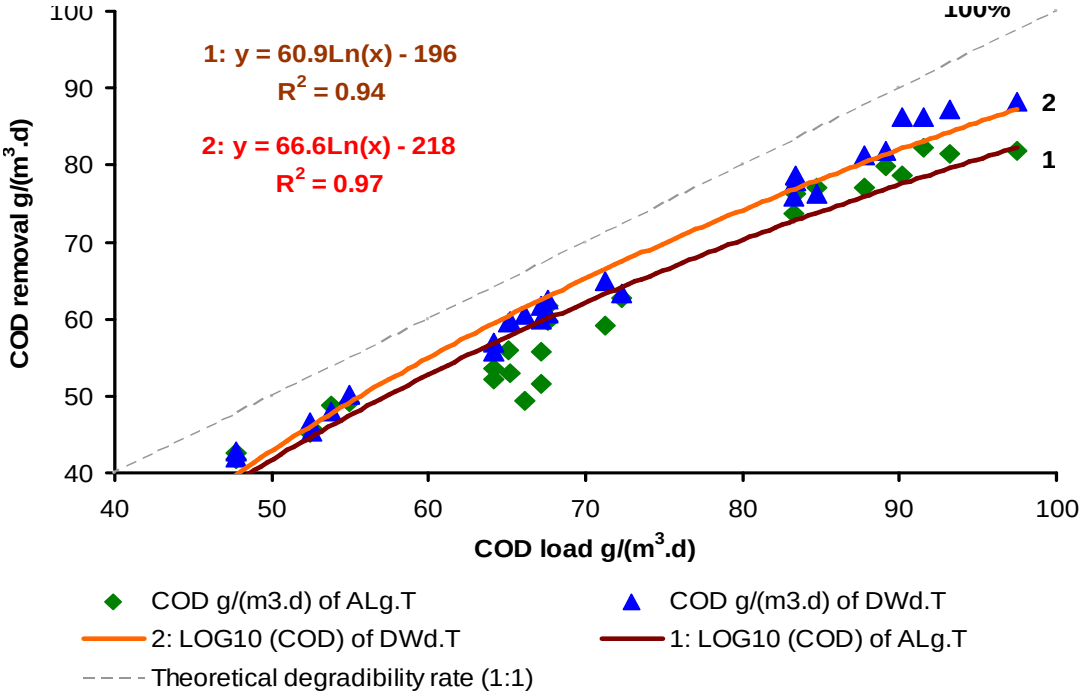


Figure 23. Comparison of COD removal with COD load into 1: Algal; 2: Duckweed experiment

The comparison of COD removal efficiency of algal/duckweed colonies developing under strong COD influent proved that these (*Lemna minor sp.*) experiments show a higher organic-matter-removal-efficiency in comparison with the reviewed literature. Under high load of COD (maximum COD observation was 780 mg/l), algae and duckweed displayed a good removal capacity for organic substances. High ammonia concentration is another factor in wastewater related to limiting the growth of algae and duckweed, as listed in the literature (Caicedo *et al.* 2000; Clement and Merlin 1995; Wang 1991). The results obtained from this research were lower than those observed using other technologies to remove COD from wastewater such as activated sludge process >95%, but is in the range of the processes such as low rate trickling filter and rotating biological contactors with approximately 90% COD removed from effluent (von Sperling 2005; DWA 2011). In comparison with other natural systems, the results of this research are higher than constructed wetland, facultative pond, as can see in the table 9 below:

Table 9. Comparison of COD removal efficiency by the algal and duckweed experiments with another process

Removal efficiency % COD		System
von Sperling *et al.* (2005)	65-80	Facultative pond
	75-80	Constructed wetland
	65-80	Facultative aerated lagoon
Nurdogan and Oswald (1995)	90	High rate algal pond
Alaerts *et al.* (1996)	90-97	Duckweed covered lagoon
DWA 2011	98	Advanced activated sludge process
Own study	86	Algal experiment
	91	Duckweed experiment

1.3.2 BOD_5

The average BOD_5 mass loading of the system from feeding tanks was 284 mg BOD_5/(l·d). In algal experiments, the effluent BOD_5 was 20 mg BOD_5/l (min: 6 mg/l, max: 62 mg/l) and 21 mg BOD_5/l was detected in duckweed experiment (minimum value of 9 mg/l, maximum: 70 mg/l). Meanwhile, the reference treatment achieved only 86.7 mg BOD_5/l during the entire length of study (Fig. 24).

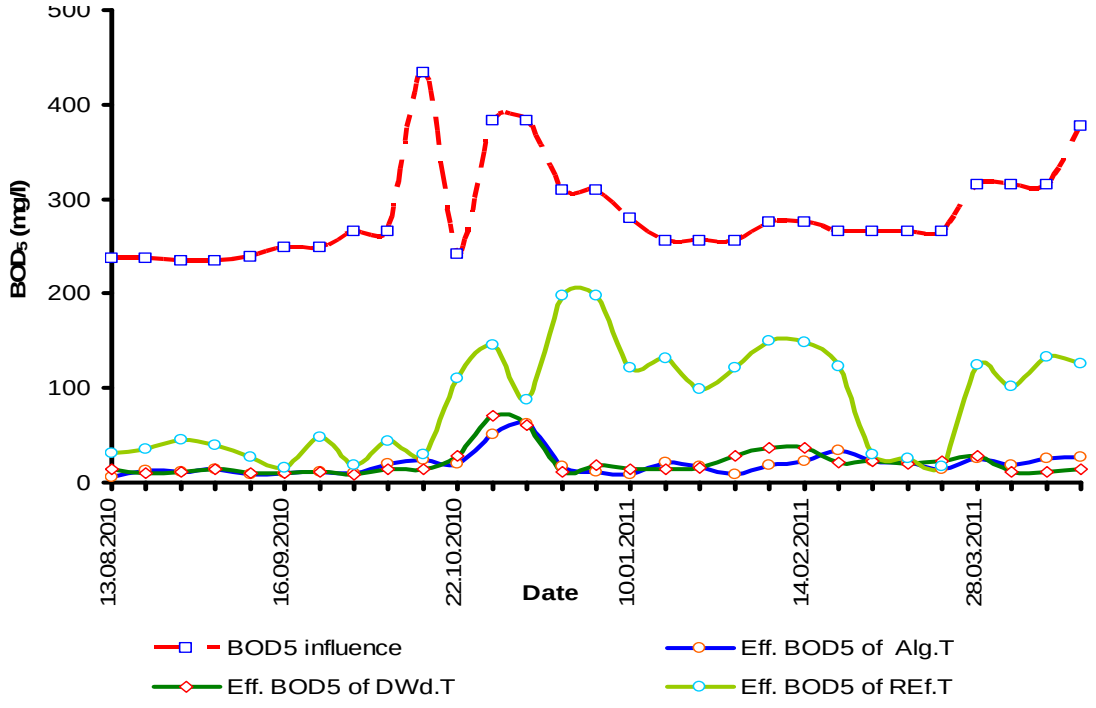


Figure 24. BOD_5 measured in the influent and at the effluent of the experiments

The results showed the performances of algal and duckweed in the systems works well with respect to the BOD_5 removal. The effluent BOD_5 concentration showed that the high concentration of organic matter removal occurred in the system due to contributions by algae and duckweed. Comparison of algal and duckweed treatment revealed that there were no significant differences between the two systems on the BOD_5 removal efficiency.

In the reference treatment system without algae or duckweed, there is a BOD_5 removal efficiency of only 70% of total BOD_5 influent, when compared to 93% BOD_5 removed by the experiments with algal and duckweed present.

The absence of algae or duckweed in the reference experiment led to a decreased dissolved oxygen concentration. In the duckweed experiment, high oxygen concentrations are produced due to the dense cover of duckweed, which may also reduce oxygen diffusion from the water to the air.

- BOD_5 removal efficiency

Based on the results of BOD_5 measurement in the effluents of the algal and duckweed experiments, it could indicate the high BOD_5 removed efficiency from the influent, with correlation factors of $R^2 = 0.98$ (shows in the Fig. 25). The linear trend was used to evaluate the result. The trend presents a better comparison between the evaluated result with the theoretical results than the $\log_{10}$ or exponential trend..

Highest removal efficiency was recorded in both experiments when the load of BOD_5 was around 30-<60 $g/(m^3 \cdot d)$. There was no observation value when BOD_5 load was over 60 $g/(m^3 \cdot d)$. The decrease in BOD_5 concentration was generally consistent with the oxygen production and ammonia reduction in setups.

Adapting several researches on BOD_5 removal by using algae and duckweed in the past, the results of this study has similar findings to these past researches and could indicate that the system had high effectiveness of BOD_5 removal or even more efficiency of nitrogen removal than other systems. This is because the experiment had high loading rate of substances and no mechanical or electrical techniques were applied to algal and duckweed setups.

Algal and duckweed experiments produced high oxygen concentrations. Hence, constant aerobic conditions remain at the surface of treatment basins. Therefore a high efficiency of BOD_5 removal can be achieved. However, the oxygen profile shows that the concentration depletes over the depth of the treatment basin.

This result is in agreement with Alaerts *et al.* (1996), where the surface load rate was 48-60 kg BOD_5/(ha·d), the concentration reduction was 90-97% for COD, 95-99% for BOD_5, and 74-77% for Kjeldahl-N and total phosphorus (aeration through the surface was 3-4 g O_2/m^2·d). Alaerts *et al.* (1996) studied the performance of duckweed in a full-scale duckweed-covered sewage lagoon in Bangladesh with a HRT of 20 days. Mandi (1994) reported a BOD_5 removal efficiency of 60-70% in a pilot plant, which was operated in *Lemna gibba* at a hydraulic retention time of about 7 days.

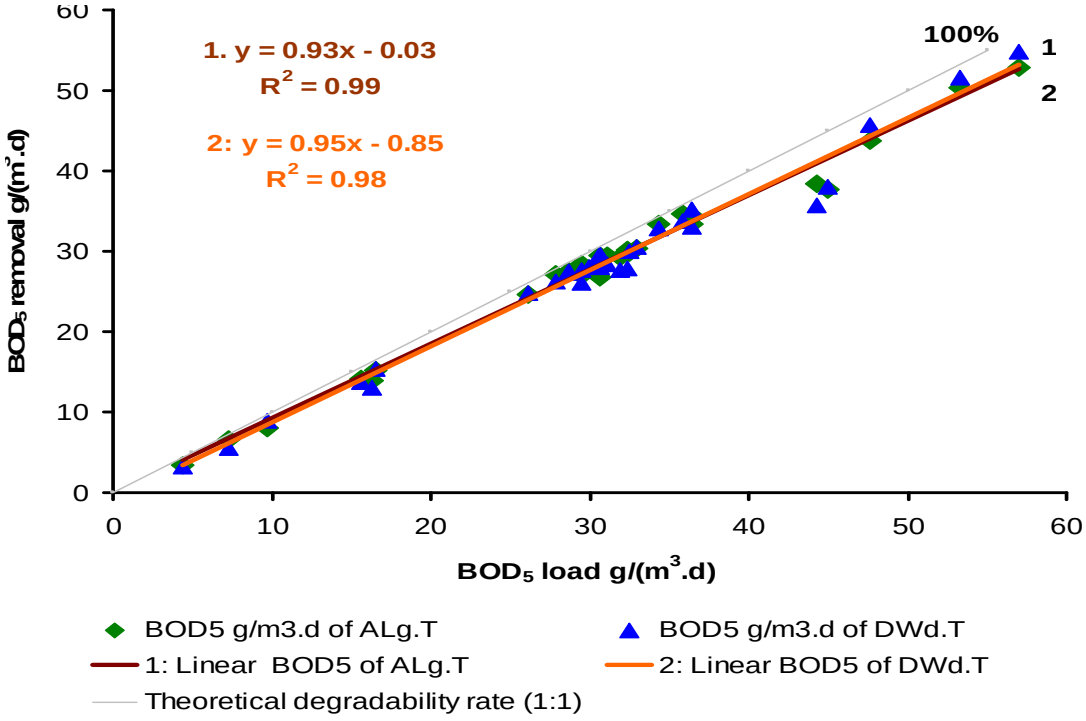


Figure 25. BOD_5 removal with BOD_5 load into 1: Algal; 2: Duckweed experiment

The BOD_5 degradation in the two experiments probably increases due to the growth of nitrifiers resulting from more attaching surface and high oxygen concentration. Muttamara and Puetpaiboon (1997), McLean *et al.* (2000) pointed out that ponds providing more attachment-surface lead to high ammonia removal rates. Zimmo (2003) prevailed that in facultative conditions, high BOD_5 removal efficiency can be achieved (46-50%).

It can be assumed that, BOD_5 removal efficiency of duckweed treatment was slightly lower than of algal treatment. It could be explained by duckweed communities developing and covering the whole surface of water. It then reduces and prevents light penetration into the treatment unit and hence reduces the photosynthetic production of oxygen by phytoplankton. The results of this study are in accordance with several results obtained from advanced

activated sludge process (DWA 2011), low rate trickling filter (von Sperling 2005), as can see in the table 10 below.

Table 10. Comparison of BOD_5 removal efficiencies by the algal and duckweed experiments with another result

Removal efficiency % BOD		System
von Sperling *et al.* (2005)	75-85	Facultative pond
	85-90	Constructed wetland
	75-85	Facultative aerated lagoon
	80-90	Low rate trickling filter
Alaerts *et al.* (1996)	95-99	Duckweed covered sewage lagoon
Grönlund (2004)	90	High rate agal ponds (HRAP)
DWA 2011	>98	Advanced activated sludge process
Own study	93	Algal experiment
	93	Duckweed experiment

1.3.3 Oxygen production

The oxygen created in the three experiments is shown in the Fig. 26. The maximum produced oxygen was recorded to be about 6.3 mg O_2/l (min: 4 mg/l) in the algal and 6.4 mg O_2/l (min: 3 mg/l) in the duckweed experiment (equivalent to 70-73% DO saturation).

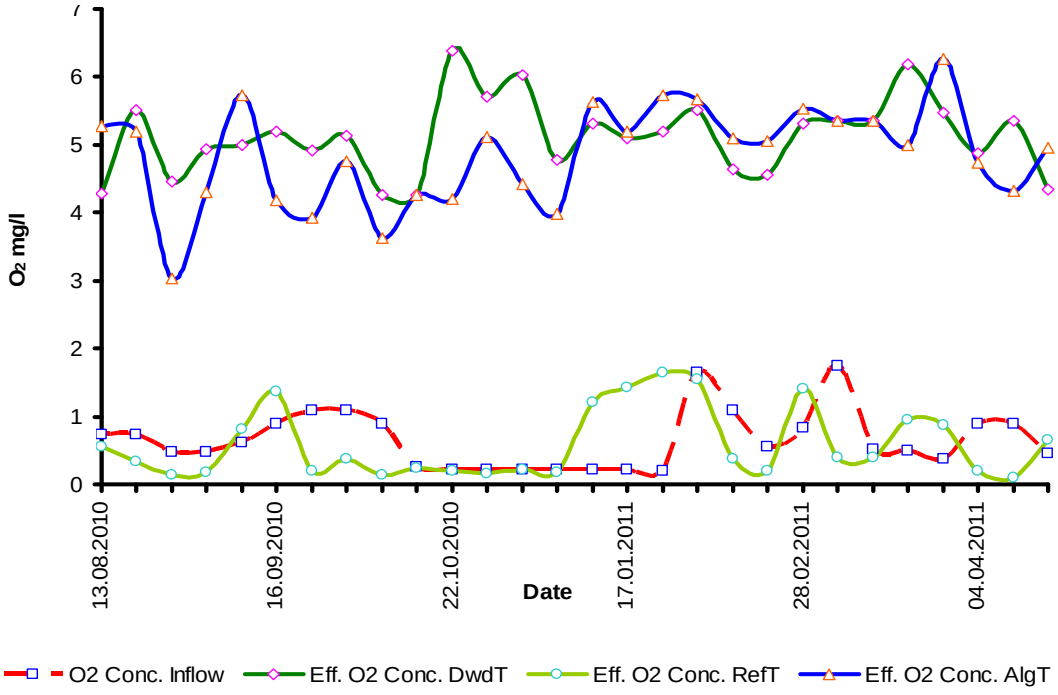


Figure 26. The oxygen concentration influent and effluent of the experiments

An average oxygen concentration created by the algal and duckweed experiments was approximately 5 mg O_2/l (the minimum value of the oxygen production in both treatments was: 3-4 mg/l). Oxygen producing rate of the algal and duckweed experiments was around 0.5 g O_2/(m^3·d). In contrast, the oxygen concentration in the discharge into system was at a significantly low value of 0.7 mg O_2/l. Oxygen production in treatments was high and it could maintain good conditions to support nitrifier bacteria growth, leading to an increase in the nitrification process. In the activated sludge process the requirement of oxygen was around 2 mg O_2/l to guarantee complete nitrification.

As the results show, the amount of oxygen concentration in the reference treatment was lowest because here, neither algae nor duckweed operated. It can be assumed that in the reference treatment, the oxygen concentration was not created. The effect of oxygen created in the experiments on ammonia nitrogen removal from wastewater will be discussed together with the results of ammonia nitrogen removal in ther section **1.3.4** of this chapter.

- Oxygen profile in the algal and duckweed experiments

For both experiments of algae and duckweed, oxygen concentrations show a decreasing trend from top to bottom of the processing unitsnits. The Fig. 27 describes the procedure for measuring the oxygen in an experiment.

Significant differences in oxygen concentrations were also found between the depths of 0.2 m, 0.5 m and 0.9 m within the same processing unit (height:100cm). The processing unit 1 is the first and processing unit 5 stands for the last unit where the sampling took place. The comparison between algal and duckweed experiments show that more oxygen was generated up to a depth of 0.2 m than at deeper layers.

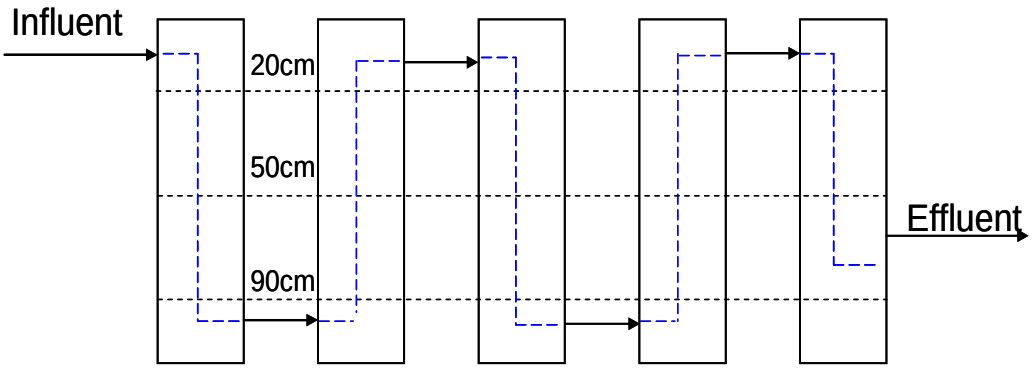


Figure 27. Schematic of the oxygen measurement in the sub-unit of the experiments

Oxygen concentrations in both algal and duckweed treatments decreased significantly with the distance from the water surface. In all experiments, oxygen concentration was close to zero at the lower 50 cm of the water column (processing unit 1 to 4). The results are shown in the table 11.

Table 11. The oxygen concentration measured at different depths of the processing unit (*n=4*)

Material	Processing unit	Oxygen concentration (O_2 mg/l)		
		20cm	50cm	90cm
Algae	1	0.48	0.30	0.23
	2	1.74	0.24	0.21
	3	2.03	0.26	0.16
	4	3.28	0.28	0.17
	5	4.09	0.33	0.17
Duckweed	1	1.23	0.38	0.19
	2	1.87	0.21	0.15
	3	2.34	0.24	0.13
	4	3.05	0.18	0.12
	5	3.89	0.76	0.17

At the last processing unit, the oxygen concentration is often higher than in the first unit due to an overload of substances in the first processing unit. Moreover, in the duckweed unit 1, oxygen concentration was higher than in the algal unit, because duckweed can produce more oxygen than algae.

It could be argued that, at certain times, oxygen concentrations at a depth of 0.2 m of the last two processing units of algal and duckweed experiments lead to good oxygen availability (approximately 4 mg O_2/l). Favourable conditions to support both nitrification and denitrification processes occur in this zone.

This result agrees with several researches about the oxygen created in treatments employing algae and duckweed. Zimmo (2003) reported that in the algal and duckweed based ponds, the oxygen at the surface of the basins was around 4-5 mg/l.

Alaerts *et al.* (1996) pointed that, average dissolved oxygen gradually increased along the surface of the lagoon's length from 2 to 4-5 mg/l, with the lagoon being constructed in a serpentine of 500 m length. Yeh *et al.* (2010) found out that algal growth in oxidation ponds could produce over 11 mg O_2/l at the temperature of 20-25^{o}C. Gutzeit (2006) indicated that in algal photobioreactor, the oxygen concentration can be approached more than 10 mg/l.

1.3.4 Nitrogen removal

- Ammonia nitrogen (*NH_4^+*-N mg/l)

The comparisons of NH_4^+-N concentrations and removal for the three setups are shown in the Fig. 28. The figure describes the trend of ammonium inflow and outflow of the experiments in different stages. At the first stage of treatment period, the ammonia concentration was probably higher due to the environmental adaption phases of algae and duckweed with wastewater discharges.

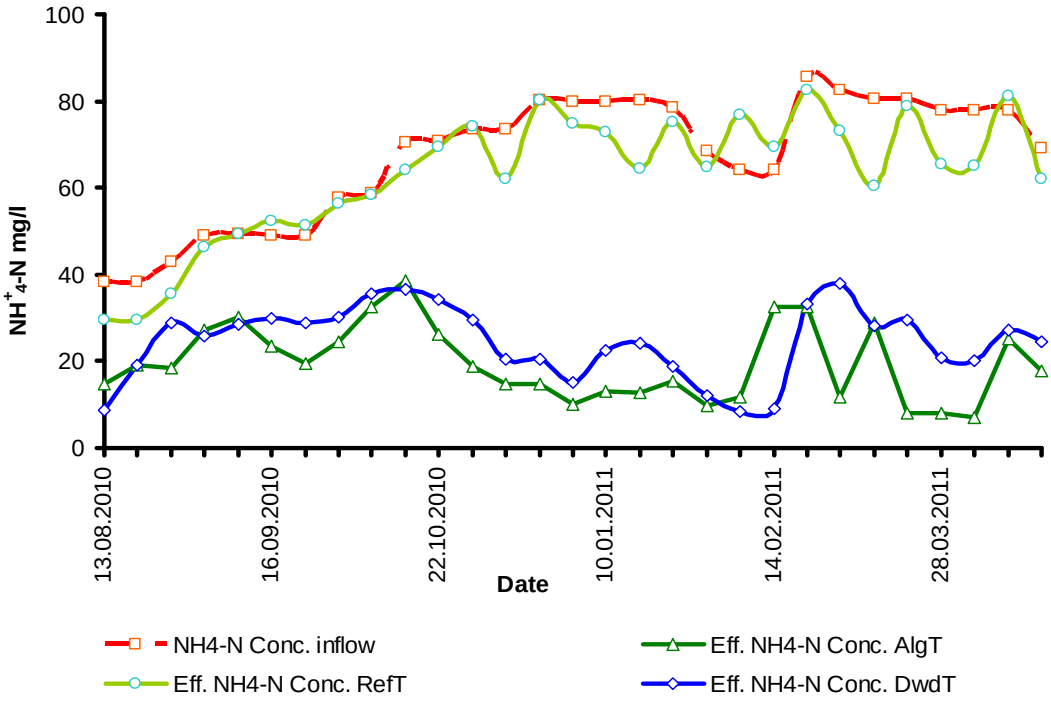


Figure 28. NH_4^+-N measured in the influent and at the effluent of the experiments

The high removal efficiency of ammonia nitrogen by algae and duckweed were recorded at the second stage of the treatment period (from 07.10.2010 to 14.02.2011). The ammonia concentration at the effluence of the treatments after 14.02.2011 changed due to the changed in/outflow rates (from 15 litres per day to 20 litres per day).

As the result shows, the ammonia measured in the influent of the three setups is 67 mg NH_4^+-N/l on average. The weekly analysis shows that an average of 73% of influent ammonia was removed by algal (min: 39%, max: 91%), 69% by duckweed experiment (min: 33%, max: 86%) during the entire period of the study.

The concentration of ammonium in the effluents of the algal experiment was 20 mg NH_4^+-N/l and was 21 mg NH_4^+-N/l at the effluence of the duckweed experiment. For the reference

experiment it was only 58 mg NH_4^+-N/l. It can be observed that there is a drop of ammonium nitrogen in the algal and duckweed systems. Based on the results, it can be assumed that the algal and duckweed experiments perform better towards ammonia removal than the reference experiment. In this setup, there was only heterotrophic bacteria growth. Since there is no oxygen produced, nitrification process may not take place, which reduces the nitrification efficiency.

- NH_4^+-N removal efficiency

As the results in the Fig. 29 show, an NH_4^+-N removal efficiency of about 70-80% for the algal and duckweed experiments was obtained. The NH_4^+-N loading rate was approximately 8-<12 g NH_4^+-N/(m^3·d), with the relative correlation obtained from the effluent being around R^2=0.63 for the algal, R^2=0.61 for the duckweed experiment. For a load rate over 12 g NH_4^+-N/(m^3·d), there was no observation number of removal efficiency. In this case, the trend of $\log_{10}$ was used. The trend shows a better correlation between the evaluated results and the theoretical results than linear or exponential trend.

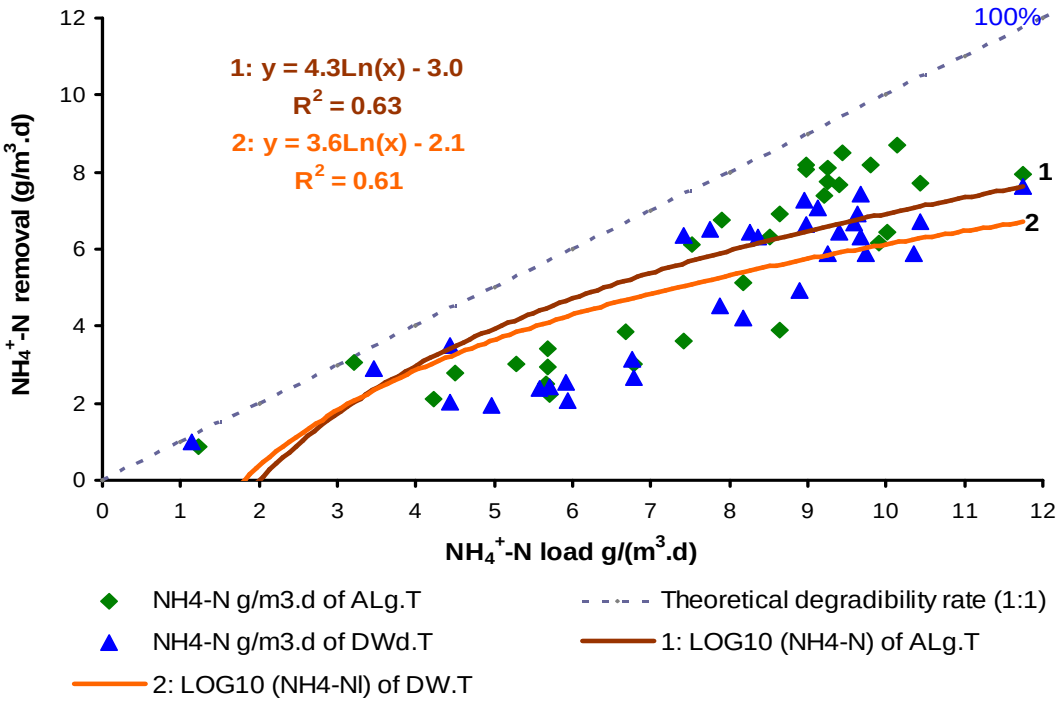


Figure 29. NH_4^+-N removal with NH_4^+-N load into 1: Algal; 2: Duckweed experiment

The result of this research is in accordance with several researches, such as Kurosu (2001) who achieved a nitrogen removal efficiency of 59% via microalgae-bacterial treatment ponds, Ilaria Di Termini *et al.* (2011) who obtained ammonia nitrogen removal efficiency of 90% in

the algal photobioreactor system with total volume more than 15.000 cm^3. On adding more process units and higher loading rates, the algal and duckweed experiments should have high functional efficiency of nitrogen removal from wastewater as well as degradation of another substances such as COD, BOD_5, PO_4-, etc. Körner and Vermaat (1998) used algae and duckweed material to remove ammonia. They reported that nitrogen removal coefficients obtained from duckweed treatment plants are higher than those from algal plants (influent ammonia concentration of 72 to 85 mg N/l). Middlebrooks et al. (1982) described that in an algal wastewater stabilization pond a reduction of up to 95% of ammonia nitrogen could be obtained.

The difference in the ammonia concentrations of 50-60 mg NH_4^+-N/l between influent and effluent may be the result of the following transformation processes: ammonia into nitrate nitrogen, incorporation in the new algal cells, development of new duckweed populations, or/and involvement in the nitrification and/or denitrification processes. Therefore, it could be also be assumed that in both treatment options nitrification/denitrification processes occur. Surplus oxygen at the first phase of both setups could support autotrophic bacteria growth to assimilate ammonium. Thus, it increases ammonia removal efficiency. On the other hand, algae can directly absorb ammonium from water for biomass growth (as reported in the literature). The result of ammonia removal efficiency of this research is lower than the results obtained from the activated sludge process (over 82% of total nitrogen removed from influent - DWA 2011), but higher than in the natural treatment system such as constructed wetland or facultative aerated ponds as shown in the table 12 below (Kapoor *et al.* 2003, von Sperling 2005).

Table 12. Comparison NH_4^+-N removal efficiency by the algal and duckweed experiments with another process

Removal efficiency % NH_4^+-N		**System**
Von Sperling *et al.* (2005)	< 50	Facultative pond
	< 50	Constructed wetland
	< 30	Facultative aerated lagoon
	65-85	Low rate trickling filter
DWA (2011)	1.12 mg/l at the effluent	Advanced activated sludge process
Nurdogan and Oswald (1995)	90	High rate algal pond
Own study	73	Algal experiment
	69	Duckweed experiment

- Oxygen production and the efficiency of ammonium-nitrogen removal

The systematic correlation between ammonia removal rates and created oxygen is found (shown in the Fig. 30). The systematic correlation between ammonia removal rates and created oxygen is found. The efficiency correlation factor for the oxygen and the ammonia removal is R^2=0.4 for duckweed and R^2=0.1 for algal treatment, together with 69-73% of ammonium removed from influent. The oxygen concentrations at the effluence of plants were around 4.5-6.5 mg O_2/l. The log_{10} trend used in this case is more suitable to evaluate the measured result than the other trends.

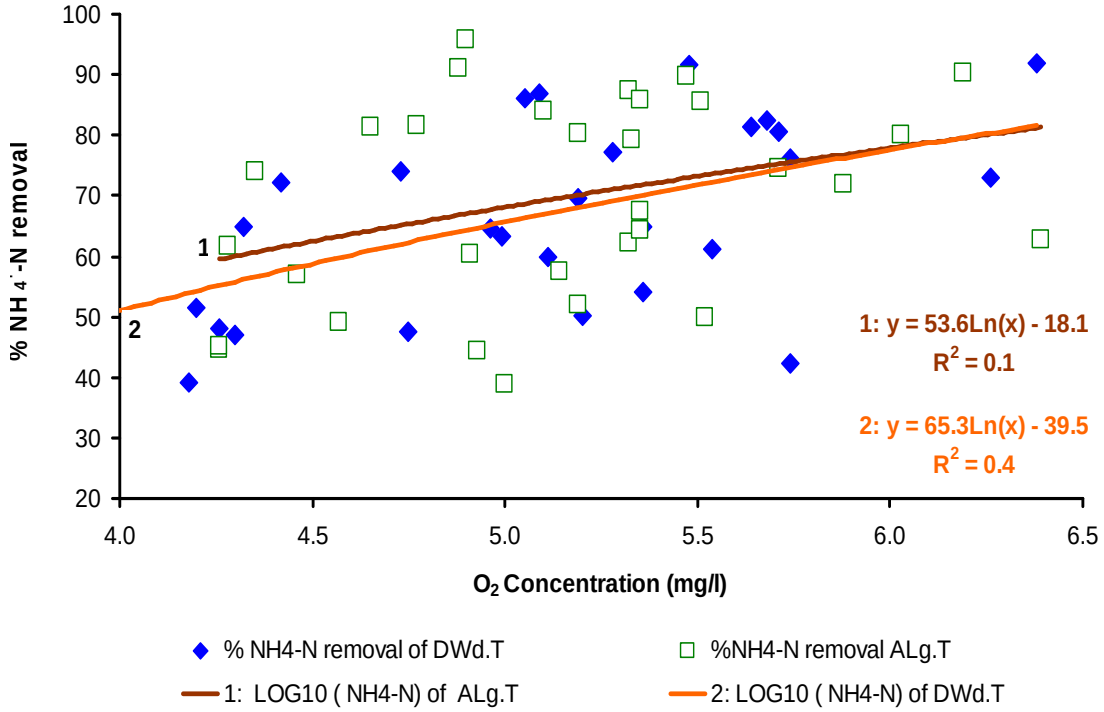


Figure 30. The relationship between O_2 mg/l generation and % NH_4^+-N removal
1: Algal; 2: Duckweed experiment

It can be seen that there is a good correlation between oxygen production and the ammonia removal in the experiments. Middlebrooks *et al.* (1982) and Silva *et al.* (1987) pointed out that a system employing algae could reach up to 95% ammonia removal efficiency from wastewater by increasing the oxygen concentration.

The growth of autotrophic nitrificants requires about 4.3 g O_2 to oxidize 1g of ammonia nitrogen. Based on the obtained result of oxygen availability (6.4 mg/l) from the research, it can be assumed that the treatments provide enough oxygen for nitrifier growth and the produced oxygen in the experiment could be higher than 6 mg/l. Hence, all the available ammonium in water will be transformed (Wolf *et al*. 2007). From the above explanations, one

of the mechanisms suggested to cause a decrease of ammonia in both treatments was ammonia oxidation. The other mechanism was direct ammonia nitrogen uptake by the algae to grow biomass (Mostert and Grobbelaar 1987; Graham and Wilcox 2000; Verdegem *et al*. 2005). Unfortunately, the study did not measure the biomass of duckweed and algae. Therefore, the growth rate of duckweed and algae could not be determined.

The growth of biomass could explain why low nitrate concentrations were observed during the algal experiment (average 2 mg NO_3^--N/l). The most probable explanation is that the denitrification occurred in the deeper part of the algal processing unit (anoxic zone). This is also the conclusion of Kuenen & Robertson (1994) and Revsbech *et al*. (2005). They also suggested that algal uptake is the second mechanism to remove ammonia after nitrification.

1.3.5 Nitrate nitrogen

Nitrate concentration in the influent for the experiments during the period of the study was 0.3 mg NO_3^--N/l. The nitrate concentration in the effluent of the plants with algae was 2.0 mg/l and for duckweed it was approximately 5 mg/l as can see in the Fig. 31. The nitrate concentrations in this research were lower in comparison with other researches and the literature. It may indicate that at the nitrification and denitrification processes coexist. The reference experiment showed less nitrate formation than the algal and duckweed experiments.

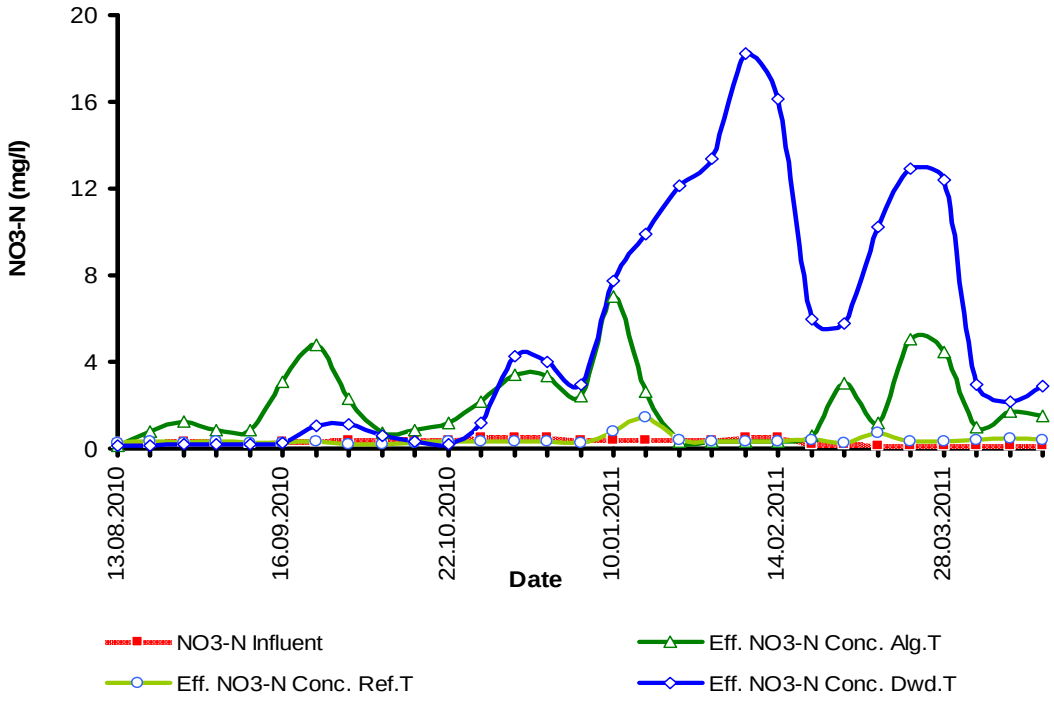


Figure 31. NO_3^--N measured in the influent and at the effluent of the experiments

A high nitrate concentration produced in the experiment might support the denitrification processes. In the activated sludge process nitrates concentration at the effluent was 6.2 mg/l (DWA 2011) and for rotating biological contactors it was lower than 2 mg/l (Metcalf and Eddy 1991).

- The NO_3^--N formation in processing units.

The test had been done with the algal experiment. In each processing unit, the same amount of algal concentration (measured by chlorophyll-α content) was added to each processing unit. The initial nitrate concentration in wastewater was 0.1 mg/l, approximately. As the results shown in the Fig. 32, the nitrate concentrations in the test follow the trend of NO_3^--N. Evidence of nitrate formation in the algal experiment in this test was indicated by the concentration of nitrates along the processing unit of the systems. It was observed that the nitrate concentration increased from the first to the last processing unit. In the first unit, the nitrate concentration was lower (0.29 mg/l) than in the unit 3, 4 and 5. The nitrate concentration at the last processing unit was 4.8 mg/l, approximately.

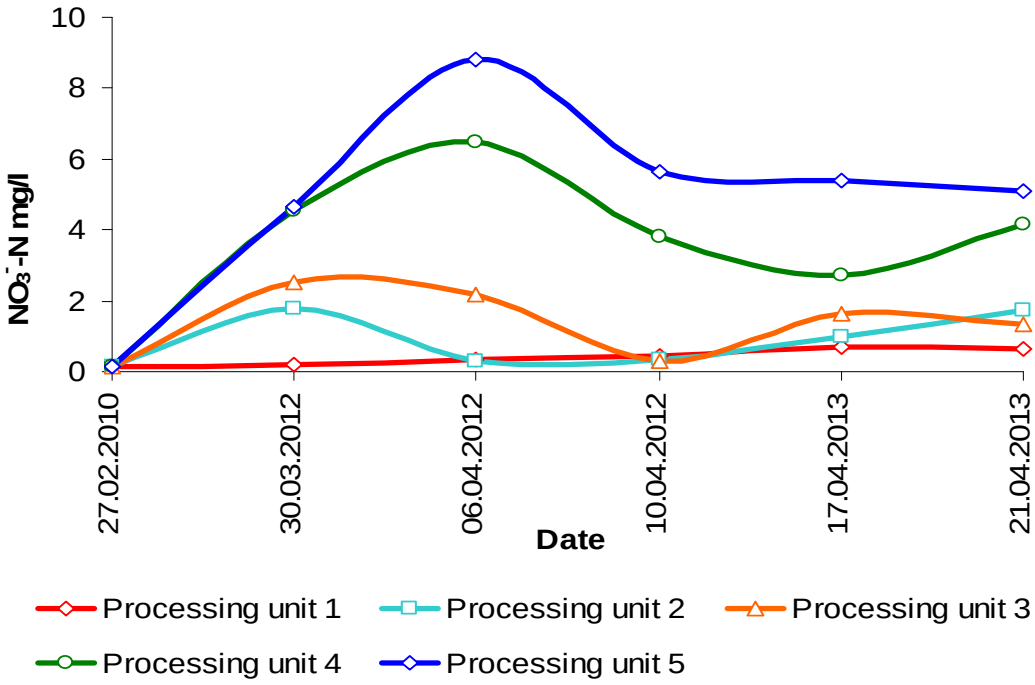


Figure 32. NO_3^--N production in algal processing units

Due to the large amount of substance discharged directly into unit 1, algae could not develop under the overload of substrates and turbulence conditions. From the observed results, the nitrate concentration increased in the subsequent units over time. There were several reasons to consider. First, the concentration of substances will be reduced by several processes in

unit 1. Second, the algal population develops faster in the next units. Third, it can be assumed that the nitrification and denitrification process exist together in unit 1 and 2 and 3 due to the low oxygen production.

1.3.6 Nitrification and denitrification efficiencies

The calculation of a nitrogen balance for the algal experiment shows that the assimilation efficiency in algae is around 70%, the nitrification efficiency is 71% while the nitrification rate is 4.4 mg/(l·d) and the denitrification rate is 4.2 mg/(l·d). It could be assumed that around 46-47 mg/l of nitrogen was lost due to the growth of algae and duckweed biomass, assimilation, and nitrification and denitrification processes.

The important role of the nitrification and denitrification processes in wastewater treatment has been reviewed in literature. Both oxygen and pH have a strong effect on the nitrification/denitrification processes (Barnes and Wilson 1978, Metcalf and Eddy 1991). In the algal and duckweed experiments, the pH was in the range of 7-7.6 (optimum rate of pH 7.2-<9.0). According to Moorhead and Reddy (1988), high oxygen production (0.5 mg/l) together with suitable pH conditions strongly support the nitrification and denitrification processes. Moreover, the oxygen produced by algae and duckweed is neither an inhibiting nor limiting factor for the nitrification.

Increasing the surface for attached growth of nitrifiers should be a solution to enhance the ammonia nitrogen removal efficiency. Nitrifiers can only develop their community while being attached to the surface of carriers. They cannot survive as freely suspended bacteria (Hammer and Knight, 1994).

McLean *et al.* (2000) obtained a high nitrification rate and ammonia removal efficiency from the attached surfaces in the lagoon when they studied a lagoon with a high density of suspended algae. Zimmo *et al.* (2000) said that without the attachment surface the nitrifiers-denitrifiers could not exist in the algal ponds, therefore the removal efficiency of ammonia nitrogen is limited.

1.3.7 Nitrogen balance in algal experiment

- Mass balance of total nitrogen organic nitrogen

Nitrogen organic can be determined by total autotrophic and heterotrophic bacteria (included algae). According to the accepted papers of Szetela *et al.* (1990), Henze *et al.* (2000a), Ziglio

et al. (2001), Roeleveld and Loosdrecht (2002), Myszograj and Sadecka (2004), Bornemann *et al.* (1998), the mass balance of total nitrogen can be calculated as:

- Mass Balance of total nitrogen (***ΔN***)

$$\mathbf{\Delta N = TN_{inflow} - TN_{outflow}}$$

Q_{inflow}	15.80	l/d
$TKN_{influent}$	83.80	mg/l
NH_4^+-$N_{influent}$	67.2	mg/l
NO_3^--$N_{influent}$	0.3	mg/l
$BOD_{5\ influent}$	284	mg/l
$Q_{outflow}$	15.60	l/d
$TKN_{effluent}$	*Not measured*	
NH_4^+-$N_{effluent}$	17.8	mg/l
NO_3^--$N_{effluent}$	2	mg/l
Hydraulic retention time (HRT)	10	day

By missing measured parameter: TKN effluent and N-org in sludge, so it is assumed:

$C_{o,\ N\text{-}org\ effluent}$: 3 mg/l; $C_{N\text{-}org\ in\ sludge} = 0.05 \times C_{o,\ BOD5\ influent} = 14.2$ mg/l

Mass $C_{N\text{-}Org\ in\ sludge} = C_{N\text{-}org\ in\ sludge} \times Q_{inflow} = 0.05 \times C_{o,\ BOD5\ influent} \times 15.8$ l/d

Mass $C_{N\text{-}Org\ in\ sludge} = 224$ g/d (= 0.22 g/d)

- Mass balance of total nitrogen inflow

Mass $TN_{influent} = (C_{o,\ TKN\ influent} + C_{o,\ NO3\text{-}N\ influent}) \times Q_{inflow} = 1.33$ (g/d)

- Mass balance of total nitrogen out

Mass TN_{out} = Mass $TN_{effluent}$ + Mass N-org $_{in\ sludge}$

Mass $TN_{effluent} = (C_{e,\ N\text{-}org} + C_{e,\ NH4\text{-}N} + C_{e,\ NO3\text{-}N}) \times Q_{outflow} = 0.36$ (g/d)

Mass N-org $_{in\ sludge} = 0.22$ (g/d)

Then: Mass balance of TN_{out} = 0.58 (g/d)

- Efficiency of assimilation

$E_{Mass\ TN\ assimilation}$ = (Mass $TN_{influent}$ – Mass $TN_{effluent}$) / Mass $TN_{influent}$

Mass $TN_{effluent}$ = 0.36 (g/d)

$E_{Mass\ TN\ assimilation}$ = (1.33 - 0.36)/1.33 = 0.73

- Efficiency of nitrification

E_{Nitri} = (Mass TN_{Nitri} – Mass $_{NH4\text{-}N\ effluent}$)/ Mass TN_{Nitri}

Mass TN_{Nitri} = Mass $TN_{influent}$ – Mass $TN_{effluent}$ = 0.97 g/d

Mass $_{NH4\text{-}N\ effluent}$ = $C_{e,\ NH4\text{-}N}$ x $Q_{outflow}$ = 0.28 (g/d). Then:

E_{Nitri} = (0.97 – 0.28)/0.97= 0.71

- Efficiency of Denitrification

$$E_{DEN} = \left(\frac{(Mass\ TN_{Nitri} - Mass_{\ NH4-N\ effluent}) - Mass_{\ NO3-N\ effluent}}{(Mass\ TN_{Nitri} - Mass_{\ NH4-N\ effluent})} \right)$$

Mass NO_3-$N_{in\ effluent}$ = $C_{e,\ NO3\text{-}N}$ x $Q_{outflow}$ = 0.03 g/d. Then: E_{DEN} = 0.95

- Nitrification rate

R_{Nitri} = ($C_{mass\ TN\ Nitri}$ – $C_{e,\ NH4\text{-}N\ effluent}$)/HRT

$C_{mass\ TN\ Nitri}$ = (Mass TN_{Nitri} x 1000 mg/g)/ $Q_{outflow}$ = 62,2 mg/l

R_{Nitri} = 4.4 (mg/l.d)

- Denitrification rate

$$R_{DEN} = \left(\frac{(C_{\ Mass\ TN\ Nitri} - C_{e,\ NH4-N}) - C_{e,\ NO3-N})}{HRT} \right) = 4.2\ (mg/l{\cdot}d)$$

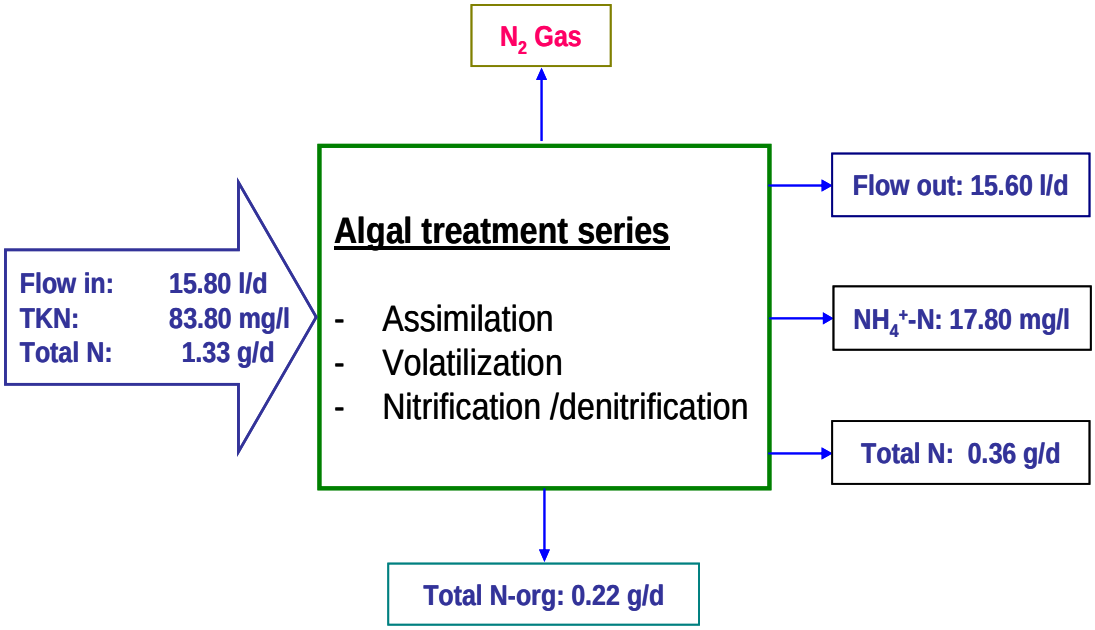


Figure 33. Mass balance of total nitrogen in algal experiment

- Efficiency of assimilation	: 73%
- Nitrification rate	: 4.4 (mg/l·d)
- Denitrification rate	: 4.2 (mg/l·d)

Short summary of algal and duckweed experiments

- High NH_4^+-N removal efficiency : 69-73%
- High COD removal efficiency: 86-91%
- High produced oxygen concentration: 5 mg/l (maximum 6 mg O_2/l)
- Algal and duckweed experiments showed the same performances
- Oxygen saturated zone was relatively small (20-25 cm from surface)
- Further measurements of TKN in effluent & N-org in sludge are needed

Reason to change to the reactor design:

- Improvement of nitrification and nitrogen removal capacity
- Obtaining higher denitrification rate
- Implementing shallower depths and bigger lightened surface to allow oxygen
- production in deeper parts of the reactor
- Testing different designs of the reactor's baffles
- Determine the performance of different designs under different light regimes

2. BAFFLED ALGAL REACTORS

2.1 Operating conditions

Table 13 describes the operating conditions for baffled algal reactors under the laboratory conditions with small-scale reactors. The observation of water evaporation had been done by monitoring the water levels in both the main reactor and collector via metric rulers. The influent of raw wastewater into the treatment reactors contained 63 mg NH_4^+-N/l, 651 mg COD/l, significantly low oxygen concentration of 0.6 mg O_2/l, TKN around 83.8 mg TKN/l.

Up to 75% of ammonia nitrogen and 25% organic nitrogen could be measured, while only trace of NO_3^--N (around 0.4 mg/l) was present. The recycle line was added with recycling ratio approximately 0.7 l/h. The three wastewater treatment reactors have no aeration systems provided, therefore the oxygen concentration was completely generated by the algae via photosynthesis. The oxygen concentration created by the algae in treatment reactors is not only for algal growth of biomass but also for nutrient and COD oxidation.

Table 13. Operating conditions for baffled algal reactors

	Quantification
- Flowing rate: -Influent - Effluent	22.50 l/d 22.30 l/d
- Evaporation from treatment reactor	0.20 l/d
- Treatment reactor (main reactor)	3 units
- Dimension - Volume capacity - Effective capacity - Baffle areas/reactor (5 baffles)	H: 60cm, W: 65cm; L: 85cm 200 litres (in volume) 180 litres (in volume) 2.4 m^2
- Collector (3 units)	H: 30cm, W.: 40cm; L: 60cm 40 litres (in volume)
- Hydraulic retention time (HRT)	10 -11 days
- Artificial light conditions	
- Light intensity	Photon flux of 0.72 x 1020 photons/m^{2}·s (120µE/m^{2}·s) Yielding: 8.000 lumen Spectral range: 400-700 nm
- Intermittent light simulations (day : night)	12 : 12 hours 06 : 18 hours
- Wastewater discharge type	Pre-settled wastewater
- Temperature on surface of treatment reactor	22^{o}C -25^{o}C (on average)
Starting up	~ 2 weeks

2.2 Experiment results

Table 14 and 15 describes the characteristics of raw wastewater without settle-able particles in the influent to the reactors, as well as the water quality at the effluent of each reactor. The determination of load removal efficiency was done following the calculation methods described in Chapter IV, part 1.1. The results show that the reactor T1 and T2 obtained high efficiency of nitrogen removal as well as COD, BOD_5 removal efficiencies. It also indicates to higher oxygen concentrations being produced in the baffled algal reactors in comparison with the un-baffled reactor.

Table 14. Characteristics of wastewater influent and effluent of baffled algal reactors (mean values, *n=52*)

	Influent concentration (mg/l)	Load rate ($g/m^3 \cdot d$)	Effluent concentration (mg/l)			Removal $g/(m^3 \cdot d)$ removal efficiency (%)		
			T_1	T_2	T_3 Ref.	T_1	T_2	T_3 Ref.
BOD_5								
12-hou light	267	27	30	37	102	24 (89%)	24 (86%)	17 (62%)
06-hour light	247	25	123	142	-	13 (50%)	11 (42%)	-
COD								
12-hour light	651	67	124	127	143	54 (81%)	54 (80%)	52 (78%)
06-hour light	595	61	178	178	-	43 (70%)	43 (71%)	-
O_2								
12-hour light	0.6		6	6	3	+0.5	+0.6	+0.30
06-hour light			4	4	1.4	+0.3	+0.3	+0.08
TKN *12-hour light*	83.8	9	24	27	44	6 (72%)	6 (68%)	4 (48%)
NH_4^+-N								
12-hour light	63	6	6	7	30	6 (90%)	6 (88%)	3 (53%)
06-hour light	68	7	20	23	-	5 (70%)	5 (67%)	-
NO_3^--N								
12-hour light	0.4		6	8	7	+0.5	+0.70	+0.50
06-hour light	0.3		1.3	0.9	0.5	+0.1	+0.06	+0.02
pH	**7.4**		**8.1**	**8.2**	**8.1**			

Note: ***Ref.***: reference algal reactor without baffles

\+ increase.

T1: vertical baffled reactor

T2: horizontal baffled reactor

T3: reference reactor (without baffles)

Table 15. Characteristics of wastewater influent and effluent of baffled algal reactors with minimum and maximum values (under 12 hours light conditions)

	Influent conconcentration (mg/l)	**Effluent concentration (mg/l)**			**removal efficiency (%)**		
		T_1	**T_2**	**T_3**	**T_1**	**T_2**	**T_3**
BOD_5							
Min	*26*	*7*	*7*	*27*	*60*	*61*	*37*
Max	*395*	*101*	*92*	*170*	*98*	*97*	*91*
COD							
Min	105	94	91	88	72	76	55
Max	803	172	167	250	86	86	88
O_2							
Min	0.2	0.3	0.3	0.3	-	-	-
Max	1.3	8.9	8.7	6.3			
NH_4^+-N							
Min	33	0.1	0.2	17	61	51	32
Max	75	33	33	49	99	99	76
pH							
Min	7.2	7.3	7.5	7.7	-	-	-
Max	7.7	8.8	9.3	8.4			

2.2.1 COD

The variation of COD in the influent and the effluent for the three reactors for the different measuring times is shown in the Fig. 34. The average values of COD in the reactors T1 and T2 were 124 and 127 mg/l respectively (loading rate was 67 g/m^3·d). The efficiency of COD removal of 81% (max: 86%) of the reactor T1 and more than 80% for reactor T2 as determined under the 12 hours/day lighting conditions.

When 6-hour lighting conditions were provided, the COD for the influent of the three reactors was around 595 mg COD/l (equal to a loading rate of 61 g COD/m^3·d). The COD in the effluents of the reactor T1 and T2 were 178 mg COD/l. The COD removal efficiency from the influent reached around 81% (max: 86 % for the reactor T1 and 75% for the reactor T2).

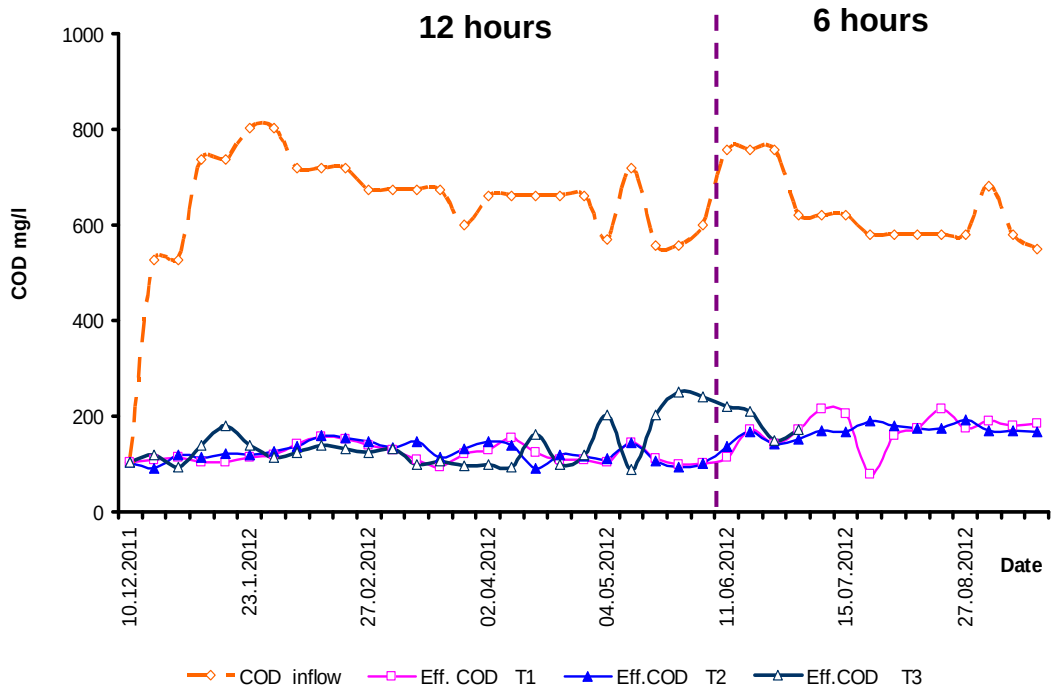


Figure 34. The comparison of COD (mg/l) in the influent and at the effluent of the three reactors with different light regimes

Inversely, it could be argued that when 12 hours of light was provided, the removal efficiency of COD was higher than the efficiency at six hours of saturated light. In the present research, the significant difference of COD removal efficiency could not be detected between the three reactors. It seems that the removal efficiency of COD in the reactors depends on other processes such as sedimentation and the activities of microorganism at the same time. Importantly, up to 82% of COD can be removed after more than three weeks. This in turn could be due to the algae achieving the maximum population and increasing biomass and

oxygen with time. In comparison, the efficiency of removal of COD in the three reactors is lower than algal and duckweed experiments, where in the algal experiment, the total COD removal efficiency was 86%.

- COD removal efficiency

The Fig. 35 shows the variation of COD in the influent and at the outflow of the different reactors at the different measuring times under 12 hour lighting conditions. The trend of $\log_{10}$ was used to detect a better trend for the evaluated result with the theoretical results.

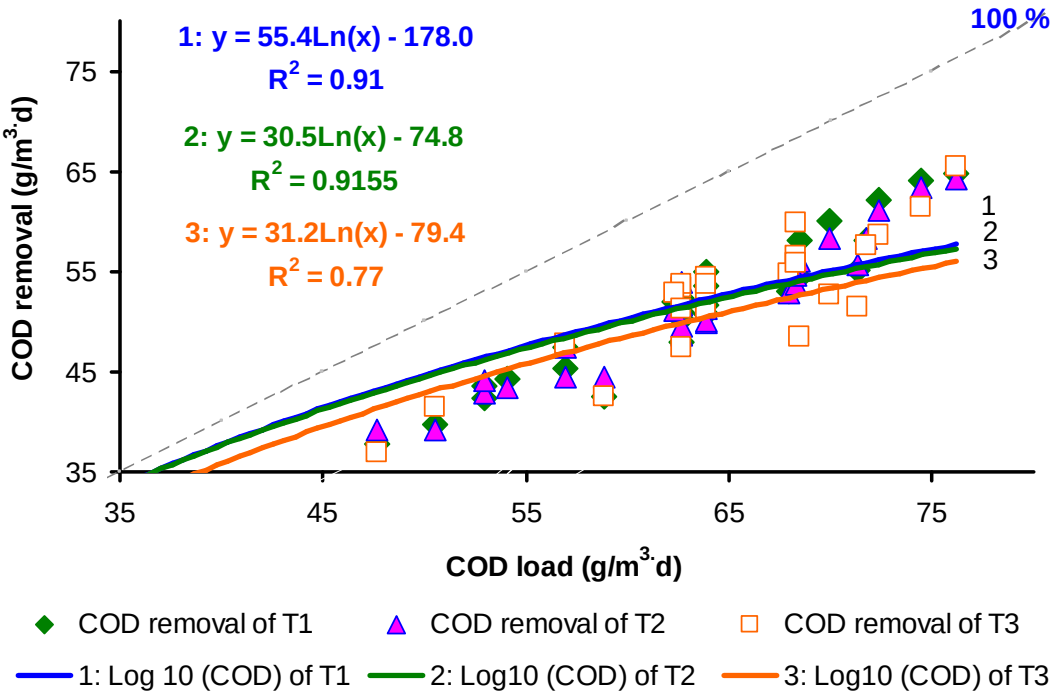


Figure 35. The comparison of the COD removal and COD load into the reactors

An average of 124-127 mg COD/l at the effluence (unfiltered samples) of the reactor T1 and T2 was measured during the entire period of the study. The high removal rates of COD when COD load rates were 60-78 g/m^{3}.d were obtained from the reactor T1, with the relative correlation factors being R^2=0.91 and R^2= 92 for the reactor T2. Meanwhile, the reactor T3 achieved only 78% COD from the influent. The relationship correlation factor of the reactor T3 was approximately R^2=0.77.

It obviously shows that for the reactors T1 and T2, the removal efficiency of COD is closer to the theoretical value than that for the reactor T3. Lower total COD removal in the reactor T3 was due to the absence of baffles. The mixed algal culture here does not have enough ability to remove COD. Moreover, the lack of the baffles (reactor T3) could make a shortcut flow

from inlet to outlet. However, the results from the three reactors showed, there were slightly different of removal efficiencies of COD between them.

Ma *et al.* (2009) as cited in Breisha (2010) found that approximately 82% of COD was removed by a bench-scale continuous flow system with Terramycin crystallization solution. Gálvez *et al.* (2000) indicated that heterotrophic denitrifying bacteria depend on the type of carbon sources while the C/N ratios were 2.5, 1.08 and 1.1 for sucrose, ethanol and methanol assays, respectively.

In comparison with other process, it could indicate that the COD removal efficiencies of the reactor obtained high efficiency. The comparison of the current study and other results obtained from different processes is describes in the table 15 below (under 12 hour light regime).

Table 16. Comparion COD removal efficiency under 12 hours light conditions by BARs with another result

Removal efficiency % COD		**System**
von Sperling *et al.* (2005)	75-85	Constructed wetland
	55-70	UASB reactor
	80-90	Conventional activated sludge
	80-90	Low rate trickling filter
	83-90	Submerged aerated biofilter + bio. N. rem.
Nurdogan and Oswald (1995)	90-95	High rate algal ponds (HRP)
	83-90	Rotating biological contactor
DWA (2011)	95	Advanced activated sludge
Own study	86	Algal experiment
	91	Duckweed experiment
	80	Vertical baffled algal reactor
	81	Horizontal baffled algal reactor

2.2.2 BOD_5

The average BOD_5 mass loading rate into the three reactors of feeding tank was 27 g $BOD_5/(m^3 \cdot d)$. Under the 12 hours light condition, the BOD_5 at the effluent of the reactors T1 and T2 reaches 30-37 mg BOD_5/l. About 89% (max: 98%) of BOD_5 removed from the influent by the reactor T1, and 86% (max: 97%) by the reactor T2 was recorded. Meanwhile,

the BOD_5 of 102 mg/l at the effluent of the reactor T3 was obtained. The measurement of BOD_5 in the different reactors is shown in the Fig. 36.

When six hours light saturation conditions were applied, the BOD_5 of the influent to the reactors was 247 mg/l. The BOD_5 at the out flow of the reactors T1 and T2 were monitored at 123-142 mg BOD_5/l. Therefore, the BOD_5 removal efficiency were 42% of total BOD_5 from the influent of reactor T2 and 52% of the reactor T1. It is quite small in comparison with the BOD_5 removal efficiency of 86-89% on average obtained in the 12 hours light saturation conditions for both the reactor T1 and T2.

It is important to recognize that the performances of the reactor T1 and T2 in the systems are working well with respect to the BOD_5 removal with long photoperiods and are reduced to an efficiency of 40-45% for short photoperiods.

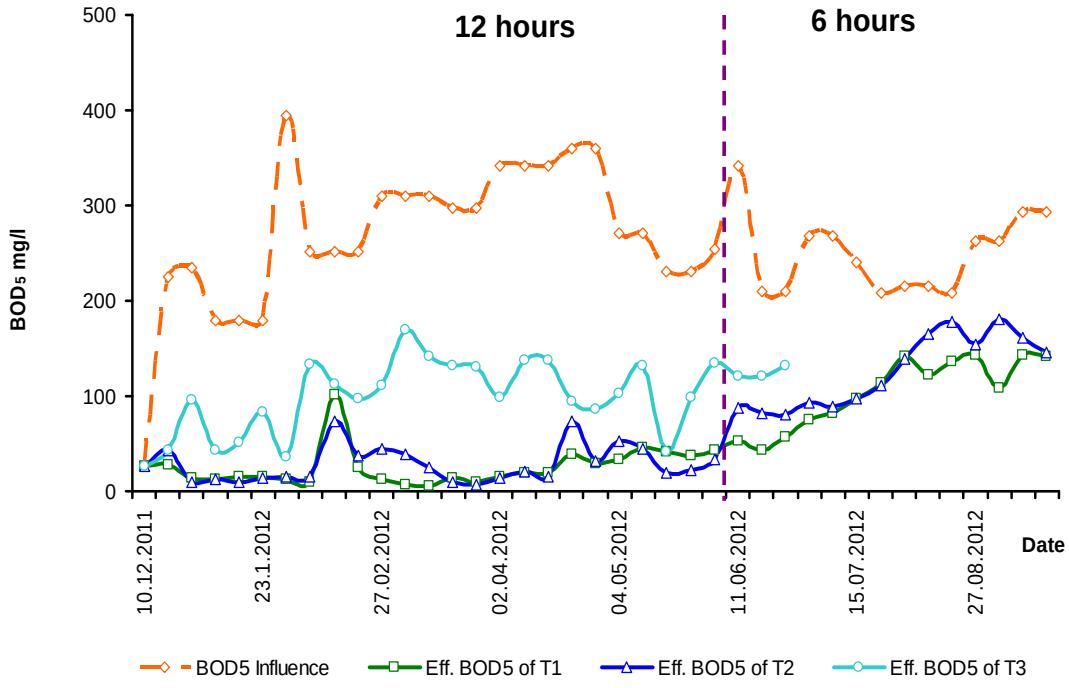


Figure 36. BOD_5 (mg/l) in the influent and at the effluent of the three reactors with different light regimes

- BOD_5 removal efficiency

The average BOD_5 mass load rate into the reactors was 27 g $BOD_5/(m^3 \cdot d)$. In the reactors T1 and T2 with light provided for 12 hours per day, the BOD_5 was detected to be about 30-37 mg BOD_5/l, respectively. Meanwhile, in the reactor T3 about 102 mg BOD_5/l at the effluent was achieved. The results showed that the performances of treatment reactor T1 and T2 are working well with respect to the BOD_5 removal. The effluents of BOD_5 concentration in the

baffled reactors showed that the highest organic matter removal occurred in the system where the baffles contributed in the reactors. The BOD_5 removal efficiency in the reactor T1 and T2 was up to 89% when 12-hour light condition was saturated with a loading rate of 30-38 g/(m^3·d). The efficiency correlation factor of the reactor T1 was R^2=0.9 and T2: R^2=0.8 (see in the Fig. 37).

There is no observation for removal efficiency when the loading rate was more than 38 g BOD_5/(m^3·d). The lowest performance of BOD_5 removal was noted in the reactor T3, where only 62% efficiency of BOD_5 removal from the influent was obtained. The BOD_5 concentration was generally corresponding to the aeration conditions and oxygen production of algae. The linear trend was used to provide a better comparison between evaluated results with theoretical result than the result obtained from the log_{10} or exponential trend.

High removal efficiencies of COD and BOD_5 in the reactors T1 and T2 could be attributed to faster algal development compared to the reactor without baffles. High BOD_5 and COD removal rates can be obtained under suitable temperature and for high biomass production by the algae (van Baalen 1962; Watanabe 1967; Lau *et al.* 1995; Tam *et al.* 1997).

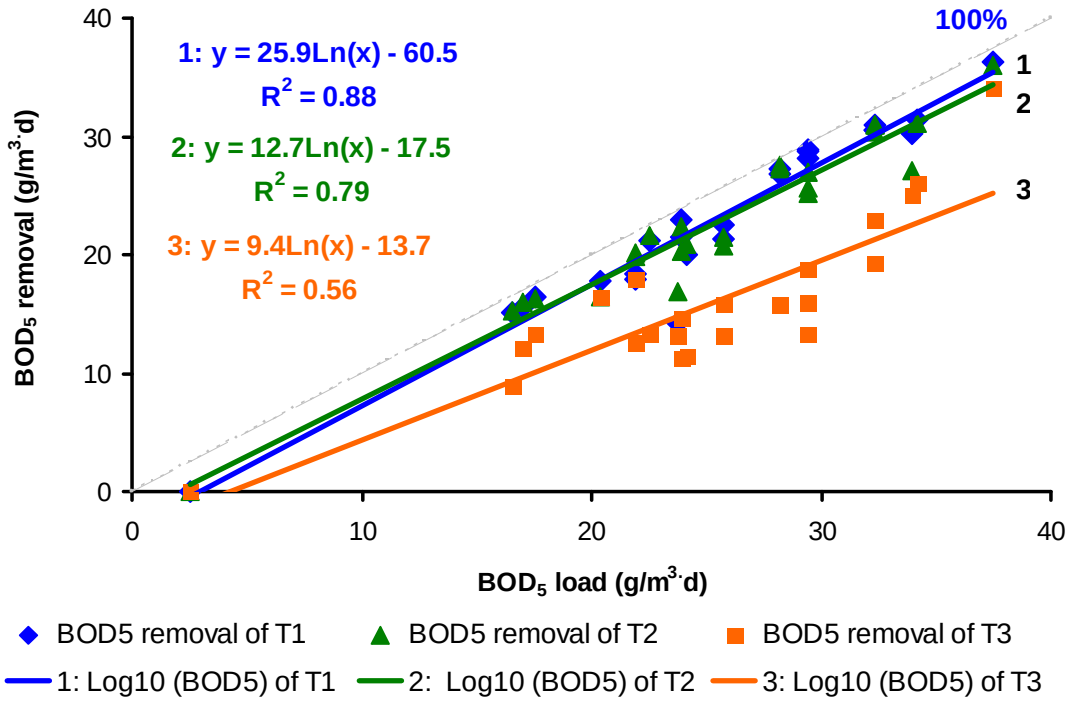


Figure 37. Comparison of BOD_5 removal with BOD_5 load into the reactors

The reason of the BOD_5 degradation in the treatment reactor due to more attaching surface and high oxygen concentration has been reviewed in the part of the experiment descriptions. The result of this research agrees with Muttamara and Puetpaiboon (1997), McLean *et al.*

(2000), Zimmo (2003). They are prevailed that high BOD_5 removal efficiency (46-50%) can be achieved in facultative conditions. Constable *et al.* (1989) and McLean *et al*. (2000) obtained the same result when they studied a pond without baffles and concluded that more efficient nitrification could be obtained from ponds with an attachment surface than from the pond without baffles. Harrison and Daigger (1987) pointed that the ammonia removal efficiency in trickling filters could reach high values when BOD loading of BOD_{bubble} 3.4-3.6 kg/(m^3·d) was present.

The BOD_5 removal efficiencies of this research were lower than the removal efficiency obtained from advanced activated sludge process 98% BOD_5 (DWA 2011), 85-93% trickling filter with low standard rate (Harrison and Daigger 1987; von Sperling 2005). Table 17 shows the comparison the result of BOD_5 removal efficiencies in this study with several the results obtained from researches using algae for nutrient removal from wastewater.

Table 17. Comparison of BOD_5 removal efficiency under 12 hours light conditions by BARs with another result

Removal efficiency % BOD_5		**System**
Alaerts *et al.* (1996)	95-99	Full-scale duckweed treatment plant
Zirschky and Reed (1988)	80	Algal and duckweed systems
Zimmo (2003)	85	Algal based waste stabilization pond
Markou and Georgakakis (2011)	90	Blue-green algae reactors with agro-industrial wastes and wastewaters
von Sperling et al. (2005)	80-90	Constructed wetland
von Sperling et al. (2005)	<50	USAB
DWA (2011)	>98	Activated Sludge process
Own study	93	Algal experiment
	93	Duckweed experiment
	89	Vertical baffled algal reactor
	86	Horizontal baffled algal reactor

Lower values of produced oxygen could be observed in reactor T3 in comparison to the reactors T1 and T2. It may be the result of lower diffusion of oxygen into the water, low space for the attached algae and shortcut flow, leading to reduced photosynthetic activity, and thus limited oxygen production.

The biomass in reactor T3 is expected to play a minor role in BOD_5 removal due to lacking space provided by baffles. Apparently, the growth rate of algae in baffled reactors is faster

than in reactor T3, and can increase their biomass in a shorter time. Zimmo (2003) suggested that an increased organic loading into the system can improve the algae's organic removal performance. However, the surplus biomass may be lost through the outflow or may contribute to the sedimentation of the die-off algae. Lai and Lam (1997) also found the relationship between organic nitrogen and the biomass production of algae. This is also reflected in higher Kjeldahl nitrogen values in the sludge. The measured results of TKN in algal sludge of this research are in agreement with this conclusion (see annex 4). In summary, BOD_5 removal efficiency in baffled algal reactors (86-89%) is higher than in the reactor without baffles (62%). Good correlation between BOD_5 removal rates and BOD_5 load was found in each reactor.

2.2.3 Oxygen production

The average oxygen mass generated in the reactor T1 and T2 was in the range of 6 mg O_2/l. The maximum 8-9 mg/l oxygen production in the rectors T1 or T2 could be achieved when 12-hour light condition was applied. This means that around 0.5-0.6 g O_2 per day can be produced in each reactor. Meanwhile, the oxygen concentration discharged into the three reactors was significantly low (0.6 mg O_2/l). As shown in the Fig. 38, the oxygen concentration in the reference reactor T3 was the lowest (3.3 mg O_2/l) in comparison with the reactors T1 and T2.

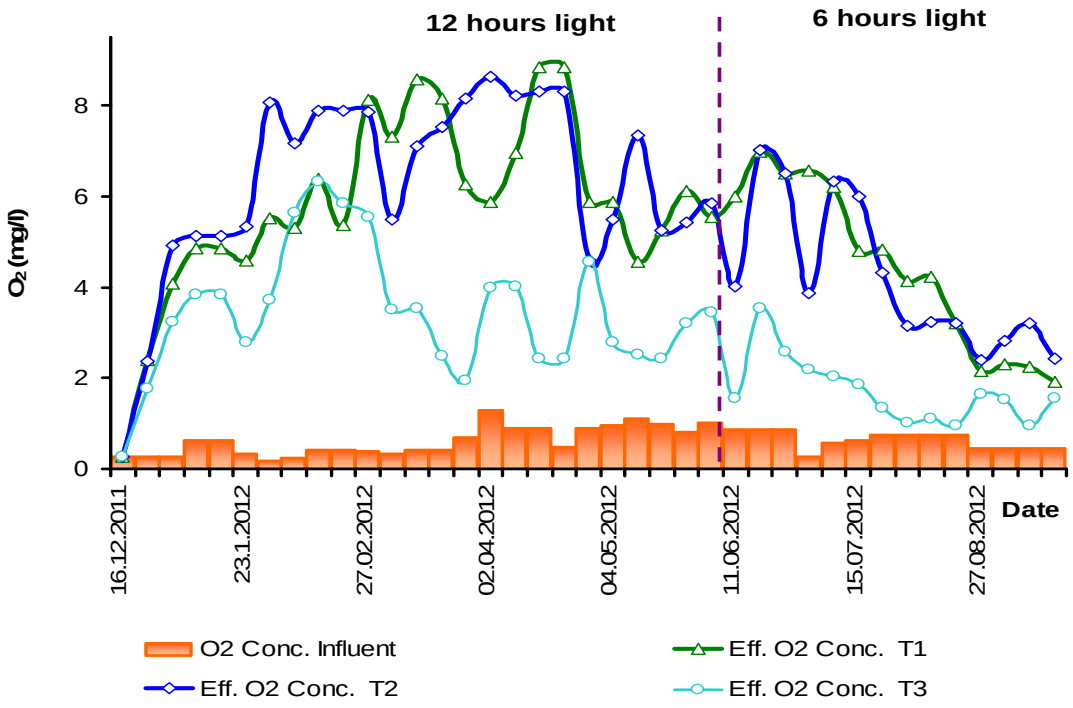


Figure 38. The variations of O_2 mg/l in the influent and the effluent of the reactors with different light regimes

In the reactor T3, there are no baffles either for algal attachment or to inhibit shortcut flow. As a result, the ammonia nitrogen efficiency as well as COD and BOD_5 removal is lower than in the other reactors. The effects of oxygen on substance removal are discussed together in the part **2.2.4** in this section). The oxygen concentrations from the effluents of the three reactors under 6 hour light conditions were monitored. About 3.6 mg O_2/l, 3.7 mg O_2/l and 1.4 mg O_2/l have been obtained from the reactors T1, T2 and T3, respectively. It is proved that, due to the light saturation conditions of six hours per day, the concentrations of oxygen produced by the three reactors decreased significantly in comparison with the conditions of 12-hour light provided. The results also points out that the reactor T3 does not have a good capacity of oxygen production.

Oxygen concentration produced by the reactors T1: 0.8 mg and T2: 1 mg O_2/l is higher than that of algal and duckweed experiments. (The discussion of the effect of oxygen on reducing substance in **BARs** will be discussed later together with the part of the result of ammonia removal). The data again highlights that the oxygen concentration produced by algae in the reactors could maintain good conditions for the nitrification process and hence lead to high ammonia removal efficiency (when compared with activated sludge process and the literature).

- Oxygen profile in treatment reactors

The Fig. 39 describes the procedure for oxygen measurement in main reactors with the number of samples, n=4.

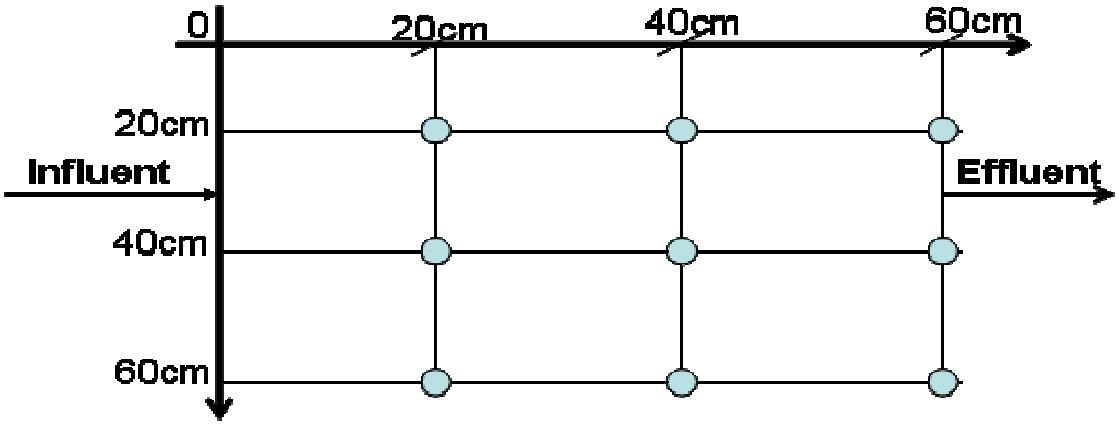


Figure 39. Schematic of oxygen measure in the reactors

Table 18 shows the oxygen measurement in each treatment system at different parts of the reactors. As shown by the results, significant differences in oxygen concentrations were found between the depths of 0.2m, 0.4m and 0.6m at same distances from the influent. It could be

seen that the oxygen concentration at a depth of 0.2 m and a distance of 0.6 m from the inlet is higher than the oxygen concentration at the distance of 0.2 m from inlet. The oxygen concentration at the bottoms of the reactors T1 and T2 (with distances from inlet of 0.2-0.4 m) shows mostly suitable conditions for denitrification to occur. The collector tank is shallow. Therefore oxygen from the air can mix more easily with the water than in the main reactor.

Table 18. Oxygen dynamic in treatment reactor (mg O_2/l, *n = 4*)

Depth (cm)	***Distance from influent (cm)***								
	T1			***T2***			***T3***		
	20	***40***	***60***	***20***	***40***	***60***	***20***	***40***	***60***
60	0.18	0.26	0.28	0.22	0.26	0.34	0.38	0.15	0.37
40	0.27	1.30	1.33	0.19	1.64	2.88	0.37	0.13	0.57
20	0.39	1.59	1.87	0.65	2.60	3.01	0.28	1.03	1.33

2.2.4 Ammonia nitrogen removal

The ammonia nitrogen concentrations in the influent and the effluent of the three reactors were monitored and are shown in the Fig. 40. The figure also shows the results obtained from three different reactors and under the different light saturation conditions.

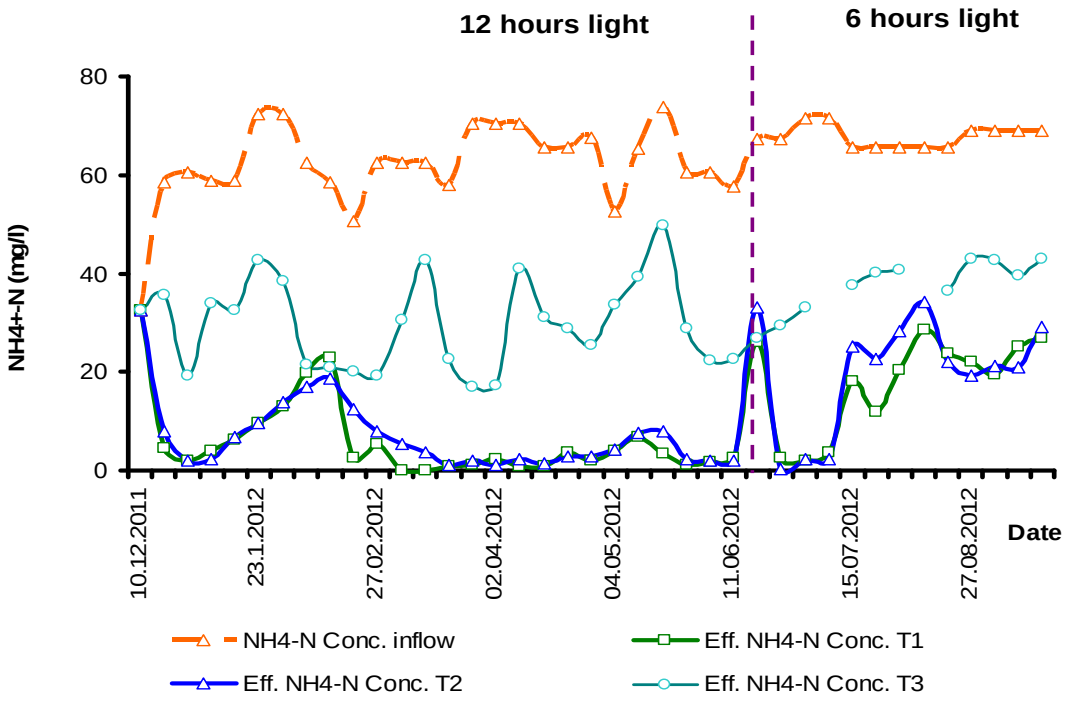


Figure 40. The comparison NH_4^+-N influence and effluence of treatment reactors with different light regimes

The ammonia nitrogen concentration discharged into treatment reactors was 63 mg NH_4^+-N/l, approximately. When 12 hours light conditions were applied, the ammonium nitrogen concentration in the effluent of the reactor T1 was approximately 6 mg NH_4^+-N/l and for that of T2 was 7 mg NH_4^+-N/l during the entire study period, except for the reactor T3, where NH_4^+-N was 30 mg/l.

Weekly sample analysis also pointed that about 90% of the influent ammonia nitrogen was removed by the reactor T1, and 88% by the reactor T2 (a maximum removal rate of 99% of ammonia was obtained for the reactors T1 and T2). The reactor T3 achieved a removal of only 53% of ammonia nitrogen from the influent. Based on the obtained result under 12 hours lighting conditions, it can be pre-concluded that both reactor T1 and T2 have performed better ammonia removal than reactor T3 (without baffles).

When 6-hour light conditions were applied, the concentrations of the ammonia nitrogen in the reactor T1 and T2 were around 23 and 20 mg NH_4^+-N/l, respectively. That means the ammonia nitrogen removal efficiencies of 67-70% were obtained from the effluents of the baffles reactors.

The concentration of ammonia in the reactor T3 was measured by quick-test from 02.07 to 17.09.2012 to roughly get the range of the ammonia concentration. These results were not considered for the evaluation. Under the same conditions (provided light, temperature), the ammonia nitrogen removal efficiencies in the baffled reactors T1, T2 were higher than for the results obtained from algal and duckweed experiments.

2.2.5 Ammonia nitrogen removal efficiency

As the results shown in the Fig. 41 indicate, NH_4^+-N removal efficiency of approximately 90% of NH_4^+-N from the influent could be achieved with loading rates of around 5-7 g NH_4^+-N /(m^3·d). The relative correlation in both baffled algal reactors were R^2 = 0.67-0.68. There was no observation number of removal efficiency at loading rates over 7 g NH_4^+-N/(m^3·d).

From the figure it could also be seen that the optimum loading rate is found to be 5-7 g NH_4^+-N /(m^3·d). In this case, the trend of $\log_{10}$ was used to evaluate the results. The trend shows a better correlation between the evaluated results and the theoretical results than the linear or exponential trend.

In the research about removal of nutrients in various types of constructed wetlands, Vymazal (2007) found that the processes that affect removal and retention of nitrogen during wastewater treatment in constructed wetlands (CWs) include NH_3 volatilization, nitrification, denitrification, nitrogen fixation, plant and microbial uptake, ammonification, nitrate reduction to ammonium, etc. Removal of total nitrogen in this study varied between 40 and 55% with removed load ranging between 250 and 630 g N/ (m^2·yr) depending on CWs type and inflow loading.

Brazil (2006) described the performance and operation of a rotating biological contactor in a tilapia recirculating aquaculture system. The system obtained an average TAN-total ammonia nitrogen areal removal rate of about 0.42 g/m^2 day. For three different applied filter medium types in commercial farms and for a range of hydraulic surface loading conditions, the highest observed TAN areal removal rate for a trickling filter was 1.1 g TAN/m^2 day, with an average TAN areal removal rate of 0.16 g/m^2·d (Eding *et al.* 2006). Lyssenko and Wheaton (2006) reported total ammonium nitrogen areal removal rates of 0.64 g/(m^2day).

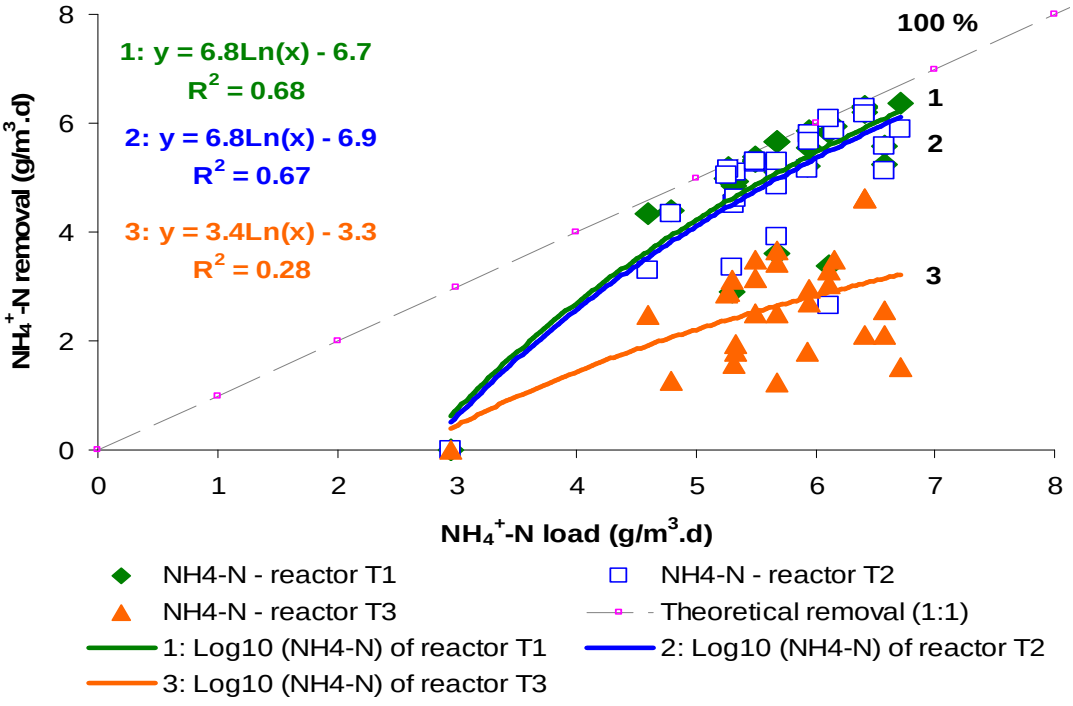


Figure 41. The evaluation of NH_4^+-N load and NH_4^+-N removal rate of the reactors

The comparison between the results achieved from this study and other results obtained from different processes are described in the table 19 below.

Table 19. Comparison of removal efficiency NH_4^+-N under 12 hours light conditions by bafflred alagl reactors with other results

Removal efficiency % NH_4^+- N		System
von Sperling *et al.* (2005)	< 50	Facultative pond
	< 50	Constructed wetland
	65-85	Low rate trickling filter
	> 80	Submerged aerated biofilter + bio.N. rem
	65-85	Rotating biological contactor
Nurdogan & Oswald (1995)	90	Algal high rate ponds (HRP)
DWA (2011)	1.2mg at the ffluent	Advanced activated sludge
Own study	73	Algal experiment
	69	Duckweed experiment
	90	Vertical baffled algal reactor
	88	Horizontal baffled algal reactor

- Effectiveness of produced oxygen on the ammonia removal efficiency.

The Fig. 42 shows, the relationship between oxygen concentration and the percentage of NH_4^+-N removal from wastewater for the reactors T1, T2 and T3.

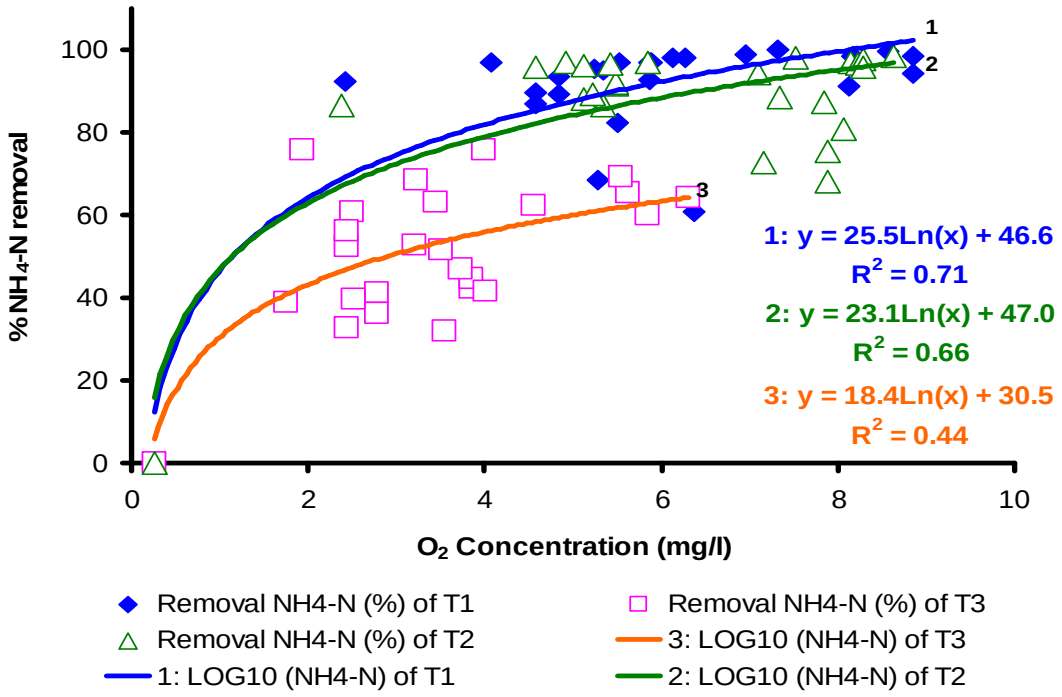


Figure 42. Log_{10} of oxygen concentration and NH_4^+-N (%) removal efficiency

Considering the impact of the oxygen produced on NH_4^+-N removal, two points must be emphasized. First, for higher oxygen concentration generated by algae (6 mg O_2/l), higher efficiency of ammonia nitrogen removal was achieved. As the results show, there is a good correlation (R^2=0.7) of the reactors T1 and T2 (higher than correlation factor of algal and duckweed experiments with R^2=0.1 to 0.4). The ammonia removal efficiency of 90% was obtained when the oxygen production reached up 4.5-9 g $O_2/(m^3 \cdot d)$.

Meanwhile, a lower correlation (R^2=0.4) is observed for the reactor T3, where the concentration of the produced oxygen was as low as 2–3 g $O_2/(m^3 \cdot d)$. Hence the removal efficiency of NH_4^+-N achievement was lower than the reactor T1 and T2. In the reactor T3, the highest value of the removal efficiency of NH_4^+-N was around 60%, with the oxygen concentration fluctuating from 2 to 4 mg O_2/l.

Additionally, a high removal efficiency was obtained from the reactors T1 and T2 corresponding to the high dissolved oxygen concentration in the bulk water. This is the result of the increased algal photosynthetic activities under the 12 hours light conditions provided. Thus, suitable conditions for autotrophic bacteria growth and its assimilating ammonia nitrogen could be created.

Silva (1982) stated firstly that the oxygen was produced by the algal treatment reactors via photosynthesis mechanisms and released into the water. Secondly, the produced oxygen can be involved in the process of oxidizing substances. As a result, the oxidized products become the nutrient sources for the growth of the nitrifying bacteria (Oswald 1973; Oron and Shelef 1982; Reed 1983; de Morais *et al.* 2007a,b; Chinnasamy *et al.* 2009).

Studying the baffled reactors (T1 and T2), a strong correlation between the ammonia removal efficiency and produced oxygen was found. It also supports the hypothesis that oxygen is the essential factor supporting to the growth of *Nitrosomonas* (Wild *et al.* 1970; Lai and Lam 1997).

Denitrifiers (Nitrosobacter) are the facultative bacteria that prefer oxygen to nitrate as the terminal electron acceptor. Thus, the limited amount of oxygen in the anoxic zone exhibited an important factor that affects the success of the denitrification. This process does not occur when the oxygen concentration in the anoxic zone is larger than 0.5 mg O_2/l.

As a result, the removal efficiency should be reduced (as reported in the literature). Ma et al. (2006) as cited in Breisha (2010) show a clear relationship between the nitrite pathway and the ammonia removal efficiency in a continuously running pilot plant. It was demonstrated

that more than 95% reduction of the oxidized nitrogen compounds could be achieved at the end of the aerobic zone via the nitrite pathway. The total nitrogen removal could be improved by about 20% and the aeration costs reduced by 24%.

- pH effects

The Fig. 43 clearly shows that as the pH approaches values of 7.5-8.5, the NH_4^+-N removal efficiency can reach a maximum of 99%. The data also indicated a high ammonia nitrogen removal efficiency in the reactors T1 and T2 (88-90%). There was no observation of removal efficiency at the pH=9. The ammonia concentration at the effluence of reactors T1 and T2 were 6.4-7.5 mg NH_4^+-N/m^3.d.

Obviously, the ammonia removal efficiencies in these reactors were higher than that in the reference reactor T3 (52%; 3.3 mg NH_4^+-N/m^3.d) at the same pH value (8.1-8.2). The correlation factor between the percentage of ammonia nitrogen removal and the range of the pH in the reactors T1 and T2 was around R^2=0.2. The application of the linear trend in this case showed the different removal efficiencies of the reactors under the effect of pH.

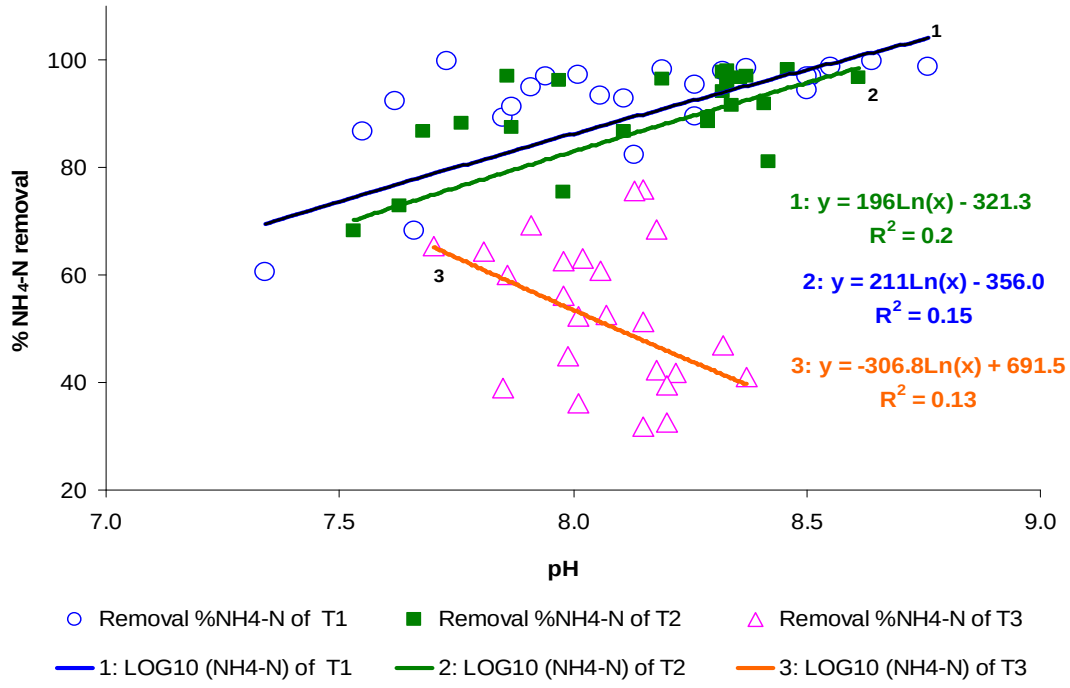


Figure 43. pH effects to NH_4^+-N removal efficiency (%) in the reactors

To obtain high growth rate of algae, the required pH conditions must be from 7 to 8.6. As a consequence, the denitrification process should occur (Henze *et al.* 1996). As shown in the

results, the pH value obtained from the reactors (pH 8.1 to 8.2 approximately) is in agreement with the optimal range of pH for bacterial growth from the literature review.

Comparing with the algal and duckweed experiments, the pH values of treatment reactors were higher than algal and duckweed experiments, i.e. pH of the reactor T1 higher by 0.2 units than the algal experiment, and 0.4 units in comparison with the duckweed experiment. The obtained data of current study is in agreement with data previously published in the literatures by Alaerts *et al.* (1996), Körner and Vermaat (1998), Vermaat and Hanif (1998), Caicedo *et al.* (2000) and Zimmo *et al.* (2000).

Several authors and the literature pointed out that when the pH is higher than 9, algae and nitrifying bacteria stop growing because pH media likely becomes a toxic agent. Hence the fraction of inorganic carbon (i.e., CO_2) decreases (Stratton 1969 as cited in Senzia et al. 2002; Zimmo *et al.* 2003). At pH values over 8.5, nitrification also decreases (van Kempen *et al.* 2001). Therefore the efficiency of nitrogen removal is significantly reduced.

Moorhead and Reddy (1988) found the principles and relationship between the dissolved oxygen and pH influence the nitrification and denitrification processes in the algal treatment plants or ponds. The efficiency of these processes in the pH range of 8-9 was higher than that at pH=7.5, as were the effects the coefficient of nutrient removal as well as COD and BOD removal (Konig *et al.* 1987; Matusiak 1977; Zimmo 2003; Kurosu, 2001).

Additionally, under high pH (>9), either the rate of ammonia volatilization or the decay of algae could increase. The large amount of organic nitrogen accumulated in the sediment contributes to this effect (Stratton 1969 as cited in Senzia et al. 2002; Zimmo *et al.* 2003; Gross *et al.* 1999; Shilton 1996).

Ferrara and Avci (1982) also pointed that sedimentation process is one of the nitrogen removal pathways. Arvin and Kristensen (1982), Szwerinski *et al. (*1986*)* Chen & Durbin (1994), Lai and Lam (1997) also pointed out that the nitrification-denitrification processes were the major mechanisms for nitrogen removal in the algae pond due to biological uptake of nitrogen by dispersing biomass, which partly settled in the sludge or was attached to the walls.

Importantly, the pH range of 7-8.5 is probably the favourable condition for algal growing population, and hence the biomass of algae increases (Konig *et al.* 1987; Matusiak 1977). In the performance of an anoxic/oxic membrane bioreactor system for the simultaneous removal of nitrogen and chemical oxygen demand, Fu *et al.* (2009) described that the process could

remove up to 87% of total nitrogen (TN) and 94% of COD, with the influent concentration of 200 mg NH_4^+-N/l. Contrary to heterotrophic denitrification, autotrophic denitrification (*Thiobacillus* and *Thiomicrospira denitrificans*) consumes alkalinity as 3.91g of $CaCO_3$ for reducing 1g NO_3^--N, as indicated by van Rijn et al. (2006) as cite by Breisha (2010).

Hunik *et al.* (1992) concluded that adjustment of pH is a possibility for improving ammonia conversion through an increase in the activity of *Nitrosomonas* bacteria. Low pH conditions primarily affect nitrifying bacterial by inhibiting enzymatic activities and secondarily affect the availability of alkalinity. At the pH range of 7.2 to 8.0, the nitrification rate is assumed to be constant, and the active nitrification processes close to neutral. The result of pH in the study (7.3 to 8.7) supported this conclusion.

In addition, Lai and Lam (1997) looked for the major pathway for nitrogen removal in WSP and pointed out that carbon dioxide was consumed by the algal photosynthesis activity affected by organic degradation, resulting in an increased pH. Khan and Yoshida (2008) studied on algae *C. vulgaris* and pointed out that in the case of pH 9.0, ammonium reduction of only 41% was achieved.

It is important to highlight that after the death the algal biomass and its sinking to the bottom of the reactor, anaerobic processes partially recycle ammonium nitrogen to the water, Therefore the removal of the dead algal sludge would complement the highly efficient algal nitrogen uptake (Camago Valero *et al.* 2010).

- pH influences to oxygen production

As the result show (see annex 6), at a pH range of 7.6-8.6, the oxygen concentration remains constant, and its highest value is in the range of 2.4-8.9 mg O_2/l, in the reactor T1. The maximum oxygen level is observed in water for a pH in the range of 8.6-8.9 for both reactors T1 and T2. This indicates that oxygen concentrations in the reactors are approaching the saturation condition (9-11 mg O_2/l).

The pH condition clearly influences the oxygen concentration in water by affecting many physico-chemical and biological environmental factors, such as substrate concentration, product concentration, dissolved oxygen and various inhibitors (Dochain and Vanrolleghem 2001).

According to Anthonisen *et al.* (1976), Sharma and Ahlert (1977), it is well known that inhibitory effects of ammonia on nitrification are not due to ammonia itself but free ammonia

(FA), which can be formed at high concentrations of total ammonia. Previous researches reported that the threshold FA concentration for inhibiting *Nitrosomonas* and *Nitrobacter* were 10–150 mg/l and 0.1–4.0 mg/l, respectively (Suthersan and Ganczarczyk 1986; Bae *et al.* 2001).

2.2.6 Oxygen effects to ammonium by different illumination phases

The ammonium nitrogen concentration could be removed from wastewater via many pathways, such as aerobic autotrophic oxidation to nitrite (the first step of nitrification), heterotrophic oxidation as a likely mechanism of removing excess intracellular reductants (Lam *et al.* 2004; Trang *et al.* 2012). The primary difference between aerobic autotrophic and heterotrophic ammonia oxidation is that the energy evolved from the former reaction is used to fix carbon dioxide - CO_2 (Lam *et al.* 2004).

It is most likely that low dissolved oxygen in the water and the high NH_4^+-N concentrations could both contribute to reducing the nitrite oxidizer activity and a consequent unstable nitrite/nitrate production in the treatment reactors (Wyffels *et al.* 2003; Wyffels 2004).

The high concentration of produced oxygen by algae under the illuminated phase was 5-8 mg O_2/l showed in the test. As a result, a high efficiency of nitrogen removal was obtained. It means that the total ammonia nitrogen could be removed up to 90%.

The removal efficiency of ammonia was reduced during the dark phase in comparison with the efficiency during the light phase. But it does not demonstrate a significant difference between the dark and illuminated phases regarding the oxygen concentration, which has a value of 5.6 mg O_2/l. A big difference in the ammonia removal efficiency could be observed at the oxygen concentration of 6.4 mg O_2/l. About 80% of ammonia was removed in the light phase while only 60% removal was observed in the dark phase.

On the other hand, several species of algae such as *C.kessleri* could chemoorganotrophically grow during the dark period since they can metabolize the organic carbons for their growth without photosynthesis (Lee *et al.* 2001*;* Janssen 2002, Geider Osbonrne 1989). This can explain why during the dark phase the oxygen concentration is still reaches a high value in the rectors. The test can serve both theoretical and practical objectives.

These results proved that this method has a great potential of using algae for the removal of nutrients from wastewater treatment in an environmentally friendly way, particularly for autotrophic compound-rich wastewaters having a low C/N ratio. The high-density algal

cultures using optimized photo-bioreactors with recycling line could also save energy and enhance the removal efficiency of organic carbon sources from wastewater.

Several studies have found a correlation between substance removal such as the nitrite and/or nitrate and oxygen limited condition, and thus could find the rate of the nitrogen removal from a particular experiment (Bernet *et al.* 2001; Han *et al.* 2001; Pollice *et al.* 2002; Ruiz *et al.* 2003; 2006).

2.2.7 Effect of light and temperature on algal growth

It is clear that light and temperature have an effect not only on simple substrates for algal growth such as glucose for yeast, but also affect the growth-limiting sources. On the other hand, it was suggested that some species of algae were able to maintain maximal growth under the dark period (Lee and Pirt 1981).

Moreover, light and temperature are almost invariably measured and controlled in experimental studies with algal material. The 'expected' effect not only focuses on the light-limited condition for algal growth but also the effectiveness of temper.

- Light limitation on algal growth and ammonia nitrogen removal

The limitation of light conditions for growth of algae is generally connected to increased chlorophyll-α content. Among the environmental factors affecting the growth rates of unicellular algae with reference to full nutrient removal from wastewater, light is frequently at an improper level because of two aspects: the durable time and the quality of light provided.

Supply of light is one of the most important factors for the photosynthetic organisms, including algae, and depends on two factors: light regimes and light intensity. Light not only affects the algal photosynthesis and productivity, but also influences cell composition and metabolic pathway (Cuaresma *et al.* 2009). Furthermore, the durable time of provided light is absolutely required for efficient production of biomass and metabolites of some algal species (Richmond 2004). The results obtained from laboratory experiments on mixed populations and algal cultures suggest that the light history of algal cells can cause a considerable variation in the rate of endogenous respiration during the ensuing dark period (as can be seen from the substances removal efficiency of BARs for different periods of light provided from the Fig. 34, 36, 38, 40).

This study pointed out that using algae could reduce ammonium nitrogen from wastewater by around 83-86% for 12 hours of provided light. In contrast, when the light condition was 6

hours per day, the efficiency of ammonia removal was reduced significantly (achieved only 67-70% of total ammonia from influent).

The result is in agreement with the researches of García *et al.* (2006), carried on the high rate pond treating urban wastewater, where 57%-73% TN was removed by algae with averaged values of chlorophyll-α content in the mixed liquor being 2.44±1.06 mg/l. Khan and Yoshida (2008) said that with 24 hour incubation, the highest reductions of algae *C. vulgaris* NTM06 in ammonium was 73%.

Powell *et al.* (2009) used acid-soluble polyphosphate (ASP) to test the uptake process by microalgae. They concluded that at the light intensity of 150 $\mu E/m^2s$, a higher amount of ASP initially accumulated in the microalgae. Lee and Lee (2001) used light dark cycles on wastewater treatment with microalgae assuming that for continuous illumination, COD removal was 89% of the initial concentration and in light/dark cycles it was 72% of total initial concentration.

Wastewater treatment in high rate algal ponds for biofuel production was studied by Park and Craggs (2010) and Park *et al.* (2011). They found that an algal concentration of 300 g TSS/m^3 will absorb almost all of the available light within the top 15 cm of high rate algal ponds (HRAP) and will remove up to 95% of soluble organic compounds (measured as $sBOD_5$).

In addition, Slamet and Hermana (2012) studied the effect of light and the performance of algae during the treatment of wastewater. They found that the reactor with natural light had a nutrient removal performance greater than the artificial light reactor. In their study, the efficiency of ammonia removal was 98% (as NH_3^+-N) at a water depth of 40cm.

It is well known that light is an essential resource often limiting the growth rate of algae and also a factor to determine the rate of photosynthesis of algae. Bouterfas *et al.* (2002) presented their study and found that the optimum light intensity varied between algal species from 90 to 400 $\mu mol/m^2 \cdot s$ and the maximum growth rate of *Selenastrum minitum* was 1.73 $\mu mol/m^2 \cdot s$ (at given temperature 35^oC).

In general, on increasing the time of light supply, the variation of the ammonium removal is in accordance with the variation of the biomass accumulation. This means that most of the ammonium nitrogen consumed by algal culture was converted into biomass. Weissman and Benemann (1978) assumed that when the net photosynthetic efficiency at maximum productivity was about 0.16, chlorophyll-α content in *Chlorella sp*. increased linearly with dry

weight (R^2 = 0.99), averaging about 2% of the ash-free dry weight. Pulz (2001) indicated that biomass concentration during production was 2–8 g/l approximately with closed system PBR (Photobioreactors).

Published data on algal productivity range from 10 to 50 g/($m^{2.}$d) by Weissman *et al.* (1988) with an average rate around 20 g/($m^{2.}$d). The maximum practical photosynthetic efficiency is still a problem (Pirt 1983). Hall (1986) found out that a theoretical photosynthetic yield of 6 to 7% of total solar energy with a biomass productivity of 35 to 70 g/($m^{2.}$d) seems possible, but it is dependent on geographic location. A photosynthetic efficiency of 10% during a period of 122 days has been reported by Laws *et al.* (1986). These experimental results are in agreement with Richmond and Beker's (1986) comments. Walker (2009) figured out that light utilization by algae is about 4.5% (12-14 g/$m^{2.}$d).

Compared with the result of this study, it can be assumed that under 12 hours of applied light, the Chlorophyll-α content has got the highest value. Hence more photosynthesis activity, higher oxygen production, and the best efficiency of ammonia removal was achieved (the Fig. 40-42 for ammonia removal and oxygen production in the reactors supports this conclusion).

- Temperature effects on algal growth and its effects on ammonia removal efficiency

The algal growth rate at different temperatures will accordingly affect nitrogen removal. This research briefly described some results of ammonia nitrogen removal due to the effect of temperature changes from 11^{o}C to 16^{o}C. As the results show, an ammonium nitrogen removal efficiency of only 54-56% was obtained in the reactors T1 and T2. The ammonia nitrogen efficiency is lower than 30-34% in comparison with the efficiency of ammonium nitrogen removal under the temperature range of 22^{o}C to 25^{o}C.

From this point of view, low temperatures might be concluded to cause lowering of algal growth. It might be assumed that under unsuitable conditions (low temperature together with the low algal photosynthesis activity), the ammonia nitrogen removal efficiency cannot achieve a high value. It is interesting to note that the growth rate of algae often changes under the effect of different temperatures (as reported in the literature). Sheehan *et al.* (1998) pointed that *Monoraphidium* could achieve a maximum growth rate 2.84/day with temperatures of 25-30^{o}C. In the same study, the lipid content in exponentially growing cells increased from 22% to 52%.

The optimal temperature measured for the conditions of the maximum algal growth rate (sufficient nutrient and light conditions) varies between algae species, but is often between 20^{o}C and 35^{o}C for many algal species (Peeter and Eilers 1978; Soeder *et al.* 1985). Eppley (1972) reported that the freshwater algal species is at 30-35°C for green algae, and above 35°C for blue-green algae. Guillard (1975) suggested that the some algal species can develop well under temperature at 28°C and ceased at 31°C.

To mention some more details of the effects of temperature conditions on algal biomass growth, Hulburt and Guillard (1968) found some species of algae closely related to *Skeletonema tropicum* had the optimum temperature range of between 25°C and 35°C, and growth ceasing both below 13°C and above 35°C. Moreover, *Chloromona* species grew well at high temperatures of above 28°C (Devos *et al.* 1998; Guillard and Ryther 1962).

The effect of temperature on the specific growth rate of algae is also supported by Imai *et al.*, 2009; Zargar *et al.* (2006) the research indicate that when *M. aeruginosa* was incubated at higher water temperatures, mean growth rates at 30 and 35°C were high μ = 0.47 and μ = 0.45, respectively.

The maximum removal efficiencies of ammonia, BOD_5, COD removal by BARs at temperatures between 22-25^{o}C in this research are suitable and in agreement with the suggestions about the temperature conditions by the authors mentioned above. Fontenot et al. (2007) as cited by Breisha (2010) indicated that the temperature range of 22-27^{o}C gave best results in terms of maximum nitrogen removal from a shrimp aquaculture wastewater. When the temperature was decreased from 20^{o}C to 17^{o}C, an approximately decrease of 15% of nitrogen occurred during denitrification (Breisha 2010).

Also, the available evidence from this research about the efficiency of ammonia removal from the reactors under low temperatures also indicate that the low temperature is close to limit of the algal population growth and hence could reduce the efficiency of ammonia nitrogen removal from wastewa.

2.2.8 Nitrate nitrogen

A comparison of nitrate concentration of the three reactors is shown in the Fig. 44. The average nitrate concentration in the influent to the reactors was 0.4 mg NO_3^--N/l, while at the effluent of reactor T1 and T2, the average nitrate concentrations were 5.5-7.5 mg/l, respectively (with 12 hours light provided).

The reference reactor showed the lowest nitrate concentration followed by reactor T1 and T2. It is interesting to note that at the time when the nitrate variations were measured, ammonia was often effectively removed in the algal treatment reactors. This may indicate to occurrence of the nitrification and/or denitrification processes.

Under the 6 hours per day saturated light conditions, the nitrate concentration in different reactors were reduced to quite small values due to less oxygen being produced in the reactors i.e. 1.3 mg NO_3^--N/l at the effluent of the reactor T1, 0.9 mg NO_3^--N/l for the reactor T2 and 0.3 mg NO_3^--N/l at the effluent of the reactor T3.

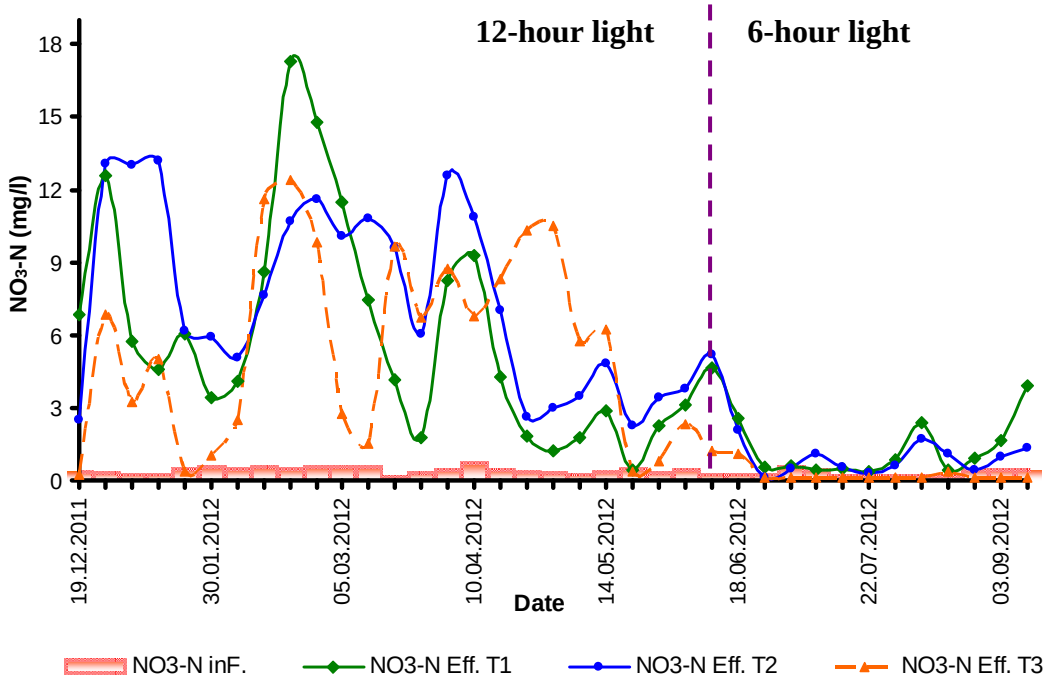


Figure 44. NO_3^--N (mg/l) in the reactors with different light regimes

The low results of NO_3^--N again highlight the influence of the reduced photosynthesis activity under the short lighting intervals. Hence, the reduction in the oxygen produced affects the nitrifying bacteria oxidizing ammonia to nitrates. Also, under the short intervals of provided light, the nutrient uptake of algae was limited. The relation between oxygen and nitrate are also discussed in secton **2.5.9** of this chapter.

Comparing with the results obtained from the algal and duckweed experiments, it could be assumed that nitrate concentrations in the reactors T1 and T2 are higher (3.5-5.4 mg/l) than algal experiment. This corresponds to higher oxygen produced in the reactors than for the algal and duckweed treatments. This result is in agreement with several results obtained from

different methods such as activated sludge process where the nitrate concentration at the effluent was maintained at 6 mg/l.

- Nitrate plays an important role for ammonia removal

Nitrate is the initial component involved in the denitrification process. The group of bacteria carrying out the denitrification process require absence of oxygen in water (Sharma and Ahlert 1977). Under aerobic conditions, the dissolved oxygen favours the activity of the nitrifying bacteria. In contrast, at low levels of oxygen i.e. concentrations less than 0.5 mg O_2/l (anoxic conditions) the biological denitrification process can be expected to occur. Both *Nitrosomonas* and *Nitrobacter* require ammonia nitrogen as the nutrient for growth.

The experiment results show that the concentrations of nitrate at the effluents of the reactors T1 and T2 were 5.5 and 7.5 mg NO_3^--N/l respectively. Additionally, the concentrations of the produced oxygen in the reactors T1 and T2 were 5.9-6.2 mg O_2/l. Therefore, the reactors T1 and T2 have the positive impact on the nitrification performance, even there was the low nitrate concentration influenced to the treatment reactors.

From the results obtained from the reactors T1 and T2 (88-90% of ammonia removal efficiency), it is clear to see that the produced nitrates contributed as an important factor to the rise of the nitrification and denitrification rates. Breisha (2010) pointed out that nitrogen tolerant bacteria include nitrate respiring bacteria and true denitrifiers.

Under the high nitrate concentration, the population of nitrate tolerant bacteria multiplies faster than nitrate intolerant bacteria. Thus, it increases the efficiency of ammonia removal. Several researchers suggested that the nitrification-denitrification process is unlikely to be the principal mechanism of nitrogen removal from wastewater on using algal ponds (Toms *et al.* 1975; Ferrara and Avci 1982; Pano and Middlebrooks 1982; Reed 1985).

However, the results obtained from this study indicate that assimilation as well as nitrification and denitrification processes were important mechanisms for the removal of nitrogen. If the detention time is long enough, there is a reduction in the concentration of oxygen in different parts of the reactors, the pH of the media is suitable and the algal population grows significantly.

2.2.9 The relationship between nitrate concentration and oxygen production

There is an assumption that oxygen is the main influencing factor for producing nitrate concentrations through the nitrification process in water. In order to see the effect of the

produced oxygen with the nitrate concentration in wastewater, the following graph (Fig. 45) for the relationship between the oxygen and the nitrate concentration. From the results obtained, it could be seen that the nitrate concentrations created in the treatment reactors were 4-10 mg NO_3^--N/l when oxygen was around 5-8.5 mg O_2/l. Another factor influencing the performance of the biological processes for nitrogen removal from wastewater is the biological degradation of COD.

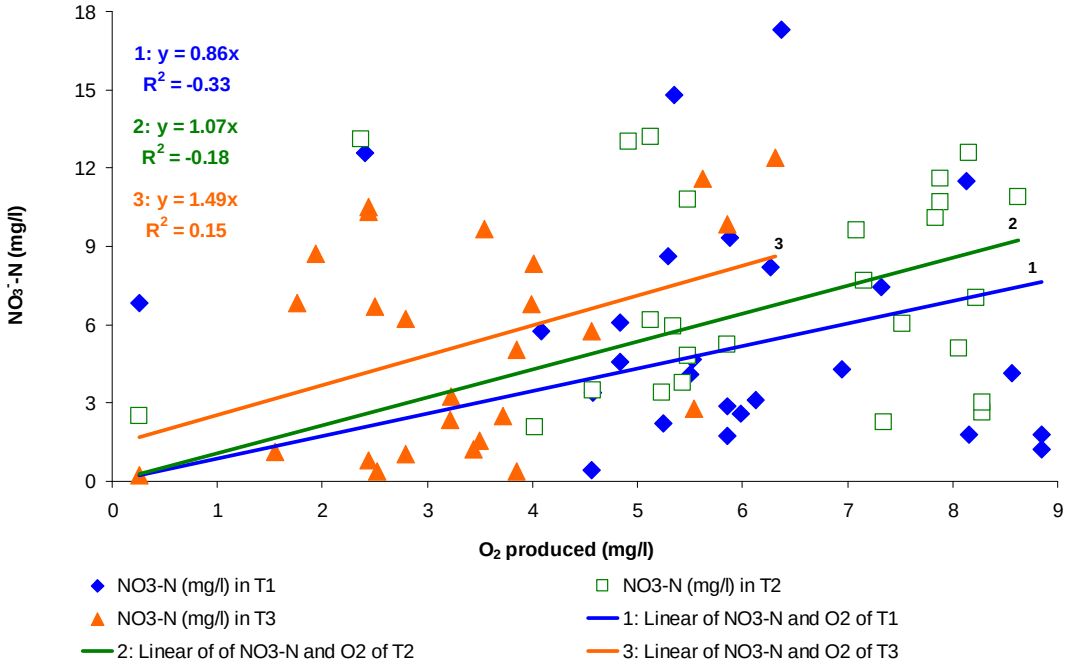


Figure 45. The linear of NO_3^--N (mg/l) and the oxygen produced in the reactors

A limited amount of COD could enhance the performance of nitrification, because the nitrate produced in water reaction could be used by heterotrophic bacteria or the algae for the growth of biomass from nitrate, rather than converting it to nitrogen gas.

Comparing the results of the research and the literature, it was found that 4.2 g of oxygen is required for each gram of NH_4^+-N removed. Therefore, it could be accepted that nitrification is not limited in the reactors T1 and T2, because the oxygen produced by the reactors is always greater than 4.0 mg/l. Moussa *et al.* (2003) showed that the ammonia nitrogen reduced significantly when the oxygen concentration was 2 mg O_2/l in comparison with 8 mg O_2/l. In their research they describe the assessment of nitrification activity using a biological oxygen monitor.

Painter (1977) found that the rate of ammonia removal in the activated sludge process depends on the population of nitrifying bacteria in the sludge, and on the growth rate of these organisms under the aerobic conditions. The ammonia oxidization in this study is reported to

be about 250 mg/(g·h). Studying nitrifying rotating biological contactors (RBC), Siegrist *et al.* (1998) observed that nearly 70% of influent nitrogen was lost due to the oxidization of ammonium. From the author's point of view, it could be concluded that in the treatment reactors of this study, the aerobic ammonium and nitrite-oxidizing organisms were the dominating population in the shallow layers of the reactor.

2.2.10 Total nitrogen Kjeldahl and its fractions

The Fig. 46-47 illustrate the variation of the TKN and its fractions in the influent, the effluent concentrations as well as the different N fractions. The average TKN concentration in the influent to the reactors was 83.80 mg TKN/l, while at the effluence of reactors T1 and T2, the average concentrations were 21 and 26 mg/l, respectively. The reference reactor showed the lowest nitrate concentration followed by reactor T1 and T2, (TKN achieved more than 46 mg/l).

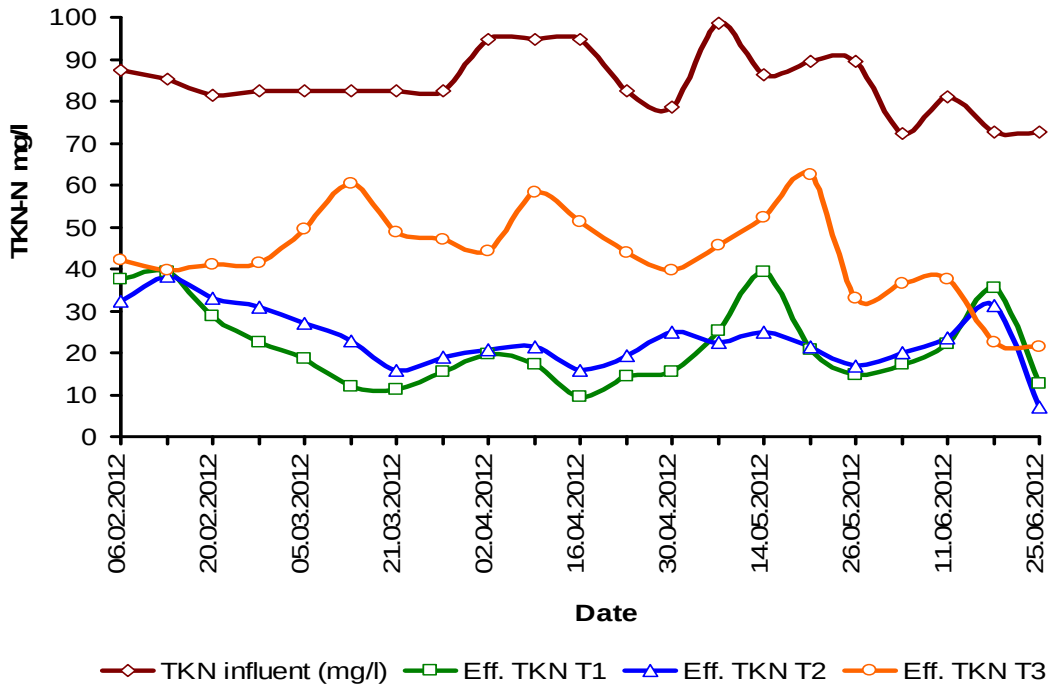


Figure 46. The variations of influent and effluent TKN concentrations in the reactors

It seems apparent that the reactor T1 performs in eliminating organic nitrogen, either by removal of the particulate biological nitrogen and/or via hydrolysis and mineralisation and further processing as ammonium. It seems apparent that the reactor T1 performs in eliminating organic nitrogen, either by removal of the particulate biological nitrogen and/or via hydrolysis and mineralisation and further processing as ammonium**.**

It is interesting to note that at the times when the nitrate variations were measured, ammonia was often effectively removed from the algal treatment reactors. This may indicate the occurrence of nitrification and/or denitrification processes in the reactors.

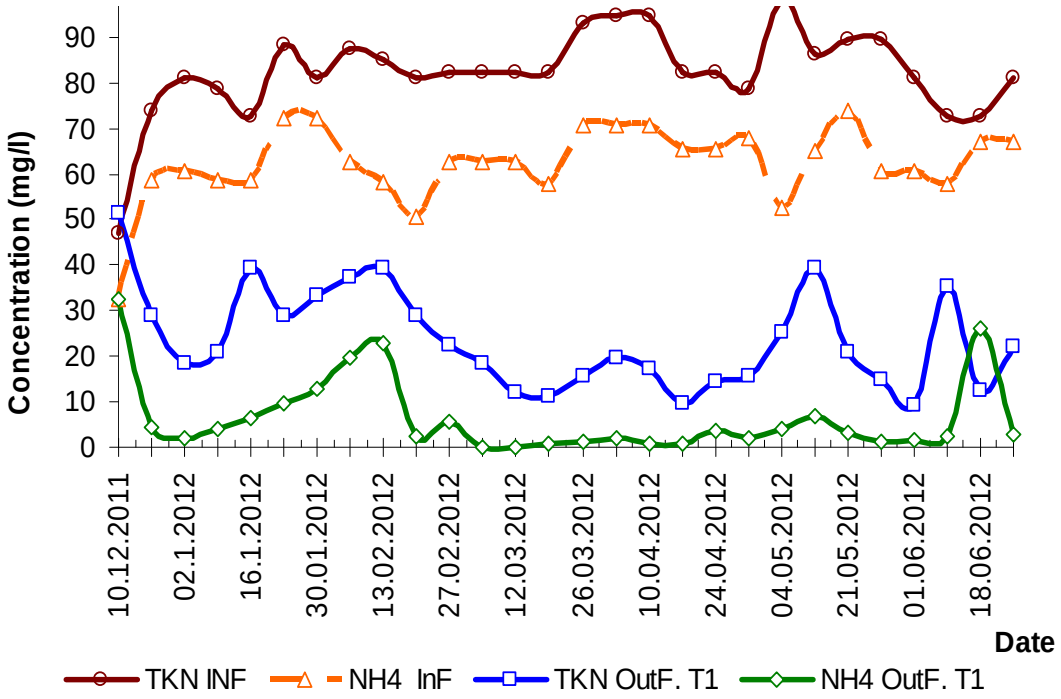


Figure 47. The variations of TKN concentrations and nitrogen fractions in reactor T1.

It is interesting to note that at the times when the nitrate variations were measured, ammonia was often effectively removed from the algal treatment reactors. This may indicate the occurrence of nitrification and/or denitrification processes in the reactors.

Regarding the overall nitrogen elimination capacity, the variation of total TNK in the influent to the reactors was observed to be 83.80 mg TKN/l. The removal effectiveness of the reactor T1 and T2 vary between 90 and 92%. Nitrogen removal is significantly reduced when the duration of the illuminated time changes to six hours. In activated sludge process total nitrogen at the effluent was around 9 mg/l and the efficiency of removal of total nitrogen was 82% (DWA 2011). This may indicate a low efficiency of ammonia nitrogen removal from the reactors in comparison.

2.2.11 Nitrogen mass balance

The basic formulae for the calculation of nitrogen mass balance and the removal efficiency of baffled algal reactors are given in part 1.3.7 of this chapter. Below, only the results from the reactor T1 are shown. The calculation for the other reactors are based the same method. The

calculation used complete parameters, except for the total solid in sludge, whose value was assumed.

Q_{inflow}	22.50	l/d
$TKN_{influent}$	83.80	mg/l
NH_4^+-$N_{influent}$	63	mg/l
NO_3^--$N_{influent}$	0.4	mg/l
$BOD_{5\ influent}$	267	mg/l

$Q_{outflow}$	22.30	l/d
$TKN_{effluent}$	24	mg/l
NH_4^+-$N_{effluent}$	6	mg/l
NO_3^--$N_{effluent}$	6	mg/l
Hydraulic retention time (HRT)	10	day

By missing measured parameter Tss in sludge, therefore N-org in sludge could be assumed:

TKN effluent = 24 mg/l

$C_{N\text{-org in sludge}} = 0.05 \times C_{o,\ BOD5\ influent} = 13.4$ mg/l

Mass $C_{N\text{-Org in sludge}} = C_{N\text{-org in sludge}} \times Q_{inflow} = 0.05 \times C_{o,\ BOD5\ influent} \times 22.5$ l/d

Mass $C_{N\text{-Org in sludge}} = 300$ g/d (= 0.3 g/d)

- Mass balance of total nitrogen inflow

Mass $TN_{influent} = (C_{o,\ TKN\ influent} + C_{o,\ NO3\text{-}N\ influent}) \times Q_{inflow} = 1.9$ (g/d)

- Mass balance of total nitrogen out

Mass TN_{out} = Mass $TN_{effluent}$ + Mass N-org $_{in\ sludge}$

Mass $TN_{effluent} = (C_{e,\ N\text{-org}} + C_{e,\ NH4\text{-}N} + C_{e,\ NO3\text{-}N}) \times Q_{outflow}$

$TKN_{effluent} = C_{e,\ N\text{-org}} + C_{e,\ NH4\text{-}N} = 24$ (mg/l)

Mass $TN_{effluent} = (24 + 0.4) \times 22.5 = 0.54$ (g/d)

Mass N-org $_{in\ sludge} = 0.3$ (g/d)

Then: Mass balance of $TN_{out} = 0.84(g/d)$

- Efficiency of assimilation

$E_{Mass\ TN\ assimilation} = (Mass\ TN_{influent} - Mass\ TN_{effluent}) / Mass\ TN_{influent}$

$Mass\ TN_{effluent} = 0.54\ (g/d)$

$E_{Mass\ TN\ assimilation} = (1.9 - 0.54)/1.9 = 0.72$

- Efficiency of nitrification

$E_{Nitri} = (Mass\ TN_{Nitri} - Mass_{NH4\text{-}N\ effluent}) / Mass\ TN_{Nitri}$

$Mass\ TN_{Nitri} = Mass\ TN_{influent} - Mass\ TN_{effluent} = 1.9 - 0.54 = 1.36\ g/d$

$Mass_{NH4\text{-}N\ effluent} = C_{e,\ NH4\text{-}N} \times Q_{outflow} = 6 \times 22.3 = 133{,}8\ mg/d\ (= 0.13g/d)$. Then:

$E_{Nitri} = (1.36 - 0.13)/1.36 = 0.90$

- Efficiency of Denitrification

$$E_{DEN} = \left(\frac{(Mass\ TN_{Nitri} - Mass_{NH4-N\ effluent}) - Mass_{NO3-N\ effluent}}{(Mass\ TN_{Nitri} - Mass_{NH4-N\ effluent})} \right)$$

$Mass\ NO_3\text{-}N_{in\ effluent} = C_{e,\ NO3\text{-}N} \times Q_{outflow} = 0.13\ g/d$. Then: $E_{DEN} = 0.89$

- Nitrification rate

$R_{Nitri} = (C_{mass\ TN\ Nitri} - C_{e,\ NH4\text{-}N\ effluent})/HRT$

$C_{mass\ TN\ Nitri} = (Mass\ TN_{Nitri} \times 1000\ mg/g)/ Q_{outflow} = 60.99\ mg/l$

$R_{Nitri} = 5.5\ (mg/l.d)$

- Denitrification rate

$$R_{DEN} = \left(\frac{(C_{Mass\ TN\ Nitri} - C_{e,\ NH4-N}) - C_{e,\ NO3-N})}{HRT} \right) = 4.9\ (mg/l{\cdot}d)$$

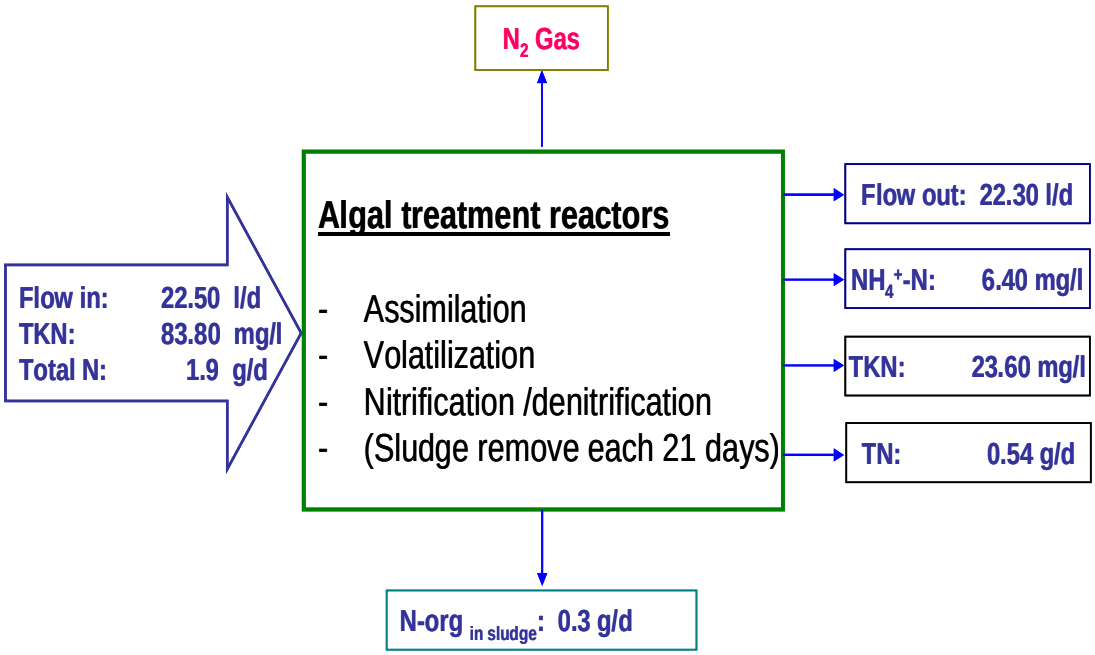


Figure 48. Mass balance of total nitrogen in baffled algal reactor T1

The comparison of removal efficiency between algal experiment and baffled algal reactors is shows in table 20.

Table 20. Comparison the nitrogen removal efficiencies of Baffled algal reactor and algal experiment

	Algal experiment (%)	**Baffled algal reactors T1 (%)**
Efficiency of assimilation	73	72
Nitrification rate	4.4 (mg/l·d)	5.5 (mg/l·d)
Denitrification rate	4.2 (mg/l·d)	4.9 (mg/l·d)

Many species of heterotrophic bacteria can transform the oxidized nitrogen compounds into nitrogen gas by biological nitrification and denitrification processes while also removing carbon. But, biological denitrification process for removing ammonia nitrogen from wastewater is usually slow. According to Wang *et al.* (1995), 10-50 mg NO_3^--N/l was removed with *Pseudomonas denitrificans* after 4-hours and denitrification of 100 mg NO_3^--N/l with the suspended bacterial cells lasted 18 hours (Lee and Daheb 1988).

Following Chen *et al.* (1972), Terry and Nelson (1975) denitrification mainly occurred in the sediment rather than in the water column due to higher amounts of organic substances, denser colonisation with denitrifiers and lower DO. Low denitrification rates were measured at lower temperatures due to the reduction in bacterial activities. The maximum depth of active denitrification within the sediment layer varies depending on the penetration of nitrate (Christensen *et al.* 1990).

Modifying the hydraulic flow patterns into upward and downward flows, as used in the treatment plants designed for this study, should be the solution to improve the efficiency of nitrification and denitrification processes. The higher the nitrate concentration is in the denitrification zone, the lesser is the ammonia nitrogen concentration at the effluent and the higher is the removal efficiency that can be obtained.

2.3 Determination of Algal biomass and Chlorophyll-α content

2.3.1 Algal growth inhibition medium test

The algal biomass production in water depends on both heterotrophic and phototrophic processes for developing their mass. One of the most important factors driving algal growth of biomass were the nutrients, fraction of substances in water and the quantification of light providing (the method use to determine Chlorophyll-α was introduced in the section on the research method).

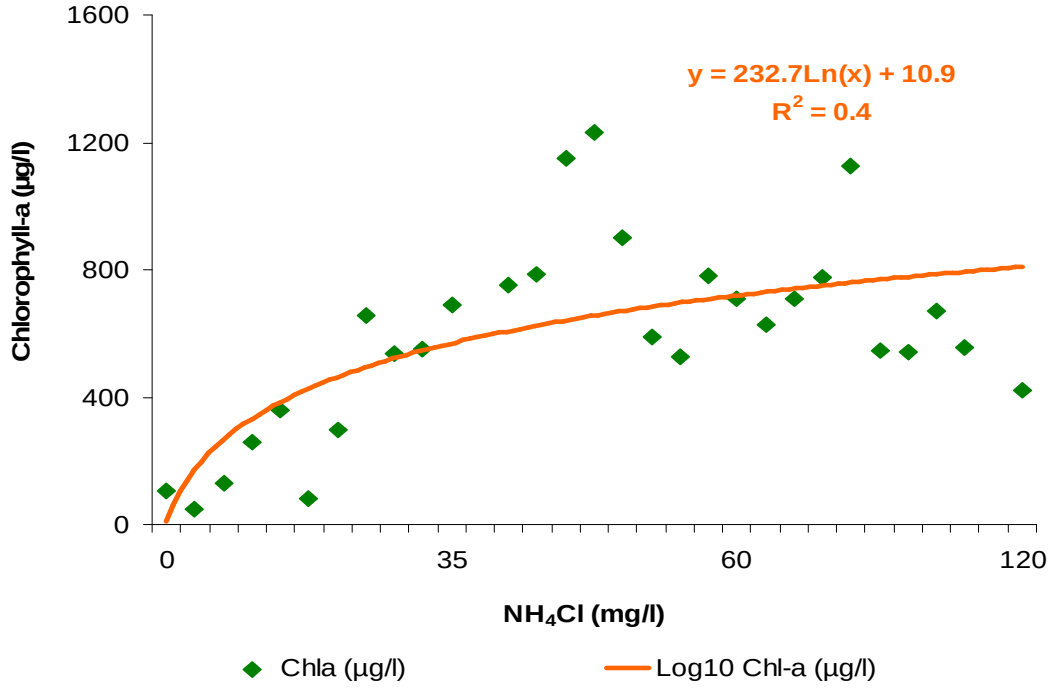


Figure 49. Growth rate medium of algae inhibition test (Chlorophyll-α content)

In order to understand the interactions between algal biomass growth and the limitation of ammonium concentration, the algal growth inhibition test with respect to ammonia chloride (NH_4Cl) concentrations had been done (as can see in the Fig. 49).

Firstly, the theoretical method was applied to obtain the algal growing inhibition in the standard environmental conditions. Secondly, a batch test involving a mixed culture of algal species was carried out. The expectation of the test was based on measuring the Chlorophyll-α content in algal cells and therefore, could actually obtain a medium growth rate of algae from wastewater.

The results showed high algal biomass growth, as the measured chlorophyll-a content was 600-1200 µg Chl-α/l and the concentration of NH_4Cl around 40-80 mg/l. The algal growth ceased if the substance exceeds 100 mg NH_4Cl/l. Based on the formula to calculate the medium growth of algal given by OECD (1984) and DIN (38 412 L16), the growth medium algal inhibition $\mu_{avg.}$ obtained from the practical analysis, could be expressed as $\boldsymbol{\mu_{avg.} = 1.38 \cdot day^{-1}}$.

2.3.2 Determination the algal specific growth rate

The relationship between the measured algal chlorophyll and the rate of algal growth inhibition were determined by the growing rate medium of the algal inhibition test. To find out how the specific growth rate of algal affects the nutrient removal, the batch tests for the growth rate of algae with raw wastewater had been done (the Fig. 50).

The test used a mixed algal culture (three replicas for each step of time in the test were used for standard deviation calculations) with 250ml of municipal wastewater (COD 600mg/l, NH_4^+-N 65mg/l, pH 7.4). The light intensity was adjusted to 9.000 Lumens, intermittent light simulation was 12:12 (day:night) and the temperature was 20^{o}C to 25^{o}C. The specific growing rate of algal inhabitant following the series-test with real wastewater could be determined by:

The results show the variation of Chlorophyll-α content of the batch test with algae cultivation during the study period (see annex 7). The Chlorophyll-α content in the initial sample started at 0.0071 g Chl.α/l. Due to the lag phase (during 24-hour cultivation), the chlorophyll content decreased shortly to 0.005 g Chl.α/l. During three-week cultivation phase, the chlorophyll-α content generally exhibited a peaking of the production with values from 0.04 to 0.044 g Chl.α/l. When the algae achieved the maximum inhibition, the growth of algae started decreasing (see the Fig. 50).

There might be two reasons for this: one, there weren't enough nutrients for their growth. Second, the decay rate of algae increased and was larger than the growth rate. Hence, although the concentration of ammonium nitrogen increased, the growth rate of algae decreased

$$\mu_{spec.} = 1.47 \cdot day^{-1}$$

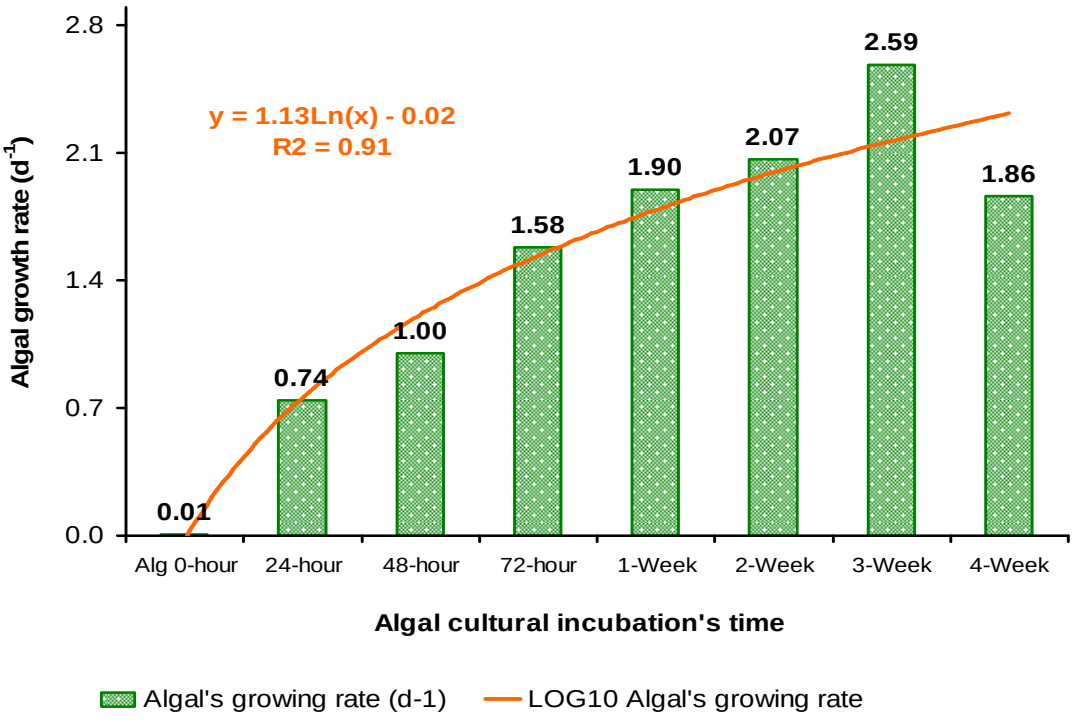


Figure 50. Determination of specific growth rate of mixed algae culture (Chlorophyll-α content)

The relationship between algal growing rate and the ammonium nitrogen utilized is shown in the Fig.49. The ammonia nitrogen reduced significantly when the algal community developed at a maximum rate of $\mu_{spec.} = 2.6 \cdot day^{-1}$, and total ammonia nitrogen utilization is 81% of total ammonia nitrogen available in wastewater.

The results obtained from this study were in the same range as several other studies about the determination of the algal growth rates. Table 21 shows the growth rates of the different algal species on changing the temperature.

Table 21. Comparison the growth rate of mixed algal culture in this study with another result

Algal species	Temp. (^{0}C)	Maximum μ_e/day	Reference
Chlorella pyrenoidosa	19	1.36	Shelef et al. (1970)
(Emerson strain)	25	1.95	Zabat (1970)
	28.5	1.84	Shelef et al. (1970)
Chlorella pyrenoidosa	35	3.94	Shelef et al. (1970)
	39.2	4.26	Shelef et al. (1970)
Chlorella sp.	25	1.88	Williams (1965)
Selenastrum capricornutum	24	1.85	Toerien et al. (1971
	27	2.45	Goldman et al. (1974)
Scenedesmus quadricauda	27	2.29	Goldman et al. (1974)
Mixed algal culture	22-25	1.47	Own study

It is evident from the results that the high Chlorophyll-a content in batch tests and the magnitude of ammonia nitrogen removal efficiency in the real treatment reactors because of NH_4^+-N consumption for the growth of algae significantly correlated with oxygen concentration and pH of the media. From this point of view, the rise of ammonia nitrogen removal in the treatment reactors coincided with the increase in Chlorophyll-a content (comparing with the batch test and at the same period of the study) as well as COD and BOD_5 removal.

The obtained results are in agreement with those of many other researches about the determination of algal growth inhibition test. Many authors have concluded that assimilation/sedimentation is the main process of nutrient removal in waste stabilization ponds. Ferrara and Avci (1982) estimated that 96% of total nitrogen removed was assimilated into algal and bacterial cells. Lai and Lam (1997) found this value to be 25% of ammonia nitrogen removal.

Chevalier and de la Noüe (1985) reported similar NH_4^+-N removal rates (100%) for carrageenan-immobilized *S. obliquus*. Lau *et al.* (1997) reported similar (95%) NH_4^+-N removal rates for alginate-immobilized *C. vulgaris* growing in artificial wastewater. Mostert

and Grobbelaar (1987) detected that the highest production rates are found at N and P concentrations exceeding 25 and 2 mg/l, respectively. Under certain N supply conditions, some species of algae such as *Scenedesmus-Chlorella* had a higher development rate for nutrient absorption. Nitrogen losses of up to 69% of the supply concentrations were measured under specific conditions.

Several reports on immobilization algal reactors found approximately 81-86% phosphorus removal and 98 -100% ammonia removals by using two biological reactors during 7 days of treatment. Wang & Huang (2003), Mohamed (2007) did research on co-immobilized *Chlorella pyrenoidosa* and activated sludge for nitrate and phosphate removal. They reported over 80% nitrate removal in all experimental periods with removal efficiency of phosphate being 88%.

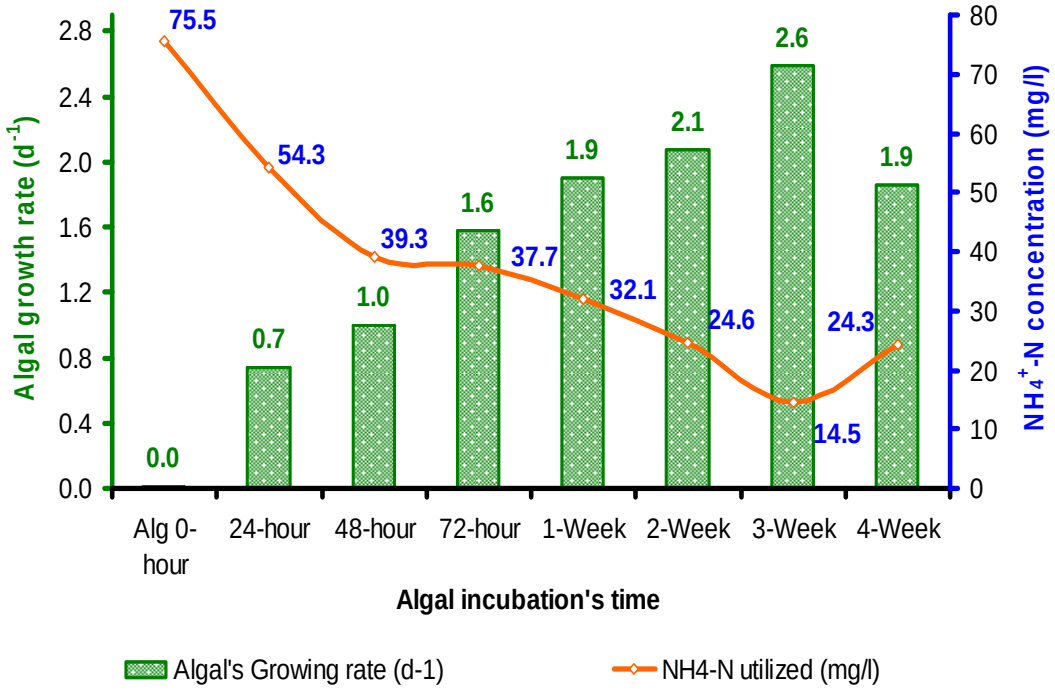


Figure 51. The algae growth rate and the ammonia nitrogen concentration utilized

Taylor *et al.* (1988) cultivated algal species *Chlamydomonas reinhardtii* in incubation with substances rich in nitrogen compounds. After 21 days of growth, *Chlamydomonas reinhardtii* consumed 83.8 and 78.7 mg N/l as NO_3^- -N and NH_4^+-N, respectively. In media in which the N source was NO_3^- -N and NH_4^+-N, phosphorus consumption was 16.97 and 16.77 mg/l resulting in the removal of 99.8 and 98.7% of the initial concentration, respectively.

These high yields of algal growth have not been obtained by any increase in the growth rate constant of the algae, but rather by the maintenance of high rates of growth in dense cultures. The growth rate constant is an expression of the potentialities of an organism. Suitable conditions for the realization of these potentialities must be provided if high yields are to be obtained (Allen and Arnon 1955). The research could assume that if the sludge is not evacuated out of the system after three weeks, the rate of ammonia nitrogen elimination in the reactors will reduce due to an increase in the rate of decay of organisms and recycling of organic matters into the water body.

Chapter V.

CONCEPTUAL MODELLING FRAMEWORK FOR INTEPRETING AMMONIA NITROGEN REMOVAL VIA ALGAL

1. MODEL-BASED DESIGN OF AMMONIA NITROGEN REMOVAL VIA ALGAL BIOLOGICAL PROCESSES

1.1 Basic information

The purpose of this study is to extend the science through advanced mathematical modelling to nitrogen removal via a Baffled Algal Reactors as well as to get more insight into and monitor the controlling biochemical processes. This study uses the mixed reactor compartment model of AQUASIM to present the behaviour of the BAR and hydrodynamics using a system that is based on a 1D description that averages all variables over horizontal cross sections.

Mathematical equations were solved for the formulations of Activated sludge model ASM1, ASM3. The completed forms of mathematical equations were set into AQUASIM for effluent quality predictions. In the model development process, the conceptual model should be established to take into account all internal parameters and to observe the interactions of all major parameters.

The components of the model equation were presented in a matrix format, making it easy to understand the outcomes of each component in the model. The main biological processes in the components of the model such as growth processes, decay processes, hydrolysis, oxygen exchange, flow rate and quality of effluent of the reactor were considered as the formulation and kinetic equations of ASM1,3. The model development also regards the mass balance equations of particulate and dissolved components that include the algal growth. The results

of the modelling can provide the important information in the development of the progression as well as calibration and validation of selected parameters.

1.2 Activated Sludge model structure ASM1 contributed in algal model.

1.2.1 COD components

Following on the study by Jeppsson (1996), in the ASM1 model, the carbonaceous material was divided into forms of the biodegradable COD the non-biodegradable COD (inert substrate) and biomass as shows in the Fig. 52. The biodegradable COD is further divided into readily biodegradable substrate ($\mathbf{S}_S$) and slowly biodegradable substrate ($\mathbf{X}_S$). The soluble component is S and a particulate component is **X**. The non-biodegradable COD is divided into soluble ($\mathbf{S}_I$) and particulate ($\mathbf{X}_I$) material.

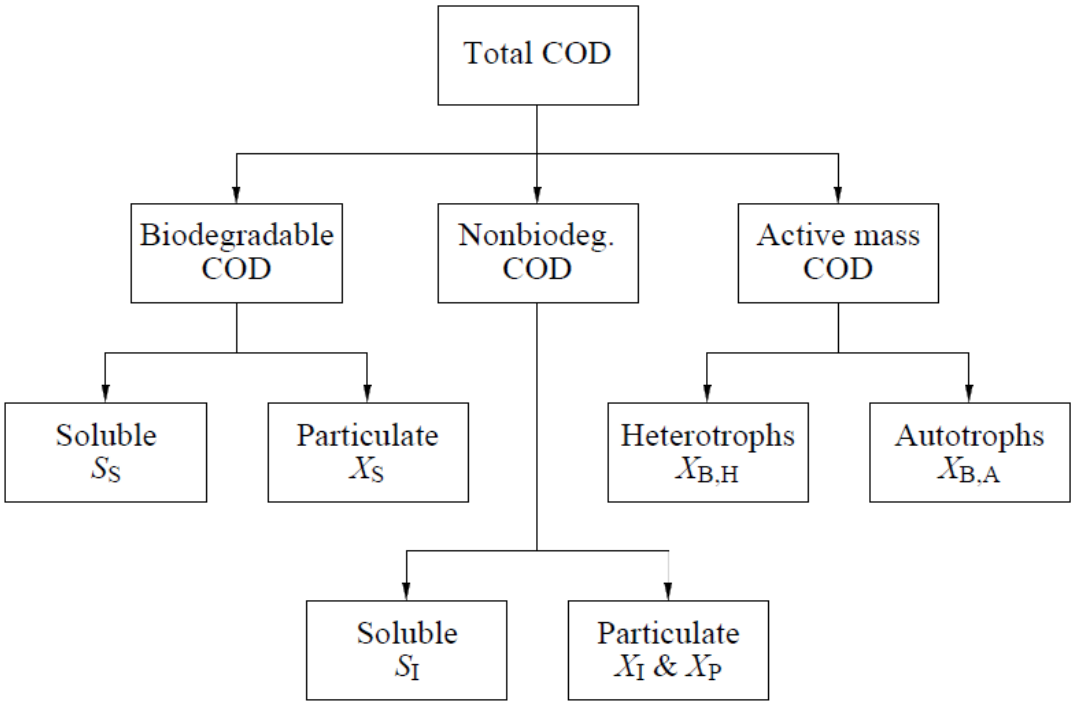


Figure 52. Wastewater characterization for carbonaceous components (Jeppsson 1996).

Additionally, the active biomass was divided into two types of the organisms: heterotrophic biomass ($\mathbf{X}_{BH}$) and autotrophic biomass ($\mathbf{X}_{BA}$). Finally, an extra state variable ($\mathbf{X}_P$) for modelling the inert particulate products arising from biomass decay is included. The balance of total COD of ASM1could be made by:

$$\mathbf{COD}_{tot} = \mathbf{S}_I + \mathbf{S}_S + \mathbf{X}_S + \mathbf{X}_{BH} + \mathbf{X}_{BA} + X_I + \mathbf{X}_P$$

1.2.2 Nitrogen components

Based on the measurements of total Kjeldahl nitrogen (TKN) in wastewater, the nitrogen compounds are divided into free and saline ammonia ($\mathbf{S_{NH}}$), organically bound nitrogen and active mass nitrogen, the fraction of the biomass which is assumed to be nitrogen.

The Fig. 53 explains the nitrogen component state variable in the model. Similar to the division of the organic material, the organically bound nitrogen is divided into soluble and particulate fractions. The particulate biodegradable organic nitrogen is shown as X_{ND}, organic nitrogen associated with the inert organic particulate products X_{NP} and the inert organic particulate matter X_{NI}. Inert soluble nitrogen is modelled as S_{NI}, the nitrate and nitrite nitrogen are combined into one variable, S_{NO}, the dissolved oxygen concentration is S_O and the alkalinity S_{ALK}.

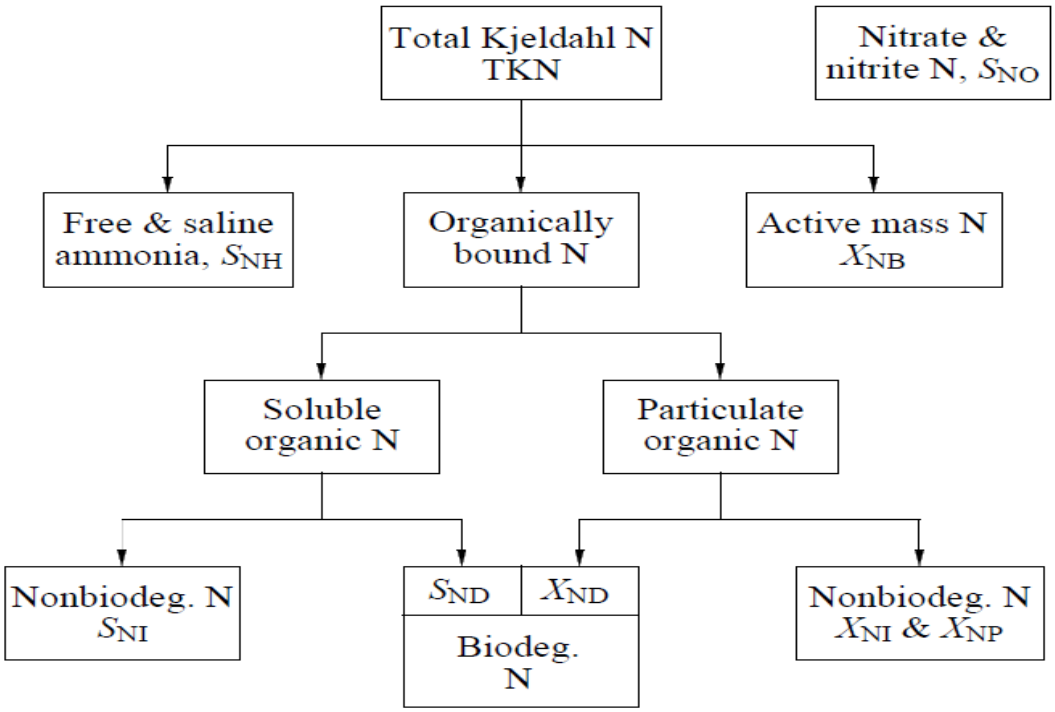


Figure 53. Wastewater characterization for nitrogenous components (Jeppsson 1996).

2. MODEL BUILDING

Most of the information about the state variables, parameters and characteristics, Monod kinetics equations, fractions or coefficients and Stoichiometric parameters, the reaction rates (ρ) that are use for developing model in this part came from the literature, ASM1 and ASM3 models, and Biofilm model. Several parameters came from the measured results or were

calculated, such as: temperature, light intensity, O_2, pH, NH_4^+-N, NO_3^--N, COD fractions, BOD_5 and specific algal growth rate ($\mu_{spec.}$).

2.1 Mass balance

In the general, the mass balance for a system describes the accumulation of mass in the system as a function input to the system. In order to account for the ammonia removal in the continuous flow system, a mass balance was performed. The mass balance is based of the basic equation:

Accumulation = **Input** – **(Output** + **Reaction or transformation)**

The components which are considered in the model and the transformation processes are characterized with the indices *i* and *j* respectively. Stoichiometric coefficients are present in the form of matrix$_{\mathbf{ji}}$. The process rate equation from the vector *j*, and the rate of production of the component *i*, r_i [$M_iL^{-3}T^{-1}$] in all parallel processes may be computed from the summation:

$$Transformation \quad (r_i) = \sum_{1}^{16} V_{i,j} \times p_j$$

Where:

- *V*: Stoichiometric coefficient from transformation matrix
- ρ: the process rate

2.2 Algal model formulation

2.2.1 Algal growth on nitrate

The growth of algae on nitrate is influenced by limiting light intensity factor. Presence of high ammonium concentrations inhibits the growth and the correction factor is incorporated in the rate equation. The kinetic equation can be expressed as:

$$(\rho) = \mu_{ALG} \times \frac{S_{NO}}{K_{NO} + S_{NO}} \times \frac{K_{NH}}{K_{NH} + S_{NH}} \times f(I) \times X_{ALG}$$

$\mu_{Algal} = \mu_{max\_algal\_20oC}$ x $1.066^{(T-20)}$

$$f(I) = \frac{I_0 / I_l}{1 + I_o / I_l}$$

I_o: Light intensity

I_l: Light efficiency

2.2.2 Algal growth on ammonia

Growth rate of algae on ammonia depends on the concentration of ammonium and the light intensity as:

$$(\rho) = \mu_{ALG} \times \frac{S_{NH}}{K_{NH} + S_{NH}} \times X_{BALG} \times f(I)$$

2.2.3 Decay of algal

Decay of algae is based on the temperature conditions, the decay rate constant and the concentration of the algal biomass. It can be formulated as:

$$(\rho) = b_{ALG} \times X_{ALG} \times \theta^{(T-30)}; \qquad b_{ALG} = b_{ALG\_20oC} \times 1.066^{(T-20)}$$

2.3 State variables

The table 22 shows the list of all state variables of the components considered in the model.

Table 22. State variables in the model matrix (cited from ASM1, ASM3)

2.4 Model matrix

	Description	Symbol	Unit
1	Soluble inert organic	$\mathbf{S_I}$	g COD/m^3
2	Readily biodegradable substrates	$\mathbf{S_S}$	g COD/m^3
3	Inert particulate organic	$\mathbf{X_I}$	g COD/m^3
4	Slow biodegradable substrates	$\mathbf{X_s}$	g COD/m^3
5	Heterotrophic biomass	$\mathbf{X_H}$	g COD/m^3
6	Autotrophic, nitrifying biomass	$\mathbf{X_A}$	g COD/m^3
7	Algal biomass	$\mathbf{X_{ALG}}$	g COD/m^3
8	Inert particulate microbial biomass products	$\mathbf{X_P}$	g COD/m^3
9	Dissolved oxygen	$\mathbf{S_O}$	g O_2/m^3
10	Nitrate nitrogen	$\mathbf{S_{NO3}}$	g N/m^3
11	Ammonia nitrogen	$\mathbf{S_{NH4}}$	g N/m^3
12	Soluble biodegradable organic nitrogen Conc.	$\mathbf{S_{ND}}$	g N/m^3
13	Slowly biodegradable organic nitrogen Conc.	$\mathbf{X_{ND}}$	g N/m^3
14	Alkalinity, bicarbonate	$\mathbf{S_{alk}}$	Mole HCO_3^- /m^3

2.4.1 Dynamic Processes and Algal model formulations

In the ASM1 structure, several processes were incorporated, such as: aerobic growth of heterotrophic biomass, anoxic growth of heterotrophic biomass, aerobic growth of autotrophic biomass, decay of heterotrophic biomass, decay of autotrophic biomass, ammonification of

soluble organic nitrogen, etc. Table 23 shows all the processes considered for developing an algal model related to AMS1 and ASM3 (**Note:** all the explanations below are cited from the research by Jeppsson 1996).

Table 23. Design of the processes rate in the model matrix

Process	**Design**
Heterotrophic growth organisms, degradation of organic material, denitrification	
1. Aerobic growth of heterotrophic	ρ1
2. Anoxic growth or denitrification	ρ2
3. Decay of heterotrophic	ρ3
Autotrophic organisms, nitrification	
4. Aerobic growth of autotrophs	ρ4
5. Ammonification of soluble organic nitrogen	ρ5
6. Decay of autotrophs	ρ6
Algal growth	
7. Algal growth on ammonia/nitrite	ρ7
8. Decay of algal	ρ8
Hydrolysis, particular and organic nitrogen material	
10. Hydrolysis of entrapped organic nitrogen	ρ9
11. Hydrolysis of entrapped organic	ρ10

2.4.2 Kinetic of biological processes

Biological processes formula based on the enzyme kinetics of Michaelis-Menten:

$$r_S = \frac{dS}{dt} = \frac{1}{Y}\mu_{\max} . \frac{S}{K_s + S} . X$$

Where:

r_s: process rate

K_s: the substrate concentration at which the reaction is half of the maximum growth rate

S: Substrate concentration

μ_{max}: Maximum growth rate

Y: The yield coefficient

X: biomass produced

Table 24. Model matrix for biological system with algal biomass

Component Processes	**X_A**	**X_{BA}**	**X_{BH}**	**X_S**	**X_P**	**X_I**	**X_{ND}**	**S_O**	**S_I**	**S_{NH}**	**S_{ND}**	**S_{NO}**	**S_S**
1. Aerobic growth of hetotrophs			1					$1-(1/Y_{BH})$		$-i_{XBH}$			$-(1/Y_{BH})$
2. Anoxic growth of heterotrophic bacteria			1							$-i_{XBH}$		$-(1-Y_{BH})/(2.86Y_{BH})$	$-(1/Y_{BH})$
3. Aerobic growth of autotrophic bacteria		1						$4.75-(1/Y_{BA})$		$-i_{XBA}-(1/Y_{BA})$		$1/Y_{BA}$	
4. Decay and recycle of heterotrophic bacteria			-1	$1-f_p$	f_p		$i_{XBH}-f_p*i_{Xp}$						
5. Decay of and recycle of autotrophs bacteria		-1		$1-f_p$	f_p		$i_{XBA}-f_{p*}i_{Xp}$						
6. Ammonification										1	-1		
7. Hydrolysis organic particular				-1									1
8. Hydrolysis organic nitrogen							$-X_{ND}/X_S$				X_{ND}/X_S		
9. Photo autotrophic algae	1							0.94		i_{XA}			
10. Mixed photo autotrophic algae	1							$1-(1/Y_A)$		$-i_{XA}$			$-1/Y_A$
11. Algae respiration	-1							-0.94					
12. Decay and recycle of algal	-1			$1-f_p$	f_p		$i_{XA}-f_p*i_{Xp}$						

3. MODELLING FORMULATION

For a model developed in AQUASIM software, the program uses the most kinetic equations of the activated sludge and biofilm models. Therefore, it should include the Monod kinetics, Stoichiometry and the wastewater fractioning such as COD, nitrogen, growth rate of algae, heterotrophic, autotrophic bacteria and the biological processes. All the considered kinetic parameters must be based on the theoretical temperature (by Arrhenius Law).

3.1 Fractions of wastewater influent and effluent characteristic could be used as the input values

3.1.1 COD mass balance (fraction of Chemical oxygen demand)

Generally, the influent containing substances such as ammonia nitrogen, phosphorus or micro pollutants can be modelled by a number of calculation methods, because they are a single agent or catalyst. According to formulations of the ASM1 and ASM3 models, the total influent COD of wastewater is a combination of several fractions. Therefore, the calculation or determination of the ratios of subcomponents of COD becomes the most important process in the model development, and that is what the models ASM1 and ASM3 are based on (Henze *et al.* 1987a,b; 1999*;* 2000a,b).

The calculation method is based on the COD components. It is includes several fractions of its major components. The chemical analysis method can separate the COD into its two forms: homogenous and filtrated (by filtration or flocculation processes). Hence, the fractions of COD are easily calculated by using a standard method (Mamais *et al.* 1993; Roeleveld and Loosdrecht 2002). On this basis, the proper assessment of fractions of COD could be calculated by the formula:

$\mathbf{COD} = \mathbf{S}_S + \mathbf{S}_i + \mathbf{X}_S + \mathbf{X}_i$ (Henze *et al.* 2000a,b; ATV A131P 2001)

$\mathbf{S}_s$: soluble easily degradable. $\mathbf{S}_s = \mathbf{COD}_{mf} - \mathbf{S}_i$

$\mathbf{S}_i$: inert soluble $0.9COD_{eff}$

$\mathbf{X}_s$: particulate slowly degradable. $\mathbf{X}_s = \mathbf{COD}_{BD} - \mathbf{S}_s$

$\mathbf{X}_i$: inert particulate. $\mathbf{X}_i = \mathbf{X}_{COD} - \mathbf{X}_s$

Whereas:

COD: chemical oxygen demand of raw wastewater.

$\mathbf{COD}_{eff}$: COD of effluent, filtrated using 0.45 μm membrane filter.

$\mathbf{COD}_{mf}$: soluble COD of raw sewage micro filtrated, using 0.45 μm membrane filters.

$\mathbf{X_{COD}}$: COD that is not degraded by biological processes

$\mathbf{X_{COD}} = (\mathbf{X_s}+\mathbf{X_i})$ – total particulate COD, hence $\mathbf{X}_{COD} = \mathbf{COD}_{tot} - \mathbf{COD}_{mf}$

$\mathbf{BOD}_{total}$: 1.47*BOD_5 (Henze *et al.* 2000a,b; ATV A131P 2001). BOD_5 determined by Oxytop method.

$\mathbf{COD}_{Biodegradable} = \mathbf{S_s}+\mathbf{X_s}$ – biodegradable fraction of COD.

$\mathbf{COD}_{Biodegradable} = \mathbf{BOD}_{tot}/(1-\mathbf{f}_{BOD})$

with $\mathbf{f}_{BOD} = 0.15$ (Roeleveld and Loosdrecht 2002)

Therefore, the practical COD balance in the reactor could be calculated by:

- COD unfiltered inflow: 651 (mg/l)
- COD_{mf} = COD influent filtration: 293 (mg/l)
- COD effluent of the practical reactor T1: 124 (mg/l)
- COD at the effluent of Schönerlinder WWTP: 35 (mg/l). To be in agreement with method developed by Henze *et al.* (2000a,b), $S_i = 0.9 \cdot$COD effluent.

$\mathbf{S_i}$	$= 0.9 \cdot COD_{eff}$	= 32 (mg/l)	4.9% COD_{total}
$\mathbf{S_S}$	$= COD_{mf} - \mathbf{S_i}$	= 261 (mg/l)	40.1% COD_{total}
$\mathbf{X_{COD}}$	$= COD_{tot} - COD_{mf}$	= 358 (mg/l)	
$\mathbf{COD_{BD}}$	$= (1.47 \times BOD_{influent})/(1-f_{BOD})$	= 460 (mg/l)	
$\mathbf{X_s}$	$= COD_{BD} - \mathbf{S_S}$	= 199 (mg/l)	30.6% COD_{total}
$\mathbf{X_I}$	$= X_{COD} - \mathbf{X_s}$	= 159 (mg/l)	24.4% COD_{total}

Therefore, the practical COD fraction is:

$$COD\ (mg/l) = 261\ mg\ \mathbf{S_S}/l + 32\ mg\ \mathbf{S_i}/l + 199\ mg\ \mathbf{X_S}/l + 159\ mg\ \mathbf{X_i}/l$$

$COD_{biodegradable}\ (S_s+X_s)/COD_{total} = 70.7\ (\%)$

$COD_{non\text{-}biodegradable}\ (S_i+X_i)/COD_{total} = 29.3\ (\%)$

Active mass **COD** $(\mathbf{X_{BH}}+\mathbf{X_{BA}})$ = Total $\mathbf{BOD}_{influent} - [(\mathbf{X_s}+\mathbf{S_s}) - (\mathbf{S_i}+\mathbf{X_i})]$

X_{BA} = 0.01 g COD/l, as adapted from the literatures for autotrophic biomass influent

3.1.2 Nitrogen mass balance

According to the ASM1 model and the accepted papers of Szetela *et al.* (1990), Henze *et al.* (2000a), Ziglio *et al.* (2001), Roeleveld and Loosdrecht (2002), Myszograj and Sadecka (2004), the fractional composition of TKN can be described as:

$$\mathbf{TKN} = \mathbf{S}_{nh} + \mathbf{S}_{nd} + \mathbf{S}_{ni} + \mathbf{X}_{nd} + \mathbf{X}_{ni}$$

In the present research, even though the volume of ammonia volatilisation in the reactor was at a small rate (A_v: 1.3 mg/l.d), but it should be in the balance equation of total nitrogen as a component. Moreover, Ferrara and Avci (1982) also found sedimentation was one of the main nitrogen removal pathway. Therefore, the equation from Bornemann *et al.* (1998) can be modified as:

$$\mathbf{S}_{NH} = \mathbf{TKN}\text{-}\mathbf{S}_{ND}\text{-}\mathbf{X}_{ND} \text{-}(0.01*\mathbf{S}_I)\text{-}(0.03*\mathbf{X}_I)\text{-}0.086*(\mathbf{X}_{BH}+\mathbf{X}_{BA})\text{-}0.063*\mathbf{X}_{PA}\text{-}(\mathbf{A}_v+ \mathbf{A}_{Sed.})$$

From measured result for TKN:

$\mathbf{S}_{NH}$ = 62.7 mg NH_4^+-N/l

$\mathbf{S}_{ND}$ = 1.7 (= 0.01*$\mathbf{S}_S$) mg NO_3^--N/l

$\mathbf{X}_{ND}$ = 8.9 (= 0.01*$\mathbf{X}_s$) mg N/l

The rest of ammonia nitrogen is contributed to the sediment and volatiles

(The abbreviation are described in the table 22 and 25)

3.2 Metabolic model

Table 25. The comparison of influent and effluent of COD fractions developed by Bornemann *et al.* (1998) and the practical results.

	Influent Theoretical	**Effluent Theoretical**	**Influent Practical result**	**Remark**
$\mathbf{S}_s$	0.15·COD	0.20·COD	**0.4**·COD	$N_{org.}=S_{ND}$
$\mathbf{X}_s$	0.45·COD	0.48·COD	**0.31**·COD	$N_{org.}=S_{ND}$
$\mathbf{S}_I$	0.05·COD	0.07·COD	**0.05**·COD	1 % total nitrogen
$\mathbf{X}_i$	0.15·COD	0.10·COD	**0.24**·COD	3 % total nitrogen
$\mathbf{X}_{BH}$	0.20·COD	0.15·COD	**0.15**·COD	8.6 % total nitrogen
$\mathbf{X}_{PA}$	-	-	**0.02**·COD	6.3 % total nitrogen
$\mathbf{X}_{BA}$	0.02 g.m^{-3}	0.01 g.m^{-3}	**0.01** g.m^{-3}	8.6 % total nitrogen
$\mathbf{X}_P$	0.02 g.m^{-3}	0.01 g.m^{-3}	**0.01** g.m^{-3}	Bornemann *et al.* 1998
$\mathbf{S}_o$	0.10 g.m^{-3}	0.10 g.m^{-3}	**0.50** g.m^{-3}	*Measure and calculation*
$\mathbf{S}_{NO}$			***Measure***	
$\mathbf{S}_{NH}$			***Measure***	
$\mathbf{S}_{ND}$	0.01· S_s	0.01· S_s	**0.01**· $\mathbf{S}_s$	Bornemann *et al.* 1998
$\mathbf{X}_{ND}$	0.03· X_s	0.03· X_s	**0.03**· $\mathbf{X}_s$	Bornemann *et al.* 1998

3.3 Stoichiometric and kinetic parameters for the algal model

Table 26. Stoichiometric and kinetic parameters for the algal model at 20ºC

		Unit	Value	Source	Select value
Stoichiometries Parameters					
$\mathbf{Y_A}$	Autotrophic yield	g COD_{microb}/ g N	0.24	Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.24
$\mathbf{Y_H}$	Heterotrophic yield	g COD_{microb}/ g $COD_{sub.}$	0.67	Bornemann *et al.* (1998) Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.67
$\mathbf{f_p}$	Fraction of microbial biomass converted to inert matter	g COD_{prodct}/ g $COD_{microb.}$	0.07-0.1	Härtel (1990) Henze *et al.* (2000a,b)	0.08
$\mathbf{i_{XB}}$	Nitrogen content of bacteria mass	g N/ g $COD_{microb.}$	0.086	Bornemann *et al.* (1998) Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.086
$\mathbf{i_{XP}}$	Nitrogen content of inerts product of decay	g N/ g $COD_{prodct.}$	0.05-0.07	Bornemann *et al.* (1998) Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.06
$\mathbf{i_{XBALG}}$	Mass of nitrogen per mass COD in algae	g N / g $COD_{microb.}$	0.06	Assumption	0.06
Kinetic parameters					
$\mathbf{\mu_H}$	Heterotrophic max.spec growth rate	day^{-1}	3-6	Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	6
$\mathbf{\mu_A}$	Autotrophic max.spec growth rate	day^{-1}	0.6- 0.8	Härtel (1990) Henze *et al.* (2000a,b)	0.8
$\mathbf{\mu_{Spec.}}$	Algae max.spec growth rate at 20^{o}C	day^{-1}	1.47	Determination	1.47
$\mathbf{K_S}$	Half saturation coefficient for heterotrophs substrate	g COD/m^3	20	Bornemann *et al.* (1998) Frizt (1979) Grau *et al.* (1996)	20

$\mathbf{K}_{OH}$	O_2 half saturation coefficient for heterotrophs	g O_2/m^3	0.2-1	Frizt (1979) Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.2
$\mathbf{K}_{OA}$	O_2 half saturation coefficient for autotrophs	g O_2/m^3	0.2-1	Bornemann *et al.* (1998) Frizt (1979) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.4
$\mathbf{K}_{NH}$	Ammonium half saturation coefficient for heterotrophs biomass	g NH_4-N/m^3	0.01-1	Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.01
$\mathbf{K}_{NO}$	Nitrate half saturation coefficient for denitrifying heterotrophs biomass	g NO^{3-}-N/m^3	0.5	Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	0.5
$\mathbf{K}_{X}$	Half saturation coefficient for hydrolysis of slowly biodegradable substrate by heterotrophs	g $COD_{sub.}$/ g COD_{microb}	0.03 -1	Bornemann *et al.* (1998) Henze *et al.* 1987a Henze *et al.* (2000 a,b)	1
$\mathbf{b}_{H}$	Decay coefficient heterotrophic biomass	day^{-1}	0.17-0.62	Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b) Lieberskind (1999)	0.62
$\mathbf{b}A$	Decay coefficient autotrophic biomass	day^{-1}	0.05-0.15	Härtel (1990) Henze *et al.* (1987a)	0.15
$\mathbf{k}_{h}$	Maximum specific hydrolysis rate	g COD_{Xs}/ g $COD_{Bio.}$	1-10	Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000 a,b)	3
$\mathbf{k}_{a}$	Maximum specific ammonification rate	g N_{org}/day	0.08	Bornemann *et al.* (1998)	0.08
$\mathbf{K}_{decp}$	First-order decomposition rate	day^{-1}	0.5	Rousseau (2005)	0.5
η_{g}	Corrector factor for anoxic growth of heterotrophs		0.4-1	Härtel (1990) Henze *et al.* (1987a) Henze *et al.* (2000a,b)	1
η_{h}	Correction factor hydrolysis and ammonification of bacteria		0.4	Henze *et al.* (2000a,b)	0.4

4. THE LIMITATIONS OF ALGAL - NITROGEN MODELLING DEVELOPMENT INCOOPERATED WITH ACTIVATED SLUDGE MODELS ASM1 AND ASM3

4.1 Parameters

- The parameters of the anaerobic, heterotrophic bacteria were mostly taken from the literature or from papers. These values are established for a model run at 30°C. Thus, using these parameters to properly model the algal development with ASM1 and 3 were not suitable.

- Information on oxygen and nitrate inhibition for the anaerobic bacteria was also lacking. The parameter values were arbitrarily put equal to the oxygen half-saturation value of 0.0002 g O_2/m^3 and the nitrate half-saturation value of 0.0005 g N/m^3. Similarly, the ammonium half-saturation constants were set equal to the ones for heterotrophic bacteria.

- The values of the growth rates of algae were obtained from this work. But, the other parameters such as the half-saturation constants, growth rate and yield, inert fraction of dead plant material came from literature or papers. There was no information about the mixed algae culture growth under the different light regimes or for the effectiveness of low temperature. The correction factor for anoxic growth was also arbitrarily set by the information collected from the literature and articles. They do not fit together.

- The algal model development in this study was based on the theoretical wastewater stabilization pond activated sludge models with a flow of 22.5 l/d. Therefore, to understand the biological processes in the reactors better, several parameters such as heterotrophic and autotrophic biomass of algae and bacteria in mixed culture should be collected from the practical results.

4.2. Final remarks

The output of the model does not represent the reality of the examined algal system, but it might provide a framework for discussion of model development results. Moreover, it is a useful tool to determine the different interactions in a biological reactor. One of the main lessons learnt from this experience is that the variables and parameters are mentioned such as the heterotrophic/autotrophic growth, decay rates should also be monitored routinely. Some important knowledge gaps were identified which might point out directions for future research.

Chapter VI.

CONCLUSIONS AND RECOMMENDATONS

1. CONCLUSIONS

During these investigations laboratory-scale algal and duckweed experiments with light control were carried out. The algal reactors have been set up with different flow patterns using different designs. The results from both experiments indicated a high potential for ammonia nitrogen, BOD_5 and COD removal together with high oxygen production and the high nitrification/denitrification ratios.

Algal and duckweed experiments showed high removal efficiencies for COD and BOD_5. However, the ammonia removal efficiency in these experiments did not approach sufficient levels, with only 67-73% of NH_4^+-N removed from the system. This is thought to be related to the limitation of surface and the depth of the experiment.

It could be concluded that the baffled algal reactors show better treatment performance for ammonia nitrogen removal with 90% of NH_4^+-N being observed to be removed without any aeration systems or CO_2 addition. Furthermore, the optimal NH_4^+-N load for ammonia removal in baffled algal reactors has been found to be 5-7 g/(m^3·d).

A comparison with other results obtained from different technologies and processes reveals the competitiveness of this approach for removing ammonia nitrogen from wastewater. This is because in this set-up, the treatment reactors can treat high substance loads without requiring either mechanical or electrical techniques. The nitrification, denitrification and assimilation processes were the major mechanisms for nitrogen removal in baffled algal reactors.

Through the result obtained from the baffled reactors, it was clear to see that nitrification and denitrification processes were the mechanisms not only for ammonia removal but also assimilation and algal uptake. Furthermore, it was observed that different hydraulic conditions lead to different nitrogen conversion performances. In this research, the optimal hydraulic retention time was found to be approximately 10 days.

The baffled reactors produced very high oxygen concentration of approximately 6 mg O_2/l in comparison with algal and duckweed experiments. The oxygen produced by the algae in the reactors was the most important factor for autotrophic, aerobic bacterial growth, oxidation of substances and decomposition of detritus. In the baffled algal reactors approximately 81% of COD and 86-89% of BOD_5 removal efficiencies were observed. The increased oxygen concentration caused by the application of baffles optimizes ammonia removal efficiency. The saturated zone reached to a depth of 40 cm.

The effects of pH on algal growth biomass and on the ammonia nitrogen removal efficiency have been studied. When produced oxygen exceeds 2.5 mg/l (till 9 mg/l) more than 90% of NH_4^+-N removal could be achieved. On the other hand, at this pH, the rate of nitrification, denitrification the algal growing rates were assumed to be constant.

Light and temperature conditions are important factors for the ecology and physiology of algae and bacteria. With the intermittent light regime of 12:12 hours (day/night) and temperatures around 22^{o}C, the highest ammonium nitrogen removal efficiency was recorded. In comparison with light conditions of 6:18 hours, the efficiency was much lower. Additionally, removal efficiency decreases with the temperature.

A specific algal growth rate, $\mu_{spec.}$ of 1.47/day was found to be at 20-22^{o}C. Furthermore the research revealed that, if the sludge is not removed from the system on time (fresh mass of produced sludge for every three weeks in baffled algal reactors were at least 1.5 l/21 days), the efficiency of ammonia nitrogen elimination will reduce. This effect could be explained by an increased decay rate of organisms and the recycling of organic matters into the water body.

The results obtained from these laboratory-scale experiments could be used to up-scale and develop new biological wastewater treatment systems. Furthermore, the influences of light and temperature seem to be the most important parameters to look at more detail in the future.

2. RECOMMENDATIONS

Biological wastewater treatment recently started to be used in many developing regions. Wastewater treatment plants constructed today aim to reduce the concentration of pollutants to an acceptable level but usually require high investment costs, skilled labour and consume a lot of energy.

Biological wastewater treatment offers major advantages over alternative treatment strategies: low operation and maintenance costs, efficient removal of organic compounds, easy operation and improved flexibility. However, the method requires large set up space and long residence times, which additionally often changes due to changes in physical conditions such as temperature, pH, etc. To reach good removal efficiency and maintain a stable state, the biological wastewater treatment needs to include other options such as growth rate, light intensity and aeration surface.

Firstly, the results proved that temperature and light conditions are the most important factors to keep algae growing and to maintain their removal capacities, especially for ammonia nitrogen. Therefore in future experiments, a variation of these factors should be examined in more details.

Secondly, it appears that the results of the algal ammonia removal efficiency in the light/dark phases do not show an unequivocal difference. Further research must be considered to get wider data about its effects.

Thirdly, the results show that a large amount of organic nitrogen accumulated in the algal sludge. Methods to store the algal sludge are necessary to recycle nutrients as well as for producing biogas and fertilizer. Future research may be needed to measure the amount of biogas producible from the algal sludge.

Fourthly, the application of a recycling line in the baffled algal reactors has proven to provide a high removal efficiency by recycling the residual degraded substances. Further researches should focus on the ratio of the recycle flow and its effects.

Fifthly, application of baffles in algal wastewater stabilisation ponds can improve its treatment effects. To obtain highest removal efficiency by the use of algae in the pre-settled wastewater, pond designers should consider a depth of the pond around 60-100cm. Use of algae as a promising biofuel should be taken in to account.

Sixthly, the transform matrix model must be based on many biological process-chains and variables to properly model the algal development. In this study, those parameters have been mostly taken from the literature. Therefore, the output of the model does not completely represent the reality of the examined algal system.

To better understand the biological processes in the reactors, those parameters should be collected from the practical results, such as the autotrophic growth, autotrophic biomass, decay rate and growth of algae on nitrates. In addition, the algal model to predict the nitrogen removal via biological processes still requires some modifications to properly model algal nitrogen removal with ASM1 and 3. Therefore, further studies focusing on this issue should be considered.

REFERENCES

Acien Fernandez, F.G., Fernandez Sevilla, J.M., Sanchez Perez, J.A., Molina Grima E. and Chisti Y. (2001). Airlift driven external loop tubular photobioreactors for outdoor production of microalgae: Assessment of design and performance. *Chemical Engineering Science*, vol. 56, pp. 2721-2732.

Acien Fernandez, F.G., Garcıa Camacho, F., Sanchez Perez, J.A., Fernandez Sevilla, J.M. and Molina Grima, E. (1998). Modelling of biomass productivity in tubular photobioreactors for microalgal cultures: effects of dilution rate, tube diameter and solar irradiance. *Biotechnol. Bioeng.*, vol. 58, pp. 605-616.

Ahlgren, G. (1987). Temperature functions in biology and their application to algal growth constants. *Oikos.*, vol. 49, pp. 177-190.

Ahmed, M., IDRIS, A., ADAM, A. (2007). Combined anaerobic-aerobic system for treatment of textile wastewater. Journal of Engineering Science and Technology, vol. 2, no. 1, pp: 55-69.

Ahn, Y.H. (2006). Sustainable nitrogen elimination biotechnologies: A review. *Process Biochemistry*, vol. 41, no. 8, pp. 1709-1721.

Akunna, J.C., Bizeau, C. and Moletta, R. (1993). Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol. *Water Research*, vol. 27, pp. 1303-1312.

Alaerts, G.J, Mahbubar, M.R. and Kelderman, P. (1996). Performance of a full-scale duckweed covered sewage lagoon. *Water Res.*, vol. 30, no. 4, pp. 843-852.

Ali Zafarzadeh, Bijan Bina, Mahnaz Nikaeen, Hossein Movahedian Attar, Mehdi Haji Khiadani (2011). Effect of dissolved oxygen and chemical oxygen demand to nitrogen ratios on the partial nitrification/denitrification process in moving bed biofilm reactors. *Irania Journal of Biotechnology*, vol. 9, no. 3, pp. 197-205.

Allen, M.B. and Arnon, D.I. (1955). Studies on nitrogen-fixing Blue-Green algae. I. growth and nitrogen fixation by *Anabaena Cylindrica lemm*. *Amer. Jour. Bot*., vol. 42, pp. 282-297.

Alley, W.M., Healy, R.W., LaBaugh, J.W. and Reilly, T.E. (2002). Flow and storage in groundwater systems. *Science*, no. 296, pp. 1985-1990.

Al-Nozaily (2001) Performance and process analysis of duckweed-covered sewage lagoon for high strength sewage. The case of Sana'a, Yemen. PHD dissertation, IHE-Unesco, Institute for water Education. The Netherland, Rotterdam A.A. Balkema printed 2001. ISBN 9054104252

Al-Nozaily, F., Alaerts, G. and Veenstra, S. (2000). Performance of duckweed-covered sewage lagoons: II. Nitrogen and phosphorus balance and plant productivity. *Water Res.*, vol. 34, no. 10, pp. 2734-2741.

Am Jang and Kim, I.S. (2004). Effect of high oxygen concentrations on nitrification and performance of high-purity oxygen A/O biofilm process. *Environmental engineering Science*, vol. 21, no. 3, pp. 273-281.

Ammann, E.C.B. and Fraser-Smith, A. (1968). Gas exchange of algae. *Applied Microbiology*, vol. 16, no. 1968, pp. 669-72.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S. and Srinath, E.G. (1976). Inhibition of nitrification by ammonia and nitrous acid. J*ournal of Water Pollution Control Federation*, vol. 48, no. 5, pp. 835-852.

APHA (2005). Standard methods for the examination of water and wastewater. American Public Health Association (APHA: 2005). Washington D.C.

Arciero, D.M. and Hooper A.B. (1993). Hydroxylamine oxidoreductase from nitrosomonas europaea is a multimer of an octaheme subunit. *Journal of Biological Chemistry*, vol. 268, no. 20, pp. 14645-14654.

Arciero, D.M., Hooper, A.B., Cai, M. and Timkovich, R. (1993). Evidence for the structure of the active site heme P460 in hydroxylamine oxidoreductase of Nitrosomonas. *Biochemistry*, vol. 32, no. 36, pp. 9370-9380.

Arvin, E. and Kristensen G.H. (1982). Effect of denitrification on the pH in biofilms. *Water Sci. Technol.*, vol. 14, no. 8, pp. 833-848.

Asadi, M.E., Clemente, R.S., Gupta, A.D., Loof, R., Hansen, G.K. (2002). Impacts of fertigation via sprinkler irrigation on nitrate leaching and corn yield in an acid-sulphate soil in Thailand. *Agric. Water Manage*., vol. 52, no. 2002, pp. 197-213.

Aslan, S. and Kapdan, I.K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater. *Ecological Eng.*, vol. 28, no. 1, pp. 64-70.

ATV A131P (2001). Dimension of biological activated treatment plant. *Wyd. Seidel-Przywecki, Warsawa* (in Polish).

Azov, Y., Goldman, J.C. (1982). Free ammonia inhibition of algal photosynthesis in intensive cultures. *Applied and Environmental Microbiology*, vol. 43, no. 4, pp. 735-739.

Bae, W., Baek, S., Chung, J., Lee Y. (2001). Optimal operational factors for nitrite accumulation in batch reactors. *Biodegradation*, vol. 12, pp. 359-366.

Barnes, D. and Bliss, P.J. (1983). Biological control of nitrogen in wastewater treatment. London: E&F. NSpon, 1983. ISBN 0419123504.

Barnes, D., Wilson, F. (1978). Chemistry and unit operations in sewage treatment. Applied Science, London.

Barton, L., Colmer, T.D. (2006). Irrigation and fertilizer strategies for minimizing nitrogen leaching from turfgrass. *Agric. Water Manage*., vol. 80, no. 1-3, pp. 160-175.

Becker, E.W. (1994). Microalgae: Biotechnology and Microbiology. UK: Cambridge University Press, 1994. ISBN 0521350204.

Benemann, J.R. (1989). The future of microalgal biotechnology, pp.317–333. in Algal and cyanobacterial biotechnology. R.C Cresswell, T.A.V Rees, and N. Shah (eds.). England, Essex, Harlow: Longman scientific & technical.

Berg, J.M., Tymoczko, J.L., Stryer, L. (1958). Biochemistry (5^{th} *edn*). New York, NY [u.a.]: Freeman, 2003. ISBN 0716746840.

Bernet, N., Dangcong, P., Delgènes, J-P. and Moletta, R. (2001). Nitrification at low oxygen concentration in biofilm reactor. *Journal of Environmental Engineering*, vol. 127, no. 3, pp. 266-271.

Bever, J. and Teichmann, H. (1990). Weitergehende Abwasserreinigung – Stickstoff und Phosphorelimination, Sedimentation und Filtration. Germany: R. Oldenbourg Verlag GmbH, München, 1990. ISBN 3486261991.

Biggs, B. and Kilroy, C. (2000). Stream periphyton monitoring manual. Prepared by NIWA for the New Zealand Ministry for the Environment, Christchurch, NZ. ISBN 0478090994.

Bishop, D.F., Heidman, J.A., Stamberg, J.A. (1976). Single-stage nitrification-denitrification. Jou*rnal of the Water Pollution Control Federation*, vol. 48, no. 3, part I (March, 1976), pp. 520-532.

Bornemann, C., Londong, J., Freund, M., Nowak, O., Otterpolh, R. and Rolfs, T. (1998). Hinweise zur dynamischen Simulation von Belebungsanlagen mit dem Belebtschlammodell Nr.1 der IAWQ. *Korrespondenz Abwasser KA*, vol. 45, no. 3, pp. 455-462. (in German).

Bouterfas, R., Belkoura, M., Dauta, A. (2002). Light and temperature effects on the growth rate of three freshwater algae isolated from a eutrophic lake. *Hydrobiologia*, vol. 489, no. 1-3, pp. 207-217.

Brazil, B.L. (2006). Performance and operation of a rotating biological contactor in a tilapia recirculating aquaculture system. *Aquac. Eng.*, vol. 34, no. 2006, pp. 261-274.

Breisha, G.Z. (2010). Bio-removal of nitrogen from wastewaters - A review. *Nature and Science*, vol. 8, no. 12, pp. 210-228.

Brindley, C., Acien, F.G., Fenandez-Sevilla, J.M. (2010). The oxygen evolution methodology affects photosynthetic rate measurement of microalgae in well-defined light regimes. *Biotechnology and Bioengineering*, vol. 106, no. 2, pp. 228-237.

Buchanan, R.E. and Gibbons, N.E. (*eds.)* (1974). Bergey's manual of determinative bacteriology (8^{th} *edn*). Baltimore: Williams & Wilkins Co., 1974. ISBN 9780683011173.

Caicedo, J.R. (2005). Comparison of performance of full-scale duckweed and algae stabilization ponds. In: Effect of operational variables on nitrogen transformations in duckweed stabilization ponds. The Netherlands: PhD Dissertation, UNESCO - IHE institute for water education and Wageningen University.

Caicedo, J.R., van der Steen, N.P., Arce, O. and Gijzen, H.J. (2000). Effect of total nitrogen concentration and pH on growth rates of duckweed *(Spirodela polyrrhiza). Water Res.*, vol. 3, no. 15, pp. 3829-3835.

Camargo Valero, M.A., Mara, D.D. and Newton, R.J. (2010). Nitrogen removal in maturation waste stabilization ponds via biological uptake and sedimentation of dead biomass. *Water Sci. Technol.*, vol. 61, no. 4, pp. 1027-1034.

Carrera, J., Baeza, J.A., Vincent, T. and Lafuente, F.J. (2003). Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system. *Water Res.*, vol. 37, no. 17, pp. 4211-4221.

Carvalho, A.P., Meireles, L.A., Malcata, X. (2006). Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol. Prog*., vol. 22, no. 6, pp. 1490-506.

Carvalho, A.P., Monteiro, C.M. and Malcata, F.X. (2009). Simultaneous effect of irradiance and temperature on biochemical composition of the microalgae *Pavlova lutheri*. *Journal of Applied Phycology*, vol. 21, no. 5, pp. 543-552.

Carvalho, A.P., Silva, S.O., Baptista, J.M. and Malcata, F.X. (2011). Light requirements in microalgal photobioreactors. *Appl Microbiol Biotechnol*., vol. 89, no. 5, pp. 1275-1288.

Charley, R.C., Hooper, D.G. and McLee, A.G. (1980). Nitrification kinetics in activated sludge at various temperatures and dissolved oxygen concentrations. *Water Res.*, vol. 14, no. 10, pp. 1387-1396.

Chen, C.Y. and Durbin, E.G. (1994). Effects of pH on the growth and carbon uptake of marine phytoplankton. *Mar. Ecol. Prog. Ser.*, vol. 89, no. 83-84, pp. 83-94.

Chen, R.L., Keeney, D.R., Graetz, D.A. and Holding, A.J. (1972). Denitrification and nitrate reduction in Wisconsin lake sediments. *J. Environ. Qual.*, vol. 1, no. 2, pp. 158-162.

Chevalier, P., de la Noüe, J. (1985). Wastewater nutrient removal with microalgae immobilized in carrageenan. *Enzyme Microbial. Technol*., vol. 7, no. 12, pp. 621-624.

Chinnasamy, S., Balasubramanian Ramakrishnan, Ashish Bhatnagar and Keshav C. Das (2009). Biomass production potential of a wastewater alga *Chlorella vulgaris ARC 1* under elevated levels of CO_2 and temperature. *Int. J. Mol. Sci.*, vol. 10, no.2, pp. 518-532. Available from: 10.3390/ijms10020518.

Christensen, S., Simkins, S. and Tiedje, M.J. (1990). Spatial variation in denitrificafion: dependency of activity centres on the soil environment. *Soil Sci. Soc. Am. J.*, vol.54, no. 6, pp. 1608-1613.

Churchwell, R.L., Kachtick, J.W. and Ford, D.L. (1980). Comprehensive analysis of nitrification of chemical processing wastewaters. *Journal WPCF*., vol. 52, no. 11, pp. 2726-2746.

Clement, B. and Merlin, G. (1995). The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Sci. Total Environ.*, vol. 170, pp. 71-79.

Codd, G.A. (1984). Toxic of freshwater cyanobacteria. *Microbiological Science*, vol. 1, no. 2, pp. 48-52.

Cohen, D. and Parnas, H. (1976). An optimal policy for the metabolism of storage material in unicellular algae. *J. Theoret. Biol.*, vol. 56, no. 1, pp. 1-18.

Cole, G.A. (1983). Chapters 11-14. In: Textbook of limnology (3rd edn.). IL: Waveland Press, Prospect Hts, 1988. ISBN 0881333786.

Constable, J.D., Conor, M.A., Scott, P.H. (1989). The comparative importance of different nitrogen removal mechanisms in 5 west lagoon, Werribee treatment complex. 13th Australian Water and Wastewater Association Conference, Canberra.

Constantine, T. (2008). An overview of ammonia and nitrogen removal in wastewater treatment. Canada: CH2M HILL. Available from: http://www.weao.org/assets/docs/new-professionals/timconstantine-nitrification_and_removal.pdf.

Copelli, M., Guetti, P.F. and Corradi, M. (1982). Rimozione di azoto e fosforo da acque retlue. Diallevamentisuinicole mediante fitodepurazione. Rimozione di azoto e fosfore seminario I.R.A.A. 22-23 October 1987, Rome, 34.

Costa, C., Dijkema, C., Fredrich, M., Garcia-Encina, P., Fernandez-Polanco, F. and Tams, A.J.M. (2000). Denitrification with methane as electron donor in oxygen limited bioreactors. *Applied Microbiology and Biotechnology*, vol. 53, no. 6, pp. 754-762.

Cuaresma, M., Janssen, M., Vilchez, C., Wijffels, R.H. (2009). Productivity of *Chlorella Sorokiniana* in a short light-path (SLP) panel photobioreactor under high irradiance. *Biotechnol. Bioeng.*, vol. 104, no. 2, pp. 352-359.

Danilov, R.A., Ekelund, N.G.A. (2001). Effects of pH on the growth rate, motility and photosynthesis in *Euglena gracilis*. *Folia Microbiol.*, vol. 46, no. 6, pp.549-554.

Davies, T.R. (1973). Isolation of bacteria capable of utilizing methane as a hydrogen donor in the process of denitrification. *Water Res.*, vol. 7 no. 4, pp. 575-579.

Davis, M.L. and Masten, S.J. (2004). Principles of environmental engineering and science (2nd *edn.*). New York: McGraw-Hill Companies, 2004. ISBN 9780073122359.

de la Noüe, J., Laliberté, G. and Proulx, D. (1992). Algae and wastewater. *J. Appl. Phycol.*, vol. 4 no. 3, pp. 247-254.

de Morais, M.G., Costa, J.A.V. (2007a). Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Con. Manage*., vol. 48, no. 7, pp. 2169-2173.

de Morais, M.G., Costa, J.A.V. (2007b). Carbon dioxide fixation by *Chlorella Kessleri, C. vulgaris, Scenedesmus obliquus* and *Spirulina sp.* cultivated in flasks and vertical tubular photobioreactors. *Biotechnol. Lett.*, vol. 29, no. 9, pp. 1349-1352.

Debabrata, M. (2004). Hybrid reactor system for wastewater treatment application and approache of modelling. *International journal of environment and pollution* (India), vol. 21, no. 2, pp. 105-131.

Debabrata, M. (2010). Simultaneous COD and ammonium nitrogen removal from a high-strength wastewater in a shaft-type Aerobic Hybrid Bioreactor. *International Journal of Environmental Science and Development*, vol. 1, no. 4, pp. 327-333.

de-Bashan, L.E., Hernandez, J.P., Morey, T., Bashan, Y. (2004). Microalgae growth-Promoting bacteria as “helpers” for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. *Water Res.*, vol. 38, no. 2, pp. 466-474.

Devos, N., Ingouff, M., Loppes, R., Matagne, R. (1998). RUBISCO adaptation to low temperatures: a comparative study in psychrophilic and mesophilic unicellular algae. *J. Phycol*., vol. 34, no. 4, pp. 665-660.

DIN EN ISO/IEC 17025: 2005-08; Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien (ISO/IEC 17025:2005); Deutsche und Englische Fassung EN ISO/IEC 17025:2005.

Dochain, D. and Vanrolleghem, P.A. (2001). Dynamical modelling and estimation in wastewater treatment processes. London: IWA Publishing, 2001. ISBN 9781900222501.

Dochain, D., Gregoire, S., Pauss, A., Schaegger, M.S. (2003). Dynamic modelling of a waste stabilization pond. *Bioprocess Biosyst. Eng.*, vol. 26, no. 1, pp. 19-26.

Doran, M.D. and Boyle, W.C. (1979). Phosphorus removal by activated algae. *Water Res.*, vol. 13, no. 8, pp. 805-812.

Duarte, P. (1994). A mechanistic model of the effects of light and temperature on algal primary productivity. *Ecological Modelling*, vol. 82, no. 1995, pp.151-160.

Eding, E.H., Kamstra, A., Verreth, J.A.J., Huisman, E.A., Klapwijk, A (2006). Design and operation of nitrifying trickling filters in recirculating aquaculture: a review. *Aquac. Eng.*, vol. 34, no. 2006, pp. 234–260.

Egli, K., Fanger, U., Alvarez, P.J.J., Siegrist, H., van der Meer, J.R., Zehnder, A.J.B. (2001). Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. *Arch. Microbiol*., vol. 175, no. 3, pp. 198-207.

Eppley, R.W. (1972). Temperature and phytoplankton growth in the sea. *Fishery Bulletin*, vol. 70, no. 4, pp. 1063-1085.

Erisman, J.W., van Grinsven, H., Grizzetti, B., Bouraoui, F., Powlson, D., Sutton, M.A., Bleeker, A., Reis, S. (2011). The European nitrogen problem in a global perspective. In: The European nitrogen assessment: sources, effects and policy perspectives. Edited by M.A. Sutton, C.M. Howard, J.W. Erisman, G. Billen, A. Bleeker, P. Grennfelt, H. van Grinsven, B.Grizzetti. UK: Cambridge University Press, 2011. ISBN 9781107006126.

Etienne Paul, Liu, Y. (*eds.*) (2012). Biological sludge minimization and biomaterials/bioenergy recovery technologies. Canada: John Wiley & Sons, Inc., 2012. ISBN 9780470768822.

F.A.O (2011). Interactions between Livestock Production Systems and the Environment - Impact Domain: concentrate feed demand . Food and Argriculture Organisation of the United Nations - FAO: 2011. Available from: http://www.fao.org/wairdocs/lead/x6123e/x6123e04.htm.

Ferrara, R.A. and Avci, C.B. (1982). Nitrogen dynamics in waste stabilisation ponds. *Jour. Wat. Poll. Contr. Fed*., vol. 54, no. 4, pp. 361-369.

Finney, B.A and Middlebrooks, E. (1980). Facultative waste stabilization ponds. *Journal of the Environmental Engineering Division. ASCE*, vol. 107, no. EE4, pp. 817-830.

Fitzgerald, G.P., Rohlich, G.A. (1964). Biological removal of nutrients from treated sewage: Laboratory experiments. *Verh. Int. Verein. Theor. Angew Limol.*, vol. XV, pp. 597-608.

Fleit, E., Melicz, Z., Sándor, D., Zrínyi, M., Filipcsei, G., László, K., Dékány, I., Király, Z. (2008). IASON-Intelligent activated sludge operated by nanotechnology-Hydrogel microcarriers in wastewater treatment. *Progr. Colloid. Polym. Sci*., vol. 135, pp. 209-217. Available from: 10.1007/2882_2008_092.

Focht, D.D., Chang, A.C. (1975). Nitrification and denitrification processes related to waste water treatment. *Adv. Appl. Microbiol*., vol. 19, pp. 153-186.

Fogg, G.E. (1975a). Algal cultures and phytoplankton ecology (3rd *edn*.). England, London: The University of Wisconsin Press, 1987. ISBN 0299105601.

Fogg, G.E. (1975b). Biochemical pathways in unicellular plants. In: photosynthesis and productivity in different environments. *Int. Bio. Programme.*, vol. 3, pp. 437-457. London-New York: Cambridge University press.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, S.F., Coe. M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C, Ramankutty, N., Snyde, P.K. (2005). Global consequences of land use. *Science*, vol. 309, no. 5734, pp. 570-574. Available from: http://www.sciencemag.org/content/309/5734/570.full.

Follett, R.F. and Hatfield, J.L. (*eds.*) (2001). Nitrogen in the environment: sources, problems and management. Elsevier B.V., 2001. ISBN 9780444504869.

Fontes, A.G., Vargas, M.A., Moreno, J., Guerrero, M.G., Losada, M. (1987). Factors affecting the production of biomass by a nitrogen-fixing blue-green alga in outdoor culture. *Biomass*, vol. 13, pp. 33-43.

Frizt, J.J., Middleton, A.C., Merredith, D.D. (1979). Dynamic process modelling of wastewater stabilization ponds. *Journal Water Pollution Control Federation*, vol. 51, no. 11, pp. 2724-2743.

Fu, Z., Yang, F., Wang, X and Liu, H. (2009). Organic carbon and nitrogen removal in anoxic/oxic membrane bioreactor treating high-strength wastewatergress and sustainable energy, vol. 28, no. 1, pp. 155-161. Available from: DOI 10.1002/ep.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R. and Vörösmarty, C.J. (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry,* vol. 70, no. 2, pp. 153-226.

Gálveza, J.M., Gómeza, M.A., Hontoria, E., González-Lópeza, J. (2000). Influence of hydraulic loading and air flow rate on urban wastewater nitrogen removal with a submerged fixed-film reactor. *Journal of Hazardous Materials*. vol. 101, no. 2003, pp. 219-229.

García, J., Green, B.F., Lundquist, T., Mujeriego, R., Hernández-Mariné, M., Oswald, W.J. (2006). Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. *Bioresource Technology*, vol. 97, no. 14, pp. 1709-1715.

García, J., Mujeriego, R. and Hernández-Mariné, M. (2000). High rate algal pond operating strategies for urban wastewater nitrogen removal. *J. Appl. Phycol.*, vol. 12, no. 3-5, pp. 331- 339.

Geider, R.J. and Osbonrne, B.A. (1989). Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth. *New. Phytol*., vol. 112, no. 3, pp. 327-341.

Gerardi, M.H. (2002). Nitrification and denitrification in the activated sludge process. New York: John Wiley & Sons, Inc., 2002. ISBN 0471065080.

Goldman, J.C. (1979). Outdoor algal mass cultures - II. Photosynthetic yield limitations. *Water Res.*, vol. 13, no. 2, pp. 119-36.

Goldman, J.C., Carpenter, E.J. (1974). A kinetic approach to the effect of temperature in algal growth. *Limnol Oceanogr*., vol. 19, no. 5, pp. 756-766.

Goldman, J.C., Graham, S.J. (1981). Inorganic carbon limitation and chemical composition of two freshwater green microalgae. *Appl. Environ. Microbiol*., vol. 41, no. 1, pp. 60-70.

Gómeza, M.A., Hontoria, E., González-López, J. (2002). Effect of dissolved oxygen concentration on nitrate removal from groundwater using denitrifying submerged filter. *Journal of Hazardous Materials*, vol. 90, no. 3, pp. 267-278.

Gons, H.J. (1977). On the light-limited growth of *Scenedesmus protuberans* Fritsch. PhD thesis, Univ. of Amsterdam, 1977.

Grady, C.P.L.Jr., Daigger, G.T. and Lim, H.C. (*eds.)* (1999). Biological wastewater treatment, 2nd edition Revised and expanded. New York, NY: Marcel Dekker, Inc., 1999. ISBN 0824889199.

Graham, L.E. and Wilcox, L.W. (2000). Algae. In Freshwater algae of North America -Ecology and classification. Edited by J.D. Wehr and R.G. Sheath. USA: Elsevier, 2003. ISBN 0127415505.

Grau, S.M., Sfinchez, A.G., Clavel, J.M., Aniorte, J.S., Grau, M.D.M. (1996). A mathematical model for waste water stabilization ponds with macrophytes and microphytes. *Ecological Modelling*, vol. 91, no. 1-3, pp. 77-103.

Gray, N.F. (2004). Biology of wastewater treatment (2nd *edn.*). Series on environmental science and management, volume 4. London: Imperial College Press, 2004. ISBN 1860943284.

Gray, N.F. (2005). Water Technology: An introduction for environmental scientists and engineers (2nd *edn.*). Oxford, Burlington, Mass: Elsevier Butterworth-Heinemann, 2005. ISBN 9780750666336.

Greenwood, N.N. and Earnshaw, A. (1997). Chemistry of the elements (2nd *edn.*). Elsevier Butterworth Heinemann 1997. ISBN 0750633654.

Grizzetti, B., Bouraoui, F. and De Marsily, G. (2008). Assessing nitrogen pressures on European surface water. *Global Biogeochemical Cycles*, 22: GB4023. In: the European nitrogen assessment: sources, effects and policy perspectives. M.A. Sutton, C.M. Howard, J.W. Erisman, G. Billen, A. Bleeker, P. Grennfelt, H. van Grinsven, B. Grizzetti (*eds.*). UK: Cambridge University Press, 2011. ISBN 9781107006126.

Grizzetti, B., Bouraoui, F., Billen, G. *et al*. (2011). Nitrogen as a threat to European water quality. In: The European nitrogen assessment: sources, effects and policy perspectives. M.A. Sutton, C.M. Howard, J.W. Erisman, G. Billen, A. Bleeker, P. Grennfelt, H. van Grinsven, B. Grizzetti (*eds.*). UK: Cambridge University Press, 2011. ISBN 9781107006126.

Grobbelaar, J.U. (1982). Potential of algal production. *Water SA.*, vol. 8, no. 2, pp. 79-85.

Grobbelaar, J.U. (1991). The influence of light/dark cycles in mixed algal cultures on their productivity. *Bioresource Technol.*, vol. 38, no. 2-3, pp. 189-194.

Groeneweg, J., Sellner, B. and Tappe, W. (1994). Ammonia oxidation in nitrosomonas at NH_3 concentrations near K_m: effects of pH and temperature. *Water Res.*, vol. 28, no. 12, pp. 2561-2566.

Grönlund, E., (2004). Microalgae at wastewater pond treatment in cold climate - an ecological engineering approach. Doctoral Thesis, Luleå University of Technology, Luleå, Sweden.

Gross, A., Boyd, C.E., Wood, C.W. (1999). Ammonia volatilisation from freshwater fish ponds. *J. Environ. Qual.*, vol. 28, pp. 793-797.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. pp. 26-60. In: Culture of marine invertebrate animals. W.L. Smith and M.H. Chanley (*eds.*). New York: Plenum Press, 1975. ISBN 0306308045.

Guillard, R.R.L. and Ryther, J.H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can.J. Microbiol.*, vol. 8, no. 2, pp. 229-239.

Gutzeit, G. (2006). Entwicklung und Modellierung eines neuartigen Abwasserreinigungsverfahrens mit symbiotischer Algen-Bakterien-Biomass. Dissertation. Germany: Technical University of Hamburg, 2006 (in German). ISBN 3930400804.

Hall, D.O. (1986). The production of biomass: a challenge to our society, p.1-24. In: Handbook of microalgae mass culture. A. Richmond (*ed.*). Boca Raton, Florida: CRC Press.

Hammer, D.A. and Knight, R.L. (1994). Designing constructed wetlands for nitrogen removal. *Water Sci. Technol.*, vol. 29, no. 4, pp. 15-27.

Han, D.W., Yun H.J., Kim, D.J. (2001). Autotrophic nitrification and denitrification characteristics of an upflow biological aerated filter. *J. Chem. Technol. Biot.*, vol. 76, no. 11, pp. 1112-1116.

Hanaki, K., Wantawin, C. and Ohgaki, S. (1990a). Nitrification at low level of DO with and without organic loading in a suspended growth reactor. *Water Res.*, vol. 2, no. 3, pp. 297-302.

Hanaki, K., Wantawin, C. and Ohgaki, S. (1990b). Effects of the activity of heterotrophs on nitritrification in suspended growth reactor. *Water Res.*, vol. 24, no. 3, pp. 289-296.

Hannan, P.J. and Patouillet, C. (1963). Gas exchange with mass cultures of algae. I. Effects of light intensity and rate of carbon dioxide input on oxygen production. *Appl. Microbiol.* vol. 11, no. 5, pp. 446-449.

Harrison, J.R., Daigger, G.T. (1997). A comparison of trickling filter media. *Journal WPCF.*, vol. 59, no. 7, pp. 679-685.

Härtel, L. (1990). Modellansätze zur dynamischen Simulation des Belebtschlammverfahrens, Schriftenreihe WAR, Band 47, Darmstadt (in German).

Hazen and Sawyer Environmental Engineers and Scientists (2009). Literature review of nitrogen reduction technologies for onsite sewage treatment systems. Task A.2 Final Report prepared for the Florida Department of Health (2009). Accessed June 6, 2011. Available from: http://www.doh.state.fl.us/environment/ostds/research/Nitrogen/Task_A Lit_Review.pdf.

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., Van Loosdrecht, M.C.M. and Heijnen, J.J. (1998). The SHARON process: an innovative method for nitrogen removal from ammonium rich wastewater. *Water Sci. Technol.*, vol. 37, no. 9, pp. 135-142.

Helmer-Madhok, C., Schmid, M., Filipov, E., Gaul, T., Hippen, A., Rosenwinkel, K.H., Seyfried, C.F., Wagner, M., Kunst, S. (2002). Deammonification in biofilm systems: population, structure and function. *Water Sci. Technol.*, vol. 46, no. 1-2, pp. 223-231.

Hemens, J. and and Mason, M.H. (1968). Sewage nutrient removal by shallow algal stream. *Water Res.*, vol. 2, no. 4, 277-287.

Henze, M., Grady, C.P.L., Gujer, W., Marais, G.V.R., Matsuo, T. (1987b). Activated sludge model No.1. IAWPRC Scientific and Technical Report 3. London, UK: IAWPRC.

Henze, M., Grady, C.P.L.Jr., Gujer, W., Marais, G.V.R. and Matsuo, T. (1987a). Activated sludge model No.1. IAWQ Scientific and Technical Report No. 1. UK: London.

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M.C., Marais, G.V.R., van Loosdrecht, M.C.M. (1999). Activated sludge model No.2d. *Water Sci. Technol.*, vol. 39, no. 1, pp. 165-182.

Henze, M., Gujer, W., Mino, T., van Loosdrecht, M. (2000a). Activated sludge models ASM1, ASM2, ASM2d AND ASM3. IWA Task group on Mathematical modelling for design and operator of biological wastewater treatment (*eds.*). Published by IWA Publishing in its Scientific and Technical Report series. IWA Publishing, 2000. ISBN 1900222248.

Henze, M., Gujer, W., Mino, T., van Loosdrecht, M. (2000b). Activated sludge model No.3. Activated sludge models ASM1, ASM2, ASM2D and ASM3, in: IWA Scientific and

Technical Report No.9, by M. Henze, W. Gujer, T. Mino, M. Van Loosdrecht (*eds*.). London: IWA, 2000.

Henze, M., Harremoës, P., la Cour Jansen, J., and Arvin, E. (1996). Wastewater Treatment Biological and Chemical Processes (3rd *edn.*). R.B. Murphy, W.H. Rulkens (*eds.*). New York: Springer-Verlag Berlin Heidelberg, 2002. ISBN 3540422285.

Henze, M., van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D. (*eds.*) (2008). Biological wastewater treatment - Principles, modelling and design. IWA Publishing, 2008. ISBN 9781843391883.

Hulburt, E.M. and Guillard R.R.L. (1968). The relationship of the distribution of the diatom Skeletonema Tropicum to temperature. *Ecology*, vol. 49, pp. 337-339.

Hunik, J.H., Meijer, H.J.G. and Tramper, J. (1992). Kinetics of *Nitrosomonas europaea* at extreme substrate, product and salt concentrations. *Appl. Microbiol. Biotechnol*., vol. 37, no. 6, pp. 802-811.

Hurse, J.T. and Cornor, A.M. (1999). Nitrogen removal from wastewater treatment lagoons. *Water Sci. Technol.*, vol. 39, no. 6, pp. 191-198.

Hyman, M.R., Murton, I.B. and Arp, D.J. (1988). Interaction of ammonia monooxygenase from nitrosomonas europaea with alkanes, alkenes and alkynes. *Applied and Environmental Microbiology*, vol. 54, no. 12, pp. 3187-3190.

Ifeanyi, V.O, Anyanwu, B.N., Ogbulie, J.N., Nwabueze, R.N., Ekezie, W. and Lawal O.S. (2011). Determination of the effect of light and salt concentrations on *Aphanocapsa* algal population. *African Journal of Microbiology Research*, vol.5, no. 17, pp. 2488-2492. Academic Journals, 2011. Available from: http://www.academicjournals.org/ajmr. DOI: 10.5897/AJMR10.734. ISSN 19960808.

Ilaria Di Termini, Annalisa Prassone, Claudia Cattaneo, Mauro Rovatti (2011). On the nitrogen and phosphorus removal in algal photobioreactors. *Ecological Engineering* vol. 37, no. 2011, pp. 976–980.

Imai, H., Chang, K-H. and Nakano, Sh-ichi. (2009). Growth responses of harmful algal species microcystis (Cyanophyceae) under various environmental conditions. interdisciplinary studies on environmental chemistry- environmental research in Asia. Y. Obayashi, T. Isobe, A. Subramanian, S. Suzuki and S. Tanabe (*eds*), pp. 269-275.

ISO (2001). Duckweed growth Inhibition, determination of toxic effect of water constituents and wastewater to duckweed (*lemna minor*). ISO/WD20079 DIN AK "Biotese", B 155c.

Jackson, B.M., Wheater, H.S., Wade, A.J., Butterfield, D., Mathias, S.A., Ireson, A.M., Butler, A.P., McIntyre, N.R., Whitehead, P.G. (2007). Catchment-scale modelling of flow and nutrient transport in the Chalk unsaturated zone. *Ecological Modelling*, vol. 209, no. 1, pp. 41-52.

Jackson, L.L., Keeney, D.R. and. Gilbert, E.M. (2000). Swine manure management plans in north-central Iowa: Nutrient loading and policy implications. *J. Spoil. Water Conserv.*, vol. 55, no. 2, pp. 205-212.

Jacob-Lopes, E., Scoparo, C.H.G., Lacerda, L.M.C.F. and Franco, T.T. (2009). Effect of light cycles (night/day) on CO_2 fixation and biomass production by microalgae in photobioreactors. *Chemical Engineering and Processing: Process Intensification*, vol. 48, no. 1, pp. 306-310.

Jalali, M. (2005). Nitrates leaching from agricultural land in Hamadan, Western Iran. *Agric. Ecosyst. Environ.*, vol. 110, no. 3-4, pp. 210-218.

Janssen M., Janssen, M., de Winter, M., Trampler, J., Mur, L.R., Sne,l J., Wijffel, R.H. (2000). Effeciency of light utilization of *Chlamydomonas reinhardtii* under medium-duration light/dark cycle. *J. Biotechnol.*, vol. 78, no. 2, pp. 123-137.

Janssen, M., Tramper, J., Mur, L.R., Wijfells, R.H. (2002). Enclosed outdoor photobioreactors: Light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnol. Bioeng.*, vol. 81, no. 2, pp. 193-210.

Janssen, M., Wijffels, R., von Stockar, U. (2007). Biocalorimetric monitoring of photoautotrophic batch cultures. *Thermocima Acta*, vol. 458, no. 1-2, pp. 54-64.

Janssen, M.G.J. (2002). Cultivation of microalgae: effect of light/dark cycles on biomass yield. PhD thesis, Wageningen University. Wageningen: Printed by Ponsen & Looijen BV, 2002. ISBN 9058085929.

Jeppsson, U. (1996). Modelling aspects of wastewater treatment processes. *Lund Institute of Technology, Dept. of Industrial Electrical Eng. and Automation 1996*. Available from: http://www.iea.lth.se/publications/point/Theses/pdf_files/LTH-IEA-1010.pdf.

Jinsoo Kim, Joo-Youp Lee (2001). Growth kinetic study of *Chlorella vulgaris* fast-growing marine diatom. *Limnol. Oceanogr.*, vol. 23, no. 4, pp. 696-703.

Jones, W.W. and Sauter, S. (2005). Distribution and abundance of *Cylindrospermopsis raciborskii* in Indiana lakes and reservoirs. *School of Public and Environmental Affairs Indiana University Bloomington*, IN.

Juliette, L.Y., Hyman, M.R. and Arp, D.J. (1995). Roles of bovine serum albumin and copper in the assay and stability of ammonia monooxygenase activity in-vitro. *Journal of Bacteriology*, vol. 177, no. 17, pp. 4908-4913.

Kadlec, R.H. and Wallace, S.D. (1996). Treatment wetlands (2nd *edn.*). Boca Raton, FL: CRC Press, Taylor & Fracis Group, 2009. ISBN 9781566705264.

Kapoor, A., Kuiper, A., Bedard, P., Gould, W.D. (2003). Use of a rotating biological contactor for removal of ammonium from mining effluents. *The European Journal of Mineral Processing and Environmental Protection*, vol. 3, no. 1, pp. 88-100.

Kayombo, S., Mbwette, T.S.A., Katima, J.H.Y. (2005). Waste stabilization pond and constructed wetlands design manual. Publication by UNEP-IETC with the Danish International Development Agency.

Keeney, D., Muller, M. (2000). Nitrogen and the upper Mississippi river. Institute for Agriculture and Trade Policy 2105 First Avenue South Minneapolis. USA: Minnesota.

Khan, M., Yoshida, N. (2008). Effect of L-glutamic acid on the growth and ammonium removal from ammonium solution and natural wastewater by *Chlorella vulgaris NTM06*. *Bioresource Technol.*, vol. 99, no. 3, pp. 575-582.

Khin, T. and Annachhatre, A.P. (2004*a*). Nitrogen removal in a fluidized bed bioreactor by using mixed culture under oxygen-limited condition. *Water Sci. Technol.*, vol. 6, no. 50, pp. 313-320.

Khin, T. and Annachhatre, A.P. (2004*b*). Novel microbial nitrogen removal processes. *Biotechnology Advances*, vol. 22, no. 7, pp. 519-532.

Khoeyi, A.Z., Seyfabadi, J. and Ramezanpour, Z. (2012). Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae *Chlorella vulgaris*. *Aquacult. Int.*, vol. 20, no. 1, pp. 41-49.

King, L. (1970). The role of carbon in eutrophication. *J. Wat. Pollut. Control Fed.*, vol. 42, no. 12, pp. 2035-2051.

Knowles, G.A., Downing, L., Barrett, M.J. (1965). Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer. *J. gen. Microbiol.*, vol. 38, pp. 263-278. In: Biological and chemical processes (3rd *edn.*). M. Henze, P. Harremoes, Jes la Cour Jansen and E. Arvin. U. Förster, R.J. Murphy, W.H. Rulkens (*eds.*). NY: Springer-Verlag-Berlin Heidenberg, 2002. ISBN 3540422285.

Konig, A., Pearson, H.W., & Silva, S.A. (1987). Ammonia toxicity to algal growth in waste stabilization ponds. *Water Sci. Technol.*, vol. 19, no. 12, pp. 115-122.

Koppe, P. and Stozek, A. (1999). Kommunals Abwasser. Seine Inhaltsstoffe nach Herkunft, Zusammensetzung und Reaktionen im Reinigungsprozeß einschließlich Klärschlaämmen, 4. Auflage. Germany, Essen: Vulkan-Verlag, 1999. ISBN 3802728335.

Körner, S. and Vermaat, J.E. (1998). The relative importance of *lemna gibba* L., bacteria and algae for nitrogen and phosphorus removal in duckweed - covered domestic wastewater. *Water Res.*, vol. 32, no. 12, pp. 3651-3661.

Krause-Jensen, D., Sagert, S., Schubert, H., Boström, C. (2008). Empirical relationships linking distribution and abundance of marine vegetation to eutrophication. *Ecological Indicators*, vol. 8, no. 5, pp. 515-529.

Kruskopf, M., Flynn, K.J. (2005). Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. *New Phytologist*, vol. 169, no. 3, pp. 525-536.

Kuai, L. and Verstraete, W. (1998). Ammonium removal by oxygen-limited autotrophic nitrification-denitrification system. *Applied and Environmental Microbiology*, vol. 64, no. 11, pp. 4500-4506.

Kuenen, J.G. and Robertson, L.A. (1985). Ecology of nitrification and denitrification. The Netherlands: TU Deflt, 1988. ISBN 0521351995.

Kuenen, J.G. and Robertson, L.A. (1994). Combined nitrification-denitrification process. *FEMS Microbiology reviews*, vol. 15, pp. 109-117.

Kuenen, J.G., Jorgensen, B.B. and Revsbech, N.P. (1986). Oxygen microprofiles of trickling filter biofilm. *Water. Res.*, vol. 20, no. 12, pp. 1589-1598.

Kurosu, O. (2001). Nitrogen removal from wastewater in microalgal-bacterial treatment ponds. Available from: http://nature.berkeley.edu/classes/es196/projects/2001final/Kurosu.pdf.

Kwangyoung, L. and Lee, C-G. (2002). Nitrogen removal from wastewater by microalgae without comsuming organic carbon sources. *J. Microbiol. Biotechnol.*, vol. 12, no. 6, pp. 979-985.

Lai, P.C.C., Lam, P.K.S. (1997). Major pathways for nitrogen removal in wastewater stabilization ponds. *Water, Air, and Soil Pollution*, vol. 94, no. 1-2, pp. 125-136.

Lam, P., Cowen, J.P. and Jones, R.D. (2004). Autotrophic ammonia oxidation in a deep-sea hydrothermal plume. *FEMS Microbiology Ecology*, vol. 47 no. 2, pp. 191-206.

Larsdotter, K. (2006). Microalgae for phosphorus removal from wastewater in a Nordic climate. A doctoral thesis from the School of Biotechnology. Sweden, Stockholm: Royal Institute of Technology. ISBN: 9171782885.

Lau, P.S., Tam, N.F.Y. and Wong, Y.S. (1995). Effect of algal density on nutrient removal from primary settled waste-water. *Environmental Pollution,* vol. 89, no. 1, pp. 59-66.

Lau, P.S., Tam, N.F.Y., Wong, Y.S. (1997). Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella Vulgaris*. *Environ. Technol.*, vol. 18, pp. 945-951.

Laws, E.A., Taguchi, S., Hirata, J., Pang, L. (1986). High algal production rates achieved in a shallow outdoor flume. *Biotech. Bio.eng.*, vol. 28, pp. 191-197.

Lee, C-G, Tim D. Fletcher and Sun, G. (2009). Nitrogen removal in constructed wetland systems. *Eng. Life Sci.*, vol. 9, no. 1, pp. 11-22.

Lee, K. and Lee, C-G. (2001). Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnology and Bioprocess Engineering*, vol. 6, no. 3, pp. 194-199.

Lee, Y.K., Pirt, S.J. (1981). Energetics of photosynthetic algal growth: Influence of intermittent illumination in short (40s) cycle. *J. gen. Microbiol*., vol. 124, pp. 43-52.

Lee, Y.W., Daheb, M.F. (1988). Kinetics of low solids bio-denitrification of water supplies. *J. Water Poll. Control Fed.*, vol. 60, pp. 1857-1861.

Lessard, P. and LeBihan, Y. (2003). Fixed film processes. Part 3 of Microbiology of wastewater treatment: Introduction to microbiological wastewater treatment. In: Handbook of water and wastewater microbiology. D. Mara and N. Horan (*eds.*). Academic Press: Elsevier, 2003. ISBN 0124701000.

Levenspiel, O. (1999). Chemical reaction engineering (3rd *edn*.). NY: John Wiley and Sons, Inc., 1999. ISBN 047125424X.

Lieberskind, M. (1999). Parameter für di dynamische Simulation kommunaler Abwasserreinigungsanlagen. Dissertation GWA171. Gewässerschutz Wasser Abwasser, Aachen (in German).

Liqa Raschid-Sally, Wim van der Hoek and Mala Ranawaka, editors (2011). Wastewater reuse in agriculture in Vietnam: water management, environment and human health aspects. Working Paper 30.

Liu, D.H.F., Liptak, B.G. (1999). Environmental engineers' Handbook CRCnetBASE. Boca Raton, FL: CRC Press LLC, 2000 Corporate Blvd., N.W., 1999. ISBN 0849321573.

Liu, Y., Tay, J.H. and Hung, Y.T. (2005). Biological nitrification and denitrification. In: Biological treatment processes, Volume 8. Edited by L.K. Wang, N.C. Pereira and Y.T. Hung. Totowa, NJ: Humnána Press, 2009. ISBN 9781588291639.

Lloyd, N.D.H, Canvin, D.T. and Culver, D.A. (1977). Photosynthesis and photorespiration in Algae. *Plant Physiol.*, vol. 59, no. 5, pp. 936-940.

Lowe, K.S., Rother, N.K., Tomaras, J.M.B., Kathleen DeJong, Tucholke, M.B., McCray, J.E., Junko Munakata-Marr (2006). Influent constituent characteristics of the modern waste stream from single sources: Literature review. *Water Environment Research Foundation.* Available from: http://www.ndwrcdp.org/documents/04-dec-1a/04dec1p1.pdf.

Lyssenko, C., Wheaton, F. (2006). Impact of positive ramp short-term operating disturbances on ammonia removal by trickling and submerged-upflow biofilters for intensive recirculating aquaculture. *Aquac. Eng.*, vol. 35, no. 2006, pp. 26-33

Macedo, M.F., Duarte, P., Ferreira, J. (2002). The influence of incubation periods on photosynthesis–irradiance curves. *Journal of Experimental Marine Biology and Ecology*, vol. 274, no. 2, pp. 101-120.

Madigan, M.T., Martinko, J.M., Parker, J. (1949). Brock biology of microorganisms (13th *edn.*). San Francisco, CA: publishing as Benjamin Cummings, 2012. ISBN 9780321649638.

Maestrini, S.Y., Robert, J.M., Leftley, J.W., Collos, Y. (1986). Ammonium thresholds for simultaneous uptake of ammonium and nitrate by oyster-pond algae. *Journal of Experimental Marine Biology and Ecology*, vol. 102, pp. 75-98.

Mallick, N. (2002). Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *Biometals*, vol. 15, no. 4, pp. 377-390.

Mamais, D., Jenkins, D., Pitt, P. (1993). A rapid physico-chemical method for the determination of readily biodegradable soluble COD in municipal wastewater. *Water Res.*, vol. 27, no. 1, pp. 195-197.

Mandi, L. (1994). Marrakesh wastewater purification experiment using vascular aquatic plants *Eichhornia crassipe mé Lemna gibba. Water Sci. Technol.*, vol. 29, pp. 283-287.

Mara, D.D., Alabaster, G.P., Pearson, H.W. and Mills, S.W. (1992). Waste stabilization oonds: A design manual for Eastern Africa. UK, Leed: Lagoon Technology International Ltd.

Markou, G., Georgakakis, D. (2011). Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. *Applied Energy*, vol. 88, no. 10, pp. 3389-3401.

Marshall, J.S. and Huang, Y. (2010). Simulation of light-limited algae growth in homogeneous turbulence. *Chemical Engineering Science*, vol. 65, no. 2010, pp. 3865-3875

Mata, T.M., Melo, A.C., Simoes, M. and Caetano, N.S. (2012). Parametric study of a brewery effluent treatment by microalgae *Scenedesmus obliquus*. *Bioresource Technology*, vol. 107, pp. 151-158.

Mata, T.M.. Martins, A.A., Caetano, N.S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, vol. 14, no. 1, pp. 217-232.

Matusiak, K . (1977). Studies on purification of wastewater from the nitrogen fertiliser industry by intensive algal cultures. *Acta Microbiologica Polonica*, vol. 25, pp. 233-242.

McCafferty, W.P. (1981). Aquatic entomology. Boston: Science Books Intl., 1981. ISBN 0867200170.

McGraw-Hill (2005). Biological Nutrient Removal (BNR) Operation in wastewater treatment plants. WEF Manual of Practice No. 29. ASCE/EWRI Manuals and reports on engineering practice No. 109. Water Environment Federation and American Society of Civil Engineers/ Environmental and Water Resources Institute. WEF Press, 2006. E-book. 0-07-146415-8. Available from: DOI: 10.1036/0071464158.

McKinney, R.E. (2004). Environmental pollution control microbiology. NY-Basel: Marcel Dekker Inc., 2004. ISBN 082475493X.

McLean B.M., Baskran, K., and Connor, M.A. (2000). The use of algal-bacterial biofilms to enhance nitrification rates in lagoons: Experience under laboratory and pilot scale conditions. *Wat .Sci. Tech.,* vol. 42, no.10-11, pp. 187-195.

Mehta, S.K., Gaur, J.P. (2005). Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Critical Reviews in Biotechnology,* vol. 25, pp. 113-152.

Melis, A. (2009). Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. *Plant Science,* vol. 177, no. 4, pp. 272-280.

Metcalf & Eddy Inc., (2003). Wastewater engineering treatment and reuse. (4th *edn.*). Revised by George Tchobanoglous, Franklin L. Burton, H. David Stensel. Boston, New York: McGraw-Hill, 2003. ISBN 0070418780.

Metcalf and Eddy, Inc. (1991). Wastewater engineering. Treatment, disposal and reuse. Edited by G. Tchobanoglous and F.L. Burton. New York: McGraw-Hill, 1991.

Middlebrooks, E.J., Middlebrooks, C.H., Reynolds, J.H., Walters, G.Z., Reed, S.C. and George, D.B. (1982). Wastewater stabilization lagoon design, performance and upgrading. New York: Macmillan; London: Collier Macmillan, 1982. ISBN 0029495008.

Milligan, A.J. and Cosper, E.M. (1997). Growth and photosynthesis of the "Brown tide" Aureococcus anophagefferens in subsaturating constant and fluctuating light. *Mar. Ecol. Prog. Ser.,* vol. 153, pp. 67-75.

Moez Bouali, Ines Zrafi, Feki Mouna and Amina Bakhrouf (2012). Pilot study of constructed wetlands for tertiary wastewater treatment using duckweed and immobilized microalgae. *African Journal of Microbiology Researc,* vol. 6, no. 31, pp. 6066-6074. Academic Journals, 2012. Available from: http://www.academicjournals.org/AJMR. ISSN 19960808.

Mohamed Sayed Abdel Hameed and Ola Hammouda Ebrahim (2007). Review biotechnological potential uses of immobilized Algae. *Int. J. Agri. Biol.*, vol. 9, no.1, pp. 183-192

Molina Grima, E., Acién Fernández, F.G., García Camacho, F., Chisti, Y. (1999). Photobioreactors: light regime, mass transfer, and scaleup. *J. Biotechnol.*, vol. 70, no. 1-3, pp. 231- 248.

Molina Grima, E., Garcia Camacho, F., Sanchez Perez, J.A., Acien Fernandez, F.G., Fernandez Sevilla, J.M. (1997). Evaluation of photosynthetic efficiency in microalgae cultures using averaged irradiance. *Enzyme Microbiol. Technol.*, vol. 21, no. 5, pp. 375-381.

Molina, Grima, E., Fernandez, J., Acien Fernandez, F.G., Chisti, Y. (2001). Tubular photobioreactor design for algal cultures. *J. Biotechnol*., vol. 92, no. 2, pp. 113-131.

Monteiro, C.M., and Castro, P.M.L., (2012). Metal uptake by microalgae: underlying mechanisms and practical applications. *Biotechnol. Prog.*, vol. 28, no. 2, pp. 299-311.

Moorhead, K.K. and Reddy, K.R. (1998). Oxygen transport through selected aquatic macrophytes. *J. Environ. Qual.*, vol. 17, no. 1, pp.138-142.

Mostert, E.S. and Grobbelaar, J.U. (1987). The Influence of nitrogen and phosphorus on algal growth and quality in outdoor mass algal cultures. *Biomass.*, vol. 13, no. 4, pp. 219-233.

Moussa, M.S., Lubberding, H.J., Hooijmans, C.M., van Loosdrecht, M.C.M. and Gijzen, H.J. (2003). Improved method for determination of ammonia and nitrite oxidation activities in mixed bacterial cultures. *Applied Microbiology & Biotechnology*, vol. 63, no. 2, pp. 217-221.

Mulder, A. (2003). The quest for sustainable nitrogen removal technologies. *Water Sci. Technol.*, vol. 48, no. 1, pp. 67-75.

Murat, S., Insel, G., Artan, N., Orhon, D. (2003). Effect of temperature on the nitrogen removal performance of a sequencing batch reactor treating tannery wastewater. *Water Sci. Technol.*, vol. 48, no. 11-12, pp. 319-26.

Muttamara, S. and Puetpaiboon, U. (1997). Roles of baffles in waste stabilization ponds. *Wat. Sci. Tech.*, vol 35, no. 8, pp. 275-284.

Myszograj, S., Sadecka, Z. (2004). COD fractions in mechanical-biological wastewater treatment on the basis of the sewage treatment plan of Sulechów (in Polish). Rocznik Ochrona Srodowiska, Koszalin. 6, 233.

Nelson, L.M., and Knowles, R. (1978). Effect of oxygen and nitrate on nitrogen fixation and denitrification by *Azospirillum brasilense* growing continuous culture. *Canadian Journal of Microbiology*, vol. 24, pp. 1395-1403.

Nielsen, L.P., Christensen, P.B., Revsbech, N.P., Sarensen, J. (1990). Denitrification and oxygen respiration in biofilms studied with a microsensor for nitrous oxide and oxygen. *Microb. Ecol.*, vol. 19, pp. 63-72.

Nurdogan, Y. and Oswald, W.J. (1995). Enhanced nutrient removal in high-rate ponds. *Water Sci. Technol.*, vol. 31, no. 12, pp. 33-43. Elsevier.

NYS Department of HealthCenter for Environmental, NY USA (2008). Cyanobacterial harmful algal blooms (cyanoHABs): Developing a public health response. *Lake and Reservoir Management*, vol. 18, pp. 20-31.

O.E.C.D (1984). Guidelines for the testing of chemicals: Algal, growth inhibition test. Organization for Economic Cooperation and Development-OECD: 1984. Available from: http://www.oecd.org/dataoecd/17/22/1948257.pdf.

Oh-Hama, T. & Miyachi, S. (1998). Chlorella, pp. 3-26. In microalgae biotechnology. Edited by M.A. Borowitzka and L.J. Borowitzka. Cambridge, UK: Cambridge University Press, 1998. ISBN 0521323495.

Oliver, R.L and Ganf, G.G (2000). Freshwater blooms, in the ecology of cyanobacteria: their diversity in time and space. B.A. Whitton and M. Potts (*eds.*). Kluwer: Dordrecht, pp. 149-194.

Omar, M.E. (2010). Improvement of detention ponds with respect to salinity. PhD dissertation, Technical university of Berlin. Germany, Aachen: Shaker Verlag, 2011. ISBN 9783832298432.

Oron, G. (1994). Duckweed culture for wastewater renovation and biomass production. *Agric. Waste Management, vol*. 26, no. 1-2, pp. 27-40.

Oron, G. and Shelef, G. (1982). Maximizing algae yield in high-rate ponds. *J. Environmental Enginerring Division. ASCE*, vol. 180, no. 4, pp. 730-738.

Osborne, B.A., Raven, J.A. (1986a). Light absorption by plants and its implication for photosynthesis. *Biological Reviews*, vol. 61, pp. 1-61.

Osborne, B.A., Raven, J.A. (1986b). Growth light level and photon absorption by cell of *Chlamydomonas reihardtii, Dunobliquus* (Chlorophyceae, Chlorococcales) and *Euglena viridis* (Euglenophyceae, Euglenales). *Br. Phycol. J.*, vol. 21, pp. 918-925.

Oswald, W.J. (1973). Complete waste treatment in ponds. Progress in waster technology. *Water Quality Management and Pollution Control*, vol. 3, pp. 153-163. London: Pergamon press.

Oswald, W.J. (1988). Micro-algae and waste-water treatment, pp. 305-328. In: Micro-algal biotechnology. M.A. Borowitzka and L.J. Borowitzka (*eds.*). Cambridge, UK: Cambridge University press, 1998. ISBN 0521323495.

Ozengin, N. and Elmaci, A. (2007). Performance of duckweed (Lemna minor L.) on different types of wastewater treatment. *Journal of Environmental Biology*, vol. 28, no. 2, pp. 307-314.

Painter, H.A. (1977). Microbial transformations of inorganic nitrogen. *Prog. Wat. Tech.*, no. 8, vol. 4/5. pp. 3-29.

Pano, A., and Middlebrooks, E.J. (1982). Ammonia nitrogen removal in facultative wastewater stabilization ponds. *Journ. Wat. Poll. Cont. Fed.*, vol. 54, no. 4, pp. 344-351.

Park, J.B.K., Cragg, R.J. and Shilton, A.N. (2011). Wastewater treatment with high rate algal ponds for biofuel production. *Bioresource teachnology*, vol. 102, no. 1, pp. 35-42.

Park, J.B.K., Craggs, R.J. (2010). Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Sci. Technol.*, vol. 61, no. 3, pp. 633-639.

Peeter, J.C.H and Eilers (1978). The relationship between light intensity and photosynthesis: a simple mathematical model. *Hydrobiol. Bull.*, vol. 12, pp. 134-136.

Petre, M. (editor). (2013). Environmental biotechnology – new approaches and prospective applications. Published by InTech, 2013. ISBN 9789535109723.

Picioreanu, C., van Loosdrecht, M.C.M. and Heijnen, J.J. (1997). Modelling of the effect of oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. *Water Sci. Technol.*, vol. 6, no. 1, pp. 147-156.

Pirt, S.J. (1983). Maximum photosynthetic efficiency: a problem to be resolved. *Biotechnol. Bioeng.*, vol. 25, pp. 1915-1922.

Pohland, F.G. and Suidan, M.T. (1978). Prediction of pH stability in biological treatment systems, pp. 441-463. In: Chemistry of wastewater technology. A.J. Rubin (*ed.*). Ann Arbor Science Publishers, 1978. ISBN 0250401851.

Pollice, A., Tandoi, V., Lestindi, C. (2002). Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. *Water Res.*, vol. 36, no. 10, pp. 2541-2546.

Posten, C. (2009). Design principles of photo-bioreactors for cultivation of microalgae. *Eng. Life Sci.*, vol. 9, no. 3, pp. 165-177.

Powell, N., Shilton, A., Chisti, Y., Pratt, S. (2009). Towards a luxury uptake process via microalgae - defining the polyphosphate dynamics. *Water Res.*, vol. 43, no. 17, pp. 4207-4213.

Prosser, J.I. (1989). Autotrophic nitrification in bacteria, pp. 125-181. In: Advances in microbial physiology, vol. 30. A.H. Edited by Rose and D.W. Tempest. New York, USA: Academic Press Inc., 1989. lSBN 0120277301.

Pulz, O. (2001). Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology*, vol. 57, pp. 287-293.

Raven, J.A. and Geider, R.J. (1988). Temperature and algal growth. *New. Phytol*., vol. 110, pp. 441-461.

Reed, S.C. (1983). Nitrogen removal in waste-water stabilization ponds. Paper presented at the *56th Annl. Conf. WPCF*. Atlanta, Oct. 2-7, 21.

Reed, S.C. (1984) Nitrogen removal in wastewater ponds. USA CRREL, Hanover, NH CRREL: Report 84-13.

Reed, S.C. (1985). Nitrogen removal in wastewater stabilization ponds. *Jour. Water Pollut. Contr. Fed.*, vol. 57, no. 1, pp. 39-45.

Reid, W.V., Mooney, H.A., Cropper, A., Capistrano, D., Carpenter, S.R., Chopra, K., Dasgupta, P., Dietz, T., Duraiappah, A.K, Hassan, R., Kasperson, R., Leemans, R., May, R.M., McMichael, A.J., Pingali, P., Samper, C., Scholes, R., Watson, R.T., Zakri, A.H., Shidong, Z., Ash, N.J., Bennett, E., Kumar, P., Lee, M.J., Raudsepp-Hearne, C., Simons, H., Thonell, J. and Zurek, M.B. (*eds*.) (2005). Ecosystems and human well-being: Synthesis. District of Columbia, Washington: Island Press, 2005. ISBN 1597260398.

Revsbech, N.R., Jacobsen, J.P., Nielsen, L.P. (2005). Nitrogen transformations in microenvironments of river beds and riparian zones. *Ecological Engineering*, vol. 24, pp. 447-455.

Richmond, A. (2000). Microalgal biotechnology at the turn of the millennium: a personal view. *J. Appl. Phycol*., vol. 12, pp. 441-451.

Richmond, A. (*ed.*) (2004). Handbook of microalgal culture: biotechnology and applied phycology. Ames, Iowa, USA: Blackwell Science, 2004 . ISBN 0632059532.

Richmond, A. and Becker, W. (1986). Technological aspects of mass cultivation - a general outline. In Handbook of microalgal culture. A. Richmond (*ed.*). Florida Boca Raton: CRC Press.

Richmond, A., Cheng-Wu, Z., Zarmi, Y. (2003). Efficient use of strong light for high photosynthetic productivity: interrelationships between the optical path, the optimal population density and cell-growth inhibition. *Biomo. Eng.*, vol. 20, no. 4-6, pp. 229-236.

Ritchie, R. (2006). Consistent sets of spectrophotometric chlorophyll equations for Acetone, Methanol and Ethanol solvents. *Photosynthesis Research*, vol. 89, no. 1, pp. 27-41.

Rittmann, B.E. and McCarty, P.L. (2001). Environmental biotechnology: principles and applications. New York: McGraw-Hill, 2001. ISBN 0072345535.

Rochet, M., Legendre, L. and Demers, S. (1986). Quality. *Journal of Experimental Marine Biology and Ecology*, vol. 101, no. 3-4, pp. 211-226.

Roeleveld, P.J., Van Loosdrecht, M.C.M. (2002). Experience with guidelines for wastewater characterization in The Netherlands. *Water Sci. Tech.*, vol. 45, no. 6, pp. 77-87.

Rousseau, D. (2005). Performance of constructed treatment wetland: model-based evaluation and impact of operation and maintenance. PhD thesis. Ghent: Ghent University, 2005.

Ruiz, G., Jeison, D., Chamy, R. (2003). Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonium concentration. *Water Res.*, vol. 37, no. 6, pp. 1371-1377.

Ruiz, G., Jeison, D., Rubilar, O., Ciudad, G. and Chamy, R. (2006). Nitrification-denitrification via nitrite accumulation for nitrogen removal from wastewaters. Bioresource Technology, vol. 97, no. 2, pp. 330-335.

Ruiz-martin, A., Mendoza, l.G., Stepheson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource technology*, vol. 101, no. 2010, pp 58-64. Elsevier.

S. and Vermaat, J.E. and Veenstra, S. (2003). The Capacity of Duckweed to Treat Wastewater. *J. Environ. Qual.*, vol. 32, no. 5, pp. 1583-1590.

Salem, S., Berends, D.H.J.G., van der Roest, H.F., van der Kuij, R.J., van Loosdrecht, M.C.M. (2004). Full-scale application of BABE technology. *Water Sci. Technol.*, vol. 50, no. 7, pp. 87-96.

Santos, M.C.R., Oliveira, J.F.S. (1987). Nitrogen transformations and removal in waste Stabilization ponds in Portugal: seasonal variations. *Water Sci. Technol.*, vol. 19, no. 12, pp.123-130.

Sattayatewa, C., Krishna Pagilla, K., Robert Sharp, R. and Pitt, P. (2010). Fate of organic nitrogen in four biological nutrient removal wastewater treatment plants. *Water Environment Research*, vol. 82, no. 12, pp. 2306-2315.

Schagerl, M. and Künzl, G. (2007). Chlorophyll a extraction from freshwater algae - A reevaluation. *Biologia.*, vol. 62, no. 3, pp. 270-275.

Schlesinger, W.H. (2009). On the fate of anthropogenic nitrogen. In: Proceedings of the National Academy of Sciences of the United States of America, vol. 106, pp. 203-208.

Sedlak, R. (*ed.)* (1991). Phosphorus and nitrogen removal from municipal wastewater, Principles and practice (2nd *edn*.). NY, USA: The Soap and Detergent Association, 1991. ISBN 0873716833.

Senzia, M.A., Mayo, A.W., Mbwette, T.S.A., Katima, J.H.Y., Jørgensen, S.E. (2002). Modelling nitrogen transformation and removal in primary facultative ponds. *Ecological Modelling*, vol. 154, no. 3, pp. 207-215.

Senzia, M.A., Mashauri, D.A., Mayo, A.W. (2003). Suitability of constructed wetlands and waste stabilisation ponds in wastewater treatment: nitrogen transformation and removal. *Physics and Chemistry of the Earth, Parts A/B/C*, vol. 28, no. 20-27, pp. 1117-1124.

Shammas N.K. (1983). Biocontactors for wastewater reuse, kinetic approach for achieving the required effluent quality. In *First Saudi Engineering Conference, May 1983*. KSA: Jeddah.

Shammas, N.K. (1971). Optimization of biological nitrification. PhD dissertation. MI, USA: Microfilm Publication, University of Michigan Ann Arbor, 1971.

Shammas, N.K. (1982). An allosteric kinetic model for the nitrification process. In *Proceedings of Tenth Annual Conference of Water Supply Improvement Association,* Honolulu, HI, pp. 1-30.

Shanab, S., Essa, A. and Shalaby, E. (2012). Bioremoval capacity of three heavy metals by some microalgae species (Egyptian Isolates). *Plant Signal Behav.*, vol.7, no. 3, pp. 392-399. Available from: doi 10.4161/psb.19173.

Sharma, B. and Ahlert, R.C. (1977). Nitrification and nitrogen removal. *Water Res.*, vol. 11, pp. 897-925.

Sheehan, J., Dunnahay, T., Benemann, J., Roessler, P. (1998). A look back at the US Department of Energy's Aquatic Species Program-Biodiesel from Algae. National Renewable Energy Laboratory, report NREL/TP of La Paz, Baja California, Mexico.

Sheppard, C.W. (1962). Chapter 1. In: Principles of the tracer method. New York: John Wiley & Son.

Shilton, A. (1996). Ammonia volatilisation from a piggery pond. *Water Sci. Technol.*, vol. 33, no. 7, pp. 183-189.

Shilton, A. (2005). Pond treatment technology. London, UK: IWA, 2005. ISBN 9781843390206.

Shilton, A. and Harrison, J. (2003). Guidelines for the hydraulic design of wastewater stabilization ponds. Wellington NZ: Ministry for the Environment. ISBN 0473087359.

Shilton, A., Wilks, T., Smyth, J. and Bickers, P. (2000). Tracer studies on a New Zealand waste stabilization pond and analysis of treatment efficiency. *Water Sci. Technol.*, vol. 42, no. 10-11, pp. 343-348.

Siegrist, H., Reithaar, S., Lais, P. (1998). Nitrogen loss in a nitrifying rotating contactor treating ammonium-rich wastewater without organic carbon. *Water Sci. Technol.*, vol. 38, no. 8-9, pp. 241-248.

Silva, S.A. (1982). On the treatment of domestic sewage in waste stabilization ponds in N.E. Brazil. PhD thesis, University of Dundee, 1982.

Silva, S.A., de Oliveira, R., Soares, J., Mara, D.D. and Pearson, H.W. (1995). Nitrogen removal in pond systems with different configurations and geometries. *Water Sci. Technol.*, vol. 31, no. 12, pp. 321-330.

Silva, S.A., Mara, D.D. and de Oliveira, R. (1987). The performance of five deep waste stabilisation ponds in northeast Brazil. *Water Sci. Technol.*, vol. 19, no. 12, pp. 61-64.

Slamet, A., Hermana, J. (2012). Effect of light exposure and wastewater depth on the performance of algae reactor during the treatment of Surabya municipal wastewater. *J. Appl. Environ .Biol. Sci.*, vol. 2 no. 12, pp. 615-619.

Soeder, C.J. (1986). An historical outline of applied algology, pp. 25-42. In CRC Handbook of microalgal mass culture. Edited by A. Richmond. Boca Raton: CRC Press, 1986. ISBN 0632059532

Soeder, C.J., Hegewald, E., Fiolitakis, E., Grobbelaar, J.U. (1985). Temperature dependence of population growth in a green microalgal: thermodynamic characteristics of growth intensity and the influence of cell concentration. *Zeitschrift für Naturforschung*, vol. 40, pp. 227-233.

Strous, M. (2000) Microbiology of anaerobic ammonium oxidation. PhD thesis. The Netherlands: TU Delft, 2000.

Suthersan, S., Ganczarczyk, J.J. (1986). Inhibition of nitrite oxidation during nitrification: some observation. *Water Pollut. Res. J. Canada*, vol. 21, pp. 257-266.

Sutton, M.A., Howard, C.M., Erisman, J.W., Gilles Billen, Albert Bleeker, Peringe Grennfelt, Hans van Grinsven, Bruna Grizzetti (*eds.*) (2011). The European nitrogen assessment: sources, effects and policy perspectives. UK: Cambridge University Press 2011. ISBN 9781107006126.

Sutton, P.M., Bridle, T.R., Bedford, W.K. and Arnold, J. (1981). Nitrification and denitrification of an industrial wastewater. *Journal of the Water Pollution Control Federation*, vol. 53, pp. 176. In: Advanced biological treatment processes - Handbook of environmental engineering series - Volume 9. Edited by L.K. Wang, N.K. Shammas, Y.Tse Hung. New York, USA: Humana Press, 2009. ISBN 9781588293602.

Szetela, R.W. (1990). Dynamic model of activated sludge wastewater treatment plant. Prace Naukowe Instytutu Inżynierii Ochrony Środowiska Politechniki Wrocławskiej (in Polish). Wrocław, 64, 32.

Szwerinski, H., Arvin, E. and Harremoës, P. (1986). pH-decrease in nitrifying biofilms. *Water Res.*, vol. 20, no. 8, pp. 971-976.

Tam, N.F.Y., Wong, Y.S. and Leung, G. (1992). Effect of exogenous carbon sources on removal of inorganic nutrients by the nitrification denitrification process. *Water Res.*, vol. 26, no. 9, pp. 1229-1236.

Tam, N.F.Y., Wong, Y.S. and Simpson, C.G. (1997). Removal of copper by free and immobilised microalgae, *Chlorella vulgaris*, pp. 17-36. In: Wastewater treatment with algae. Edited by Y.S. Wong & N.F.Y. Tam. Berlin: Springer, 1998. ISBN 3540633634.

Tang, E.P.Y. (1995). *The allometry of algal growth rates***.** *Journal of Plankton Research*, vol. 17, no. 6, pp. 1325-1335.

Taylor, R.W. and Kroken, S. (1988). Phylogenetic species, reproductive mode, and specificity of the green alga *Trebouxia* forming lichens with the fungal genus *Letharia*. *The Bryologist,* vol. 103, no. 4, pp. 645-660.

Terry, R.E. and Nelson, D.W. (1975). Factors influencing nitrate transformations in sediments. *J. Environ. Qual.*, vol. 4, no. 4, pp. 549-554.

Thalasso, F., Vallecillo, A., Garcia-Encina, P. and Fernandez-Polanco, F. (1997). The use of methane as a sole carbon source for wastewater denitrification. *Water Res.*, vol. 31, no. 1, pp. 55-60.

Third, K.A., Sliekers, A.O., Kuenen, J.G. and Jetten, M.S.M. (2001). The CANON system (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. *System Appl. Microbiol.*, vol. 24, no. 4, pp. 588-596.

Tillett, D.M. (1988). Lipid productivity and species competition in laboratory models of algae mass cultures. PhD thesis. The School of Chemical Engineering, Georgia Institute of Technology.

Toms, I.P., *et al.* (1975). Observations on the performance of polishing lagoons at a large regional works. *Water Pollution Control*, vol. 74, pp. 383-401.

Torzillo, G., Pushparaj, B., Masojidek, J., Vonshak, A. (2003). Biological constraints in algal biotechnology. *Biotechnology and Bioprocess Engineering*, vol. 8, no. 6, pp. 338-348.

Trang, T.V., Stolyar, S.M., Pinchuk, G.E., Hill, E.A., Kucek, L.A., Brown, R.N., Lipton, M.S., Osterman, A., Fredrickson, J.K., Konopka, A.E., Beliaev, A.S., Reed, J.L. (2012). Genome-scale modelling of light-driven reductant partitioning and Carbon fluxes in diazotrophic unicellular cyanobacterium *Cyanothece*sp. ATCC 51142. Available from: http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1002460.

Turkar, S.S., Bharti, D.B. and Gaikwad, G.S. (2011). Various methods involved in waste water treatment to control water pollution. J. *Chem. Pharm. Res.*, vol. 3, no. 2, pp. 58-65.

Turner, J.S. and Britaine, E.G. (1962). Oxygen as a factor in photosynthesis. *Biol. Rev*., vol. 37, pp. 130-170.

Udo Wiesmann, In Su Choi, Eva-Maria Dombrowski (2007). Fundamentals of biological wastewater treatment. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA, 2007. ISBN 9783527312191.

Underwood, G.J.C., Baker, J.H. (1991). The effect of various aquatic bacteria on the growth and senescence of duckweed (Lemnaminor). *J. Appl. Bacteriol.*, vol. 70, pp. 192-196.

USEPA (1973). Nitrification and denitrification facilities-wastewater treatment. US Environmental Protection Agency, Technology Transfer (EPA-625/4-73-004a).

USEPA (1998). Design manual constructed wetlands and aquatic plant systems for municipal wastewater treatment. USEPA 1998 - U.S Environmental Protection Agency Office of Research and Development Centre for Environmental Research Information Cincinnati, OH.

Valigore, J.M. (2011). Microbial (Microalgal-Bacterial) biomass grown on municipal wastewater for sustainable biofuel production. Doctor of Philosophy in Civil and Natural Resources Engineering at the University of Canterbury. Available from: http://ir.canterbury.ac.nz/bitstream/10092/5661/1/valigore2011.pdf.

van Baalen, C. (1962). Studies on marine blue-green algae. *Bot. Marina,* vol. 4, pp. 130-139.

van der Steen, N.P. (2000) Faecal coliform removal from UASB effluent in integrated systems of algae and duckweed. PhD thesis. Israel: Ben-Gurion University of Negev, 2000.

van Grinsven, H., Ward, M.H., Benjamin, N. and de Kok, T.M.C.M. (2006). Does the evidence about health risks associated with nitrate ingestion warrant an increase of the nitrate standard for drinking water?. *Environmental Health: A Global Access Science Source 2006*, vol. 5, pp. 5-26.

van Kempen, R., Mulder, J.W., Uijterlinde, C.A. and Van Loosdrecht, M. (2001). Overview: full scale experience of the SHARON process for treatment of rejection water of digested sludge dewatering. *Water Sci. Technol.*, vol. 44, no. 1, pp. 145-152.

Veenstra, S.F., Al-Nozaily and Alaerts, G.J. (1995). Purple non sulphur bacteria and their influence on waste water stabilization pond performance in the Yemen republic. *Water Sci. Technol.*, vol. 31, no. 12, pp. 141-149.

Verdegem, M.C.J., Eding, E.H., Sereti, V., Munubi, R.N., Santa-Reyes, R.A., van Dam, A.A. (2005). Similarities between microbial and periphytic biofilms in aquaculture systems. In: Periphyton, ecology, exploitation and management. M.E. Azim (*ed.*), UK, Oxford shire: CABI International Wallingford.

Vermaat, J.E. and Hanif, K.M. (1998). Performance of common duckweed species (Lemnaceae) and the waterfern Azolla filiculoides on different types of wastewater. *Water Res.*, vol. 32, no. 9, pp. 2569-2576.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. and Melillo, J.M. (1997). Human domination of Earth's ecosystems. *Science*, vol. 277, no. 5325, pp. 494-499.

von Sperling, M. and Carlos Augusto de Lemos Chernicharo (2005). Biological wastewater treatment in warm climate regions, volume 1. IWA Publishing, 2005. ISBN 1843390027.

Walker, D.A. (2002). The Z-scheme-down hill all the way. *Trends in Plant Science*, vol. 7, pp. 183-185.

Walker, D.A. (2009). Biofuels, facts, fantasy and feasibility. *Journal of Applied Phycology,* vol. 21, no. 5, pp. 509-517.

Wang, J.H., Baltzis, B.C., Lewandowski, G.A. (1995). Fundamental denitrification kinetic studies with *Pseudomonas* denitrificans. *Biotechnol. Bioeng*., vol. 47, pp. 26-41.

Wang, L.K., Hung, Y.T. and Shammas, N.K. (*eds.*) (2005). Advanced physicochemical treatment processes. Totowa, NJ: Humana Press, 2009. ISBN 9781597450294.

Wang, L.K., Shammas, N.K., Hung, Y.Tse (*eds*.) (2009a). Advanced biological treatment processes. In: Handbook of environmental engineering series, Volume 9. New York, USA: Humana Press, 2009. ISBN 9781588293602.

Wang, W. (1991). Ammonia toxicity to macrophytes (Common duckweed and rice) using static and renewal methods. *Environ. Toxic. Chem.*, vol. 10, pp. 173-1178.

Wang, Y. and Huang, G. (2003). Nitrate and phosphate removal by coimmobilized Chlorella *pyrenoidosa* and activated sludge at different pH. *Water Qual. Res. J. Canada*, vol. 38, pp. 541-51.

Watanabe, A. and Yamamoto, Y. (1967). Heterotrophic nitrogen fixation by the blue-green algae *Anabaenopsis circularis*. *Nature (London)*, vol. 214, pp. 738.

WEF (1998). Biological and chemical system for nutrient removal. Water Environment Federation- WEF 1998. Alexandria, VA.

WEF Technology Assessments (2000). Nitrogen removal using oxidation Ditches. Water Environment Federation-WEF 2000.

Weissman, J.C., Benemann, J.R. (1978). Biomass recycling and species control in continuous cultures. *Bioengineering and Biotechnology*, vol. 21, p. 627-648.

Weissman, J.C., Goebel, R.P. (1987). Factors affecting the photosynthetic yield of microalgae, pp. 139-168. In: FY 1986 Aquatic species program annual report. D.A. Johnson (*ed*.). Solar Energy Research Institute, Golden, Colorado, SERI/SP-231-3071.

Weissman, J.C., Goebel, R.P., Benemann, J.R. (1988). Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. *Biotechnol. Bioeng*., vol. 31, pp. 336-344.

Wentworth, M., Ruban, A.V., Horton, P. (2003). Thermodynamic investigation into the mechanism of the chlorophyll fluorescence quenching in isolated photosystem II light harvesting complexes. *Journal of Biological Chemistry*, vol. 278, pp. 21845-21850.

Wild, H.E., Sawyer, C.N. and McMahon, T.C. (1970). Factors affecting nitrification kinetics. 43rd Annual Conference. *Water Pollution Control Federation*, vol. 43, pp. 1845-1854.

Wu, X. and Merchuk, J.C. (2002). Simulation of algae growth in a bench scale bubble column. *Biotechnol. Bioeng.*, vol. 80, pp. 156-168.

Wyffels, S. (2004). Feasibility of combined autotrophic nitrogen removal processes for the treatment of highstrength nitrogen wastewaters. PhD thesis. Belgium: Faculty of Applied Biological Sciences- Ghent University, 2004.

Wyffels, S., Boeckx, P., Pynaert, K., Verstraete, W., Van Cleemput, O. (2003). Sustained nitrite accumulation in a membrane-assisted bioreactor (MBR) for the treatment of ammonium-

rich wastewater. *Journal of Chemical Technology & Biotechnology*, vol. 78, pp. 412-419. Available from: http://onlinelibrary.wiley.com/doi/10.1002/jctb.808/pdf.

Yeh, T.Y., Ke, T.Y., Lin, L. (2010). Algal growth control within natural water purification systems: macrophyte light shading effects. Springer Science+Business Media B.V. Water: *Air Soil Pollut.* Available from: DOI 10.1007/s11270-010-0447-4.

Zargar, S., Krishnamurthi, K., Saravanadevi, S., Ghosh, T.K. and Chakrabarti, T. (2006). Temperature-induced stress on growth and expression of Hsp in freshwater algal *Scenedesmus quadricauda*. *Biomedical and Environmental Science*, vol. 19, pp. 414-421.

Zeng, W., Zhang, Y., Li, L., Peng, Y., Wang, S. (2009). Control and optimization of nitrifying communities for nitritation from domestic wastewater at room temperatures. *Enzym Microb Technol.*, vol. 45, no. 2012, pp. 226-232. Available from: http://www.sciencedirect.com/science/article/pii/S0960852412011273.

Ziglio, G., Andreottola, G., Foladori, Ragazzi, M. (2001). Experimental validation of a single - OUR method for wastewater RBCOD characterisation. *Water Sci. Technol.*, vol. 43, no. 11, pp. 119-126.

Zimmo, O. (2003). Nitrogen transformations and removal mechanisms in algal and duckweed stabilization ponds. PhD dissertation. The Netherlands: UNESCO-IHE, Delft, Wageningen University, 2003.

Zimmo, O.R, Al Sa'ed, R. and Gijzen, H.J. (2000). Comparison between algae based and duckweed based wastewater treatment: differences in environmental conditions and nitrogen transformations. *Water Sci. Technol.,* vol. 42, no. 10-11, pp. 215-222.

Zimmo, O.R, van der Steen, N.P. and Gijzen, H.J. (2003). Comparison of ammonia volatilisation rates in algae and duckweed-based waste stabilization ponds treating domestic wastewater. *Water Res.*, vol. 37, no. 19, pp. 4587-4594.

Zimmo, O.R, van der Steen, N.P. and Gijzen, H.J. (2004). Nitrogen mass balance across a pilot-scale algae and duckweed based wastewater stabilization ponds. *Water Res.,* vo. 38, no. 4, pp. 913-920.

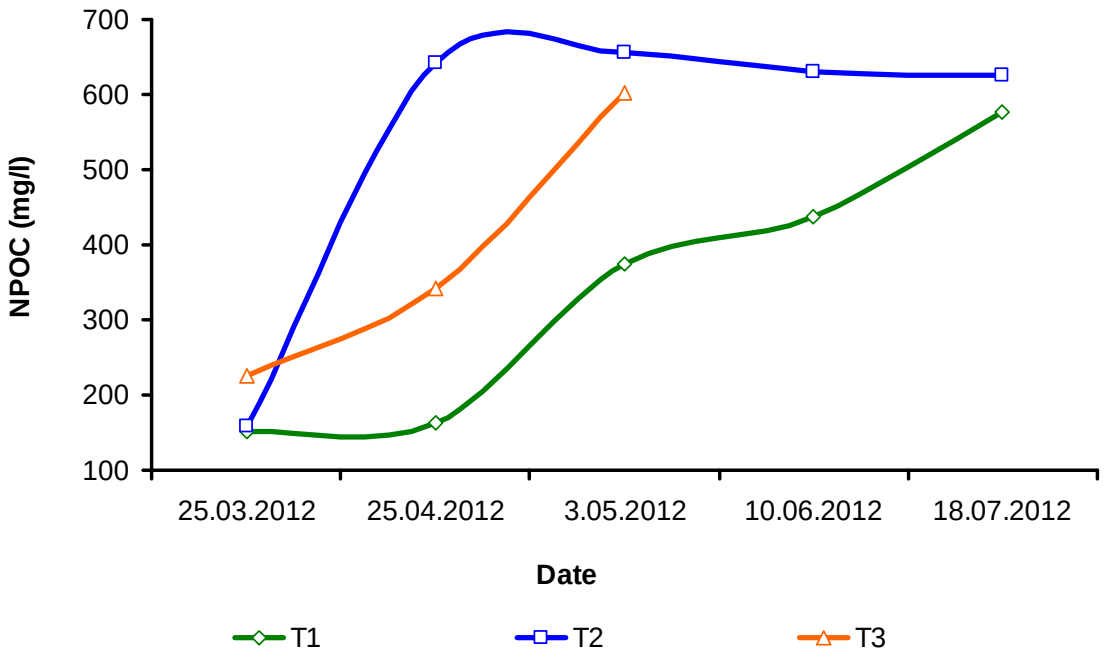
Zimmo, O.R., Al-Sa'ed, R.M., van der Steen, N.P. and Gijzen, H.J. (2002). Process performance: Assessment of algae-based and duckweed-based wastewater treatment systems. *Water Sci. Technol.*, vol. 45, no. 1, pp. 91-101.

Zirschky, J. and Reed, S.C. (1988). The use of duckweed for wastewater treatment. *J. WPCF.*, vol. 60, no. 7, pp. 1253-1258.

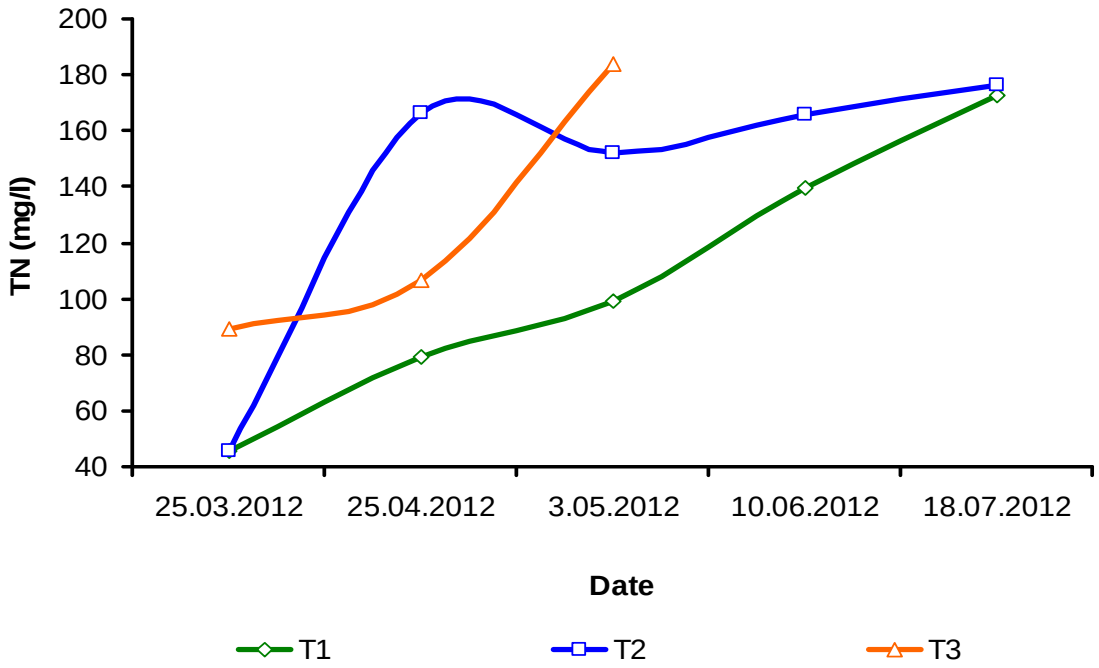
ANNEX

Annex 1. Total organic carbon (TOC) and total nitrogen (TN) in total algal sludge

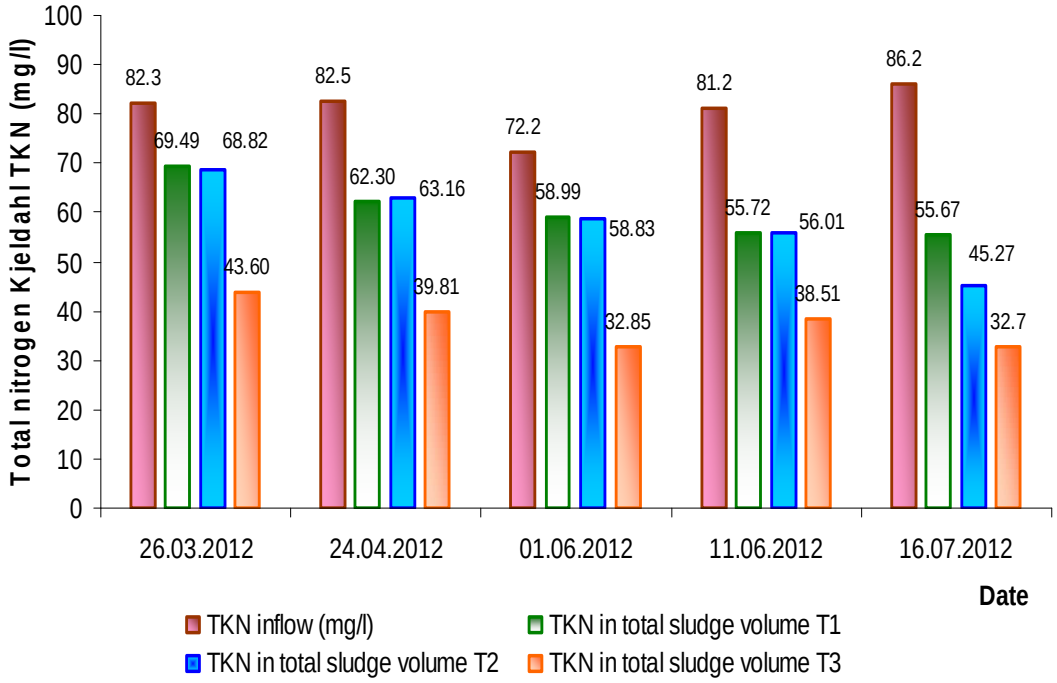
Date	Reactor	NPOC	TN	Unit	C:N Ratio BOD_5 30:1	Average	C:N Ratio BOD_5 15:1	Average
25.03.2012	T1	151.6	45.55	mg/l	9.01		4.51	
25.04.2012	T1	162.4	79.39	mg/l	14.67		7.33	
3.05.2012	T1	374.2	99.26	mg/l	7.96	10.03	3.98	5.02
10.06.2012	T1	437.3	139.3	mg/l	9.56		4.78	
18.07.2012	T1	577.3	172.4	mg/l	8.96		4.48	
25.03.2012	T2	159	45.44	mg/l	8.57		4.29	
25.04.2012	T2	641.5	166.1	mg/l	7.77		3.88	
3.05.2012	T2	655.4	152.1	mg/l	6.96	7.93	3.48	3.97
10.06.2012	T2	629.5	165.7	mg/l	7.90		3.95	
18.07.2012	T2	624.9	176.2	mg/l	8.46		4.23	
25.03.2012	T3	225.8	89.26	mg/l	11.86	10.13	5.93	5.06
25.04.2012	T3	601.4	183.9	mg/l	9.17		4.59	
3.05.2012	T3	342.8	106.8	mg/l	9.35		4.67	



Annex 2. NPOC in total algal sludge of the reactors



Annex 3. TN in total algal sludge of the reactors



Annex 4. The variations of Total Kjeldahl Nitrogen (TKN) in total algal sludge of the reactors

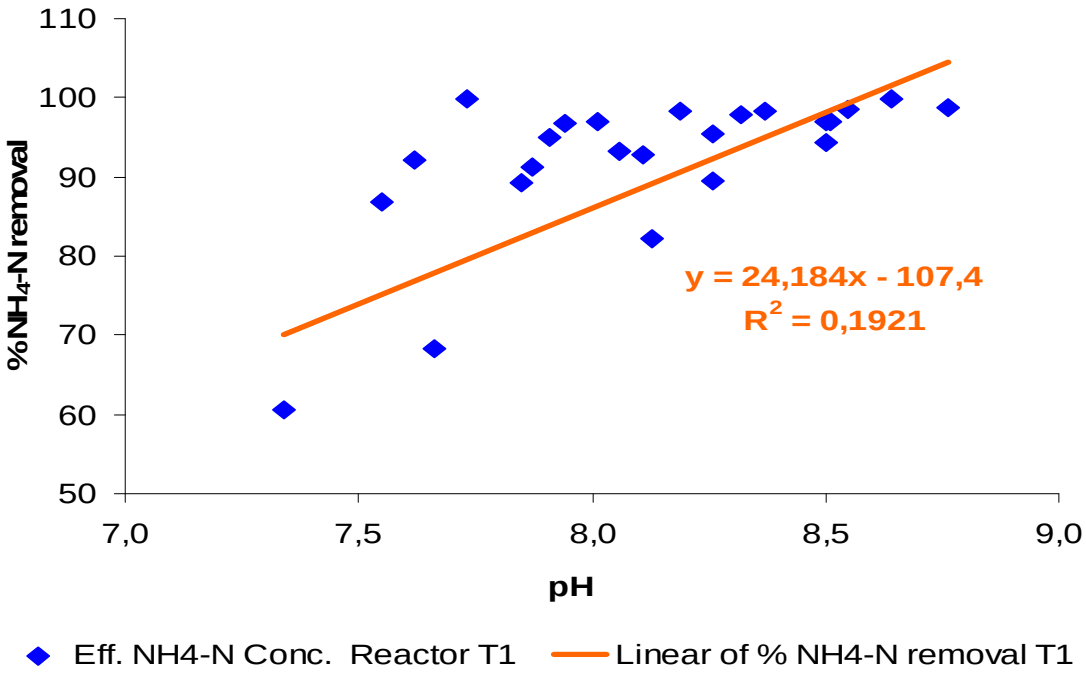
Annex 5. Diffusion coefficients for dissolved component in water

Component		Diffusion coefficient (10^{-4}·m^2·d^{-1})	Source
S_O	Dissolved oxygen	2.2	van Hulle *et al.* (2005)
S_{NO}	Nitrate or/and nitrite	1.4	van Hulle *et al.* (2005)
S_S	Readily biodegradable COD	0.58	van Hulle *et al.* (2005)
S_{ND}	Organic nitrogen	2	Beran *et al.* (2005)
S_I	Inert soluble COD	2	Beran *et al.* (2005)
S_{NH}	Ammonia nitrogen	1.12	Janse (2005)

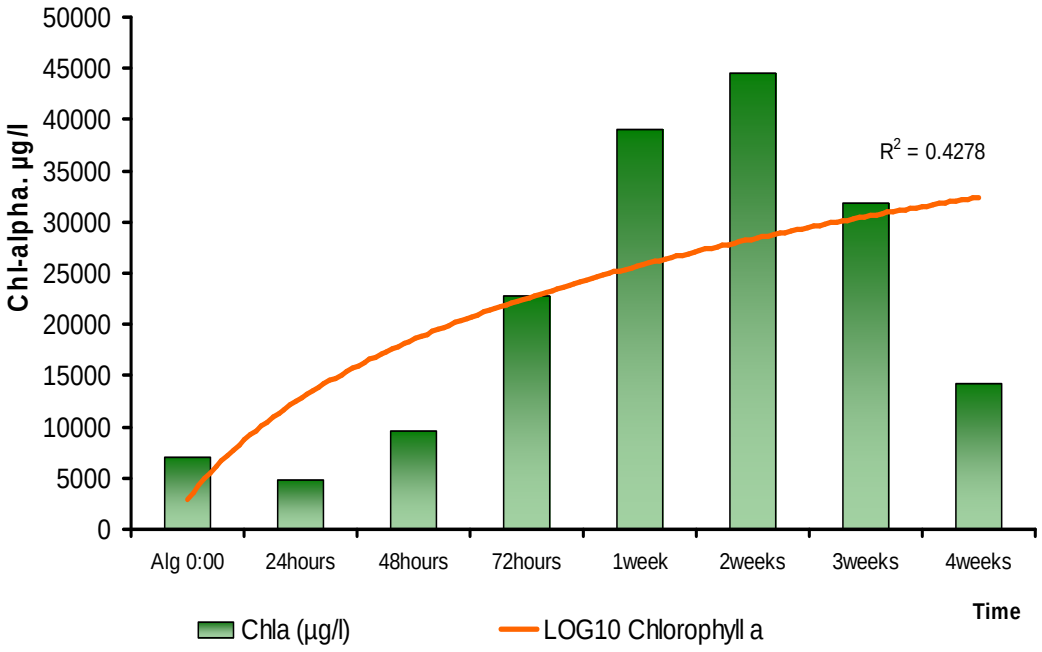
van Hulle, S.W.H., Zaher, U., Schelstraete, S., & Vanrolleghem, P.A. (2005). Titrimetric monitoring of a completely autotrophic nitrogen removal process. *Water Sci. Technol.*, vol. 53, no. 4-5, pp. 533-540.

Beran, B., Kargi, F. (2005). A dynamic mathematical model for wastewater stabilization ponds. *Ecological Modelling*, vol. 181, no. 1, pp. 39-57.

Janse, J.H. (2005). Model studies on the eutrification of shallow lakes and ditches. Ph.D thesis. The Netherlands: Wageningen University, 2005.



Annex 6. pH influets to ammonia remova efficiency



Annex 7. Chlorophyll alpha measurement in different period of testing time