# Mycological studies of the activated sludge from MBRs related to the biological activity of fungi in raw wastewater treatment

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Dedication: This thesis is firstly dedicated to the Egyptian young people who have been steadfast in the field and to the souls of the martyrs of our revolution who were killed and their blood paid the price for freedom and the salvation of the regime. Love and appreciation for my country, Egypt.

Secondly it is dedicated to my family. All through my life my parents have always been there, prayers, supporting and trusting me during those difficult times. I would like to dedicate this research and everything I do to both of them. I would like to thank my wife, my daughters; Rania, Maryam, my sons Eyad, and Omar for their patience, love, helping and enabled me to finish this work on time.

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***Mohamed Fadl Allah Abdel-Latef Awad***

# ZUSAMMENFASSUNG

Die Untersuchungen dieser Studie waren in drei Teile geteilt:

• 108 Arten von 40 Gattungen wurden aus 36 Proben isoliert. Diese Proben bestanden sowohl aus aerobem als auch anoxischem Belebtschlamm und wurden aus zwei Kläranlagen in Berlin innerhalb von zehn Monaten entnommen. Fünf Isolationsmedien wurden bei 30°C ein bis zwei Wochen lang angewendet.

• 21 Isolate wurden aus Belebtschlamm aus einem MBR isoliert und auf Eliminierung von Stickstoff und Phosphor aus unbehandeltem Abwasser in Schüttelkolben überprüft. Nach einer Inkubationszeit von 15 Tagen waren nur zwei Pilz-Isolate geeignet als Wachstumsparameter und für die Eliminierung von TN, $PO_4$, $NH_4$, $NO_3$ und CSB von unbehandeltem Abwasser. Diese waren *Aspergillus niger* und *Trichoderma viride*. Die Eliminierungswerte von Stickstoff waren 86,3 % und 88,5 % und die von Phosphor 95,0% und 96,3% für jeweils *Aspergillus niger* und *Trichderma viride*. Die Fähigkeit der anderen Isolate, Stickstoff und Phosphor zu eliminieren, war jedoch niedrig. Die besten Umgebungsbedingungen für Trockensubstanz, Eiweißgehalt und Eliminierung von Verbindungen aus unbehandeltem Abwasser durch *Aspergillus niger* in SchüttelKolben waren wie folgt: pH-Optimum 4,5 bis 6,0; 30 $^oC$ und Inkubationszeit 6 Tage für Trockenstoff, 7 Tage für Eiweißgehalt, 3 Tage für die Eliminierung von $PO_4$, 4-5 Tage für $NH_3$, TN, $NO_4$ und CSB. Dagegen waren die optimalen Bedingungen für das Wachstum von *Trichoderma viride* und den Abbau wurden pH 4,5 Temperatur 25 bis 30 $^oC$, Inkubationszeiten 4 -7 Tage.

• Pilzwachstum und Schadstoffe Entfernung aus dem ungeklärten Abwasser wurde in einem 2L Batch-Reaktor unter aeroben und anaeroben Bedingungen untersucht. Die höchsten Eliminationsraten von Verbindungen aus dem ungeklärten Abwasser durch *Aspergillus niger* und *Trichoderma viride* wurden unter aeroben Bedingungen nach 3-4 bzw 2-5 Tage erreichte. Myzel-Trockenmasse und Eiweißgehalt von *Aspergillus niger* nach 15 Tagen waren 5055.0 mg/L und 259.0 mg/L. Doch die größte Trockenmasse und des höchste Eiweißgehalt wurde von *Trichoderma viride* nach 15 Tagen 7030.0 mg/L and 295.75 mg/L erreicht. Unter anaeroben Bedingungen die höchste Eliminationsraten durch *Aspergillus niger* und *Trichoderma viride* nach 5-8 und 3-5 Tagen erreicht. Myzel Trockenmasse und Eiweißgehalt von *Aspergillus niger* und *Trichoderma viride* wurden 3040 mg/L und 217,5 mg/L sowie 4080 mg/L und 263,8 mg/L bestimmt. Der vom MBR produzierte Belebtschlamm ist reich an cycloheximidresistenten keratinophilischen Pilzen und anderen Dermatophyten. Die meisten in dieser Untersuchung gewonnenen Pilzarten können als potentielle Pathogene bezeichnet werden, einige dieser Pilzarten produzieren auch Mykotoxin. Aus diesem Grunde sollten alle Arbeitskräfte im Bereich Belebtschlammverarbeitung, Abwasserbehandlung und Landwirtschaft Pilzinfektionen entgegenwirken. Auch sollte der Produktionsbereich so konzipiert sein, dass die Ausbreitung von Pilzkrankheitserregern in das Umfeld kontrolliert werden kann. Bei Pilzen wurde nachgewiesen, dass ein Potential für die Abwasserbehandlung unter bestimmten Laborbedingungen wie ein niedriger pH-Wert, Temperatur und Belüftung besteht. Wegen seines hohen Proteingehalts könnte Pilzbiomasse auch als Nahrungsquelle für Tier oder Mensch dienen.

# ABSTRACT

The investigations of this study were divided into three parts:

• 108 species belonging to 40 genera were isolated in the present investigation from 36 samples of each aerobic and anoxic activated sludge collected from 2 wastewater treatment plants in Berlin during 10 months. Five isolation media were used at 30 $^{o}$C for 1-2 weeks.

• Twenty-one isolates were screened for the elimination of compounds from raw wastewater in shaker flasks at 30 $^{o}$C for 15 days. Two isolates were the best for growth parameters and elimination of TN, $PO_4$, $NH_4$, $NO_3$, and COD from raw wastewater. These were *Aspergillus niger* and *Trichoderma viride*. The elimination values of nitrogen were (86.3 % and 88.5 %) and phosphorous (95.0 % and 96.3 %) for *Aspergillus niger* and *Trichoderma viride*, respectively. While the ability of other isolates for elimination of nitrogen and phosphorous was low. Thus in the following section these isolates were selected to examine their activity under different conditions. The best environmental conditions for dry matter, protein content and elimination of compounds from raw wastewater by *Aspergillus niger* in shacked flasks were as follows: optimum pH 4.5 - 6.0; 30 $^{o}$C and incubation periods, 6 days for dry matter, 7 days for protein content, 3 days for elimination of $PO_4$, 4-5 days for $NH_3$, 4 for TN, $NO_4$ and 4 days for COD. Whilst the optimum conditions for growth and elimination of compounds by *Trichoderma viride* were pH 4.5; 25 - 30 $^{o}$C and 4 -7 days.

• Fungal growth and elimination of compounds from raw wastewater was showed in 2L batch reactor under aerobic and anaerobic conditions. The highest elimination rate of compounds from raw wastewater by *Aspergillus niger* and *Trichoderma viride* under aerobic condition was reached after 3-4 and 2-5 days, respectively. The mycelium dry matter and protein content of *Aspergillus niger* after 15 days were 5055mg/L and 259.0 mg/L, respectively. However the highest values of dry matter and protein content of *Trichoderma viride* after 15 days were 7030.0 mg/L and 295.75 mg/L, respectively. Under anaerobic conditions the highest elimination rate of compounds by *Aspergillus niger* and *Trichoderma viride* were attained after 5-8 and 3-5 days, respectively. The mycelium dry matter and protein content of *Aspergillus niger* and *Trichoderma viride* were (3040.0 mg/L and 217.5 mg/L) and (4080.0 mg/L and 263.8 mg/L), respectively. Most fungi recovered in the present investigation can be considered as potential pathogens and some of these fungi also produce mycotoxins. Therefore, all workers in the field of activated sludge process, wastewater treatment and farm operation should be careful to avoid mycotic infections and the productions must be adapted to control the spread of pathogenic fungi in the environment. Also these experiments illustrate the possible health risk problems that may arise in the use of sludge for land reclamation and fertilization. Fungi were proved to have potential for wastewater treatment under special laboratory conditions such as low pH, temperature and aeration. Also fungal biomass produced could be used as a source of food for animal or human consumption (as enzymes), due to its high protein content.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **BNR** | Biological nutrient removal |
| **BOD** | Biochemical oxygen demand |
| **BOD5** | The amount of dissolved oxygen consumed in five days |
| **BWB** | Berliner Wasserbetriebe |
| **COD** | Chemical oxygen demand |
| **CZ** | Czapek Dox |
| **DM** | Dry matter |
| **% F** | Percentage frequency of occurrence |
| **H** | High occurrence |
| **L** | Low occurrence |
| **M** | Moderate occurrence |
| **MBRs** | Membrane bioreactors |
| **MEA** | Malt extract agar |
| **N** | Nitrogen |
| **NCI** | Number of cases of isolation |
| **OOMW** | Olive oil mill wastewater |
| **OR** | Occurrence remarks |
| **P** | Phosphorus |
| **PC** | protein content |
| **PDA** | Potato dextrose agar |
| **R** | Rare occurrence |
| **RBA** | Rose Bengal Agar |
| **SCC** | Sabouraoud's dextrose agar with Cycloheximide and Chloramphenicol |
| **SDA** | Sabouraoud's Dextrose Agar |
| **STP** | Sewage Treatment Plant |
| **TN** | Total nitrogen |
| **TSS** | Total suspended solid |
| **V** | Volume |
| **VSS** | Volatile suspended solid |
| **W1** | Weighted before using |
| **W2** | Weighted again after cooling in an exsiccator |
| **WRF** | White rot fungi |
| **WWTP** | Wastewater treatment plant |

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# 1. INTRODUCTION AND OBJECTIVES OF THE STUDY

Water crisis at present is the biggest problem according to the United Nations. Almost 32 countries of the World are facing the water crisis. Even other several parts of the world are facing the varied levels of the water crisis. So we are in urgent need to wastewater treatment. Because of acute shortage of water, the food problems are getting aggravated. About 40 million people in Africa are facing the problem of food shortage. It is expected that if the similar conditions will persist then there will be 500 million till 2025 who will suffer from these problems.

Wastewater contains countless numbers of living organisms, most of them too small to be visible except when viewed under a microscope, which is why they are called "microorganisms". Typically, wastewater prior to entering the treatment plant will contain from 100,000 to 1,000,000 microorganisms per milliliter. These microbes have their origin from two general sources: sanitary wastes and the soil. Both wastewaters and soils contain large numbers of microorganisms. Generally the microorganisms can be regarded as a natural living part of the organic matter found in wastewaters and their presence is most important because they serve a primary function in the degradation of wastes in biological wastewater treatment. In a sense the successful operation of a biological wastewater treatment plant is dependent upon knowledge of the activities of the microorganisms.

Efficient treatment then depends on understanding the requirements for optimal growth as well as recognizing unfavorable conditions. Wastewater treatment professionals face daily exposure to a wide variety of pathogens. These include viruses, bacteria, fungi, protozoa, and helminthes, as well as allergins, endotoxins, and exotoxins. While generally minimal, potential health hazards are still cause for concern. Sewage wastes is used widely as land fertilizer, therefore knowledge related to mycoflora is very important due to distribution of pathogenic fungal species in the environment. Few investigations are conducted and present valuable information. However every fungal study on the raw sewage or activated sludge, where sludge was used, adds information about environment pollution with dangerous microorganisms.

Biological wastewater treatment generates large amounts of low value bacterial biomass. The treatment and disposal of this excess bacterial biomass, also known as waste activated sludge, accounts for about 40-60% of the wastewater treatment plant operation cost. A different form of biomass with a higher value of protein content could significantly change the economics of wastewater treatment. Fungi could offer this benefit over bacteria in wastewater treatment processes. The biomass produced during fungal wastewater treatment has, potentially, a much higher value than that from the bacterial activated sludge process. The fungi can be used to derive

valuable biochemicals and can also be used as a protein source. Various high-value biochemicals are produced by commercial cultivation of fungi under aseptic conditions using expensive substrates. Food-processing wastewater is an attractive alternative as a source of low-cost organic matter and nutrients to produce fungi with concomitant wastewater purification.

The main objectives of this study are summarized in the following:

- Mycological survey of aerobic and anoxic activated sludge samples from two wastewater treatment plants with MBR (Amedeus pilot plant in Berlin/Wedding and BWB plant in Berlin/Margaretenhöhe) during a period of 10 months on five isolation media and identification of some pathogenic fungi (dermatophytes and keratinophilic).

- Screening of fungal species for elimination of nitrogen, phosphorous and COD from raw wastewater of Wassmannsdorf BWB wastewater treatment plants in Berlin.

- A study of the impact of environmental factors on the biological activity of selected fungi and their ability to eliminate compounds from raw wastewater in shaking flasks and in a batch reactor under aerobic and anaerobic systems.

# 2. REVIEW OF LITERATURE

## 2.1. Wastewater

### 2.1.1 General introduction

Wastewater is not just sewage. All the water used in the home that goes down the drains or into the sewage collection system is wastewater. This includes water from baths, showers, sinks, dishwashers, washing machines, and toilets. Small businesses and industries often contribute large amounts of wastewater to sewage collection systems; others operate their own wastewater treatment systems. In combined municipal sewage systems, water from storm drains is also added to the municipal wastewater stream. Wastewater is about 99 percent water by weight and is generally referred to as influent as it enters the wastewater treatment facility. Domestic wastewater comes primarily from individuals, and does not generally include industrial or agricultural wastewater. Many countries strive to reduce the emissions of nitrogen compounds (ammonia, nitrate and NOx) and phosphorus to surface water and atmosphere. Since mainstream domestic wastewater treatment systems are usually already overloaded with ammonia, a dedicated nitrogen removal from concentrated secondary or industrial wastewaters is often more cost-effective than the disposal of such wastes to domestic wastewater treatment. The cost-effectiveness of separate treatment has increased dramatically in the past few years, since several processes for the biological removal of ammonia from concentrated waste streams have become available (Bitton, 2005).

Wastewater generated from urban and rural areas after domestic use is a large source of water. It is mainly comprised of water (99.9%) together with relatively small concentrations of suspended and dissolved organic and inorganic solids (Mara and Cairncross, 1989; UN Department of technical cooperation for development, 1985). Among the organic substances present in sewage are carbohydrates, lignin, fats, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from process industries. Wastewater typically contains a complex mixture of components which are degraded by a diverse of microbial cells in biological reactions. As biological, biochemical and physical phenomena all influence the nutrient removal. Biological wastewater treatment involves the transformation of dissolved and suspended organic contaminants to sludge and evolved gases ($CO_2$, $CH_4$, $N_2$ and $SO_2$) which are separable from treated water (Low and Chase, 1999).

### 2.1.2. Wastewater treatment

The major aim of wastewater treatment is to remove as much of the suspended solids as possible before the remaining water, called effluent, is discharged back to the environment. As solid

material decays and destroys or inactivates a large number of pathogens, it uses up oxygen, which is needed by the plants and animals living in the water.

Grady *et al*. (1999) reported that the principal objective of wastewater treatment is generally to allow human and industrial effluents to be disposed without danger to human health or unacceptable damage to the natural environment. Irrigation with wastewater is both disposal and utilization and indeed is an effective form of wastewater disposal (as in slow-rate land treatment). However, some degree of treatment must normally be provided to raw municipal wastewater before it can be used for agricultural, landscape irrigation or for aquaculture. The quality of treated effluent used in agriculture has a great influence on the operation and performance of the wastewater-soil-plant or aquaculture system. Wastewater treatment methods are categorized into three sub-divisions, physical, chemical and biological.

#### 2.1.2.1. Physical wastewater treatment

The use of gross chemicals or executing biological changes is strictly avoided. A prominent physical water treatment method is sedimentation, wherein coarse screening of waste water is done to remove contaminating objects after allowing them to settle at the base. Once the contaminants have settled, the cleared effluent or waste stream is removed. Sedimentation is one of the most common methods, quite often used at the beginning and the end of many water treating processes. Aeration is another physical water treatment method used, wherein air is added to wastewater physically to provide oxygen. In yet another method known as filtration, sewage is passed through filters to separate the contaminating solids from the water. Sand filter is a common filter used in this process. In a number of wastewater treatment methods, semi-solid contaminants like grease and oil are allowed to float on the surface of the water, and then they are physically removed (Spellman 2008).

#### 2.1.2.2. Chemical wastewater treatment

Chemicals are used to treat wastewater in chemical water treatment. The most common method to treat water using chemicals is chlorination, wherein chlorine, a strong oxidizing chemical is used to kill the pathogenic microorganisms which lead to decomposition of water. Ozone, an oxidizing disinfectant, is another oxidizing agent used to treat polluted water. These oxidizing agents affect the biological growth process of microorganisms, thus making the water usable. A chemical process called neutralization is commonly used in industrial wastewater treatment. In this process, acid or base is added to the water to adjust its pH value back to neutral level. A common base used in this process is lime, which is mostly used to neutralize acid wastes.

Polyvalent metals, metals having more than one valence, are very often used as coagulating chemicals in sewage treatment. Lime, iron containing compounds like ferrous chloride, ferrous sulfate, and (alum) are some commonly used coagulants. There are a few water treatment processes which can be categorized as physical as well as chemical (Spellman 2008).

#### 2.1.2.3. Biological wastewater treatment

In the biological water treatment process, microorganisms are used to biochemical decompose the wastewater and stabilize the end product. Because of drawbacks of physical and chemical treatment processes such as Increased (aquatic toxicity, sludge production, filamentous growth and cost) and decreased (sludge settle ability and sludge dewatering characteristics), the biological treatment of wastewater is advocated in the last few decades. All biological wastewater treatment processes take advantage of the ability of microorganisms to use diverse wastewater constituents to provide the energy for microbial metabolism and the building blocks for cell synthesis (Schultz, 2005). The common wastewater treatment processes include trickling filters, lagoons, stabilization ponds, constructed wetlands and activated sludge processes (EPA, 1993; Gray, 2002).

Nitrogen is essential nutrient for plant growth. Nitrogen undergoes a number of transformations in soil which together form the nitrogen cycle. These reactions are largely mediated by microorganisms (Kilham, 1994). Various common heterotrophic soil fungi are capable of oxidizing reduced forms of nitrogen in soil (Killham, 1994; Mekki *et al*., 2006). The ability of fungi to nitrify ammonium has also been demonstrated (Alexander, 1977; Atlas and Bartha, 1997; Guest and Smith, 2002).

Nitrogen appears in wastewater as ammonia, nitrite, nitrate and organic nitrogen. Organic nitrogen is decomposed to ammonia, which in turn on one hand is assimilated to microbial cells, leading thus to net growth, on the other hand is oxidized to nitrite and nitrate. In a second step, nitrate is converted to gaseous nitrogen and is removed from the wastewater. Denitrification is known to proceed as conversion of nitrates to nitrites and subsequent conversion of nitrites to nitric oxide, nitrous oxide and nitrogen gas (Guest and Smith, 2002).

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Nitrogen removal is one of the main concerns in modern wastewater treatment. Currently, the most widely applied technology for N-removal from municipal wastewater with activated sludge systems uses nitrification combined with denitrification (Mulder, 2003). Biological nitrogen removal from wastewater is based on the nitrification and denitrification processes at the biological treatment plant with the activated sludge. Biological phosphorus removal is based on special

microorganisms–polyphosphate accumulating organisms with accumulated phosphate in excess to their metabolic requirement for growth. Usually, schemes of nitrogen removal are combined with phosphorus removal schemes. Biological phosphorus and nitrogen removal are closely linked, both in a positive and negative sense. Nitrates are usually considered to have an adverse effect on phosphorus removing capacity of activated sludge (Bitton, 2005; Jenicek *et al*., 2004). Exchange of the anaerobic and aerobic conditions is necessary for biological phosphorus removal. Phosphorus is an essential element in all living systems, and is also limiting nutrient in nutrition and production of most plants. Microbial activity in the soil is important in the cycling of phosphorus. A wide variety of heterotrophic fungi and bacteria have been shown to be capable of solubllizing insoluble phosphate (Alexander, 1977; Ali *et al*., 1986; Killham, 1994; Kucery *et al*., 1989).

Phosphorus is an essential nutrient for all organisms. It is widely used in the biosynthesis of cellular components, such as nucleic acids, phospholipids, and proteins. Therefore, phosphorus is added to plant fertilizers and animal feeds. However, phosphorus accumulation in the environment due to runoff from land treated with fertilizers and to the discharge of industrial and domestic waste is a global problem which causes eutrophication of lakes, bays, and other surface waters. Considerable attention is therefore being paid to the removal of phosphorus from wastewaters (Kornberg *et al*., 1999).

Phosphorus appears in wastewater as orthophosphate, polyphosphate and organically bound phosphorus, the last two components accounting usually for up to 70 percent of the influent phosphorus. Microbes utilize phosphorus during cell synthesis and energy transport. As a result, 10 to 30 percent of the influent phosphorus is removed during traditional mechanical/biological treatment (Mulder and Rensink, 1987; Metcalf and Eddy, 1991; Henze, 1996; Wenzel and Ekama, 1997). When enhanced phosphorus removal is desired, the process is modified, so that the sludge is exposed to both anaerobic and aerobic conditions. Then certain microorganisms, capable of storing phosphorus (in the form of polyphosphates), metabolize it for energy production and cell synthesis, resulting in the removal of phosphorus from the system through the waste activated sludge.

Phosphorus and nitrogen are two most important elements helping the growth of microorganisms and aquatic plants in rivers, lakes, and shallow embayed areas of the marine environment. Attempting to protect the surface water quality, nitrogen and phosphorus have been identified as the limiting nutrients for microorganisms' growth. Limiting the discharge of phosphorus, however, has been recognized as the more effective method of these two to prevent eutrophication (Vabolienė *et al*., 2007).

Biological nitrogen and phosphorus removal from wastewater is an essential treatment to avoid unpleasant conditions for natural resources (Pitman, 1982; Horan, 1999; Schutte and Van der Post, 2003). Enhanced biological phosphorus removal has been considered to depend on polyphosphate microorganisms that are able to accumulate polyphosphate by storing more phosphorus than they need for growth (Lydia, 2006). Although nitrifying microorganisms are known to oxidize ammonia-nitrogen to nitrate-nitrogen in a two-stage conversion process, very little energy is derived from these oxidation reactions. In fact, energy required to converting $CO_2$ to cellular carbon and nitrifying microorganisms represent a small percentage of total population of microorganisms in activated sludge (Choubert *et al*., 2005).

Ramothokang *et al*. (2006) reported that the availability of excess nutrients (phosphorus and nitrogen) in wastewater systems causes many water quality problems. These problems include eutrophication whereby algae grow excessively and lead to depletion of oxygen, death of the aquatic life and bad odours. Biological phosphorus removal has gained attention because the condition of wastewater is manipulated in order to facilitate nutrient removal by the microbial communities in the wastewater. It has been reported that filamentous bacteria are capable of removing P at a similar or higher rate to that of heterotrophic bacteria. It has also been reported that conditions that facilitate biological nitrogen removal promote bulking in a biological nutrient removal system.

The concentration of oxygen must be enough in the aerobic zone for microorganisms that have accumulated phosphorus for complete or near complete nitrification. The concentration of oxygen must be 2–6 $mg/L^{-1}$, no less than 1 mg/L in the aerobic zone, because otherwise the phosphates will split from the microorganisms to wastewater (Henze *et al*., 1997; Droste, 1996). Phosphates' emission from microorganisms to wastewater is possible in the sludge sedimentation phase.

Biological wastewater treatment generates large amounts of low value bacterial biomass. The treatment and disposal of this excess bacterial biomass, also known as waste activated sludge, accounts for about 40-60 % of the wastewater treatment plant operation cost. A different form of biomass with a higher value could significantly change the economics of wastewater treatment. Fungi could offer this benefit over bacteria in wastewater treatment processes. The biomass produced during biological wastewater treatment with fungi has, potentially, a much higher value than that from the bacterial activated sludge process. Fungi involved in the removal of carbonaceous contaminants from wastewater require nitrogen and phosphorous for growth and reproduction. Fungi require nitrogen to form proteins, cell wall components, and nucleic acids (Maier, 1999).

**2.1.2.3.1. Activated sludge process**

The activated sludge process is a biological method of wastewater treatment that is performed by a variable and mixed community of microorganisms in an aerobic aquatic environment. These microorganisms derive energy from carbonaceous organic matter in aerated wastewater for the production of new cells in a process known as synthesis, while simultaneously releasing energy through the conversion of this organic matter into compounds that contain lower energy, such as carbon dioxide and water, in a process called respiration. As well, a variable number of microorganisms in the system obtain energy by converting ammonia nitrogen to nitrate nitrogen in a process termed nitrification. This consortium of microorganisms, the biological component of the process, is known collectively as activated sludge (Water Environment Association 1987).

Presently, the activated sludge system is the most widely used biological treatment process for both domestic and industrial wastewaters. The system is a biological method that is performed by a mixed community of microbes and uses the metabolic reactions of the microbes to produce high-quality effluent in an aquatic environment (Water Environment Association, 1987; Muyima *et al*., 1995; EPA, 2002). This is achieved by converting and removing substances that have an oxygen demand (Water Environment Federation, 1996; Eikelboom and Draaijer, 1999; Gray, 2002).

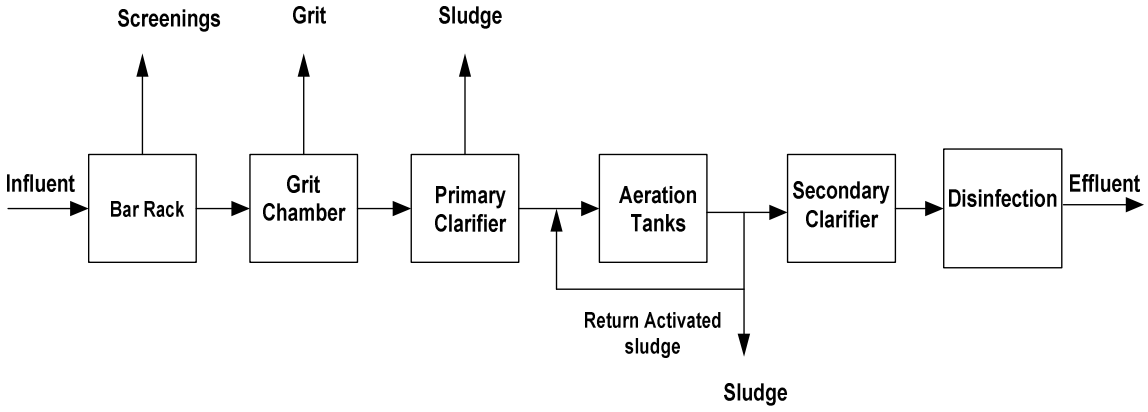



**Fig. 2.1:** Simplified flow diagram for a biological wastewater treatment with activated sludge process.

The activated sludge process comprises 2 liquid stream processing units-the aeration basin (biological reactor) and the secondary clarifier. The aeration basin provides the environment for transformation and removal of pollutants by a mixed variable consortium of micro and macro-organisms termed activated sludge. The microorganisms include eubacteria, filamentous bacteria, algae, fungi, protozoa and rotifers. Flocs are the basic ecological units of activated sludge. Fungal

hyphae are often associated with flocs, but rarely predominate under normal operating conditions (Jenkins *et al*., 1993).

Current mainstream technologies for wastewater treatment, such as the activated sludge process with N and P removal, are too costly to provide a satisfactory solution for the growing wastewater problems in developing regions. Recently, using physicochemical and biological methods to remove nitrogen, phosphorus, and organic pollutants efficiently has also been reported. However, because of the disadvantages of high operation costs, high chemical demand that easily leads to secondary pollution, and deposition of excess sludge, these methods can not be widely applied. Although it is accepted that microorganisms are directly responsible for the effectiveness and success of the activated sludge treatment process, the complexity of microbiological populations is often under estimated during design of the latter. Full understanding of the ecological, physiological and biochemical activities of the microflora is necessary for optimal control of the process (Adamse *et al*., 1984).

## 2.2. Occurrence of fungi in activated sludge

Activated sludge is rich in organic matter and provides ideal conditions for growth of many groups of fungi. Abdel-Hafez and El-Sharouny (1990) reported that numerous microorganisms are present in large numbers in sewage or soil amended with activated sludge. Several fungi previously isolated from these substrates are known to be pathogenic to plants, animals and humans. Sewage sludge (wastewater) typically contains a complex mixture of components which are degraded by a diverse range of microbial cells; therefore, it is a good environment for the growth of fungi. Both wastewater and sewage sludge are habitats for saprotrophic fungi as well as typical pathogens, which could unfavorably affect human health (Baran, 1998).

The occurrence of a certain amount of fungi can be stated in all kinds of waste water, where conditions for the growth of living organisms are retained. All can be classified as belonging to the ecological group called by numerous authors `sewage fungi', `polluted fungi', 'Abwasserpilze' etc. (Feldman, 1957; Cooke, 1963). Mostly therewith are not meant organisms specialized exclusively on aqueous media but organisms generally found also in other habitats. Thirty-eight species of fungi were identified in pure culture after isolation from activated sewage sludge by serial dilution. Nine species and genera were identified that had not been previously reported (Diener *et al*., 1976).

Häuslerova (1976) noted that the fifteen species of micromycetes belonging to 10 genera were isolated and identified and in addition several representatives of Saccharomycetaceae, which were not identified from activated sludge. Generally, the filaments in the activated sludge are ascribed to filamentous bacteria and as long as fungi were isolated from activated sludge their

presence has been reported only in the form of spores. Millner *et al*. (1977) mentioned that the *Aspergillus fumigatus*, a medically important fungal opportunist and respiratory allergen, was isolated from sewage sludge. Cook and Schlitzer (1981) reported that the *Candida albicans* was isolated from sewage influent but occurred commonly in low numbers**.** De Bertoldi *et al*. (1983) in his studies on the composting of sewage sludge, reported that the 60 fungal species belonging to 49 genera from thermpophilic and mesophilic fungi were isolated during the composting of the organic fraction of urban solid waste mixed with sewage sludge.

Ulfig and Korcz (1983) reported that the six species from kerainophilic fungi (*Microsporum gypseum, Trichophyton terrestre, T. ajelloi, Chrysosporium pannorum, C. keratinophilum,* and *C. pruinosum*) were recovered from five mixed samples of sewage sludges which were collected from 4 wastewater treatment plants in Katowice, Poland. Hiremath *et al* (1985a) mentioned that the 29 species belonging to 16 genera were isolated from sewage sludge of wastewater. Abdel-Hafez and EL-Sharouny (1987) noted that eighty six saprophytic fungal species representing 30 genera were isolated from soil samples irrigated by sewage effluents during January 1985-December 1986 and the most prevalent species were *Acremonium stricturn, Aspergillus fumigatus, A. niger, A. sydowii, Chaetomium globosum, Fusarium solani, Mucor hiemalis, Penicillium chrysogenurn*, and *Stachybotrys chartarum.* El-Shafei *et al*. (1989) isolated eight different *Streptomyces* species, two *Mucor rouxii* and *Aspergillus flavus* from sewage sludge. Forty-three species representing 22 genera, 17 species of which were dermatophyte and 26 species of cycloheximide resistant fungi were isolated from 40 sewage sludge samples collected from Upper Egypt (Abdel-Hafez and EL-Sharouny, 1990).

Bux and Kasan (1994) mentioned that the twenty-four fungal genera (filamentous and yeast) were isolated from 10 activated sludge plants in South Africa on three isolation media, casilone glycerol yeast extract agar, rose Bengal chloramphenicol agar and yeast malt extract agar, respectively. Bień and Nowak (1995) reported that in stabilized sewage sludge from municipal wastewater treatment plant in Czestochowa the following fungi were identified: *Aspergillus sp*., *Fusarium sp*., *Mucor mucedo*, *Penicillium sp*., *Geotrichum candidum, Candida sp*., *Rhodotorula rubra*, *Microsporum sp*., and *Torulopsis glabrata*. Hashem (1995) reported that the nineteen species belonging to 16 genera were isolated from 25 sewage sludge samples on Czapeks agar at 27 $^{o}$C for one week, *Alternaria alternate* and *Aspergillus flavus* were with 80 % frequency.

Dave *et al***.** (1996) isolated *Geotrichum sp* and *Trichoderma sp* from petrochemical effluent treatment plant activated sludge. Ulfig *et al*. (1996) used 4 different isolation media for isolation of keratinolytic fungi from sewage sludge, reported that the 185 fungal appearances belonging to 10 species were observed.

Ali-Shtayeh *et al*. (1999) in his studies on soil receiving raw city wastewater reported that the 57 CH-resistant fungal species belonging to 18 genera were recovered, of which 49 species were recovered from soil habitats and 28 species from raw city wastewater. Ali-Shtayeh and Jamous (2000) isolated 55 species belonging to 21 genera from raw city sewage and reported that the species most commonly found in raw city sewage include *Alternaria alternata*, *Aspergillus candidus*, *Geotrichum candidum* and *Paecilomyces lilacinus*. Field soils receiving raw city sewage or normal irrigation water were shown to be rich in pathogenic and potentially pathogenic keratinophilic fungi, including dermatophytes.

Boszczyk-Maleszak *et al*. (2002) mentioned that the five fungal strains were isolated and identified from activated sludge. All isolated strains appeared to be Moniliales from the class of fungi Imperfecti (*Candida sp*., *Monosporium sp.,* and *Trichospoon sp.*)**.** Molla *et al*. (2002) reported that the twenty seven filamentous fungal strains representing 5 genera; *Aspergillus, Penicillium, Trichoderma, Myriodontium,* and *Pleurotus* were isolated from domestic wastewater sludge cake from Indah Water Konsortium. Muhsin and Hadi (2002) mentioned that the four fungal species including two dermatophytes and two saprophytes were isolated from sewage sludge samples at Basrah (Iraq) they were tested for their degradative ability towards three types of keratin substrates.

Ulfig (2003) mentioned that the 343 keratinophilic fungal strains from 9 species were isolated from the sludge. The total isolation frequency was 94.4 %. *Fusarium solani*, *Phialophora melinii*, *Aspergillus virsicolor*, and *Fusarium oxysporum* predominated at 23 and 26 $^{o}$C. However, the last species was also recorded frequently at 33 $^{o}$C. Faryal and Abdul Hameed (2005) mentioned that the fungal strains were isolated from textile effluent samples revealed the presence of 11 fungal species belonging to 7 genera on Sabouraoud's Dextrose agar. Al-Zubeiry (2005) reported that the sixty five species belonging to 23 genera from raw sewage and 60 species belonging to 25 genera were isolated from dewatered sludge (manure) of secondary effluent on Sabouraoud's dextrose agar without or with cycloheximide. Six strains of *Aspergillus niger* were isolated from STP sludge on Rose Bengal Agar medium Jamal *et al*. (2005).

Kacprzak *et al*. (2005) in his studies on the wastewater and sewage sludge reported that 52 species of microscopic fungi were isolated from studied samples. From 34 species in raw wastewater, 32 species in treated wastewater to 29 species in sewage sludge was noted**.** Paiva *et al*. (2005) mentioned that the twelve strains of filamentous fungi, most of them belonging to the Deuteromycetes class, were isolated from activated sludge. Ulfig (2005) isolated 10 species belonging to 6 genera (keratinolytic fungi) and 13 species belonging to 9 genera (keratinophilic fungi) from sewage sludge. Soomoro *et al*. (2007) reported that fifteen species of fungi were isolated from sludge on Sabouraoud's dextrose agar (SDA) supplemented with chloramphenicol.

Ulfig *et al*. (2007) mentioned that the composition of keratinolytic fungi that grow above or below a 1- cm sludge blanket (under oxic or anoxic condition) was 107 keratinolytic fungi occurrences belonging to at least nine species were observed above a sludge planked, whereas only 55 occurrences representing sight species were noticed below this blanket. While the composition of non-keratinolytic fungi in sludges under oxic or anoxic conditions was 133 non-keratinolytic fungi occurrences belonging to seven species were observed under oxic conditions, whereas 119 occurrences representing six species were identified under anoxic conditions.

Hedayati and Mirzakhani (2009) noted that the 326 fungal colonies belonging to 7 species were isolated from 35 sludge samples cultured on Sabouraoud's dextrose agar with cycloheximide and chloramphenicol. *Geotrichum* 59.5 %, *Cladosporium* 13.8 %, *Alternaria* 11.3 %, and *Penicillium* 10.7 % species were the most prevalent. Shah *et al.* (2009) reported that *Fusarium sp* was isolated from sewage sludge and grew rapidly on Sabouraoud's dextrose agar. Weber *et al*. (2009) isolated 41 fugal isolates from aerobic sewage granules, these isolates were assigned to the taxonomic groups *Pleosporaceae*, *Xylariales*, *Theleobolaceae*, *Claviceps*, *Aureobasidium*, *Candida boleticola*, and *Tremellomycetes.*

Wemedo *et al*. (2009) mentioned that the fungal species were isolated from oilfield wastewater include the *Aspergillus fumigatus*, *A. niger*, *Fusarium sp*., *Mucor sp*., *Penicillium sp*., *Rhizopus sp*. and *Saccharomyces* on Potato dextrose and oil agar medium. Shugaba *et al*. (2010) reported that the four species of fungi (*Aspergillus flavus*, *A. niger*, *A. parasiticus* and *Penicillium roqueforti*) were isolated from sludge obtained from the factory.

Wastewater and sewage sludge contain high quantities of keratin remnants which have particular physicochemical and microbiological characteristics. It can be expected, therefore, that keratinophilic fungi and dermatophytes occur abundantly in the sludge environment and the influence of environmental factors on their qualitative and quantitative composition can be observed more easily than in other habitats. Besides, sewage sludge is increasingly being used to fertilize agricultural and forest areas and to reclaim devastated terrains. Hence, the recognition of the distribution of pathogenic fungal species in such sludge is important from a public health point of view. Several investigations have been made on Keratinophilic fungi in soils receiving raw city wastewater, sewage and activated sludge in different parts of the globe (Ali-Shtayeh, 1988; Abdel-Hafez and EL-Sharouny, 1990; Abdullah and Hassan, 1995; Ali-Shtayeh and Jamous, 2000; Ali-Shtayeh *et al*., 1999; Kacperzak *et al*., 2005; Soomro *et al*., 2007; Hedayati and Mirzachani, 2009; Ulfig, 2000; 2003; 2005; 2006; Ulfig and Korcz, 1983; 1991; 1994; Ulfig and Ulfig, 1990; Ulfig *et al*., 1996; 1997; 2007)

Among the organic substances present in sewage are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from process industries (Al-Zubeiry, 2005). Due to high micro- and macro-element contents sewage sludge is often used for reclamation of agricultural, forest and devastated soils. However, sludges also contain many harmful chemicals, e.g., heavy metals and pathogenic organisms. Therefore, each time sludge land use must be preceded with sanitary analyses and health risk evaluation. The occurrence and survival of bacteria, viruses, protozoa and helminthes in sewage sludge have been relatively well recognized (Straub *et al*., 1993; USEPA, 1999).

Sewage sludge also contains pathogenic macro and microorganisms, which can give rise to potential hazard (Abderrahman and Shahlam, 1991) to the health of humans, animals and plants. The health risk associated with wastewater is a major deterrent in wastewater reuse for irrigation. Health risk is associated with pathogens, which may spread diseases through being directly or indirectly ingested into the human body (Dudley *et al*., 1980; WHO, 1981; 1989; Feachem *et al*., 1983; FAO, 1992; Shuval, 1991; Shuval *et al*., 1986) and fungi (Velez and Diaz, 1985; Bunes and Merk, 1992).

Pathogens pose the greatest threat to public health; especially when the receiving water is used for domestic recreation on agricultural purpose (Tchobanogeuos, 1979). There are many conditions, which may increase the health risk of wastewater reuse in agriculture. The first of these conditions is survival time of pathogenic microorganisms.

The natural survival time of pathogenic organisms depends on the carrying medium and the environment. The survival time is a time during which pathogens are capable of causing diseases if they came into contact with a host under favorable condition. The second of these conditions are pathogenic fungi capable of causing diseases which can be found in foods contaminated with sewage water (Kowal *et al*., 1980; Rosas *et al*., 1984). They also can be found harmful to the soil, crops and grazing animals. On the other hand Pathogenic microorganisms can be transferred from raw sewage and secondary effluent during the irrigation process, directly or indirectly to the plants, animals and humans also make various infectious diseases. Different authors have proved that 5 vegetables are contaminated with microorganisms, when they are irrigated with sewage water and when the soil is fertilized with manure because both usually contain great amounts of pathogenic organisms (Epstein *et al*., 1982; Larkin *et al*., 1978).

## 2.3. The activity of fungi in biological wastewater treatment (elimination of nitrogen, phosphorus and COD)

Discharging the effluents from wastewater treatment into the environment causes many problems due to the richness of these effluents in nutritive substances ($NH_4$, $NO_3$, and $PO_4$), leading to the eutrophication of natural water bodies (Lavoie and de la Noüe 1985). Many studies suggested the using of microorganisms to remove nutrient substances from wastewater rich in nitrogenous and phosphate compounds (Williams, 1981; Kunikane *et al*., 1984, Rodrigues and Oliveira, 1987; Tam and Wong, 1989).

There are many reasons for using fungi in biological wastewater treatment processes. Another is the capacity of these organisms to grow at pH values below 5 (Lilly and Barnett, 1951; Cochrane, 1958; Thanh and Simard 1973a; Karim and Sistrunk, 1985a), thus offering some hope of controlling competing organisms by conducting fermentations at low pH values. Fungi also have been utilized for removal of eutrophication agents and bioremediation of metal contaminated wastewater streams (Thanh and Simard, 1973a; Akthar and Ghaffar, 1986; Akthar and Mohan, 1995; Bosshard *et al*., 1996). There is current interest in the use of microorganisms for the removal of nitrogen, phosphorus, and metals from commercial and municipal waste (Cassidy *et al*., 1996). Two different white rot fungi have been used in the pretreatment of OMW prior to anaerobic digestion, with quite different results. *P. chrysosporium* reduced the COD of OMW, (Gharsallah *et al*., 1999). However, pretreatment with *Geotrichum candidum* reduced the COD of OMW and increased substrate uptake during anaerobic digestion (Martin *et al*., 1993).

Fungi are heterotrophic and able to utilize a wide range of organic materials. They are mostly aerobic wastewater microorganism species. Fungi could offer this benefit over bacteria in wastewater treatment processes. The biomass produced during fungal wastewater treatment has, potentially, a much higher value than that from the bacterial activated sludge process. The fungi can be used to derive valuable biochemicals and can also be used as a protein source. Various high-value biochemicals are produced by commercial cultivation of fungi under aseptic conditions using expensive substrates. Food-processing wastewater is an attractive alternative as a source of low-cost organic matter and nutrients to produce fungi with concomitant wastewater purification (Sankaran, *et al*., 2010).

Shoun and Tanimoto (1991) demonstrated filamentous fungi also had a biochemical pathway to perform denitrification. Based on this discovery, Guest and Smith (2002) proposed that fungi have significant advantages over bacteria denitrifiers; including fungi have both biochemical pathways for nitrification and denitrification, higher rates of nitrification-denitrification, greater

resistance to toxic inhibitory compounds, lower oxygen and carbon source concentration requirements.

Rezende *et al*. (2004) mentioned an excellent potential for the use of yeast in the soil as a source of nitrate and available P for plant nutrition. Phosphate release may also take place in fully aerobic conditions if the aerobic phase is prolonged. The reason for this can be that the carbon store in the cells becomes depleted before the sludge reaches the end of the aerobic zone, and degradation of polyphosphates results in the beginning of a new carbon store build-up (Casey, 1997; Matuzevičius and Paulauskienė, 1998).

Researchers recognized the potential use of fungi in wastewater treatment during the late 1950s to early mid 1960s (Guest and Smith, 2002). Fungi usually are saprophytic organisms and are classified by their mode of reproduction. As saprophytes they obtain their nourishment from the degradation of dead organic matter. Most fungi are free-living and include yeast and molds. Most fungi are strict aerobes and can tolerate a low pH and a low nitrogen environment. Although fungi grow over a wide range of pH values (2.0–9.0), the optimum pH for most species of fungi is 5.6, and their nitrogen nutrient requirement for growth is approximately one-half as much as that for bacteria. During the late 1950s to early mid 1960s researchers started to recognize the potential for fungi to carry out wastewater treatment.

Cooke (1976) carried out a number of surveys of receiving water bodies, trickling filter, activated sludge and anaerobic digester for the various types of microorganisms found in the processes. The studies found fungi occurring in all the systems; however, no quantitative enumerations were performed**.** Cooke (1976) advocated the use of fungi in wastewater treatment because fungi appeared to show higher rates of degradation and showed a much greater ability to degrade cellulose, hemi-cellulose and lignin materials. However, Cooke's research did not move beyond a survey of the populations to creating a wastewater treatment system based on fungi. Fungi have been indicated as the dominant or secondary bulking filament in approximately 1 % and 2 % of wastewater treatment plants in the U.S.A. Some researchers have argued the numbers of fungi may be higher than reported due to the fact that analysts are not looking for fungi and that fungi have typical forms in wastewater (Cooke, 1976; Wanner, 1994).

The fungi along with the bacteria partake in degrading organic substances and some inorganic ones contained in the wastewater. Compared to the bacteria the fungi have the advantage of being able to grow also on a weakly moist medium of a low pH-value, low nitrogen content (deficient for the bacteria), low temperature etc. On the basis of these properties a mass growth of fungi is frequently observed in a place, where the bacteria find no more vital conditions. This

concerns above all the purification of some industrial waste water where extreme conditions for other organisms can exist. During the hydrobiological investigation of some treatment plants for sewage and industrial waste water mainly in the territory of Bohemia, we usually find fungi which were a regular part of the biocenosis. They often increase much in number in a certain plant unit or in the whole purification plant. Their mass development becomes apparent by a conspicuous coloration of the growth on the trickling filter bed or on other bases. So e.g. an imperfect fungus of the genus *Fusarium* creates, because of the carotenoid pigment content in the mycelium and substrate, a pink till orange colored growth. Some genera of fungi form white, bush-like coats; other ones form dark till black coats. When analyzing the growth biologically, the centrifugation or the activated sludge, spores or mycelium fragments can very frequently be observed. They grow also on plates by the cultivation of other microorganisms. Therefore, if we do not want to commit inaccuracies in our conclusions, the fungi must be considered as a part of the biocenosis of the respective habitat (Sladka and Ottova, 1968).

Until recently the rationale of wastewater treatment has been restricted to the destruction of pathogens and preservation of acceptable oxygen balance in receiving waters (La Riviere, 1977). Considerable attention is given to the removal of waste material before discharging effluents: compounds of carbon, nitrogen, phosphorus and sulfur present in most municipal and agricultural wastes are important causes of eutrophication (Wuhrman, 1968; La Riviere, 1977) and their control may therefore have considerable impact on the quality of natural waters.

The use of fungi and yeasts in biological treatment of domestic and industrial wastewater has been studied since 1970. Thanh and Simard (1971) studied the biological treatment of domestic wastewater with different yeast strains. All the tests were carried out with shaken 500 mL flasks at 26-28 $^{o}$C for 3 days. The initial pH was adjusted to 5.0. The result indicated that the yeast strains which gave high ammonia-nitrogen and COD removal efficiency were *Rhodotorula marina* (85 % NH3-N and 67 % COD removals) and *Candida krusei* (91 % NH3-N and 72 % COD removals). Especially, yeast strain *Rhodotorula glutinis* and *Trichothecium roseum* could completely remove phosphorous compounds in domestic wastewater (Simard and Thanh, 1973). However, COD reduction was not as high as had initially been expected. The authors analyzed the cause to be the result of rapid uptake of phosphorous and nitrogen compounds before the organics could be assimilated.

Church and Nash (1970) reported that a significant reduction in BOD of wastewater by *Trichoderma viride* at pH range between 3 and 4. Similarly Cook *et al.* (1956) reported the use of pure and mixed cultures of fungi in the removal of BOD from domestic waste waters at 4 pH. Since nitrogen containing compounds greatly influence the BOD of surface waters, an understanding of

nitrification dynamics in waste water treatment systems is of special significance (Downig *et al*., 1964).

Deocadiz (1977) studied yeast treatment of mixture of domestic and paper mill white wastewater. Two yeast strains *Candida utilis* and *Rhodotorula glutinis* were cultured in shaking flasks. Approximately 80% of COD, 50 % of N and 62 % of P were removed after 24 h. *Rhodotorula* yeast strain also gave the highest removal efficiency for the biological treatment of potato chips wastewater. The COD, N and P removals were 80 %, 96 % and 57 %, respectively (Simard *et al.*, 1973).

Greben *et al*. (2007) reported that the fungal consortium, consisting of six hyphomycetous isolates were used for biological nitrate removal from synthetic wastewater, some of which belong to the genera *Fusarium*, *Mycor*, and *Penicillium*, was able to remove a significant portion of the nitrate from treated water. Akhtar and Ghaffar (1986) reported that the nine species of fungi, *Aspergillus niger*, *A. flavus*, *A. terreus*, *Fusarium solani*, *Mucor sp*., *Neurospora crassa*, *Penicillium janthinellum*, *Trichoderma harzianum* and *Trichothecium roseum* were evaluated for their potential to remove $NH_3$-N from domestic waste water. Of the fungi tested, *A.flavus* was found to be the most effective in the removal of $NH_3$-N. Maximum reduction (92 %) of NH3-N by this organism was observed at pH 8.0 and at 20 $^oC$.

Olive mill wastewater normally contains high concentration of COD (100-200 g/L). Scioli and Vollaro (1997) reported that *Yarrowia lipolytica* cultured in the 3.5 L-aerated fermenter was capable of reducing the COD level of Olive oil processing wastewater by 80 % in 24 hrs. Useful biomass (40 % protein) could also be obtained in this process.

Fungi have been attracting a growing interest for the biotreatment (removal or destruction) of wastewater ingredients such as metals, inorganic nutrients and organic compounds (Akthar and Mohan, 1995; Feijoo and Lema, 1995; Field *et al*., 1993; Palma *et al*., 1999; Coulibaly, 2002). Thirty-five strains of filamentous fungi were screened for bioconversion of domestic wastewater sludge; all of the tested strains were able to grow in wastewater sludge. But the strains (*Penicillium sp.*, *Aspergillus sp*., *Trichoderma sp.*, and *Phanerochaete chrysosporium*) were selected for the bioconversion of domestic wastewater sludge (Alam *et al*., 2004).

Domestic sewage contains carbon and nutrient sources that can be removed by fungal biomass. In an early investigation, Thanh and Simard (1973a) demonstrated the capacities of seventeen fungal biomasses to remove phosphates (84.1 %), ammonia (73.3 %), total nitrogen (68.1 %) and chemical oxygen demand (COD) (39.3 %) from domestic wastewater incubated in Bellco flasks on shaker at 300 rpm and pH 5.0. They obtained fungal growth on this effluent with

an accumulation of biomass (451.2 mg $L^{-1}$) that contained protein (47 % g g-1). There was variability in fungal capacities as to the removal of pollutants. In fact, *Trichothecium roseum* was the best in phosphate removal (97.5 %), whilst *Epicoccum nigrum*, *Geotrichum candidum*, and *Trichoderma sp.* were the best in the removal of ammonia (84.0 %), total nitrogen (86.8 %) and COD (72.3 %), respectively. Concerning cell-protein production, *Paecilomyces carneus* had the highest ratio of protein to biomass (92.5 %). However, this fungus did not grow very well on domestic sewage.

Domestic wastewater pretreatment by a strain of *A. niger* has been investigated under transient conditions (stirred tanks reactor in series). This fungal biomass removed about 72 % of COD and 65.4 % of N-total (Coulibaly, 2002). Despite the differences between the bioprocess investigated in these two studies, COD and protein removal rates are in the same order. The chemical oxygen demand is a measure of oxygen equivalent of the organic matter as well as microorganisms in the wastewater (Pipes and Zumda, 1997). Thus, the COD is a very important factor in evaluating the organic content of wastewater. Garcia *et al*., (2000) noted that *Geotrichum candidum* removed COD. However, by optimizing Olive mill wastewater (OMW) composition (COD: N: S = 100:5:2) for *G. candidum* growth.

Fungal pretreatment of agroindustrial effluents under aerobic conditions makes it possible to obtain biochemical oxygen demand (BOD) reduction up to 85.4 %, (Vinciguerra *et al*., 1995; Yesilada *et al*., 1995; 1999; Garcia *et al*., 1997, 2000; D'Annibale *et al*., 1998; Setti *et al*., 1998; Gharsallah *et al*., 1999; Robles *et al*., 2000; Kissi *et al*., 2001).

Hu (1989) used ten different yeast strains in cultures to treat industrial wastewater which contains high concentration of BOD ranging from 24,000 to 44,000 mg/L. These yeast strains were screened from 391 colonies isolated from soil samples. Most could grow well within pH range of 3.0-5.0, with pH 4.0 being the optimum. The result shows that the two strains could reduce soluble COD by 92 % at 7 days. Arnold *et al.* (2000) investigated the ability of selected yeast strains (*Candida utilis* and *Galactomyces geotrichum*) to purify silage effluent on the shaker-flask scale. High removal efficiencies of ammonia (85-99 %), COD (74-95 %) and phosphate (82-99 %) were obtained after 24 hrs at initial pH values 3.7-5.8. Marwaha *et al.* (1999) investigated the ability of two yeast strains *Candida parapsilosis* and *Candida haemulonii* for treatment of dairy effluents. All tests were conducted with shaker-flasks and incubated at 30 $^{o}$C for 24 hrs. The result indicated that maximum BOD (90 %) and COD (82 %) removals were obtained at pH 5.5.

Church *et al*. (1973) reported that fungi Imperfecti have the ability to convert dissolved and suspended organic matter into a mycelium that not only has a high protein content to be valuable as

an animal feed supplement, but also forms large enough particulates (flocs) that can be readily recovered by simple filtration or screening. They reported that the fungus *Trichoderma viride* was used in an aerated lagoon and an oxidation ditch to treat corn and pea canning wastes, and greater than 95.0 % COD removal of the wastes was achieved. Church and Nash (1970) have also indicated that the waste load of acid effluents from vegetable processing can be reduced by fermentation with species of fungi Imperfecti at pH between 3 and 4. Besides reducing the COD of corn by 98.0 %, production of fungal biomass was 50.0 to 60.0 g of dry mycelium per 100 g of COD utilized.

Thanh and Simard (1973b) reported the treatment of domestic wastewater by various yeast species. The focus of the work was to produce food yeast (animal feed/single cell protein) on a cheap available medium. The study screened 27 yeast strains for their ability to produce a high biomass, while maximizing reduction of phosphate, ammonia and organic matter. The studies were conducted in batch 500-mL baffled culture shake flasks containing 150 mL of sterile wastewater. Reported phosphate removal ranged from 12 to 100 %, total nitrogen removal from 22.0 to 93.0 %, ammonia nitrogen from 27.0 to 90.0 % and COD removal from 0.0 to 72.0 %. Growth of *Neurospora sitophila* NRRL 2884 on alkaline effluents from rutabaga and potato, after adjusting the pH to 5.6, was studied by Beuchat *et al.* (1978). They mentioned that the fungus was able to reduce the COD values from 42.0 to 68.0 % of the initial values of wastes after 4 days under submerged fermentation.

Hang and Woodams (1979) described a process for the assimilation of baked bean processing wastewater by the mycelium of *Aspergillus foetidus* NRRL 337. They reported that the fungus is capable of rapidly digesting over 80.0 % of the BOD and produces no foul odors. The mycelium recovered had a crude protein content of greater than 50 % and was readily harvested by simple filtration. The optimal conditions for the fungal process are pH 3.3; temperature 30 °C; incubation time 24 h; and aeration rate, 2 $mMO_2$/liter/h.

Hiremath *et al.* (1985b) performed a similar study except they tested seven fungal species isolated from a wastewater stabilization pond. The major goal of the study was to maximize biomass production of fungi as a food source for animal or human consumption. The trials were conducted in 2-L conical flasks containing 1.5 L of sterile fresh wastewater. Flasks were inoculated with pure cultures of fungi and incubated at room temperature for 10 d. The culture flasks were gently agitated twice a day. The study reported BOD5 removal between 53.0 and 72.0 %, phosphate removal from 34.0 to 77.0 %, and ammonia nitrogen removal between 49.0 and 77.0 %. Due to the experimental design, the cultures were most likely completely anaerobic for the entire 10-d incubation. Both studies were performed under non-ideal conditions with no process optimization of the parameters reported. Although the data shows fungi will treat wastewater, it does not give

any indication of the maximum removal efficiencies. However, both studies indicate promising results for removal of nitrogen and phosphorus. Additional evidence, although not conclusive, that fungi are capable of wastewater treatment was found during the study of onsite sphagnum peat wastewater treatment system.

Brooks (1988) found excellent removal of BOD, organic nitrogen (90.0 to 95.0 %) and ammonia nitrogen (95.0 to 99.0 %). Based on standard bacterial and fungi enumeration techniques, the ratio of fungi to bacteria was 8:1 during winter and 2.4:1 during the summer. This led her to hypothesize fungi had a large role in removal of nitrogen from the wastewater. However, due to the use of standard enumeration techniques it is very likely the populations were underestimated due to the fastidious nature of environmental microbes. Furthermore, a direct comparison ratio of fungi to bacterial colony forming units is not appropriate. This ratio employs the flawed assumption that each bacterium and fungus is equal in degradation rate and range (BOD, ammonia nitrogen, and organic nitrogen) of the wastewater. A different experimental design and enumeration is needed before comparisons can be made.

Several white rot fungi were evaluated for their ability to remove COD from Olive mill waste-waters (OMW). Among these, *Phanerochaete chrysosporium* showed the highest potential for the biological depollution of OMW. Approximately 73 % of the chemical oxygen demand (COD) was removed by *P. chrysosporium* strain HD. *Phlebia radiata*, *Dichomitus squalens*, *Polyporus frondosus* and *Coriolus versicolor* could also remove COD but to a lesser extent (Sayadi and Ellouz, 1993). The highly dewaterable fungal biomass produced from wastewater treatment can be used as a source of protein and biochemicals (Stevens and Gregory, 1987; Jin *et al*., 1999b; Barbesgaard *et al*., 1992; Huang *et al*., 2003).

Karim and Sistrunk (1985b) reported that among the fungi (*Aspergillus oryzae* ATCC 9362, *A. foetidus* NRRL 337, and *Neurospora sitophila* NRRL 2884) used for the fermentation study on the steam peeled potato effluent, *N. sitophila* was the most effective in reducing the COD. The COD of the fermented activated wastewater from steam peeled potatoes inoculated with N. sitophila, which had a portion of the wastewater replaced daily, was reduced by 69.0 and 90.0 % of the original COD value after 24 and 48 h of fermentation, respectively. The fungal biomasses produced from the wastewater were 1.30 and 165.0 g/L (dry wt.) of mycelium, respectively.

Vabolienė *et al*. (2007) reported that microorganisms that have accumulated polyphosphates grow at the aerobic and anaerobic zone; they can accumulate a large quantity of phosphates in their own cells. Twenty two fungal isolates were studied to determine the potential for development of fungal nitrogen treatment technology for municipal wastewater. Fungi were screened for nitrogen

treatment potential using an aerobic 3 day ammonium concentration decrease test. Seven fungi were found to have potential for nitrogen or phosphate treatment in Erlenmeyer flasks at 21°C (Guest and Smith 2007).

In the process of fungal treatment of wastewater streams, the selected fungus must be able to reduce nitrogen and phosphorus compounds to a low level and the mycelium can serve as a feed or food product (Church and Nash, 1970; Church *et al*., 1973). Successful wastewater control by use of fungi has been reported by previous workers (Lilly and Barnett, 1951; Cochrane, 1958; Gray *et al*., 1963; 1964, Thanh and Simard, 1973 a,b; Beuchat *et al*., 1978; Lemmel *et al*., 1979; Barker and Worgan, 1981; Karim and Sistrunk, 1985 a,b; Suwandi and Mohdmmed, 1984; Shoun *et al*., 1992; Yesilada *et al*., 1995; 1999; Guest and Smith, 2002; Truong *et al*., 2004; Greben *et al*., 2007).

Borja *et al*. (1998) compared anaerobic digestion of OMW pretreated by two different fungi and a bacterium: *Geotrichum candidum*, *Aspergillus terreus* and *Azotobacter chroococcum*. These organisms decreased the COD concentration of OMW by 59.0 %, 87.0 % and 79.0 %, respectively. Subsequently, the kinetics of anaerobic digestion of OMW pretreated by *G. candidum*, *A. terreus* and *A. chroococcum* were enhanced 2.5-, 4.2- and 4.0-fold, respectively (McNamara *et al*., 2007). *Candida bidinii*, *Geotrichum candidum*, *Penicillium sp* and *Aspergillus niger* were used in reduction of COD in Olive oil mill wastewaters, these strains were reduced 45.0 -78.0 % of COD (Aissam *et al*., 2007).

## 2.4. Role of fungi in membrane bioreactor (MBR)

Combining membrane technology with biological reactors for the treatment of wastewaters has led to the development of three generic membrane bioreactors (MBRs): for separation and retention of solids; for bubbles less aeration within the bioreactor and for extraction of priority organic pollutants from industrial wastewaters. Membrane, when coupled to biological processes, are mostly used as a replacement for sedimentation i.e., for separation of biomass (Stephenson *et al*., 2000). Application of the membrane bioreactor (MBR) concept in wastewater treatment offers the possibility of overcoming low biodegradation rate and poor sludge settling in the secondary sedimentation tank. MBR process can be operated at high MLSS and thus organic removal can be improved. This results in sludge wastage and plant size reduction (Visvanathan *et al.*, 2000). Moreover, the selection of microorganisms present in the membrane bioreactor is no more dependent on their ability to form biological flocs and settling characteristics.

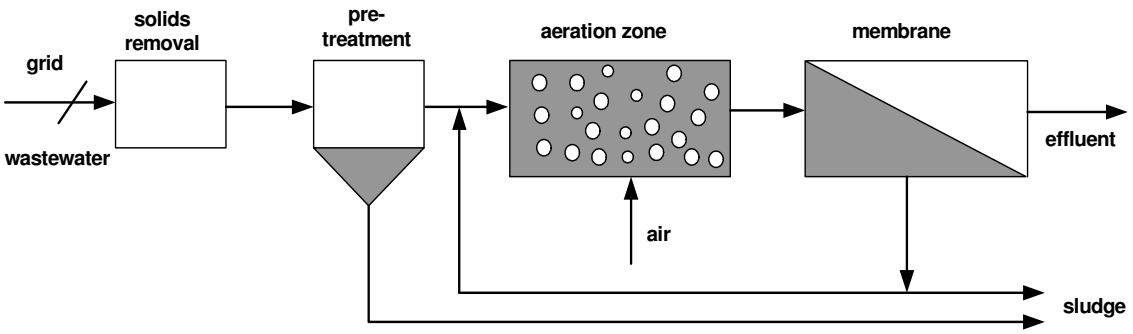



**Fig. 2.2:** Schematic of wastewater treatment process with membrane bioreactor

Microbial cells are the most important of the available catalysts for degradation of the compounds present in water and sewage. This is so because this catalyst type has high specificity of enzymes, but at the same time is not inactivated and from the point of view of process economy is similar to a chemical catalyst. The only condition to make this degradation process efficient is to set up conditions suitable for microbial cell growth (pH, temperature, the range of carbon source concentration, the effect of other substances present in the medium) and a proper substrate stream. The process is effective when the concentration of active biomass is high. Hence the use of a microfiltration membrane which retains cells in the biodegradation zone is highly recommended. A high concentration of a biocatalyst allows use to carry out the process at an increased feed flow (Senthuran *et al*., 1999), while the high reaction rate obtained causes that the sewage undergoes an advanced treatment.

Recent technical innovation and significant membrane cost reduction have pushed membrane bioreactors (MBRs) to become an established process option to treat wastewaters. The combination of membrane separation with a suspended growth bioreactor is now widely used for municipal and industrial waste treatment (Judd, 2006). When used with domestic wastewater, MBR processes could produce effluent of high quality enough to be discharged to coastal, surface or brackish waterways or to be reclaimed for urban irrigation. Other advantages of MBRs over conventional processes include small footprint, easy retrofit and upgrade of old wastewater treatment plants into MBRs. The membrane bioreactor is based on the conventional wastewater process, but the separation of micro-organisms is performed by filtration with membranes (Judd, 2006).

The membrane bioreactor was applied to biodegradation of many types of organic (Poeton *et al*., 1999; Koma *et al*., 2001) and inorganic pollutants (Chuichulcherm *et al*., 2001; Kolmert and Johnson, 2001). If the difference between particle sizes of substrate and product is big, it is

desirable that beside microbial cells retained by the membrane, also high-molecular substrate is caught and the product is removed they can be separated by size exclusion membrane filtration with ultra- or nanofiltration. This is used on industrial scale for the production of enzymes. This is a special case of the microbial membrane bioreactor.

In recent years, membrane bioreactors were used widely with fungi in microbial biotechnology, such as biological wastewater treatment, production of enzymes, proteins, organic acids and other metabolites. Fungi are similar to bacteria but are multicellular wastewater microorganism species. The fungi are larger than the bacteria and cannot compete with the bacteria or other microorganisms in wastewater for organics under normal environmental conditions. The fungi tend to be filamentous and present too much mass per surface area. Wastewater fungi are strict aerobes and can grow slowly in the absence of oxygen. Municipal wastewaters contain fungi spores, primarily from the soil. Fungi have a vegetative structure known as mycelium. The mycelium consists of a rigid, branching system of tubes, through which flows a multinucleate mass of cytoplasm. A mycelium arises by the germination and outgrowth of a single reproductive cell, or spore. *Y*easts are exceptional fungi that cannot form a mycelium, so are unicellular. Fungi are heterotrophs and are able to utilize a wide range of organic materials. They are mostly aerobic wastewater microorganism species (More *et al*., 2010).

Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints of wastewater. For example, *Aspergillus niger* is the prototypical fungus for the production of citric acid (Lal, 1980; Grewal and Kalra, 1995; Kareem, *et al*., 2010; Watanapokasin, *et al*., 2010).

At present, using fungi bioreactor to treat the wastewater is regarded as the main development and the continuous production of metabolites, such as lignin, enzymes, acids and others. Various reactor systems have been used, including submerged membrane bioreactor (Solomon and Petersen 2002; Hai *et al*., 2006; 2008; Sheldon *et al*., 2008) and continuous membrane bioreactor (Gao *et al*., 2009).

A submerged membrane fungi reactor, coupling the excellent degradation capability (due to non-specific extracellular oxidative enzymes) of white-rot fungi with the inherent advantages of an MBR, was envisaged as an efficient system for treatment of textile wastewater. A submerged microfiltration membrane bioreactor implementing the white-rot fungus *Coriolus versicolor* was developed for the treatment of textile dye wastewater different fouling-prevention techniques

(Hai *et al*., 2006). Membrane bioreactors with fungi are being increasingly used in enzymatic catalysed transformations from wastewater (López *et al*., 2002).

Hai and Yamamoto (2010) reported that a membrane bioreactor (MBR) utilizing a mixed microbial community dominated by fungi was explored for treatment of a synthetic textile dye wastewater containing dye, starch and other nutrients. Preliminary batch tests confirmed the superior decolouration capacity of a pure fungus culture due to simultaneous biosorption and biodegradation in contrast to mainly biosorption in case of conventional activated sludge.

The application of fungi in large- scale waste treatment, however, has been impeded owing to the lack of an appropriate reactor system capable of coping with rather slow fungal degradation, loss of the extracellular enzymes and mediators with discharged water, and excessive growth of fungi (Moreira *et al*., 1998; Zhang and Yu 2000; Zhang *et al*., 1999 ). In this context, a feasible system may be envisaged by coupling the excellent degradation capability of the white-rot fungi with the inherent advantages of a membrane bioreactor (MBR), i.e, suspended solids and macro-colloidal material free permeate, retention of high biomass concentration requiring a small footprint and allowing the process to be operated at a low F/M ratio, hence, yielding reduced excess sludge production.

## 2.5. Factors affecting on biological activity of fungi for wastewater treatment

### 2.5.1. Effect of different pH values

Cook *et al*. (1956) reported the use of pure and mixed cultures of fungi in the removal of COD from domestic wastewaters at pH 4. Similarly Church and Nash (1970) noted that a significant reduction in COD of waste water by *Trichoderma viride* at pH range between 3 and 4. Since nitrogen containing compounds greatly influence the COD of surface waters, an understanding of nitrification dynamics in waste water treatment systems is of special significance (Downig *et al*., 1964). Thanh and Simard (1973a) mentioned that the optimum pH for the highest reduction rate of phosphate and ammonia from wastewater by *Trichoderma roseum* was 3.5 and 4.0, respectively.

Akhtar and Ghaffar (1986) observed that of the fungi tested, *A. flavus* was found to be the most effective in the removal of $NH_3$-N from domestic wastewater incubated in Erlenmeyer flasks with 0.5 % sugar adjusted to pH 5.0. Maximum reduction 92.0 % of NH3-N by this organism was observed at pH 8.0. Jaouani *et al*. (2005) mentioned that the highest reduction rate of COD in Olive oil mill wastewater was obtained around pH 3.5 by using *Pycnoporus coccineus*. Alaoui *et al*. (2008) screening 4 white rot fungi isolates (*Phanerochaete chrysosporium*, *Trametes versicolor*,

*Coriolopsis polyzona*, and *Pycnoporus coccineus*) for Olive oil mill wastewater treatment and reported that the initial pH before treatment was 5.0. Final value obtained with the free form of *Coriolopsis polyzona* was 5.5 pH, whereas was apparently only slightly affected by others type of cultivation for this particular strain. A significant pH decrease of 0.5 units was observed for *T. versicolor*; *P. chrysosporium* and *P. coccines* under immobilized form. The more important pH drops were observed for *P. coccineus* and *P. chrysosporium* growing under free form, respectively attaining values 4.25 and 4.5 of pH.

### 2.5.2. Effect of incubation temperature

A temperature range between 15 and 30 $^{o}$C was reported by Thanh and Simard (1973a) for biological treatment of domestic wastewater by *Trichoderma viride* which was incubated in Erlenmeyer flasks for 72 hours. Akhtar and Ghaffar (1986) mentioned that the optimum $NH_3$-N removal from domestic waste water by *Aspergillus flavus* was observed at 20 $^{o}$C. Yesilada *et al.* (1999) mentioned that the seven fungal species (*Coriolus virsicolor*, *Funalina trogii*, *Lentinus ligrinus*, Laetiporus sulphoureus (brown rot fungus), *Phanerochaete chrysosprium*, *Pleurotus ostreatus* and *P. sajor*, were screened for treatment of Olive oil mill wastewater in Erlenmeyer flasks at 30 $^{o}$C with agitation (150 rpm) for 6 days, while *Phanerochaete chrysosporium* incubated at 40 $^{o}$C. These fungi are able to reduce COD but the best results can be obtained with *Coriolus versicolor*, *Funalina trogii* and *Pleurotus sajorcaju* (63.0 -70.0 %). A relatively low level of COD removal was obtained with *Pleurotus ostreatus* 17.0 %. High biomass yield 0.616 g/50 mL was obtained after 3 days of growth.

Robles *et al.* (2000) reported that the seven strains of *Penicillium sp.* isolated from local OMW disposal ponds were used in Olive mill wastewate treatment, the best results were obtained by using strain P4, which formed 21.50 g (dry weight) of biomass per liter of undiluted wastewater after 20 days of cultivation at 28 °C and in an orbital shaker at 120 rpm. This and other strains also carried out an outstanding reduction of the chemical oxygen demand (COD) of OMW, as well as a pH raise. Kissi *et al*. (2001) studied the role of two white- rot basidomycete (*Phanerochaete chrysosporium and Pleurotus ostreatus*) for reduction of Olive mill wastewater COD in shaken flasks at 20–37 $^{o}$C for 15 days, and reported that the highest decrease in COD was observed at 28$^{o}$C after 6 days incubation with *Phanerochaete chrysosporium*. Khanongnuch *et al*. (2006) reported that the white rot fungus *Coriolus versicolo* reduced 67.0 % of COD from textile wastewater at 37 °C on 120 rpm orbital shaker for 4 days. Gonçalves *et al*. (2009) screened seven isolates from *Yeast* for biological treatment of Olive mill wastewater in Erlenmeyer baffled flasks 100 ml and lab-scale bioreactor was 240 rpm and 400 rpm at 27 $^{o}$C for 150 hr. The highest reduction value of COD (70.0 %) was found with *Candida cylindracea* CBS 7869.

### 2.5.3. Effect of incubation period

Abdel Karim *et al*. (1989) mentioned that *Trichoderma viride* reduced more than 95.0 % COD from Palm oil mill effluent in shaker flasks at 125, 28 ± 2 $^{o}$C for 14 days. The biomass (dry weight) of mycelium produced from *T. viride* was 1.37-1.42 g/L and the protein content 37.6-40.0 %. Hamdi *et al*. (1991) screened *Aspergillus niger* for bioconversion of Olive mill wastewater with two incubation system. Small–scales were carried out in Erlenmeyer flasks on a rotary shaker at 150 rpm and aerobic batch fermentations were performed in 1.5 L of wastewater medium at an agitation speed of 300 rpm. All experiments were carried out at 35 $^{o}$C for 80 hr. The removal rate of COD by *Aspergillus niger* (61.6 % in flasks and 52.5 % in fermentor) and soluble protein in filtrate obtained from flasks 3.75 g/L and 4.95 g/L from fermentor were determined after 72 hr incubation time.

Garcia *et al*. (2000) reported that *Geotrichum candidum* removed 25.0-38.0 % COD from wastewater after 20 days in shaker flasks. Blanquez *et al*. (2002) mentioned that the white rot fungus *Phanerochaete flavido-alba* removed 75.0 % COD from Olive mill wastewater after 6 days in batch fermenter. Guest and Smith (2007) screened seven fungal isolates belonging to *Geotrichum, Mucor, Penicillium, Phoma*, and *Yeast* for reduction of $NH_4$, $PO_4$, and COD from wastewater by using batch reactor (2 L) at 21°C for three days, the highest decrease ratio 7.3:1 (97.0 %) of COD: $NH_4$ was noted by *Geotrichum candidum*.

Jaouani *et al*. (2003) mentioned that fifty eight fungal isolates were screened for reduction of COD from Olive oil mill wastewater, *Pycnoporus coccineus*, *Pleurotus sajor caji*, *Coriolopsis polyzona*, and *Lentinus tigrinus* from 13 white rot fungi, were very active to remove COD from (OOMW) incubated in static and agitated culture flasks for 20 days. Jasti *et al*. (2006) reported that the maximum chemical oxygen demand (COD) removal of 78.0 % from corn processing wastewater was achieved at a 5 h with a biomass yield of 0.44 g/L by *Rhizopus oligosporus* in biofilm reactor. Four white rot fungi (WRF) strains, *Phanerochaete chrysosporium*, *Trametes versicolor*, *Coriolopsis polyzona* and *Pycnoporus coccineus*, were screened for reduction of COD and decolourization of Olive oil mill wastewater in Erlenmeyer flask at 28 $^{o}$C for 15 days (Alaoui *et al*., 2008).

# 3. MATERIAL AND METHODS

## 3.1. Fungal survey of activated sludge

### 3.1.1. Collection of activated sludge samples

Thirty six from each aerobic and anoxic activated sludge samples were collected from wastewater treatment plants with MBRs from two places (Amedeus pilot plant in Berlin/Wedding and BWB plant in Berlin/Margaretenhöhe). These samples were withdrawn monthly (during a period of 10 months from August/08 to May/09) from plants. Samples were put in clean and sterile bottles sealed and transferred to the laboratory and stored at 4 $^{o}$C, where fugal analysis was made.

**Table 3.1:** Physicochemical characteristics of activated sludge

| Parameter | Concentration | | | |
|---|---|---|---|---|
| | Amedeus pilot plant in Wedding | | BWB plant in Margaretenhöhe | |
| | aerobic | anoxic | aerobic | anoxic |
| pH | 7.2 | 7.0 | 7.5 | 7.3 |
| TSS (mg/L) | 380.0 | 380.5 | 377.0 | 382.0 |
| VSS (mg/L) | 260.0 | 260.0 | 261.0 | 259.4 |
| $PO_4$ (mg/L) | 3.6 | 3.6 | 3.7 | 3.4 |
| TN (mg/L) | 60.0 | 60.3 | 60.5 | 59.7 |
| $NH_4$-N (mg/L) | 24.6 | 25.0 | 24.4 | 24.2 |
| $NO_3$-N (mg/L) | 3.5 | 3.5 | 3.3 | 3.5 |
| COD (mg/L) | 818.5 | 820.3 | 816.5 | 817.6 |

### 3.1.2. Estimation of fungi

Aliquots of 0.1 mL homogenized activated sludge (Bux and Kasan, 1994) were put into Petri-dish followed by 20 mL from isolation medium. Fifteen plates were used for each activated sludge sample (3 plates for each type of isolation media). Plates were incubated at 30 $^{o}$C for 1-2 weeks and the developing fungi were counted, isolated and identified. Pure cultures were transferred to test tube slants and reservation in fridge.

### 3.1.3. Isolation media

Five types of media were used for isolation of various genera and species or various groups of fungi and these were summarized in Table 3.2.

The hair-baiting technique (Ulfig, 2003) was used for determination of keratinophilic fungi in activated sludge. Samples were dewatered by centrifuging at 4000 rpm for 15 min. Petri dishes were

filled with 40 g of dewatered sludge and covered each with 0.4 g of detergent-defatted, fine cut and autoclaved children's hair, and incubated in the dark at room temperature for four months. Three dishes responded to each sludge sample. During incubation, stable moisture conditions (ca. 40 %) were maintained in the dishes. The presence of keratinophilic fungi was confirmed by low-power microscopic examination. The plates were inspected daily for up to 5 weeks before being discarded. Fragments of colonized hair were inoculated onto slopes of Sabouraud's dextrose agar with cycloheximide and chloramphenicol (SCC), and incubated for 2 weeks at 30 $^{o}$C.

**Table 3.2:** Types and compositions of isolation media

| Isolation media | Composition of media |
|---|---|
| 50 % Sucrose Czapek-Dox agar | Sucrose 20.0 g/L, $NaNO_3$, 3.0 g/L, KCL, 0.5 g/L, $MgSO_4.7H_2O$, 0.5 g/L, $FeSO_4.7H_2O$, 0.01 g/L, KH2PO4, 1.0 g/L, agar, 15.0 g/L, chloramphenicol, 0.1 g/L and distilled water 1000 mL |
| Malt extract agar (MEA) | Malt extract, 30.0 g/L, mycological peptone, 5.0 g/L, agar, 15.0 g/L, chloramphenicol, 0.1 g/L and distilled water 1000 mL |
| Rose bengal chloramphenicol agar (RBCA) | Dextrose, 10 g/L, agar, 15.5 g/L, $MgSO_4.7H_2O$, 0.5 g/L, KH2PO4, 1 g/L, mycological peptone, 5 g/L, rose bengale, 0.05 g/L, chloramphenicol, 0.1 g/L and distilled water 1000 mL |
| Sabouraoud's dextrose agar (SDA) | Dextrose, 40 g/L, casein peptone, 5 g/L, peptic digest of animal tissue, 5 g/L, agar, 15 g/L, chloramphenicol 0.05 mg/L and distilled water 1000 mL |
| Sabouraoud's dextrose agar with cycloheximide and chloramphenicol (SDACC) | Dextrose, 40 g/L, meat peptone, 5.0 g/L, casein peptone, 5.0 g/L, cycloheximide, 0.5 g/L, chloramphenicol, 0.05 g/L, agar, 15 g/L and distilled water 1000 mL. |

### 3.1.4. Purification and identification of fungal genera and species

After the development of pigment on colonies to facilitate complete differentiation of fungal types, repeated sub-culturing on isolation media was necessary to obtain pure cultures. Sporulation was induced by subjecting cultures to ultraviolet light. Isolates were characterized according to morphological features, cultural characteristics such as pigmentation of the mycelium and direction of

growth of the hypha, whether aerial or lateral, microscopic observation of structures involved in asexual reproduction e.g., conidia or spores and in sexual reproduction and the presence of fruiting bodies. Light photomicrographs were made mostly from slide cultures. Slide cultures were made by removing a small cylinder of the agar medium by a cork borer and inserting it on the surface of the same agar inside a Petri-dish. The top cylinder is inoculated with the fungus and covered with a sterilized cover slip. After few days, the fungus growing on the cover slip is gently stained with cotton blue and mounted in lactophenol. Identification was accomplished using appropriate taxonomic techniques (Raper and Fennell, 1965; Ellis, 1971; Booth, 1977; Frey *et al*., 1979; Pitt, 1988; Rippon, 1988; Moubasher, 1993; Domsch *et al*., 1995; Kane *et al*., 1997; De Hoog *et al*., 2000; Watanabe, 2002; Leslie and Summerell, 2006; Pitt and Hocking, 2009; AUMC, 2010).

## 3.2. Screening of fungal isolates for elimination of nitrogen, phosphorus and COD from raw wastewater

### 3.2.1. Collection of wastewater samples

Raw wastewater samples from Wassmannsdorf BWB wastewater treatment plants in Berlin were collected in clean and sterile bottles sealed, transferred to the laboratory and stored at 4 $^{o}$C, where screening was made.

### 3.2.2. Determination of the properties of raw wastewater

#### 3.2.2.1. Physical properties

TS and VSS were measured according to German standard methods (DIN 38409-1). 50 mL of sludge or raw wastewater were filled in a ceramic crucible which was weighted before using (W1). The crucible was then dried at 105 °C for 24 h and weighted again after cooling in an exsiccator (W2). The difference of W1 and W2 is the TS weight. For VSS determination, the crucible was placed in a 550 °C muffle furnace for at least 2 h to oxidize all carbon compounds and weighted for the last time after cooling. The mass difference before and after the combustion is interpreted as VSS weight.

#### 3.2.2.2. Chemical properties

Total nitrogen (TN), ammonium ($NH_4$), nitrate ($NO_3$), phosphorus ($PO_4$) and chemical oxygen demand (COD) determinations were carried out by cuvette test kits (Hach-Lange). The pH of the medium was measured using a pH meter (WTW M340), Table 3.3.

**Table 3.3:** Physicochemical characteristics of raw wastewater

| Parameter | Concentration |
|---|---|
| pH | 7.5 |
| TSS (mg/L) | 235.0 |
| VSS (mg/L) | 193.5 |
| TN (mg/L) | 103.3 |
| $PO_4$ (mg/L) | 8.5 |
| $NH_4$ (mg/L) | 60.9 |
| $NO_3$ (mg/L) | 4.3 |
| COD (mg/L) | 1185.86 |

The photometric determination was conducted on an Isis 9000 photometer (Hach-Lange). Depending on the tests, the standard deviation is between 5 % and 10% according to the manufacturer. Details about the used tests are given in Table 3.4.

**Table 3.4:** Cuvette test kits (Hach-Lange) were used

| Parameter | Test numbers |
|---|---|
| TN | LCK 338 |
| $PO_4$ | LCK 350 |
| $NH_4$-N | LCK 302 |
| $NO_3$-N | LCK 339 |
| COD | LCK 515 |

### 3.2.3. Fungal stock

Twenty-one fungal species belonging to 12 genera were isolated from activated sludge of two wastewater treatment plants in Berlin with MBR were screened for elimination of nitrogen and phosphorus from raw wastewater Table 3.5.

### 3.2.4. Preparation of spore suspension

Fungal spore suspensions were prepared from potato dextrose agar (PDA) slants on Petri dishes. The slants were incubated at 28 °C for 4 days. Spores were harvested from the surface of each slant into 10 mL of sterile water. This suspension containing $1x10^7$–$1x10^8$ spores per mL determined by haemocytometer counts was used as inoculum. Preparation of preculture was carried out in shake flask culture. The preculture medium was used, inoculated with 3.0 % (v:v) spore suspensions and then incubated on an orbital shaker with a shaking rate of 150 rpm at 30 °C for 24 h. The suspended fungal cultures were used for inoculation.

### 3.2.5. Cultures and incubation

One hundred mL of raw wastewater adjusted at pH 4.5 (adjusted by 1N HCl or NaOH before sterilization) were put in a 250 mL Erlenmeyer flask. A set of triplicate flasks was used for each isolate. All flasks were sterilized at 120 °C for 20 min and inoculated with 2 mL of fungus spore suspension. The cultures were incubated in a shaker incubator (GFL Model No. 1092, Technische Universität Berlin) at 300 rpm, at 30 $^{o}$C for 15 days. All flasks, filter paper, distilled water were sterilized prior to use.

**Table 3.5:** Twenty-one fungal isolates were screened for elimination of nitrogen, phosphorus and COD from raw wastewater

| Isolates |
|---|
| *Aspergillus flavus var. flavus* |
| *Aspergillus niger* |
| *Aspergillus oryzae* |
| *Aspergillus terreus var. terreus* |
| *Aspergillus versicolor* |
| *Aspergillus ustus* |
| *Cladosporium cladosporioide* |
| *Doratomyces stemonitis* |
| *Fusarium oxysporum* |
| *Geotrichum candidum* |
| *Gibberella accuminata* |
| *Mucor circinelloides* |
| *Penicillium brevicompactum* |
| *Penicillium chrysogenum* |
| *Penicillium citrinum* |
| *Penicillium oxalicum* |
| *Rhizopus arrhizus* |
| *Syncephalastrum racemosum* |
| *Trichoderma hamatum* |
| *Trichoderma viride* |
| *Ulocladium chartarum* |

### 3.2.6. Analytical measurements

The culture samples were centrifuged daily at 1300 rpm for 10 min (Jouan MR23i Thermo electron corporation centrifuge, Technical University Berlin). The supernatants were removed and analyzed for TN, $NH_4$, $NO_3$, $PO_4$ and COD (by cuvette test kits). The residues were used for determination of dry matter (DM) and protein content (PC).

#### 3.2.6.1. Dry matter

The residues were washed three times with distilled water and then pipetted into evaporation plates and dried overnight at 90 °C (6 inches of vacuum) for dry matter determination (Thanh and Simard, 1973a).

### 3.2.6.2. Determination of total protein

This was made according to the method of Lowry *et al*. (1951) using bovine serum albumin as a standard.

**Reagents**

I- Solution A: 2 % of $Na_2CO_3$ (2g in 100 mL) in 0.1 N NaOH (4 g in 100 mL distilled water).

II- Solution B: 05 % of $CuSO_4$ (0.5 g in 100 mL) in 1 % sodium or potassium tartarate (1 g in 100 mL distilled water).

III- Solution C: 50 mL of solution A were mixed with 1mL of solution B. Mixing of the two solutions was done just before the protein determination.

IV- Solution D: this solution was prepared by diluting of Folin reagent with distilled water in the proportion of 1:3 (v/v).

**Procedure**

1. In test tube, 0.1 mL of the extract of each fungus tested was added to 5 mL of solution C.
2. The contents of the tube were mixed and left for 10 min at room temperature.
3. Then 0.5 mL of solution D was rapidly added and mixed with the tube contents.
4. The absorbance (Abs) of color was measured after 30 minutes incubation at room temperature at a wavelength of 750 nm.
5- The calculation of total protein concentration was carried out using the following general formula for the colorimetric determination:

$$g\ \% = \frac{\text{Abs.of sample}}{\text{Abs.of standard}} \text{ X concentration of standard}$$

Bovine serum albumin (Albumin fraction V 1.12018.0025 Merck Germany) was used as a standard; its concentration was determined spectrophotometrically by Specord 200, Analytic Jena Spectrophotometer Technische Universität Berlin.

### 3.2.7. Environmental factors affecting on the biological activity of *Aspergillus niger* and *Trichoderma viride* for nitrogen, phosphorus and COD elimination from raw wastewater

The effect of different environmental factors on elimination of phosphorus and nitrogen by tow fungal isolates (*Aspergillus niger* and *Trichoderma viride*) was studied; these isolates were the most active for nitrogen and phosphorus elimination from raw wastewater.

#### 3.2.7.1. Effect of different pH values

One hundred mL wastewater was charged into each conical flask of 250 ml capacity. The wastewater media was adjusted to different pH values (2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0), by using 1N of HCl or NaOH. All flasks were sterilized, then inoculated with 2 mL of fungus suspension and incubated at 30 $^{o}$C and 300 rpm for 15 days. A set of triplicate flasks was used for each pH. The total nitrogen, nitrate, ammonium, phosphate, COD, total protein and dry matter content were determined daily.

#### 3.2.7.2. Effect of incubation temperature

This experiment was carried out in order to determine the effect of different incubation temperatures on the elimination of nitrogen and phosphorus from raw wastewater. The wastewater medium was adjusted to pH 4.5 by addition of 1N HCl or NaOH before sterilization. One hundred mL wastewater was charged into each conical flask of 250 mL capacity. A set of triplicate flasks was used for each particular incubation temperature. The flasks were sterilized and then inoculated with 2 mL of fungus suspension. The inoculated flasks were incubated at different temperatures (5, 10, 15, 20, 25, 30, 35, 40 and 45 $^{o}$C) for 15 days in shaking incubator adjusted at 300 rpm. The total nitrogen, nitrate, ammonium, phosphate, COD, total protein and dry matter content were determined daily.

#### 3.2.7.3. Effect of incubation period

The inoculated flasks as mentioned before were incubated at 30 $^{o}$C and 300 rpm for 1 to 15 days. Cultures were removed at different periods of incubation (daily) and 8 h (three times each day). The total nitrogen, nitrate, ammonium, phosphates, COD, dry matter and total protein content were determined.

## 3.3. Elimination of nitrogen, phosphorous and COD from raw wastewater nutrients in Batch reactor systems by *Aspergillus niger* and *Trichoderma viride*

For a standard batch reactor test 2 L raw wastewater from Wassmannsdorf wastewater treatment plant in Berlin after adjusted to pH 4.5 and sterilization was filled into the batch reactor. The schematic of batch reactor presented in Figure 3.1. Six experiments were made (4 aerobic phases and 2 anaerobic phases). In aerobic case the incubated samples were provided with a stable source of air (flow rate 68 L/h). While in anaerobic the samples connected with a nitrogen cylinder (flow rate 68 L/h). 50 mL of fungus spore suspension of *Aspergillus niger* and/or *Trichoderma viride* were inoculated either in aerobic or anaerobic conditions. The batch reactor test was setup at 300 rpm and 30 $^{o}$C for 15 days. One sample every day and one sample every 8 h (three time each day) was withdrawn to determine the reduction degree of TN, $NH_4$, $NO_3$, $PO_4$ and COD. At the end of incubation periods the yield of batch reactor was harvested to determine the dry matter (DM) and protein content (PC). The flow rate of oxygen and nitrogen was determined by flowmeter (Rotameter Platon Co.).

## 3.4. Effect of pH 7.5 on the growth and elimination activites of *Aspergillus niger* and *Trichoderma viride* in raw wastewater (aerobic batch)

This experiment was carried out in order to determine the effect of pH 7.5 of raw wastewater on the ability of *Aspergillus niger* and *Trichoderma viride* for growth and elimination of nutrients. The aerobic batch reactor as mentioned before was filled with the raw wastewater at pH 7.5 and inoculated with each isolate separately. Through the incubation period (8 days) the pH was adjusted to 7.5 by adding NaOH. The growth and elimination degree (%) for TN, $PO_4$, $NH_4$, $NO_3$ and COD were measured.

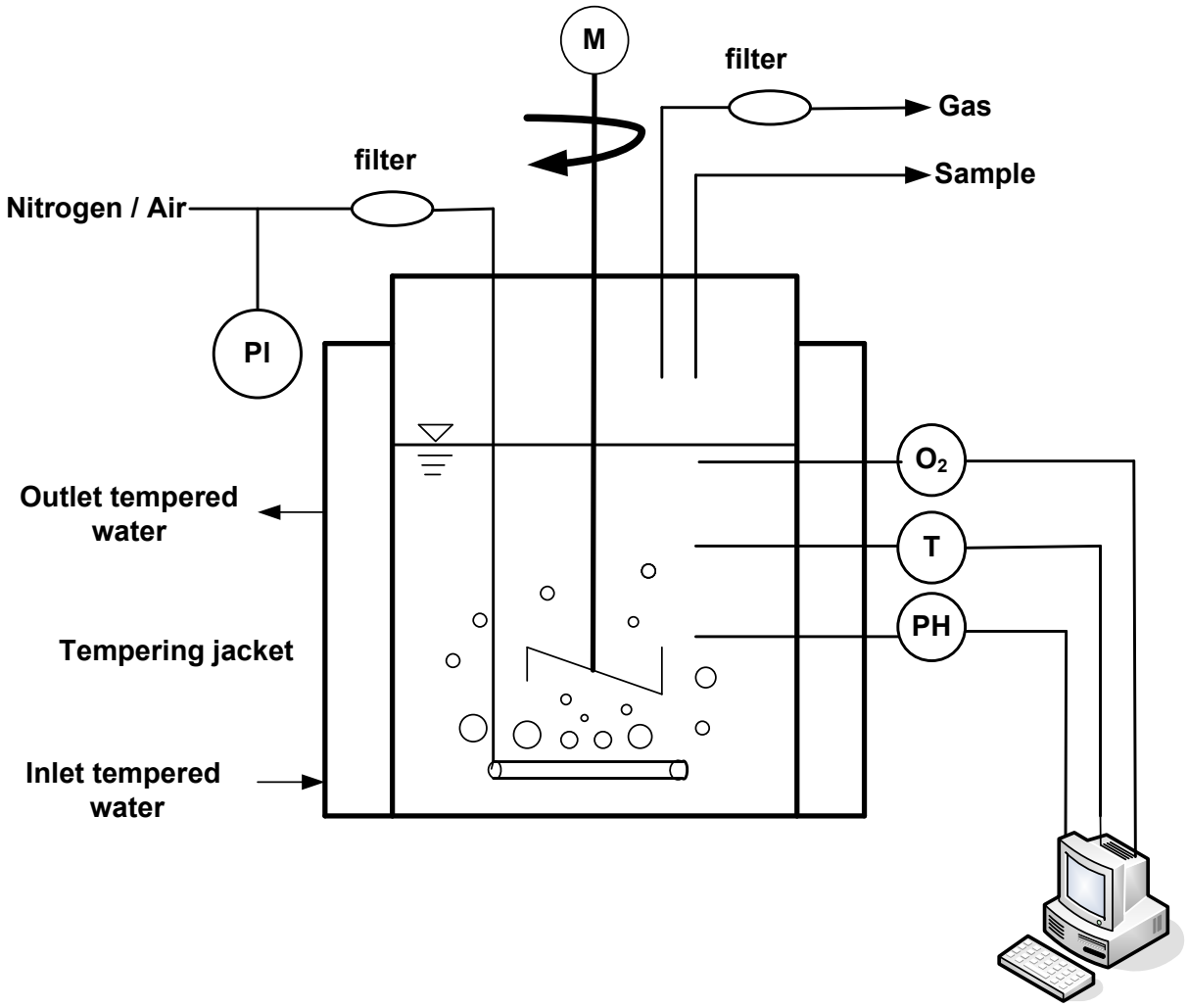



**Fig. 3.1:** Schematic of batch reactor

# 4. RESULTS AND DISCUSSION

## 4.1. Fungi recovered from activated sludge

One hundred-eight species belonging to 40 genera were collected in the present investigation from 36 samples of each aerobic and anoxic activated sludge of two wastewater treatment plants with membrane bioreactors on five isolation media 50 % Sucrose Czapek-Dox agar (28 genera and 62 species), Malt extract agar (26 genera and 60 species), Rose bengal Cloramphenicol agar (27 genera and 60 species), Sabouraud's dextrose agar media (29 genera and 61 species) and Sabouraud's dextrose agar with cycloheximide and chloramphenicol (21 genera and 46 species) media at 30 $^{o}$C for 1-2 weeks (Table 4.6 to 4.10 and Figure 4.1 to 4.5).

*Aspergillus* and *Penicillium* contributed the broadest spectra of species. *Aspergillus* was represented by 18 species belonging to 4 subgenera and 11 sections described by Klich and Pitt (1992) as shown in Table 4.1. Also, the previous *Aspergillus* species were belonging to 11 groups described by (Raper and Fennell, 1965) as recorded in Table 4.2.

From all isolates of *Penicillium*, 15 species were identified which represent four subgenera described by (Pitt, 1988; 2009; Frisvad and Samson, 2004) as follows: Subgenus Aspergilloides, 1 species; Subgenus Biverticillium, 4 species; Subgenus Furcatum, 4 species, Subgenus *Penicillium*, 6 species; and Table 4.3.

The previous isolates of *Penicillium* representing 15 species belonging to three sections and three subsections described by (Raper and Thom, 1949) as follows: Monoverticillata 1 species; Biverticillata symetrica 4 species; Asymetrica velutina subsection, 6 species; Asymetrica divaricata subsection, 1 species; and Asymetrica fasciculat subsection, 3 species Table 4.4. Seventy-five species of fungi other than *Aspergillus* and *Penicillium* belonging to 34 genera were collected in this study Table 4.5.

## 4.2. Isolation media

### 4.2.1. 50 % Sucrose Czapek-Dox agar

Sixty-two species representing 28 genera were isolated from both aerobic and anoxic activated sludge on 50 % Sucrose Czapek-Dox agar at 30 $^{o}$C for 1-2 weeks. The obtained data show that, *Aspergillus* was found at 94.4 % followed by *Penicillium* 61.1 %, *Fusarium* 61.1 %, *Trichoderma* 44.4 % and *Geotrichum* 41.6 % genera were the most prevalent in all activated sludge

samples (Table 4.6 and Figure 4.1). For more clarification the results were described in both aerobic and anoxic activated sludge as follows.

**Table 4.1:** Different subgenera and sections of *Aspergillus* species isolated during this investigation according to Klich and Pitt (1992)

| Subgenus | Section | Species |
|---|---|---|
| **I. Aspergillus** | **1- Aspergillus** | Eu. Chevalieri (L. Mangin) Thom & Church |
| **II. Circumdati** | **2-Circumdati** | *A. alutaceus* var. *alutaceus* Berkely and Curtis = *A. ochraceus* Wilhelm |
| | **3- Candidi** | *A. candidus* Link |
| | **4- Flavi** | *A. flavus* Raper & Fennell *var. columnaris*<br>*A. flavus var. flavus* Link<br>*A. oryzae* (Ahlburg) Cohn<br>*A. parasiticus* Speare |
| | **5- Nigri** | *A. niger* van Tieghem |
| **III- Fumigati** | **6- Fumigati** | *A. fischerians* Samson & W. Gams<br>= *Neosartorya fischeri* (Wehmer) Malloch & Cain<br>*A. fumigatus* Fresenius |
| **IV-Nidulantes** | **7. Flavipides** | *A. carneus* Blochwitz |
| | **8- Nidulantes** | *Em. nidulans* (Eidam) Vuillemin |
| | **9-Versicolores** | *A. sydowii* (Bainier and Sartory) Thom and Church<br>*A. versicolor* (Vuillemin)Tiraboschi |
| | **10- Terrei** | *A. terreus* Thom |
| | **11- Usti** | *A. ustus* (Bainier) Thom & Church |

**Table 4.2:** Different groups of *Aspergillus* species isolated during this investigation according to Raper and Fennell (1965)

| Species | Group |
|---|---|
| *A. alutaceus* Berkely and Curtis var. *alutaceus* | *A.ochraceus* |
| *A. candidus* Link | *A. candidus* |
| *A. carneus* Blochwitz | *A. flavipes* |
| *A. flavus* Raper & Fennell var. *columnaris* | *A. flavus* |
| *A. flavus* Link var. *flavus* | |
| *A. oryzae* (Ahlburg) Cohn | |
| *A. parasiticus* Speare | |
| *A. fischerians* Samson & W. Gams | *A. fumigatus* |
| *A. fumigatus* Fresenius | |
| *A. niger* van Tieghem | *A. niger* |
| *A. sydowii* (Bainier and Sartory) Thom and Church | *A. versicolor* |
| *A. versicolor* (Vuillemin) Tiraboschi | |
| *A. terreus* Fennell & Raper var. *africanus* | *A. terreus* |
| *A. terreus* Thom & Raper var. *aureus* | |
| *A. terreus* Thom var. *terreus* | |
| *A. ustus* (Bainier) Thom & Church | *A. ustus* |
| *Em. nidulans* (Eidam) Vuillemin | *A. nidulans* |
| *Eu. chevalieri* (L. Mangin) Thom & Church | *A. chevalieri* |

**Table 4.3:** Classification of Penicillium species isolated in this investigation according to Pitt 1988; 2009; Frisvad and Samson 2004

| **Subgenus: Aspergilloides Dierckx** | **Subgenus: Biverticillium Dierckx** | **Subgenus: Furcatum Pitt** | **Subgenus: *Penicillium* Pitt** |
|---|---|---|---|
| **Section:** *Aspergilloides* Dierckx<br><br>**Series:** *Glabra P. glabrum* (Wehmer) Westling<br>= *P. Frequentans* Westling | **A. Section:** *Coremigena*<br><br>**Series:** *Duclauxii*<br>*P. declauxii* Delacroix | **A. Section:** Divaricatum Raper & Thom and Pitt<br><br>**Series:** *Canescentia*<br>*P. janczewskii* Zaleski<br>= *P. nigricans* (Bainier) Thom | **Section:** Penicillium Pitt<br><br>**Series:** *Urlicicola*<br>*P.brevicompactum* Dierckx<br>*P. verrucosum var. verrucosum* Dierckx<br><br>**Series:** *Expansa*<br>*P. chrysogenum* Thom<br>*P. expansum* Link<br><br>**Series:** *Viridicala*<br>*P. puberulum*<br>= *P. auranliogriseum* Dierckx<br>*P. roquefortii* Thom |
| | **B. Section:** *Simplicia* Pitt<br><br>**Series:** *Miniolulea*<br>*P. funiculosum* Thom<br>*P. purpurogenum* Stoll<br><br>**Series:** *Islandica*<br>*P. islandicum* Sopp | **B. .Section:** Furcatum Pitt<br><br>**Series:** *Cilrina*<br>*P. citrinum* Thom<br>*P. corylophilum* Dierckx<br><br>**Series:** *Oxalica*<br>*P. oxalicum* Currie & Thom | |

**Table 4.4:** Different groups of *Penicillium* species isolated in this investigation according to the key of Raper and Thom (1949)

| **Monoverticillata** | **Asymmetrica** | | | **Biverticillata symmetrica** |
|---|---|---|---|---|
| *P. glabrum* (Wehmer) Westling | Fasciculata | Divaricata | Velutina | *P. declauxii* Delacroix |
| | *P. brivicompactum* Dierckx | *P. nigricans* (Bainier) Thom | *P. expansum Link* | |
| | *P. chrysogenum* Thom | | *P. puberulum* Bainier | *P. funiculosum* Thom |
| | *P. citrinum* Thom | | P. *verrucosum var. verrucosum* Dierckx | *P. islandicum* Sopp |
| | *P. corylophilum* Dierckx | | | *P. purpurogenum* Stoll |
| | *P. roquefortii* Thom | | | |
| | *P. oxalicum* Currie & Thom | | | |

**Table 4.5:** List of fungi other than *Aspergillus* and *Penicillium* isolated in the present investigation

| Genera and Species |
|---|
| *Acremonium curvulum* W. Gams1971 |
| *A. recifei* (Leão & Lôbo) W. Gams 1971 |
| *A. rutilum* W. Gams1971 |
| *A. strictum* W. Gams1971 |
| *Alternaria alternata* (Fr.) Keissl. 1912 |
| *A. brassicae* (Berk.) Sacc. 1880 |
| *A. chlamydospora* Mouch. 1973 |
| *Aurobasidium pullulans* (de Bary) Arnaud 1918 |
| *Botryodiplodia theobronae* Pat. 1892 |
| *Candida albicans* (C.P. Robin) Berkhout 1923 |
| *Chaetomum cochliodes* Palliser 1910 |
| *C. globosum* Kunze: Fries 1817 |
| *Chrysosporium asperatum* J.W. Carmich. 1962 |
| *C. georgii* (Varsavsky & Ajello) Oorschot 1980 |
| *C. indicum* (H.S. Randhawa & R.S. Sandhu) Garg 1966 |
| *C. keratinophilum* D. Frey ex J.W. Carmich. 1962 |
| *C. pannorum* (Link) S. Hughes 1958 |
| *C.tropicum* J.W. Carmich. 1962 |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries 1952 |
| *C. herbarium* (Pers.) Link 1816 |
| *C. oxysporum* Berk. & M.A. Curtis 1868 |
| Cochliobolus lunatus R.R. Nelson & F.A. Haasis 1964 |
| *Doratomyces stemonitis* (Pers.) F.J. Morton & G. Sm. 1963 |
| *Epicoccum nigrum* Link ex Fr 1815 |
| *Fusarium dimerum* Penz. 1882 |
| *F. lichenicola* (Cylindrocarpon lichenicola) C. Massal. 1903 |
| *F. oxysporium* Schltdl. 1824 |
| *F. roseum* Link 1832 |
| *F. solani* (Mart.) Sacc. 1881 |
| *Geosmithia lavendula* (Raper & Fennell) Pitt 1979 |
| *Geotrichum candidum* Link 1809 |
| *Gibberella accuminata* C. Booth 1971 |
| *G. avenacea* R.J. Cook 1967 |
| *G. fujikuroi* var. *fujikuroi* (Sawada) Wollenw. 1931 |
| *Gliocladium roseum* Bainier 1907 |
| *G. viride* Matr. 1893 |
| *Gymnoascus reesii* Baran. 1872 |
| *G. reticulatus* Zukal 1887 |
| *Microsporum Cookei* Ajello 1959 |
| *M. ferrugineum* M. Ota 1921 |
| *M. gypseum* (E. Bodin) Guiart & Grigoraki 1928 |
| *Mucor circinelloides* Tiegh. 1875 |
| *M. hiemalis* Wehmer 1903 |
| *Myrothesium cinctrum* (Corda) Sacc. 1886 |
| *Oidiodendron griseum* Robak 1934 |
| *Paecilomyces lilacinus* (Thom) Samson 1974 |
| *P. marquandii* (Massee) S. Hughes 1951 |
| *P. variotii* Bainier 1907 |
| *Phialophora verrucosa* Medlar 1915 |
| *Rhinocladiella atrovirens* Nannf. 1934 |
| *Rhizopus arrhizus* A. Fisch. 1892 |
| *R. oryzae* Went & Prins. Geerl. 1895 |
| *Rhodotorula rubra* (Schimon) F.C. Harrison 1928 |
| *Scopulariopsis asperula* (Sacc.) S. Hughes 1958 |
| *S. brevicaulis* (Sacc.) Bainier 1907 |
| *S. brumptii* Salv.-Duval 1935 |
| *Setosphora rostrata* K.J. Leonard 1976 |

**Table 4.5:** continued

| Genera and Species |
|---|
| *Sporothrix schenkii* Hektoen & C.F. Perkins 1900 |
| *Stachybotrys chrtarum* (Ehrenb.) S. Hughes 1958 |
| *S. elegans* (Pidopl.) W. Gams 1980 |
| *Stemphylium vesicarium* (Wallr.) E.G. Simmons 1969 |
| *Syncephalastrum racemosum* Cohn ex J. Schröt. 1886 |
| *Trichoderma hamatum* (Bonord.) Bainier 1906 |
| *T. koningii* Oudem. 1902 |
| *T. viride* Pers. 1794 |
| *Trichophyton ajelloi* var. *ajelloi* (Vanbreus.) Ajello 1968 |
| *T. equinunm* Gedoelst 1902 |
| *T. mentagrophytes* (C.P. Robin) Sabour. 1895 |
| *T. terrestre* Durie & D. Frey 1957 |
| *Trichospoon pullulans* (Lindner) Diddens & Lodder 1942 |
| *Ulocladium chartarum* (Preuss) E.G. Simmons 1967 |
| *U. microsporum* Moub. & Abdel-Hafez 1977 |
| *Verticillium chlamydosporium* Goddard 1913 |
| *V. lecanii* (Zimm.) Viégas 1939 |
| *yeasts* |

#### 4.2.1.1. Fungi recovered from aerobic activated sludge samples

Fifty-eight species representing 28 genera were collected from 36 aerobic samples on 50 % Sucrose Czapek-Dox agar at 30 $^{o}$C for 1-2 weeks (Table 3.6 and Figure 3.1). The total count of fungi in aerobic activated sludge ranged between 17-62 colonies/ml activated sludge and the highest count was estimated in sample No. 23. Also, the data in Table 3.6 determined the *Aspergillus* was the most common genus and was recovered in high frequency of occurrence 94.4 % of samples constituting 21.1 % of total fungi. The count of *Aspergillus* ranged between 1-16 colonies/mL activated sludge. It was represented by 11 species of which *A. fumigatus* was isolated in high frequency and *A. niger* was isolated in moderate frequency. They emerged in 55.5 % and 41.6 % of the samples matching 58.8 % and 44.1 % of total *Aspergillus* and 6.0 % and 4.6 % of total fungi, respectively. *A flavus* var. *columnaris*, *A. flavus* var. *flavus*, *A. alulaceus*, *A. carneus*, *A. nidulans (Emericella nidulan)*, *A. oryzae*, *A. terreus* var. *africanus*, *A. terreus* var. *terreus* and *A. ustus* were isolated in moderated, low or rare frequency (Plats A.6, 8, 10-14 and 17-19). They emerged in 19.4 %, 30.5 %, 5.5 %, 2.7 %, 5.5 %, 11.1 %, 8.3, 2.7 and 5.5 % of samples matching 20.6 %, 32.4 %, 5.9 %, 2.9 %, 5.9 %, 11.7 %, 8.8 %, 2.9 % and 5.9 % of total *Aspergillus*, respectively.

Data in Table 4.6 and Figure 4.1 showed that *Fusarium* occupied the second place in the number of cases of isolation and was recovered in high frequency of occurrence 61.1 % of samples constituting 6.2 % of total fungi. Its counts ranged between 1-6 colonies/mL activated sludge. *Fusarium* was represented by 4 species of which *F. dimerum*, *F. oxysporum*, *F. solani*, and *F. roseum* (Plates A.35, 37 and 38), were isolated in moderate and low frequency and emerged in

27.7 %, 13.8 %, 11.1 % and 8.3 % of samples matching 45.4 %, 22.7 %, 18.2 %, and 13.63 % of total *Fusarium* and 3.3 %, 1.8 %, 1.0 % and 0.95 % of total fungi, respectively.

*Penicillium* was also common and ranked third according to their total counts. It was encountered in 55.5 % of samples constituting 12.0 % of total fungi. The genus counts ranged between 1-9 colonies/mL activated sludge giving maximum in sample No. 9 (9 colonies). It was represented by 6 species of which *P. chrysogenum* and *P. citrinum* (Plates A. 55 and 56), were isolated in low frequency and emerged in 19.4 % and 22.2 % of the samples matching 35.0 % and 40.0 % of total *Penicillium,* respectively. *Penicillium brevicompactum*, *P. corylophilum*, *P. oxalicum* and *P. roqueforti* were isolated in rare frequency, (Plates A.54, 57, 63 and 65). They emerged in 8.0 %, 2.0 %, 8.0 %, and 2.0 % of samples and 15.0 %, 5.0 %, 5.0 % and 15.0 % of total *Penicillium,* respectively (Table 4.6 and Figure 4.1).

*Alternaria* was isolated in moderate frequency and was recovered in 36.1 % of samples and represented by 2 species, *A. alternata* and *A. chlamydospora* (Plates A.4 and 5), were recovered from 8.3 % and 27.7 % of samples matching 23.1 % and 76.9 % of total *Alternaria,* respectively. *Geotrichum candidum* (Plate A.40), was recovered in 36.1 % of samples and 4.9 % of total fungi. *Scopulariopsis* was isolated in moderate frequency and emerged in 36.1 % of the samples matching 5.48 % of total fungi and represented by *S. asperula* and *S. brevicaulis* (Plate A.70), and were recovered from 19.4 % and 8.3 % of samples matching 53.8 % and 46.2 % of total *Scopulariopsis* matching 3.1 % and 2.3 % of total fungi, respectively.

*Trichoderma* was isolated in moderate frequency and emerged in 33.3 % of the samples matching 6.5 % of total fungi. Three species were identified *T. hamatum* and *T. koningii*, *T. viride* (Plates A. 77, 78 and 79) and were recovered from 11.1 % and 13.8 % of samples matching 46.6 %, 40.0 % and 26.6 % of total *Trichoderma* and 1.7 %, 2.0 % and 2.9 % of total fungi, respectively. Unidentified *yeasts* were recovered from 27.7 % of samples matching 6.8 % of total fungi (Plates A.89 and 90).

*Cladosporium* was isolated in moderate frequency and emerged in 25.0 % of samples and 3.39 % of total fungi. Three species were identified *C. cladosporioides, C. herbarium* and *C. oxysporum* (Plates A.30 and 31) and were recovered from 11.1 % and 5.5 % of samples matching 44.4 % and 22.2 % of total *Cladosporium* and 0.5 %, 1.6 % and 1.3 % of total fungi, respectively (Table 3.6 and Figure 3.1). *Doratomyces stemonitis* (Plate A.33) was isolated in low frequency and emerged in 22.2 % of samples matching 3.1 % of total fungi. *Rhizopus* was isolated in low frequency and emerged in 19.4 % of samples and 3.1 % of total fungi and represented by *R. arrhizus* and *R. oryzae* were recovered from 13.8 % and 5.5 % of samples matching 80.0 % and

60.0 % of total *Rhizopus* and 2.3 % and 0.7 % of total fungi, respectively. *Mucor* was represented by *M. circinelloides* (Plate A.49) and recovered from 19.4 % of samples matching 2.6 % of total fungi.

The presented data in Table 4.6 and Figure 4.1 shows that *Acremonium*, *Chaetomum*, *Gibberella* and *Paecilomyces* were recovered from 16.6 % of samples matching 2.3 %, 3.7 %, 2.3 % and 2.1 % of total fungi, respectively. *Acremonium* was represented by *A. curvulum* and *A. strictum* (Plates 1 and 3) were recovered from 8.3 % and 11.1 % of samples matching 50.0 % and 66.6 % of total *Acremonium* and 1.3 % and 1.0 % of total fungi, respectively; *Chaetomum* was represented by *C. cochliodes* and *C. globosum* (Plates A. 24 and 25), were recovered from 11.1 % and 5.5 % of all samples matching 66.6 % and 33.3 % of total *Chaetomum,* 2.3 % and 1.3 % of total fungi, respectively, *Gibberella* was represented by *G. fujikuroi* var. *fujikuroi* (Plate A.43) and *Paecilomyces* was represented by *P. lilacinus* and *P. variotii* (Plates A.51 and 53), were recovered from 8.3 % and 11.1 % of samples.

*Chrysosporium* was recovered from 13.8 % of samples and represented by *C. georgii* and *C. tropicum* (Plates A.26 and 29) and were recovered from 11.1 % and 5.5 % of samples matching 80.0 % and 40.0 % of total *Chrysosporium* and 1.8 % of total fungi, respectively. *Stachybotrys* and *Ulocladium* ware isolated in rare frequency and emerged in 11.1 % of samples and 1.5 % of total fungi; *Stachybotrys* was represented by *S. chartarum* (Plate A.74) and *S. elegans* and were recovered from 5.5 % and 2.7 % of samples. *Ulocladium* was represented by *U. chartarum* (Plate A.85).

*Cochliobolus lunatus, Geosmithia lavendula*, *Gliocladium roseum* and *Syncephalastrum racemosum* (Plates A.32, 39, 44, and 76) were isolated in rare frequency of occurrence matching collectively 8.3 % of all samples. *Aurobasidium pullulans*, *Botryodiplodia theobromae*, *Phialophora verrucosa*, *Setosphaeria rostrata* and *Trichophyton ajelloi* var. *ajelloi* (Plates A.21, 22, 67, 72 and 80) were isolated in rare frequency of occurrence matching collectively 2.7 % of all samples (Table 4.6 and Figure 4.1).

#### 4.2.1.2. Fungi recovered from anoxic activated sludge samples

Fifty-two species representing 26 genera were collected from 36 samples of anoxic activated sludge on 50 % Sucrose Czapek-Dox agar at 30 $^{o}$C for 1-2 weeks as presented in Table 4.6 and Figure 4.1. The total count of fungi in anoxic activated sludge ranged between 12-58 colonies/mL activated sludge and the highest count was estimated in sample No. 18.

**Table 4.6:** Numbers of cases of isolation (NCI) out of 36, percentage frequency and occurrence remarks (OR) of fungal genera and species recovered from each aerobic and anoxic activated sludge samples with MBRs on 50 % Sucrose Czapek-Dox agar media at 30 °C

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| *Acremonium* | 6 | 16.6 | L | 2 | 5.5 | R |
| *A. curvulum* W. Gams | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. strictum* W. Gams | 4 | 11.1 | R | 1 | 2.7 | R |
| *Alternaria* | 13 | 36.1 | M | 5 | 13.8 | L |
| *A. alternata* (Fr.) Keissl. | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. brassicae* (Berk.) Sacc. | - | - | - | 2 | 5.5 | R |
| *A. chlamydospora* Mouch. | 10 | 27.7 | M | 5 | 13.8 | L |
| *Aspergillus* | 34 | 94.4 | H | 28 | 77.7 | H |
| *A. alutaceus* Berk. & M.A. Curtis var. *alutaceus* | 2 | 5.5 | R | - | - | - |
| *A. carneus* Blochwitz | 1 | 2.7 | R | - | - | - |
| *A. fischerianus* Samson & W. Gams | - | - | - | 1 | 2.7 | R |
| *A. flavus* Raper & Fennell var. *columnaris* | 7 | 19.4 | R | 5 | 13.8 | L |
| *A. flavus* Link var. *flavus* | 11 | 30.5 | M | 7 | 19.5 | L |
| *A. fumigatus* Fresen. | 20 | 55.5 | H | 5 | 13.8 | L |
| *A. nidulans (Emericella nidulan)* (Eidam) G. Winter | 2 | 5.5 | R | - | - | - |
| *A. niger* sensu auct. pro parte, pre | 15 | 41.6 | M | 10 | 27.7 | M |
| *A. oryzae* (Ahlb.) E. Cohn | 4 | 11.1 | R | 1 | 2.7 | R |
| *A. terreus* Fennell & Raper var. *africanus* | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. terreus* Thom var. *terreus* | 1 | 2.7 | R | 1 | 2.7 | R |
| *A. ustus* (Bainier) Thom & Church | 2 | 5.5 | R | - | - | - |
| *Aurobasidium pullulans* (de Bary) Arnaud | 1 | 2.7 | R | - | - | - |
| *Botryodiplodia theobromae* Pat. | 1 | 2.7 | R | 2 | 5.5 | R |
| *Chaetomum* | 6 | 16.6 | L | 3 | 8.3 | R |
| *C. cochliodes* Palliser | 4 | 11.1 | R | 3 | 8.3 | R |
| *C. globosum* Kunze | 2 | 5.5 | R | - | - | - |
| *Chrysosporium* | 5 | 13.8 | L | 4 | 11.1 | R |
| *C. georgii* (Vasravsky&Ajello) Oorschot | 4 | 11.1 | R | 2 | 5.5 | R |
| *C. tropicum* J.W. Carmich. | 2 | 5.5 | R | 3 | 8.3 | R |
| *Cladosporium* | 9 | 25.0 | M | 4 | 11.1 | R |
| *C. cladosporioides* (Fresenius) de Vries | 4 | 11.1 | R | 3 | 8.3 | R |
| *C. herbarum* (Pers.) Link | 2 | 5.5 | R | - | - | - |
| *C. oxysporum* Berk. & M.A. Curtis | 4 | 11.1 | R | 1 | 2.7 | R |
| *Cochliobolus lunatus* R.R. Nelson & F.A. Haasis | 3 | 8.3 | R | 1 | 2.7 | R |
| *Doratomyces stemonitis* (Pers.) F.J. Morton & G. Sm | 8 | 22.2 | L | 5 | 13.8 | L |
| *Fusarium* | 22 | 61.1 | H | 20 | 55.5 | H |
| *F. dimerum* Penz. | 10 | 27.7 | M | 7 | 19.4 | L |
| *F. oxysporum* Schltdl. | 5 | 13.8 | L | 11 | 30.5 | M |
| *F. roseum* Link | 3 | 8.3 | R | - | - | - |
| *F. solani* (Mart.) Sacc. | 4 | 11.1 | 4R | 4 | 11.1 | R |
| *Geosmithia lavendula* (Raper & Fennell) Pitt | 3 | 8.3 | R | 1 | 2.7 | R |
| *Geotrichum candidum* Link | 5 | 13.8 | L | 7 | 19.4 | L |
| *Gibberella fujikuroi* (sawada) Wollenweber. var. *fujikuroi* | 6 | 16.6 | L | 8 | 22.2 | L |
| *Gliocladium roseum* Bainier | 3 | 8.3 | L | - | - | - |
| *Mucor circinelloides* Tiegh. | 7 | 19.4 | L | 5 | 13.8 | L |
| *Paecilomyces* | 6 | 16.6 | L | 9 | 25.0 | M |
| *P. lilacinus* (Thom) Samson | 3 | 8.3 | R | 2 | 5.5 | R |
| *P. marquandii* (Massee) S. Hughes | - | - | - | 2 | 5.5 | R |
| *P. variotii* Bainier | 4 | 11.1 | R | 5 | 13.8 | L |
| *Penicillium* | 20 | 55.5 | H | 22 | 61.1 | H |
| *P. brevicompactum* Dierckx | 3 | 8.3 | R | 2 | 5.5 | R |
| *P. chrysogenum* Thom | 7 | 19.4 | L | 7 | 19.4 | L |
| *P. citrinum* Thom | 8 | 22.2 | L | 7 | 19.4 | L |

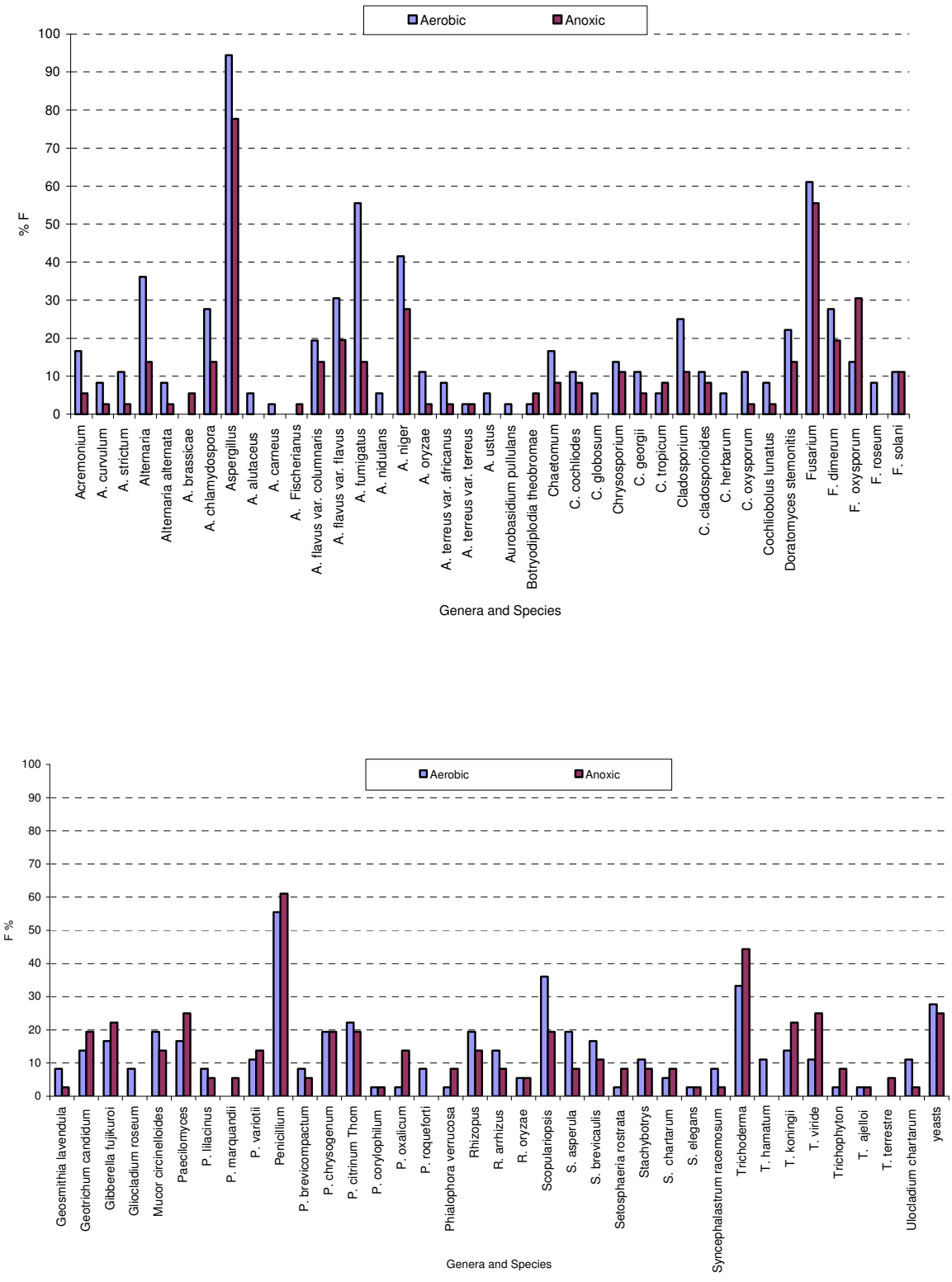
**Table 4.6:** continued

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| *P. corylophilum* Dierckx | 1 | 2.7 | R | 1 | 2.7 | R |
| *P. oxalicum* Currie & Thom | 1 | 2.7 | R | 5 | 13.8 | L |
| *P. roqueforti* Thom | 3 | 8.3 | R | - | - | - |
| *Phialophora verrucosa* Medlar | 1 | 2.7 | R | 3 | 8.3 | R |
| *Rhizopus* | 7 | 19.4 | L | 5 | 13.8 | L |
| *R. arrhizus* Fischer | 5 | 13.8 | L | 3 | 8.3 | R |
| *R. oryzae* Went & Prinsen-Geerligs | 2 | 5.5 | R | 2 | 5.5 | R |
| *Scopulariopsis* | 13 | 36.1 | M | 7 | 19.4 | L |
| *S. asperula* (Sacc.) Hughes | 7 | 19.4 | L | 3 | 8.3 | R |
| *S. brevicaulis* (saccardo) Bainier | 6 | 16.6 | L | 4 | 11.1 | R |
| *Setosphaeria rostrata* Leonard | 1 | 2.7 | R | 3 | 8.3 | R |
| *Stachybotrys* | 4 | 11.1 | R | 3 | 8.3 | R |
| *S. chartarum* (Ehrenberg) Hughes | 3 | 5.5 | R | 3 | 8.3 | R |
| *S. elegans* (Pidopl.) W. Gams | 1 | 2.7 | R | 1 | 2.7 | R |
| *Syncephalastrum racemosum* Cohn ex Schöter | 3 | 8.3 | R | 1 | 2.7 | R |
| *Trichoderma* | 12 | 33.3 | M | 16 | 44.4 | M |
| *T. hamatum* (Bonorden) Bainier | 4 | 11.1 | R | - | - | - |
| *T. koningii* Oudemans | 5 | 13.8 | L | 8 | 22.2 | L |
| *T. viride* Persoon | 4 | 11.1 | R | 9 | 25.0 | M |
| *Trichophyton* | 1 | 2.7 | R | 3 | 8.3 | R |
| *T. ajelloi* (Vanbreuseghem) Ajello var. *ajelloi* | 1 | 2.7 | R | 1 | 2.7 | R |
| *T. terrestre* Durie & Frey | - | - | - | 2 | 5.5 | R |
| *Ulocladium chartarum (*Preuss) Simmons | 4 | 11.1 | R | 1 | 2.7 | R |
| *yeasts* | 10 | 27.7 | M | 9 | 25.0 | M |
| Number of genera = 28 | 28 | | | 26 | | |
| Number of species = 62 | 58 | | | 52 | | |

NCI = Number of cases of isolation (out of 36)
% F = Percentage frequency of occurrence (calculated per 36 samples)
OR = Occurrence remarks: [H= High occurrence, isolated more than 18 cases (out of 36 samples)
M = Moderate occurrence, from 9 to 18 cases
L = Low occurrence, from 5 to 8 cases
R = Rare occurrence, less than 5 cases

Data in Table 3.6 and Fig. 3.2 illustrated the *Aspergillus* was the most common genus and was recovered in high frequency of occurrence in 77.7 % of samples constituting 20.98 % of total fungi. The count of *Aspergillus* ranged between 1-14 colonies/mL activated sludge. It was represented by 9 species of which *A. niger* was isolated in moderate frequency and *A. flavus* var. *flavus* was isolated in low frequency and emerged in 27.7 % and 19.5 % of the samples matching 35.7 % and 25 % of total *Aspergillus* and 8.2 % and 3.9 % of total fungi, respectively.

*Penicillium* occupied the second place in the number of cases of isolation and was recovered from 61.1 % of samples constituting 13.7 % of total fungi. *Penicillium* was represented by 5 species of which *P. chrysogenum*, *P. citrinum* and *P. oxalicum* were isolated in low frequency and emerged in 19.4 % and 13.8 % of the anoxic samples matching 31.8 % and 22.7 % of total *Penicillium* and 3.6 %, 5.2 % and 2.3 % of total fungi, respectively (Table 4.6 and Figure 4.1).




% F = Percentage frequency of occurrence (calculated per 36 samples)

**Fig.4.1:** The comparison between percentage frequencies of fungal genera occurrence in aerobic and anoxic activated sludge on 50% Sucrose Czapek-Dox agar media

*Fusarium* occupied the third place in the number of cases of isolation and was recovered in high frequency of occurrence 55.5 % of samples constituting 10.2 % of total fungi. It was represented by 3 species of which *F. dimerum*, *F. oxysporum* and *F. solani* were isolated in moderate and low frequency and emerged in 19.4 %, 30.5 % and 11.1 % of samples matching 35.0 %, 55.0 % and 20.0 % of total *Fusarium* and 3.6 %, 4.2 % and 2.3 % of total fungi, respectively.

Also, data in Table 4.6 and Fig. 4.2 indicate that *Trichoderma* occupied the fourth place in the number of cases of isolation and was recovered from 44.4 % of samples constituting 8.8 % of total fungi. *Trichoderma* was represented by 2 species *T. koningii* and *T. viride* recovered from 22.2 % and 25.0 % of all anoxic samples matching 50.0 % and 56.0 % of total *Trichoderma* and 3.6 % and 5.2 % of total fungi, respectively. Unidentified *yeasts* were isolated in moderate frequency and recovered 25.0 % of samples matching 5.6 % of total fungi.

*Gibberella fujikuroi* var. *fujikuroi* was isolated in low frequency and emerged in 22.2 % of samples constituting 4.2 % of total fungi. *Geotrichum candidum* was isolated in low frequency and emerged in 19.4 % of samples constituting 5.9 % of total fungi. *Paecilomyces* was recovered 16.6 % of samples constituting 3.2 % of total fungi. It was represented by *P. lilacinus*, *P. marquandii* (Plate A.52) and *P. variotii* recovered from 5.5 %, 5.5 % and 13.8 % of samples matching 22.2 %, 22.2 % and 55.5 % of total *Paecilomyces* and 0.6 %, 0.9 % and 1.6 % of total fungi, respectively.

*Alternaria (*represented by *A. alternata*, *A. brassice* and *A. chlamydospora) Doratomyces stemonitis, Mucor circinelloides* and *Rhizopus* (represented by *R. arrhizus* and *R. oryzae*) ware isolated in low frequency of occurrence matching collectively 13.8 % of samples and 3.0 %, 2.3 %, 2.3 % and 3.0 % of total fungi, respectively. *Chrysosporium (*represented by *C. tropicum* and *C. georgii)* and *Cladosporium (*represented by *C. cladosporioides* and *C. oxysporum)* were isolated in low frequency and emerged in 11.1 % of samples matching 2.3 % and 1.6 % of total fungi, respectively.

*Chaetomum cochliodes*, *Phialophora verrucosa*, *Setosphaeria rostrata* (Plate A.72), *Stachybotrys* (represented by *S. chartarum* and *S. elegans*) and *Trichophyton terrestre* (Plate A.83) were isolated in rare frequency and emerged in 8.3 % of anoxic samples. *Acremonium* (represented by *A. curvulum* and *A. strictum*) and *Botryodiplodia theobromae* were isolated in rare frequency and emerged in 5.5 % of anoxic samples. *Cochliobolus lunatus*, *Geosthmithia lavendula*, *Syncephalastrum racemosum*, and *Ulocladium chartarum* were isolated in rare frequency and emerged in 2.7 % of the anoxic samples (Table 4.6 and Figure 4.1).

### 4.2.2. Malt extract agar (MEA)

Sixty species representing 26 genera were collected from 36 aerobic and anoxic activated sludge samples on Malt extract agar at 30 $^{o}$C for 1-2 weeks. The obtained data in Table 4.7 show that, *Geotrichum* was found at 94.4 % followed by *Aspergillus* 88.8 %, *Penicillium* 88.8 %, *Fusarium* 86.1 % and unidentified *yeasts* 75.0 % genera were the most prevalent in all activated sludge samples (Figure 4.2). For more clarification the results were described in both aerobic and anoxic activated sludge as follows.

#### 4.2.2.1. Fungi recovered from aerobic activated sludge samples

Fifty- seven species representing 25 genera were collected from 36 aerobic samples on Malt extract agar at 30 $^{o}$C for 1-2 weeks (Table 4.7 and Figure 4.2). The total count of fungi in anoxic activated sludge ranged between 8-63 colonies/mL activated sludge and the highest count was estimated in sample No. 11. *Geotrichum candidum* was the most common genus and recovered in high frequency of occurrence in 94.4 % of samples constituting 11.7 % of total fungi. The count of *Geotrichum candidum* ranged between 4-35 colonies/mL activated sludge.

*Penicillium* occupied the second place in the number of cases of isolation and was recovered from 88.8 % of samples constituting 19.2 % of total fungi. It was represented by 9 species of which *P. brevicompactum*, *P. chrysogenum*, *P. citrinum* and *P. roqueforti* were isolated in moderate frequency and emerged in 27.7 %, 50.0 %, 44.4 % and 25.0 % of samples matching 31.3 %, 62.1 %, 55.2 % and 31.0 % of total *Penicillium,* 0.7 %, 5.7 %, 3.9 %, and 2.9 % of total fungi, respectively (Plates A. 54-60, 62 and 64-65).

Also, the data in Table 4.7 and Fig. 4.2 reveals that, *Aspergillus* occupied the third place in the number of cases of isolation and was recovered in high frequency of occurrence 83.3 % of aerobic samples constituting 16.1 % of total fungi. The count of *Aspergillus* ranged between 1-18 colonies/mL activated sludge. It was represented by 13 species of which *A. flavus* var. *flavus* and *A. fumigatus* were isolated in high frequency and emerged in 61.1 and 52.7 % of samples matching 73.3 % and 63.3 % of total *Aspergillus* matching 3.4 % and 3.5 % of total fungi, respectively.

*Fusarium* was recovered in high frequency of occurrence in 80.5 % of aerobic samples constituting 7.8 % of total fungi. It was represented by 3 species of which *F. dimerum*, *F. solani* and *F. oxysporum* were isolated in moderate and low frequency and emerged in 44.4 %, 50.0 % and 22.2 % of the aerobic samples matching 55.2 %, 62.0 %, and 27.6 % of total *Fusarium* and 2.8 %,

1.5 %, and 3.5 % of total fungi, respectively. Unidentified *yeasts* were isolated in high frequency and emerged in 75.0 % of all samples constituting 8.8 % of total fungi.

*Trichoderma* was recovered in moderate frequency of occurrence 50.0 % of samples and represented by 3 species *T. koningii*, *T. hamatum* and *T. viride* were recovered from 25.0, 13.8 % and 11.1 % of samples matching 50.0 %, 27.8 % and 22.2 % of total *Trichoderma* and 0.9 %, 1.4 % and 0.9 % of total fungi, respectively. *Doratomyces stemonitis* was isolated in moderate frequency and recovered in 47.2 % of samples constituting 3.9 % of total fungi (Table 4.7 and Figure 4.2). *Alternaria (*represented by *A. alternata* and *chlamydospora)* and *Gibberella* represented by *G. acuminata* (Plate A.41) and *G. fujikuroi* var. *fujikuroi*, were isolated in moderate frequency and emerged in 41.6 % of samples and 3.2 % and 3.5 % of total fungi, respectively.

*Gymnoascus roseum* was isolated in moderate frequency and emerged in 30.5 % of samples and 4.9 % of total fungi. *Acremonium* (represented by *A. curvulum* and *A. strictum*) was isolated in moderate frequency and emerged in 25.0 % of samples matching 1.8 % of total fungi.

The results in Table 4.7 and Figure 4.2 indicated the *Rhodotorula rubra* (Plate A.69) was isolated in moderate frequency and emerged in 25.0 % of samples and 4.9 % of total fungi. *Candida albicans* was isolated in low frequency and emerged in 22.2 % samples matching 2.1 % of total fungi. *Mucor* (represented by *M. circinelloides* and *M. hiemalis*), *Cladosporium* (represented by *C. cladosporioides* and *C. oxysporum*) and *Rhizopus* (*R. arrhizus* and *R. oryzae*) were isolated in low frequency and emerged in 22.2 %, 16.6 % and 16.6 % of samples matching 1.9 %, 1.6 % and 2.3 % of total fungi, respectively.

*Chaetomum* (represented by *C. cochliobolus* and *C. globosum*), *Paecilomyces* (represented by *P. lilacinus* and *P. variotii*) were isolated in low frequency and emerged in 13.8 % of aerobic samples, respectively. *Chrysosporium tropicum* and *Scopulariopsis brevicaulis* were isolated in rare frequency and emerged in 11.1 % of aerobic samples, respectively.

*Gliocladium roseum* and *Phialophora verrucosa* (Plate A.67) were isolated in rare frequency and emerged in 5.5 % of the aerobic samples. While *Epicoccum nigrum*, *Oidiodendron griseum* and *Syncephalastrum racemosum* (Plates A. 34, 50, and 76), were isolated in rare frequency and emerged in 2.7 % of aerobic samples, respectively (Table 4.7 and Figure 4.2).

#### 4.2.2.2. Fungi recovered from anoxic activated sludge samples

Fifty-three species representing 25 genera were collected from 36 anoxic activated sludge samples on Malt extract agar at 30 $^{o}$C for 1-2 weeks (Table 4.7 and Figure 4.2).

**Table 4.7:** Numbers of cases of isolation (NCI) out of 36, percentage frequency and occurrence remarks (OR) of fungal genera and species recovered from each aerobic and anoxic activated sludge samples with MBRs on Malt extract agar media at 30 °C

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| *Acremonium* | 9 | 25.0 | M | 7 | 19.4 | L |
| *A. curvulum* W. Gams | 2 | 5.5 | R | 3 | 8.3 | R |
| *A. strictum* W. Gams | 7 | 19.4 | L | 4 | 11.1 | R |
| *Alternaria* | 15 | 41.6 | M | 6 | 16.6 | L |
| *A. alternata* (Fr.) Keissl. | 5 | 13.8 | L | 2 | 5.5 | R |
| *A. chlamydospora* Mouch. | 12 | 33.3 | M | 4 | 11.1 | R |
| *Aspergillus* | 30 | 83.3 | H | 32 | 88.8 | H |
| *A. alutaceus* Berk. & M.A. Curtis var. *alutaceus* | 4 | 11.1 | R | 6 | 16.6 | L |
| *A. candidus* Link | 3 | 8.3 | R | 2 | 5.5 | R |
| *A. flavus* Raper & Fennell var. *columnaris* | 10 | 27.7 | M | 5 | 13.8 | R |
| *A. flavus* Link var. *flavus* | 22 | 61.1 | H | 13 | 36.1 | M |
| *A. fumigatus* Fresen. | 19 | 52.7 | H | 21 | 58.3 | H |
| *A. niger* sensu auct. pro parte, pre | 11 | 30.5 | M | 15 | 41.6 | M |
| *A. oryzae* (Ahlb.) E. Cohn | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. sydowii* (Bainier & Sartory) Thom & Church | 1 | 2.7 | R | - | - | - |
| *A. terreus* Fennell & Raper var. *africanus* | 5 | 13.8 | R | 3 | 8.3 | R |
| *A. terreus* Thom & Raper var. *aureus* | 2 | 5.5 | R | - | - | - |
| *A. terreus* Thom var. *terreus* | 6 | 16.6 | R | 3 | 8.3 | R |
| *A. ustus* (Bainier) Thom & Church | 1 | 2.7 | R | 2 | 5.5 | R |
| *A.versicolor* (Vuill.) Tirab. | 1 | 2.7 | R | - | - | - |
| *Candida albicans* (C.P. Robin) Berkhout | 8 | 22.2 | L | 6 | 16.6 | L |
| *Chaetomum* | 5 | 13.8 | L | 3 | 8.3 | R |
| *C. cochliodes* Palliser | 2 | 5.5 | R | 1 | 2.7 | R |
| *C. globosum* Kunze | 2 | 5.5 | R | 3 | 8.3 | R |
| *Chrysosporium tropicum* J.W. Carmich. | 4 | 11.1 | R | 3 | 8.3 | R |
| *Cladosporium* | 6 | 16.6 | L | 4 | 11.1 | R |
| *C. cladosporioides* (Fresen.) G.A. de Vries | 3 | 8.3 | R | 2 | 5.5 | R |
| *C. oxysporum* Berk. & M.A. Curtis | 4 | 11.1 | R | 2 | 5.5 | R |
| *Doratomyces stemonitis* (Pers.) F.J. Morton & G. Sm. | 17 | 47.2 | M | 11 | 30.5 | M |
| *Epicoccum nigrum* Link | 1 | 2.7 | R | - | - | - |
| *Fusarium* | 29 | 80.5 | H | 31 | 86.1 | H |
| *F. dimerum* Penz. | 16 | 44.4 | M | 7 | 19.4 | L |
| *F. oxysporum* Schltdl. | 8 | 22.2 | L | 14 | 38.8 | M |
| *F. solani* (Mart.) Sacc. | 18 | 50.0 | M | 12 | 33.3 | M |
| *Geotrichum candidum* Link | 34 | 94.4 | H | 31 | 86.1 | H |
| *Gibberella* | 15 | 41.6 | M | 9 | 25.0 | M |
| *G. acuminata* Wollenw. | 7 | 19.4 | L | 3 | 8.3 | R |
| *G. fujikuroi* (Sawada) Wollenw. var. *fujikuroi* | 12 | 33.3 | M | 8 | 22.2 | M |
| *Gliocladium roseum* Bainier | 2 | 5.5 | R | 4 | 11.1 | R |
| *Gymnoascus reesii* Baran. | 11 | 30.5 | M | 9 | 25.0 | M |
| *Mucor* | 8 | 22.2 | L | 4 | 11.1 | R |
| *M. circinelloides* Tiegh. | 7 | 19.4 | L | 4 | 11.1 | R |
| *M. hiemalis* Wehmer | 2 | 5.5 | R | - | - | - |
| *Oidiodendron griseum* Robak | 1 | 2.7 | R | 4 | 11.1 | R |
| *Paecilomyces* | 5 | 13.8 | L | 8 | 22.2 | L |
| *P. lilacinus* (Thom) Samson | 2 | 5.5 | R | 3 | 8.3 | R |
| *P. variotii* Bainier | 5 | 13.8 | L | 5 | 13.8 | L |
| *Penicillium* | 32 | 88.8 | H | 25 | 69.4 | H |
| *P. brevicompactum* Dierckx | 10 | 27.7 | M | 7 | 19.4 | L |
| *P. chrysogenum* Thom | 18 | 50.0 | M | 5 | 13.8 | L |
| *P. citrinum* Thom | 16 | 44.4 | M | 9 | 25.0 | M |
| *P. corylophilum* Dierckx | 4 | 11.1 | R | 1 | 2.7 | R |

**Table 4.7:** Continued

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| *P. duclauxii* Delacr. | - | - | - | 1 | 2.7 | R |
| *P. funiculosum* Thom | 3 | 8.3 | R | - | - | - |
| *P. glabrum* (Wehmer) Westling | 3 | 8.3 | R | 1 | 2.7 | R |
| *P. Janczewskii* K. M. Zalessky | - | - | - | 2 | 5.5 | R |
| *P. oxalicum* Currie & Thom | 5 | 13.8 | L | 2 | 5.5 | R |
| *P. purpurogenum* Stoll | 3 | 8.3 | R | 1 | 2.7 | R |
| *P. roqueforti* Thom | 9 | 25.0 | M | 2 | 5.5 | R |
| *Phialophora verrucosa* Medlar | 2 | 5.5 | R | 5 | 13.8 | L |
| *Rhizopus* | 6 | 16.6 | L | 2 | 5.5 | R |
| *R. arrhizus* A. Fisch. | 6 | 16.6 | R | 2 | 5.5 | R |
| *R. oryzae* Went & Prins. Geerl. | 1 | 2.7 | R | - | - | - |
| *Rhodotorula rubra* (Schimon) F.C. Harrison | 9 | 25.0 | M | 11 | 30.5 | M |
| *Scopulariopsis brevicaulis* (Sacc.) Bainier | 4 | 11.1 | R | 3 | 8.3 | R |
| *Syncephalastrum racemosum* Cohn ex J. Schröt. | 1 | 2.7 | R | 3 | 8.3 | R |
| *Trichoderma* | 18 | 50.0 | M | 25 | 69.4 | H |
| *T. hamatum* (Bonord.) Bainier | 5 | 13.8 | L | 6 | 16.6 | L |
| *T. koningii* Oudem. | 9 | 25.0 | M | 10 | 27.7 | M |
| *T. viride* Pers. | 4 | 11.1 | R | 18 | 50.0 | M |
| *Trichophyton terrestre* Durie & D. Frey | - | - | - | 1 | 2.7 | R |
| *yeasts* | 27 | 75.0 | H | 24 | 66.6 | H |
| Number of genera = 26 | 25 | | | 25 | | |
| Number of species = 60 | 57 | | | 53 | | |

NCI = Number of cases of isolation (out of 36)
% F = Percentage frequency of occurrence (calculated per 36 samples)
OR = Occurrence remarks: [H= High occurrence, isolated more than 18 cases (out of 36 samples)
M= Moderate occurrence, from 9 to 18 cases
L = Low occurrence, from 5 to 8 cases
R = Rare occurrence, less than 5 cases

The total count of fungi in anoxic activated sludge ranged between 9-71 colonies/mL activated sludge and the highest count was estimated in sample No. 18. *Aspergillus* was the most common genus and recovered in high frequency of occurrence 88.8 % of samples constituting 17.7 % of total fungi. It was represented by 10 species of which *A. fumigatus* was isolated in high frequency. *A. niger* and *A. flavus* var. *flavus* were isolated in moderate frequency. They emerged in 58.3 %, 41.6 % and 36.1 % of the samples matching 65.6 %, 46.8 % and 40.6 % of total *Aspergillus* and 5.5 %, 2.4 % and 2.1 % of total fungi, respectively. *A. alutaceus* var. *alutaceus*, *A. candidus*, *A. flavus* var. *columnaris*, *A. oryzae*, *A. terreus* var. *africanus*, *A. terreus* var. *terreus* and *A. ustus* were isolated in low or rare frequency. They emerged in 16.6 %, 16.6 %, 13.8 %, 2.7 %, 8.3 %, 8.3 % and 11.1 % of samples matching 18.7 %, 18.7 %, 15.6 %, 3.1 %, 9.3 %, 9.3 % and 12.5 % of total *Aspergillus*, respectively.

*Fusarium* occupied the second place in the number of cases of isolation and was recovered in high frequency of occurrence 86.1 % of sample constituting 6.4 % of total fungi. *Fusarium* was represented by 3 species of which *F. dimerum*, *F. oxysporum,* and *F. solani* were isolated in low

and moderate frequency and emerged in 19.4 %, 38.8 % and 33.3 % of samples matching 22.6 %, 45.2 % and 38.7 % of total *Fusarium* and 1.4 %, 2.3 % and 2.6 % of total fungi respectively.

*Geotrichum candidum* occupied the third place in the number of cases of isolation and was recovered in high frequency of occurrence 86.1 % of anoxic samples constituting 12.9 % of total fungi. *Penicillium* occupied the fourth place in the number of cases of isolation and was recovered from 69.4 % of samples constituting 11.6 % of total fungi. It was represented by 10 species of which *P. citrinum* was isolated in moderate frequency and *P. brevicompactum* and *P. chrysogenum* were isolated in low frequency emerged in 25.0 %, 19.4 % and 13.8 % of samples matching 36.0 %, 28.0 % and 20.0 % of total *Penicillium* and 2.3 %, 3.1 % and 3.76 % of total fungi (Table 4.7 and Figure 4.2).

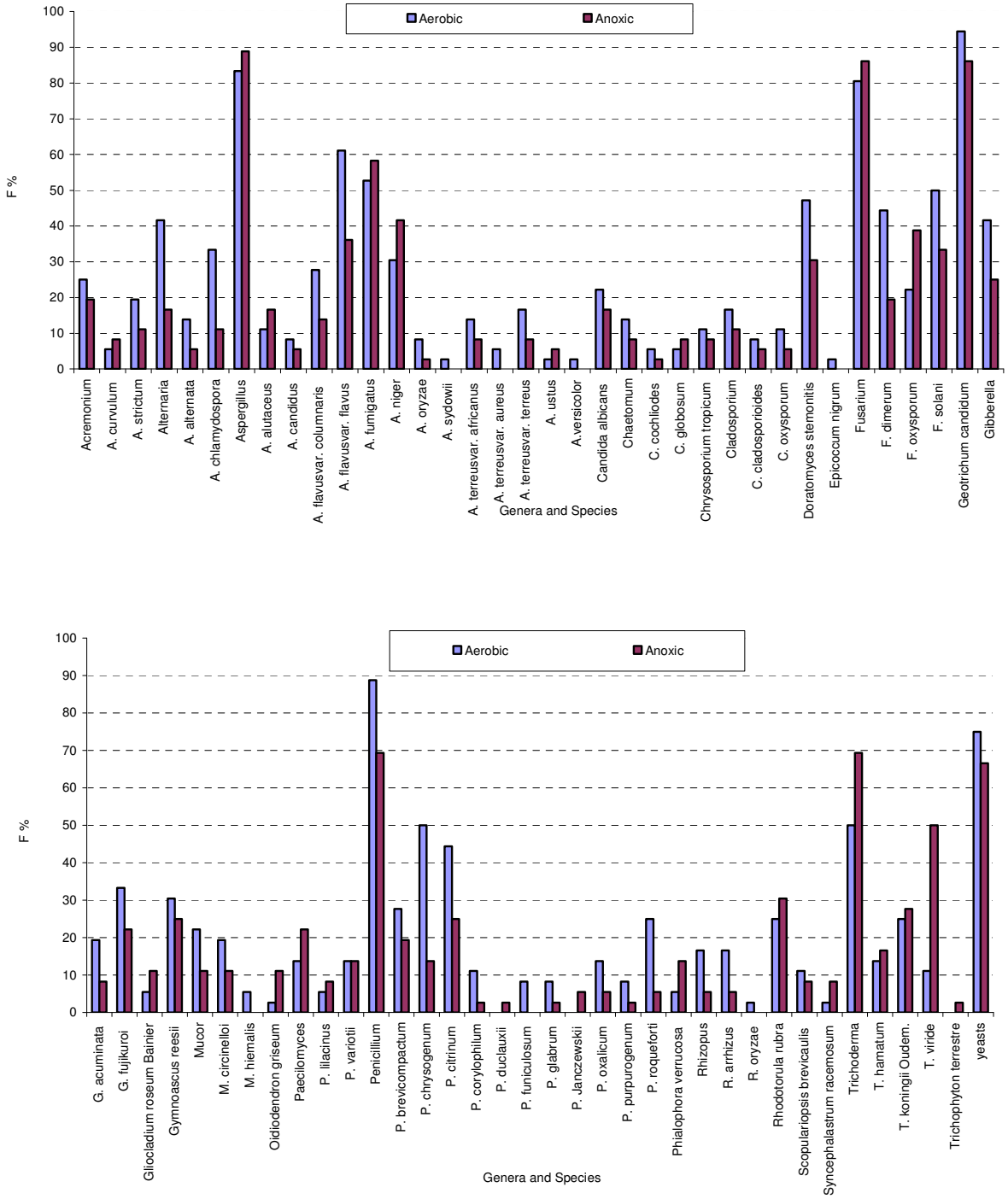
*Trichoderma* was recovered in high frequency of occurrence 69.4 % of samples constituting 7.5 % of total fungi and represented by 3 species. *T. hamatum, T. koningii* and *T. viride* were recovered from 16.6 %, 27.7 % and 50.0 % of samples matching 24.0 %, 40 % and 72.0 % of total *Trichoderma* and 1.6 %, 2.5 % and 3.4 % of total fungi, respectively. Unidentified *yeasts* were isolated in high frequency and recovered in 66.6 % of samples constituting 11.1 % of total fungi. *Doratomyces stemonitis* and *Rhodotorula rubra* were isolated in moderate frequency and recovered in 30.5 % of samples and constituting 3.1 % and 4.3 % of total fungi, respectively.

Also, the data presented in Table 4.7 indicate that *Gibberella* (represented by *G. acuminata* and *G. fujikuroi* var. *fujikuroi)* and *Gymnoascus reesii* were isolated in moderate frequency and emerged in 25.0 % of samples constituting 2.3 % and 3.9 % of total fungi, respectively. *Paecilomyces* (represented by *P. lilacinus* and *P. variotii*) and *Acremonium* (represented by *A. curvulum* and *A. strictum*) were isolated in low frequency and emerged in 22.2 % and 19.4 % of samples and 1.6 % and 2.4 % of total fungi, respectively. *Candida albicans* (Plate A.23) was isolated in low frequency and emerged in 16.6 % of samples constituting 2.5 % of total fungi (Table 4.7 and Figure 4.2).

*Alternaria (represented* by *A. alternata* and *chlamydospora)* and *Phialophora verrucosa* were isolated in low frequency and emerged in 16.6 % and 13.8 % of samples and 1.9 % and 1.1 % of total fungi, respectively. *Cladosporium* (*C. cladosporioides* and *C. oxysporum*), *Gliocladium roseum*, *Mucor circinelloides* and *Oidiodendron griseum* were isolated in rare frequency and emerged in 11.1 % of the anoxic samples and 1.4 % , 1.1 %, 0.9 % and 0.8 % of total fungi, respectively.

*Chaetomum* (represented by *C. cochliobolus* and *C. globosum*), *Chrysosporium tropicum, Scopulariopsis brevicaulis* and *Syncephalastrum racemosum* were isolated in rare frequency and

emerged in 8.3 % of anoxic samples, respectively. *Rhizopus arrhizus*, *Stemphylium vesicarium* (Plate A.75) and *Trichophyton terrestre* were isolated in rare frequency and emerged in 5.5 % and 2.7 % of samples, respectively (Table 4.7 and Figure 4.2).




% F = Percentage frequency of occurrence (calculated per 36 samples)

**Fig. 4.2:** The comparison between percentage frequencies of fungal genera occurrence in aerobic and anoxic activated sludge on Malt extract agar media

### 4.2.3. Rose bengal chloramphenicol agar (RBCA)

Sixty species belonging to 27 genera were collected from 36 aerobic and anoxic activated sludge samples on (RBCA) medium at 30 $^{o}$C for 1-2 weeks Table 4.8 and Figure 4.3. The *Geotrichum* was found at 88.8 % followed by unidentified *yeasts* 77.1 %, *Penicillium* 75.0 % and *Trichoderma* 55.5 % genera were the most prevalent in all activated sludge samples. For more clarification the results were described in both aerobic and anoxic activated sludge as follows.

#### 4.2.3.1. Fungi recovered from aerobic activated sludge samples

Fourty-six species representing 25 genera were collected from 36 samples on rose bengal cloramphenicol agar at 30 $^{o}$C for 1-2 weeks Table 4.8 and Figure 4.3. The total count of fungi in aerobic activated sludge ranged between 12-115 colonies/mL activated sludge and the highest count was estimated in sample No. 24. *Geotrichum candidum* was the most common genus and was recovered in high frequency of occurrence in 88.8 % of samples constituting 14.7 % of total fungi.

The results in Table 4.8 reveal that *Penicillium* occupied the second place in the number of cases of isolation and was recovered from 75.0 % of samples constituting 12.7 % of total fungi. It was represented by 10 species of which *P. citrinum*, *P. brevicompactum* and *P. chrysogenum* were isolated in moderate and low frequency and they emerged in 30.5 %, 16.6 % and 19.4 % of samples matching 40.7 %, 22.2 %, and 25.9 % of total *Penicillium* and 3.8 %, 2.2 % and 2.2 % of total fungi (Plates A.54-61, 63-66).

Unidentified *yeasts* occupied the third place in the number of cases of isolation and were recovered from 65.7 % of samples constituting 13.0 % of total fungi. *Trichoderma* occupied the fourth place in the number of cases of isolation and was recovered from 55.5 % of samples constituting 8.2 % of total fungi. *T. hamatum* and *T. viride* were isolated in moderate and low frequency and recovered from 36.3 % and 22.2 % of samples matching 65.0 % and 40 % of total *Trichoderma* and 4.4 % and 3.6 % of total fungi, respectively (Table 4.8 and Figure 4.3).

*Aspergillus* was isolated in moderate frequency and comprised 47.2 % of samples constituting 12.0 % of total fungi. *Aspergillus* was represented by 8 species of which *A. flavus* var. *columnaris, A. fumigatus* and *A. niger* were isolated in moderate and low frequency. They emerged in 25.0 %, 22.2 % and 16.6 % of samples matching 52.9 %, 47.0 % and 35.3 % of total *Aspergillus* and 3.8 %, 2.8 % and 3.0 % of total fungi, respectively (Plates A.7, 16, and 20).

Also results presented in Table 4.8 and Figure 4.3 attained the *Doratomyces stemonitis*, *Candida albicans, Gymnoascus reesii* and *Rhodotorula rubra* were isolated in moderate frequency and comprised 47.2 %, 41.6 %, 41.6 % and 33.3 % of samples constituting 6.2 %, 4.6 %, 5.4 % and

6.4 % of total fungi. *Fusarium* (represented by *F. dimerum* and *F. solani*) was isolated in moderate frequency and comprised 27.7 % of samples 4.0 % of total fungi. *F. dimerum* and *F. solani* were isolated in low incidence, emerging in 13.8 % and 16.6 % of samples matching about 50.0 % and 60.0 % of total *Fusarium* and 1.4 % and 2.6 % of total fungi, respectively.

*Alternaria chlamydospora* was isolated in low frequency and comprised 25.0 % of samples constituting 2.2 % of total fungi. *Gibberella* (represented by *G. acuminata*, *G. avenacea* (Plate A.42) and *G. fujikuroi*) and *Mucor circinelloides* were isolated in low frequency and comprised 13.8 % of samples constituting 2.4 and 1.6 % of total fungi, respectively. *Trichophyton* [represented by *T. ajelloi* var. *ajelloi* and *T. equinum* (Plate A.81)] was isolated in low frequency and comprised 8.3 % of samples constituting 0.8 % of total fungi. *Chrysosporium tropicum*, *Cladosporium cladosporioides*, *Paecilomyces lilacinus*, *Rhizopus arrhizus*, *Stachybotrys elegans* and *Ulocladium microsporum* (Plate A.86) were isolated in rare frequency and comprised 5.5 % of samples matching, respectively. *Aurobasidium pullulans*, *Gliocladium roseum*, *Myrothesium cinctrum*, *Oidiodendron griseum*, *Scopulariopsis brevicaulis* and *Syncephalastrum racemosum* were isolated in rare frequency and comprised 2.7 % of samples (Table 4.8 and Figure 4.3).

#### 4.2.3.2. Fungi recovered from anoxic activated sludge samples

Fourty-two species belonging to 21 genera were collected from anoxic sludge samples during this investigation (Table 4.8 and Figure 4.3). The total count of fungi in aerobic activated sludge ranged between 25-134 colonies/mL activated sludge and the highest count was estimated in sample No. 14.

Unidentified *yeasts* were the most common genus and were recovered in high frequency of occurrence in 77.1 % of samples constituting 19.4 % of total fungi. *Geotrichum candidum* occupied the second place in the number of cases of isolation and was recovered from 75.0 % of samples constituting 9.1 % of total fungi.

Data in Table 4.8 showed that *Penicillium* was the third place in the number of cases of isolation and recovered from 61.1 % of samples constituting 18.9 % of total fungi. It was represented by 10 species of which *P. chrysogenum* and *P. citrinum* were isolated in low and moderate frequency emerged in 19.4 % and 41.6 % of samples matching 31.8 % and 68.2 % of total *Penicillium* and 4.5 % and 6.2 % of total fungi, respectively.

*Aspergillus* was isolated in moderate frequency and comprised 41.6 % of the samples constituting 14.1 % of total fungi. Seven species were identified from *Aspergillus* of which *A. flavus* var. *flavus* and var. *columnaris* were isolated in low frequency and recovered in 13.8 % and 19.4 %

of samples matching 25.0 % and 46.6 % of total *Aspergillus* and 2.8 % and 4.7 % of total fungi, respectively. *Doratomyces stemonitis* was isolated in moderated frequency and recovered from 30.5 % of samples constituting 5.0 % of total fungi (Table 4.8 and Figure 4.3).

**Table 4.8:** Numbers of cases of isolation (NCI) out of 36, percentage frequency and occurrence remarks (OR) of fungal genera and species recovered from each aerobic and anoxic activated sludge samples with MBRs on Rose bengal cloramphenicol agar media at 30 $^{o}$C

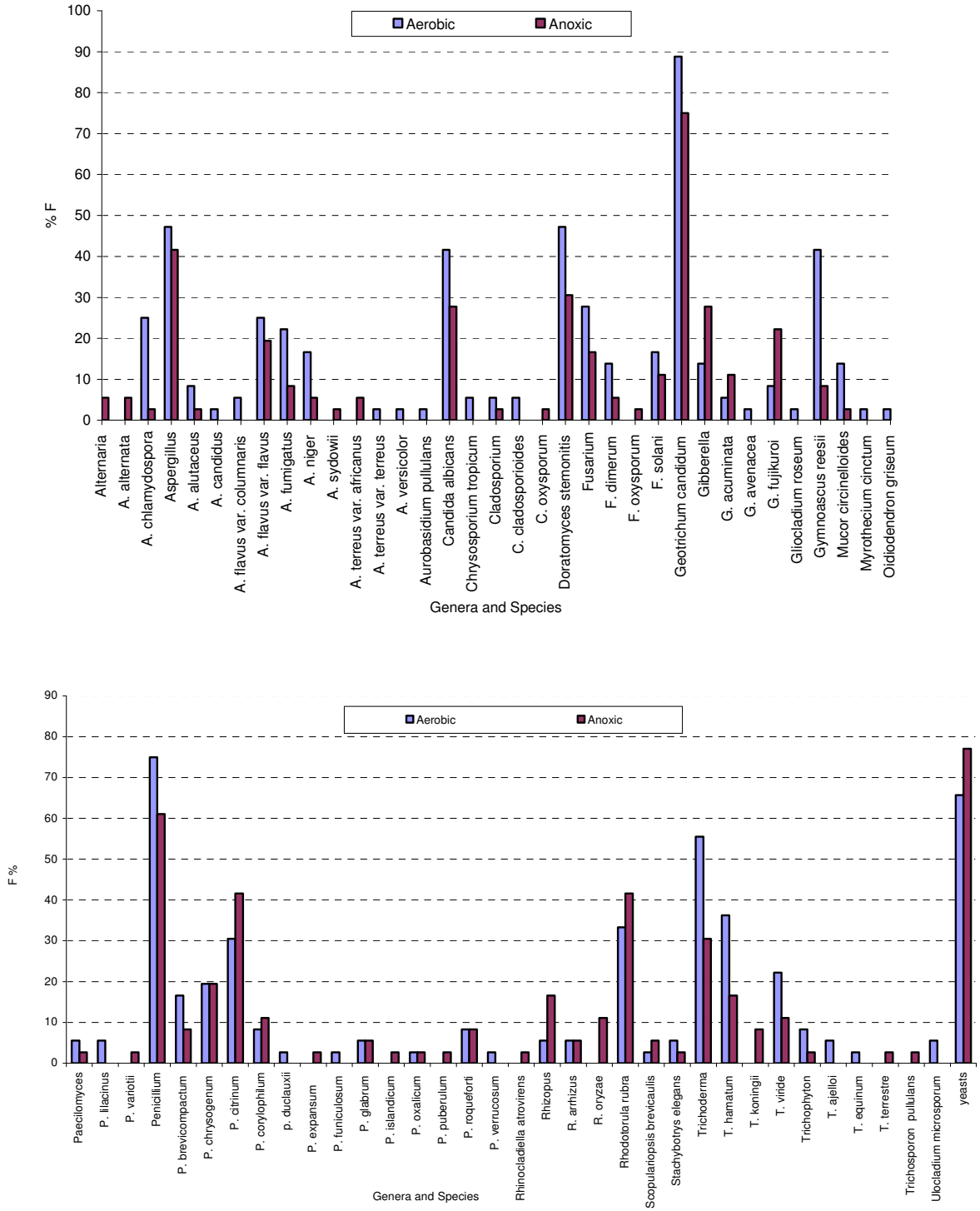
| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| *Alternaria* | 9 | 25.0 | M | 2 | 5.5 | R |
| *A. alternata* (Fr.) Keissl. | - | - | - | 2 | 5.5 | R |
| *A. chlamydospora* Mouch. | 9 | 25.0 | M | 1 | 2.7 | R |
| *Aspergillus* | 17 | 47.2 | M | 15 | 41.6 | M |
| *A. alutaceus* Berk. & M.A. Curtis var. *alutaceus* | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. candidus* Link | 1 | 2.7 | R | - | - | - |
| *A. flavus* Raper & Fennell var. *columnaris* | 2 | 5.5 | R | - | - | - |
| *A. flavus* Link var. *flavus* | 9 | 25.0 | M | 7 | 19.4 | L |
| *A. fumigatus* Fresen. | 8 | 22.2 | L | 3 | 8.3 | R |
| *A. niger* Tiegh. | 6 | 16.6 | L | 2 | 5.5 | R |
| *A.* sydowii (Bainier & Sartory) Thom & Church | - | - | - | 1 | 2.7 | R |
| *A. terreus* Fennell & Raper var. *africanus* | - | - | - | 2 | 5.5 | R |
| *A. terreus* Thom var. *terreus* | 1 | 2.7 | R | - | - | - |
| *A. versicolor* (Vuill.) Tirab. | 1 | 2.7 | R | - | - | - |
| *Aurobasidium pullulans* (de Bary) Arnaud | 1 | 2.7 | R | - | - | - |
| *Candida albicans* (C.P. Robin) Berkhout | 15 | 41.6 | M | 10 | 27.7 | M |
| *Chrysosporium tropicum* J.W. Carmich. | 2 | 5.5 | R | - | - | - |
| *Cladosporium* | 2 | 5.5 | R | 1 | 2.7 | R |
| *C. cladosporioides* (Fresen.) G.A. de Vries | 2 | 5.5 | R | - | - | - |
| *C. oxysporum* Berk. & M.A. Curtis | - | - | - | 1 | 2.7 | R |
| *Doratomyces stemonitis* (Pers.) F.J. Morton & G. Sm. | 17 | 47.2 | M | 11 | 30.5 | M |
| *Fusarium* | 10 | 27.7 | M | 6 | 16.6 | L |
| *F. dimerum* Penz. | 5 | 13.8 | L | 2 | 5.5 | R |
| *F. oxysporum* Schltdl. | - | - | - | 1 | 2.7 | R |
| *F. solani* (Mart.) Sacc. | 6 | 16.6 | L | 4 | 11.1 | R |
| *Geotrichum candidum* Link | 32 | 88.8 | H | 27 | 75.0 | H |
| *Gibberella* | 5 | 13.8 | L | 10 | 27.7 | M |
| *G. acuminata* Wollenw. | 2 | 5.5 | R | 4 | 11.1 | R |
| *G. avenacea* R.J. Cook | 1 | 2.7 | R | - | - | - |
| *G. fujikuroi* var. *fujikuroi* (Sawada) Wollenw. | 3 | 8.3 | R | 8 | 22.2 | L |
| *Gliocladium roseum* Bainier | 1 | 2.7 | R | - | - | - |
| *Gymnoascus reesii* Baran. | 15 | 41.6 | M | 3 | 8.3 | R |
| *Mucor circinelloides* Tiegh. | 5 | 13.8 | L | 1 | 2.7 | R |
| *Myrothecium cinctum* (Corda) Sacc. | 1 | 2.7 | R | - | - | - |
| *Oidiodendron griseum* Robak | 1 | 2.7 | R | - | - | - |
| *Paecilomyces* | 2 | 5.5 | R | 1 | 2.7 | R |
| *P. lilacinus* (Thom) Samson | 2 | 5.5 | R | - | - | - |
| *P. variotii* Bainier | - | - | - | 1 | 2.7 | R |
| *Penicillium* | 27 | 75.0 | H | 22 | 61.1 | H |
| *P. brevicompactum* Dierckx | 6 | 16.6 | L | 3 | 8.3 | R |
| *P. chrysogenum* Thom | 7 | 19.4 | L | 7 | 19.4 | L |
| *P. citrinum* Thom | 11 | 30.5 | M | 15 | 41.6 | M |

**Table 4.8:** Continued

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| *P. corylophilum* Dierckx | 3 | 8.3 | R | 4 | 11.1 | R |
| *p. duclauxii* Delacroix | 1 | 2.7 | R | - | - | - |
| *P. expansum* Link | - | - | - | 1 | 2.7 | R |
| *P. funiculosum* Thom | 1 | 2.7 | R | - | - | - |
| *P. glabrum* (Wehmer) Westling | 2 | 5.5 | R | 2 | 5.5 | R |
| *P. islandicum* Sopp | - | - | - | 1 | 2.7 | R |
| *P. oxalicum* Currie & Thom | 1 | 2.7 | R | 1 | 2.7 | R |
| *P. puberulum* Bainier | - | - | - | 1 | 2.7 | R |
| *P. roqueforti* Thom | 3 | 8.3 | R | 3 | 8.3 | R |
| *P. verrucosum* Dierckx var. *verrucosum* | 1 | 2.7 | R | - | - | - |
| *Rhinocladiella atrovirens* Nannf. | - | - | - | 1 | 2.7 | R |
| *Rhizopus* | 2 | 5.5 | R | 6 | 16.6 | R |
| *R. arrhizus* A.Fisch. | 2 | 5.5 | R | 2 | 5.5 | R |
| *R. oryzae* Went & Prins. Geerl. | - | - | - | 4 | 11.1 | R |
| *Rhodotorula rubra* (Schimon) F.C. Harrison | 12 | 33.3 | M | 15 | 41.6 | M |
| *Scopulariopsis brevicaulis* (Sacc.) Bainier | 1 | 2.7 | R | 2 | 5.5 | R |
| *Stachybotrys elegans* (Pidopl.) W. Gams | 2 | 5.5 | R | 1 | 2.7 | R |
| *Trichoderma* | 20 | 55.5 | H | 11 | 30.5 | M |
| *T. hamatum* (Bonord.) Bainier | 13 | 36.3 | M | 6 | 16.6 | L |
| *T. koningii* Oudem. | - | - | - | 3 | 8.3 | R |
| *T. viride* Pers. | 8 | 22.2 | L | 4 | 11.1 | R |
| *Trichophyton* | 3 | 8.3 | R | 1 | 2.7 | R |
| *T. ajelloi* (Vanbreus.) Ajello *var. ajelloi* | 2 | 5.5 | R | - | - | - |
| *T. equinum* Gedoelst | 1 | 2.7 | R | - | - | - |
| *T. terrestre* Durie & D. Frey | - | - | - | 1 | 2.7 | R |
| *Trichosporon pullulans* Lodder(Lindner) Diddens & Lodder | - | - | - | 1 | 2.7 | R |
| *Ulocladium microsporum* Moub. & Abdel-Hafez | 2 | 5.5 | R | - | - | - |
| *yeasts* | 23 | 65.7 | H | 27 | 77.1 | H |
| Number of genera = 27 | 25 | | | 21 | | |
| Number of species = 60 | 46 | | | 42 | | |

NCI = Number of cases of isolation (out of 36)
% F = Percentage frequency of occurrence (calculated per 36 samples)
OR = Occurrence remarks: [H= High occurrence, isolated more than 18 cases (out of 36 samples)
M = Moderate occurrence, from 9 to 18 cases
L = Low occurrence, from 5 to 8 cases
R = Rare occurrence, less than 5 cases

*Trichoderma* was isolated in moderate frequency and comprised 30.5 % of samples constituting 6.7 % of total fungi. From the genus 3 species were identified *T. hamatum*, *T. koningii,* and *T. viride*. They emerged in 16.6 %, 8.3 % and 11.1 % of the samples matching 54.4 %, 22.3 % and 36.4 % of total *Trichoderma* and 2.6 %, 1.6 % and 2.3 % of total fungi, respectively. *Candida albicans* and *Gibberella* (represented by *G. acuminata* and *G. fujikuroi* var. *fujikuroi*) were isolated in moderate frequency occurrence matching collectively 27.7 % of samples and 4.3 % and 3.5 % of total fungi, respectively.




% F = Percentage frequency of occurrence (calculated per 36 samples)

**Fig. 4.3:** The comparison between percentage frequencies of fungal genera occurrence in aerobic and anoxic activated sludge on Rose bengal cloramphenicol agar media

*Fusarium* (represented by *F. dimerum, F. oxysporum* and *F. solani*) and *Rhizopus* (represented by *R. arrhizus* and *R. oryzae*) were isolated in low frequency occurrence matching collectively 16.6 % of samples, 2.2 % and 3.4 % of total fungi, respectively. *Gymnoascus reesii* was recovered from 8.3 % of samples constituting 1.2 % of total fungi. *Alternaria* (represented by *A. alternata* and *A. chlamydospora*) and *Scopulariopsis brevicaulis* were isolated in rare frequency and comprised 5.5 % of all samples.

*Cladosporium oxysporum*, *Mucor circinelloides*, *Paecilomyces variotii*, *Rhinocladiella atrovirens* (Plate A.68), *Stachybotrys elegans*, *Trichophyton terrestre* and *Trichosporon pullulans* (Plate A.84) were isolated in rare frequency and comprised 2.7 % of the anoxic activated sludge samples (Table 4.8 and Figure 4.3).

### 4.2.4. Sabouraoud's dextrose agar (SDA)

Sixty-one species belonging to 29 genera were collected from 36 of each aerobic and anoxic activated sludge samples on Sabouraoud's dextrose agar medium at 30 $^{o}$C for 1-2 weeks. The obtained data show that, *Geotrichum* was found at 100 % followed by *Fusarium* 91.6%, *Aspergillus* 77.7 %, unidentified *yeasts* 63.8 % and *Penicillium* 50.0 %. These genera were the most prevalent in all activated sludge samples (Table 4.9 and Figure 4.4). The results were described in both aerobic and anoxic activated sludge as follows.

#### 4.2.4.1. Fungi recovered from aerobic activated sludge samples

Fifty-four species representing 26 genera were collected from 36 samples on Sabouraoud's dextrose agar media at 30 $^{o}$C for 1-2 weeks (Table 4.9 and Figure 4.4). The total count of fungi in aerobic activated sludge ranged between 15-141 colonies/mL activated sludge and the highest count was estimated in sample No. 18. *Geotrichum candidum* was the most common genus and was recovered from all samples constituting frequency of occurrence 100 % of samples constituting 15.3 % of total fungi and the highest count was estimated in sample No. 27.

The results obtained from aerobic activated sludge (Table 4.9 and Figure 4.4) indicate that *Fusarium* occupied the second place in the number of cases of isolation and was recovered from 72.2 % of samples constituting 9.2 % of total fungi. It was represented by 3 species, *F. dimerum*, *F. oxysporum* and *F. solani* which were isolated in moderate and low frequency occurrence matching collectively 33.3 %, 27.7 % and 19.4 % of samples, 46.2 %, 38.5 % and 26.9 % of total *Fusarium*, 4.7 %, 2.4 % and 2.0 % of total fungi, respectively. Unidentified *yeasts* occupied the third place in the number of cases of isolation and were recovered from 61.1 % of samples constituting 14.8 % of total fungi.

*Aspergillus* (represented by 11 species) was isolated in moderate frequency of occurrence and emerged in 55.5 % of samples constituting 9.7 % of total fungi. *A. flavus* var. *columnaris* was recovered 30.5 % frequency of samples, 55.0 % of total *Aspergillus* and 4.67 % of total fungi.

*Penicillium* (represented by 7 species) was isolated in moderate frequency of occurrence and emerged in 50.0 % of samples constituting 13.83 % of total fungi. *P. chrysogenum* and *P. citrinum* were isolated in moderate frequency of occurrence and emerged in 25.0 and 27.7 % of samples, 50.0 % and 55.5 % of total *Penicillium* and 4.1 % and 3.5 % of total fungi, respectively (Table 4.9 and Figure 4.4).

Also, the data resulted (Table 4.9) in aerobic case showed that *Trichoderma* was recovered from 41.6 % of samples constituting 5.4 % of total fungi. *T. koningii*; *T. hamatum* and *T. viride* were isolated in low and rare frequency occurrence matching collectively 11.1 %, 19.4 % and 16.6 % of samples matching 26.6 %, 46.6 % and 40 % of total *Trichoderma* and 1.0 %, 1.4 % and 2.8 % of total fungi, respectively. *Doratomyes* stemonitis was recovered from 33.3 % of samples constituting 3.4 % of total fungi.

*Alternaria* (represented by *A. alternata* and *A. chlamydospora*) and *Scopulariopsi*s (represented *S. brevicaulis* and *S. asperula*) were recovered from 27.2 % of the samples matching 3.2 % and 2.8 % of total fungi, respectively. *A. chlamydospora* was isolated in low incidence, emerging in 22.2 % of samples, matching about 80 % of total *Alternaria* and 2.2 % of total fungi. While *Scopulariopsis brevicaulis* was recovered from 16.6 % of samples, matching about 60.0 % of total *Scopulariopsis* and 1.9 % of total fungi (Table 4.9 and Figure 4.4).

*Gibberella* (represented by *G. acuminata* and *G. fujikuroi* var. *fujikuroi*) was recovered from 25 % of samples and 2.9 % of total fungi. *G. accuminata* and *G. fujikuroi* var. *fujikuroi* were isolated in low incidence, emerging in 16.6 % and 13.8 % of samples matching 66.6 % and 55.5 % of total *Gibberella* and 1.7 % and 1.3 % of total fungi. *Chrysosporium tropicum* is a dermatophyte fungus that was isolated in low frequency and comprised 19.4 % of samples and 1.6 % of total fungi.

Results in Table 4.9 reveal that, *Acremonium* (represented by *A. curvulum*, *A. rutilum,* and *A. strictum*), *Cladosporium* (represented by *C. cladosporioides* and *C. oxysporum*), *Gymnoascus reesii* (Plate A.46) and *Ulocladium chartarum* (Plate A.85) were isolated in low frequency of occurrence matching collectively 16.6 % of samples constituting 2.9 %, 2.0 %, 2.8 % and 1.5 % total fungi, respectively. *Chaetomum* (represented by *C. cochliodes* and *C. globosum*) and *Rhizopus*

(represented by *R. arrhizus* and *R. oryzae*) were recovered from 13.8 % of the samples matching 1.3 % and 1.9 % of total fungi, respectively.

*Mucor circinelloides, Paecilomyces variotii* and *Phialophora verrucosa* were isolated in rare frequency and comprised 11.1 %, 11.1 % and 8.3 % of samples, respectively. *Aurobasidium pullulans*, *Chaetomum cochliodes*, *Geosmithia lavendula* (Plate A.39), *Gliocladium roseum*, *Stachybotrys chartarum*, *stemphylum vesicarium* and *Syncephalastrum racemosum* were less frequently recovered and had a frequency between 2.7-5.5 % of samples (Table 4.9 and Figure 4.4).

#### 4.2.4.2. Fungi recovered from anoxic activated sludge samples

Thirty-nine species belonging to 21 genera were collected from aerobic samples during this investigation (Table 4.9 and Figure 4.4). The total count of fungi in aerobic activated sludge ranged between 9-127 colonies/mL activated sludge and the highest count was estimated in sample No. 26.

*Geotrichum candidum* was the most common genus and was recovered from samples, constituting frequency of occurrence 94.4 % of samples constituting 18.6 % of total fungi. *Fusarium* occupied the second place in the number of cases of isolation and was recovered from 91.6 % of samples constituting 10.7 % of total fungi. It was represented by 3 species of which *F. dimerum*, *F. oxysporum* and *F. solani* were isolated in moderate and low frequency in 25.0 %, 47.2 % and 22.2 % of samples matching 27.3 %, 51.5 % and 24.2 % of total *Fusarium* and 3.2 %, 5.5 %, and 2.0 % of total fungi, respectively.

Results recorded in Table 4.9 show that, *Aspergillus* occupied the third place in the number of cases of isolation and was recovered from 77.7 % of samples constituting 13.6 % of total fungi. From the genus 6 species were isolated of which *A. flavus* var. *flavus*, *A. flavus* var. *columnaris*, *A. fumigatus* and *A. niger* were recovered in 13.8 %, 19.4 %, 25.0 % and 27.7 % of frequency of all samples, 17.6 %, 25.0 %, 32.1 % and 35.7 % of total *Aspergillus* and 2.3 %, 4.0 %, 1.9 % and 4.9 % of total fungi, respectively.

Unidentified *yeasts* occupied the fourth place in the number of cases of isolation and were recovered from 63.8 % of samples constituting 17.78 % of total fungi. *Penicillium* was present in moderate frequency and recovered from 50.0 % samples constituting 10.04 % of total fungi. From the genus 6 species were isolated of which *P. chrysogenum* and *P. citrinum* were recovered in 16.6 % and 13.8 % of the samples, matching 33.3 % and 27.7 % of total *Penicillium* and 2.9 % and 2.3 % of total fungi, respectively.

**Table 4.9:** Numbers of cases of isolation (NCI) out of 36, percentage frequency and occurrence remarks (OR) of fungal genera and species recovered from each aerobic and anoxic activated sludge samples with MBRs on Sabouraoud's dextrose agar media at 30 °C

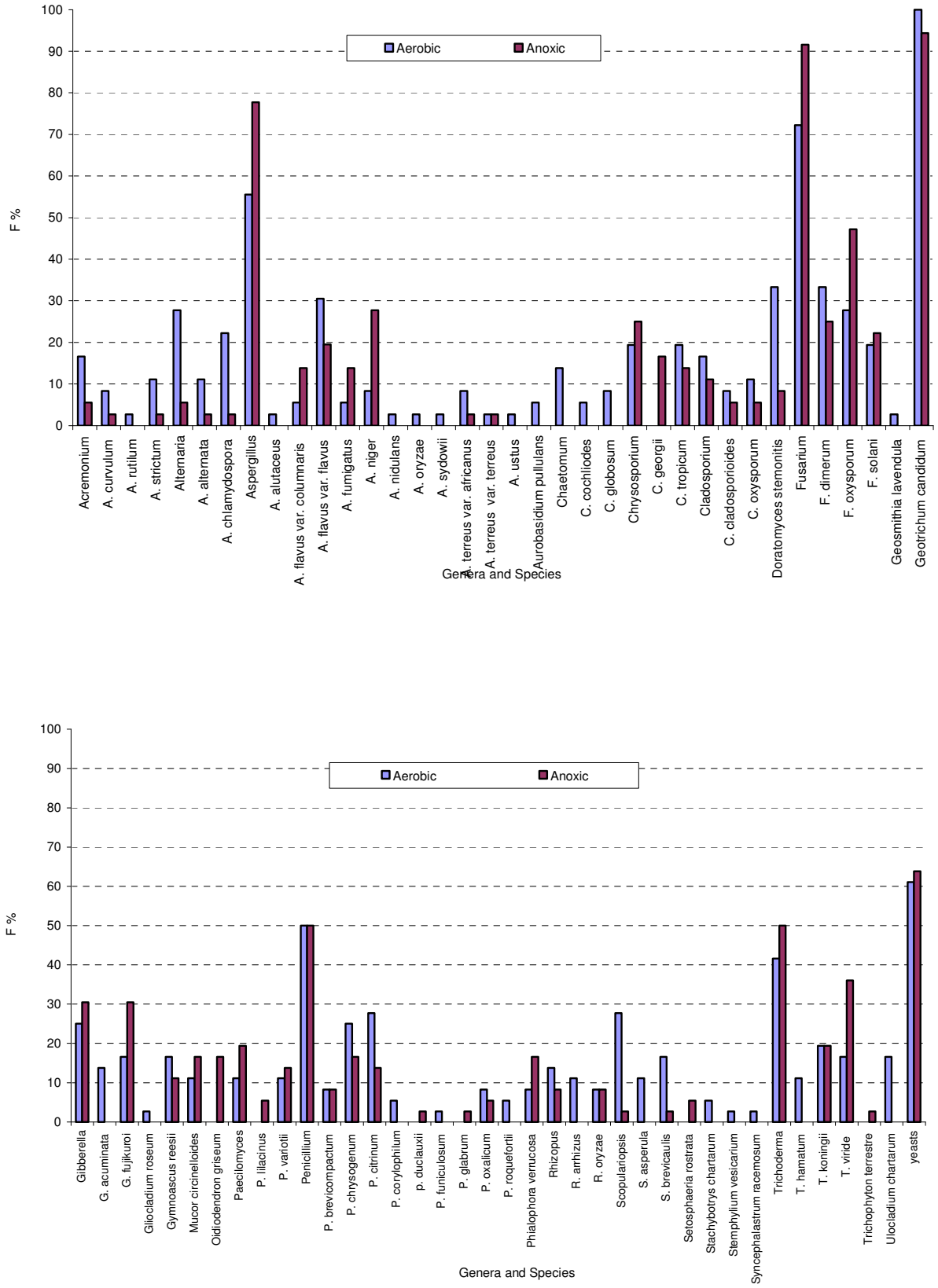
| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| *Acremonium* | 6 | 16.6 | L | 2 | 5.5 | R |
| *A. curvulum* W. Gams | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. rutilum* W. Gams | 1 | 2.7 | R | - | - | - |
| *A. strictum* W. Gams | 4 | 11.1 | R | 1 | 2.7 | R |
| *Alternaria* | 10 | 27.7 | M | 2 | 5.5 | R |
| *A. alternata* (Fr.) Keissl. | 4 | 11.1 | R | 1 | 2.7 | R |
| *A. chlamydospora* Mouch. | 8 | 22.2 | L | 1 | 2.7 | R |
| *Aspergillus* | 20 | 55.5 | H | 28 | 77.7 | H |
| *A. alutaceus* Berk. & M.A. Curtis var. *alutaceus* | 1 | 2.7 | R | - | - | - |
| *A. flavus* Raper & Fennell var. *columnaris* | 2 | 5.5 | R | 5 | 13.8 | L |
| *A. flavus* Link var. *flavus* | 11 | 30.5 | M | 7 | 19.5 | L |
| *A. fumigatus* Fresen. | 2 | 5.5 | R | 5 | 13.8 | L |
| *A. niger* Tiegh. | 3 | 8.3 | R | 10 | 27.7 | M |
| *A. nidulans (Emericella nidulans)* (Eidam) G. Winter | 1 | 2.7 | R | - | - | - |
| *A. oryzae* (Ahlb.) E. Cohn | 1 | 2.7 | R | - | - | - |
| *A. sydowii* (Bainier & Sartory) Thom & Church | 1 | 2.7 | R | - | - | - |
| *A. terreus* Fennell & Raper var. *africanus* | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. terreus* Thom var. *terreus* | 1 | 2.7 | R | 1 | 2.7 | R |
| *A. ustus* (Bainier) Thom & Church | 1 | 2.7 | R | - | - | - |
| *Aurobasidium pullulans* (de Bary) Arnaud | 2 | 5.5 | R | - | - | - |
| *Chaetomum* | 5 | 13.8 | L | - | - | - |
| *C. cochliodes* Palliser | 2 | 5.5 | R | - | - | - |
| *C. globosum* Kunze | 3 | 8.3 | R | - | - | - |
| *Chrysosporium* | 7 | 19.4 | L | 9 | 25.0 | M |
| *C. georgii* (Varsavsky & Ajello) Oorschot | - | - | - | 6 | 16.6 | L |
| *C. tropicum* J.W. Carmich. | 7 | 19.4 | L | 5 | 13.8 | L |
| *Cladosporium* | 6 | 16.6 | L | 4 | 11.1 | R |
| *C. cladosporioides* (Fresen.) G.A. de Vries | 3 | 8.3 | R | 2 | 5.5 | R |
| *C. oxysporum* Berk. & M.A. Curtis | 4 | 11.1 | R | 2 | 5.5 | R |
| *Doratomyces stemonitis* (Pers.) F.J. Morton & G. Sm. | 12 | 33.3 | M | 3 | 8.3 | R |
| *Fusarium* | 26 | 72.2 | H | 33 | 91.6 | H |
| *F. dimerum* Penz. | 12 | 33.3 | M | 9 | 25.0 | M |
| *F. oxysporum* Schltdl. | 10 | 27.7 | M | 17 | 47.2 | M |
| *F. solani* (Mart.) Sacc. | 7 | 19.4 | L | 8 | 22.2 | L |
| *Geosmithia lavendula* (Raper & Fennell) Pitt | 1 | 2.7 | R | - | - | - |
| *Geotrichum candidum* Link | 36 | 100.0 | H | 34 | 94.4 | H |
| *Gibberella* | 9 | 25.0 | M | 11 | 30.5 | M |
| *G. acuminata* Wollenw. | 5 | 13.8 | L | - | - | - |
| *G. fujikuroi* (Sawada) Wollenw. var. *fujikuroi* | 6 | 16.6 | L | 11 | 30.5 | M |
| *Gliocladium roseum* Bainier | 1 | 2.7 | R | - | - | - |
| *Gymnoascus reesii* Baran. | 6 | 16.6 | L | 4 | 11.1 | R |
| *Mucor circinelloides* Tiegh. | 4 | 11.1 | R | 6 | 16.6 | L |
| *Oidiodendron griseum* Robak | - | - | - | 6 | 16.6 | L |
| *Paecilomyces* | 4 | 11.1 | R | 7 | 19.4 | L |
| *P. lilacinus* (Thom) Samson | - | - | - | 2 | 5.5 | R |
| *P. variotii* Bainier | 4 | 11.1 | R | 5 | 13.8 | L |
| *Penicillium* | 18 | 50.0 | M | 18 | 50.0 | M |
| *P. brevicompactum* Dierckx | 3 | 8.3 | R | 3 | 8.3 | R |

**Table 4.9:** Continued

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | **NCI** | **%F** | **OR** | **NCI** | **%F** | **OR** |
| *P. chrysogenum* Thom | 9 | 25.0 | M | 6 | 16.6 | L |
| *P. citrinum* Thom | 10 | 27.7 | M | 5 | 13.8 | L |
| *P. corylophilum* Dierckx | 2 | 5.5 | R | - | - | - |
| *p. duclauxii* Delacroix | - | - | - | 1 | 2.7 | R |
| *P. funiculosum* Thom | 1 | 2.7 | R | - | - | - |
| *P. glabrum* (Wehmer) Westling | - | - | - | 1 | 2.7 | R |
| *P. oxalicum* Currie & Thom | 3 | 8.3 | R | 2 | 5.5 | R |
| *P. roquefortii* Thom | 2 | 5.5 | R | - | - | - |
| *Phialophora verrucosa* Medlar | 3 | 8.3 | R | 5 | 16.6 | L |
| *Rhizopus* | 5 | 13.8 | L | 3 | 8.3 | R |
| *R. arrhizus* A. Fisch. | 4 | 11.1 | R | - | - | - |
| *R. oryzae* Went & Prins. Geerl. | 3 | 8.3 | R | 3 | 8.3 | R |
| *Scopulariopsis* | 10 | 27.7 | M | 1 | 2.7 | R |
| *S. asperula* (Sacc.) S. Hughes | 4 | 11.1 | R | - | - | - |
| *S. brevicaulis* (Sacc.) Bainier | 6 | 16.6 | L | 1 | 2.7 | R |
| *Setosphaeria rostrata* K.J. Leonard | - | - | - | 2 | 5.5 | R |
| *Stachybotrys chartarum* (Ehrenb.) S. Hughes | 2 | 5.5 | R | - | - | - |
| *Stemphylium vesicarium* (Wallr.) E.G. Simmons | 1 | 2.7 | R | - | - | - |
| *Syncephalastrum racemosum* Cohn ex J. Schröt. | 1 | 2.7 | R | - | - | - |
| *Trichoderma* | 15 | 41.6 | M | 18 | 50.0 | M |
| *T. hamatum* (Bonord.) Bainier | 4 | 11.1 | R | - | - | - |
| *T. koningii* Oudem. | 7 | 19.4 | L | 7 | 19.4 | L |
| *T. viride* Pers. | 6 | 16.6 | L | 13 | 36.1 | M |
| *Trichophyton terrestre* Durie & D. Frey | - | - | - | 1 | 2.7 | R |
| *Ulocladium chartarum* (Preuss) E.G. Simmons | 6 | 16.6 | L | - | - | - |
| *yeasts* | 22 | 61.1 | H | 23 | 63.8 | H |
| Number of genera = 29 | 26 | | | 21 | | |
| Number of species = 61 | 54 | | | 39 | | |

NCI = Number of cases of isolation (out of 36)
% F = Percentage frequency of occurrence (calculated per 36 samples)
OR = Occurrence remarks: [H= High occurrence, isolated more than 18 cases (out of 36 samples)
M= Moderate occurrence, from 9 to 18 cases
L = Low occurrence, from 5 to 8 cases
R = Rare occurrence, less than 5 cases

*Trichoderma* (represented by *T. koningii* and *T. viride*) was isolated in moderate frequency and recovered from 50 % of the samples constituting 7.2 % of total fungi. *T. koningii* and *T. viride* were isolated in low and moderate frequency of occurrence, emerging in 19.4 and 36.1 % of samples, matching about 38.8 % and 72.2 % of total *Trichoderma* and 2.78 % and 4.5 % of total fungi, respectively. *Chrysosporium* (represented by *C. tropicum* and *C. georgii*) was isolated in moderate frequency and recovered from 25.0 % of samples and 2.5 % of total fungi. *C. tropicum* and *C. georgii* were isolated in low frequency of occurrence, emerging in 13.8 % and 16.6 % of the samples, matching about 55.5 % and 66.6 % of total *Chrysosporium* and 1.2 % of total fungi, respectively (Table 4.9 and Figure 4.4).




% F = Percentage frequency of occurrence (calculated per 36 samples)

**Fig. 4.4:** The comparison between percentage frequencies of fungal genera occurrence in aerobic and anoxic activated sludge on Sabouraoud's dextrose agar media

*Gibberella fujikuroi* var. *fujikuroi* and *Paecilomyces* (*P. lilacinus* and *P. variotii*) were isolated in low frequency and comprised 30.5 %, 25.0 % and 19.4 % of samples matching 3.8 % and 1.9 % of total fungi, respectively. *Mucor circinelloides*, *Phialophora verrucosa* and *Oidiodendron griseum* were isolated in rare frequency of occurrence, matching collectively 16.6 % of samples, 1.6 %, 1.6 % and 1.4 % of total fungi.

*Cladosporium* (represented by *C. Cladosporium* and *C. oxysporum*), *Gymnoascus reesii*, *Doratomyces stemonitis* and *Rhizopus oryzae* were isolated in rare frequency and comprised 11.1 %, 11.1 %, 8.3 % and 8.3 % of samples and 1.0 %, 2.3 %, 2.7 % and 0.6 % of total fungi, respectively.

Also, data in Table 4.9 and Figure 3.4 indicate that *Acremonium (*represented by *curvulum*, *A. strictum*), *Alternaria* (*A. alternata*, *A. chlamydospora*), *Cladosporium (C. cladosporioides*, *C. oxysporum*), *Oidiodendron griseum*, *Paecilomyces lilacinus*, *Scopulariopsis brevicaulis*, *Setosphaeria rostrata*, and *Trichophyton terrestre* were less frequent and recovered from 2.7-5.5 % of all anoxic activated sludge samples.

### 4.2.5. Sabouraoud's dextrose agar with cycloheximide and chloramphenicol (SDACC)

Fourty-six of keratinophilic species representing 21 genera were collected from 36 of each aerobic and anoxic activated sludge samples on this medium.The obtained data show that, *Aspergillus* was found at 55.5 % followed by *Chrysosporium* 41.6 %, *Penicillium* 36.1 % and *Geotrichum* 30.5 %, of samples. These genera were the most prevalent in all activated sludge samples (Table 4.10 and Figure 4.5). The results were described in both aerobic and anoxic activated sludge as follows.

#### 4.2.5.1. Fungi recovered from aerobic activated sludge samples

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Fourty- one species belonging to 21 genera were collected from aerobic samples during this investigation (Table 4.10 and Figure 4.5). *Aspergillus* was the most common genus and occupied the first place in the number of cases of isolation and was recovered in 55.5 % of the samples. From the genus 7 species were isolated of which *A. flavus* var. *flavus* and *A. fumigatus* were present in moderate and low frequency and recovered in 25.0 % and 13.8 % of the samples matching 45.0 and 25.0 % of total *Aspergillus* (Plates A.6-15).

The results in Table 4.10 indicate that, *Chrysosporium* occupied the second place in the number of cases of isolation and was recovered in 41.6 % of samples. From this genus 5 species were isolated of which, *C. asperatum*, *C. georgii*, *C. indicum*, *C. keratinophilum* and *C. tropicum*

(Plates A.26-29) were recovered in 8.3 %, 8.3 %, 19.4 % and 5.5 % of samples matching 20.0 %, 20.0 %, 46.0 % and 13.3 % of total *Chrysosporium*, respectively.

Also, results in Table 4.10 and Figure 3.5 showed that *Penicillium* occupied the third place in the number of cases of isolation and was recovered from 30.5 % of samples. From this genus 3 species were isolated of which *P. chrysogenum*; *P. citrinum* and *P. funiculosum* were recovered in 11.1 %, 5.5 % and 11.1 % of samples matching 36.4 %, 18.8 %, and 36.4 % of total *Penicillium*, respectively.

*Geotrichum candidum* was present in moderate frequency and was recovered from 22.2 % of samples. *Candida albicans* and *Trichophyton* were isolated in low frequency and recovered from 19.4 % of samples. Two species were isolated from *Trichophyton* of which *T. ajelloi* var. *ajelloi* and *T. mentagrophytes* var. *interdigitale* (Plate A.82) were recovered in 11.1 % and 8.3 % of samples matching 57.1 % and 50.0 % of total *Trichophyton*, respectively.

*Microsporum* was present in low frequency and recovered from 16.6 % of all samples. Three species were isolated from *Microsporum* of which *M. cookei*, *M. ferrugineum* and *M. gypsum* (Plates A.47 and 48) were recovered in 2.7 %, 5.5 % and 8.3 % of samples matching 16.6 %, 33.3 % and 50.0 % of total *Microsporum,* respectively. *Alternaria alternata*, *Paecilomyces variotii* and *Trichoderma* (represented by *T. koningii* and *T. viride*) were found in low frequency and recovered from 13.8 % of samples (Table 4.10 and Figure 4.5).

*Acremonium* (represented by *A. curvulum* and *A. recifei*), *Cladosporium cladosporioides*, *Fusarium* represented by *F. lichenicola* (Plate A.36) and *F. solani*, *Gliocladium viride* (Plate A.45), *Gymnoascus reticulatus, Mucor circinelloides*, *Scopulariopsis* represented by *S. brevicaulis* and *S. brumptii* (Plate A.71) and *Verticillum chlamydosporium* (Plate A.87) isolated in rare frequency and were recovered from 8.3 % of samples. While *Chaetomum cochliodes*, *Sporothrix schenkii* (Plate A.73) and unidentified *yeastss* were recovered from 2.7 %, 5.5 %, and 5.5 % of all aerobic samples, respectively (Table 4.10 and Figure 4.5).

#### 4.2.5.2. Fungi recovered from anoxic activated sludge samples

Thirty-six species belonging to 19 genera were collected from anoxic samples during this investigation (Table 4.10 and Figure 4.5). *Aspergillus* was the most common genus and occupied the first place in the number of cases of isolation and was recovered from 50 % of samples. From the genus 4 species were isolated of which *A. flavus* var. *flavus*, *A. fumigatus, A. niger* and *A. oryzae* were present in moderate and low frequency and recovered in 30.5 %, 25.0 %, 16.6 % and 2.7 % of samples matching 61.1 %, 50.0 %, 33.3 and 11.1 % of total *Aspergillus,* respectively.

Results in Table 4.10 and Figure 4.5 reveal that, *Chrysosporium* occupied the second place in the number of cases of isolation and was recovered from 38.8 % of the samples. From this genus 4 species were isolated of which, *C. asperatum*, *C. indicum*, *C. keratinophilum, C. tropicum* and *C. pannorum* were recovered in 5.5 %, 13.8 %, 8.3 %, 2.7 % and 8.3 % of samples matching 14.3 %, 45.0 %, 27.3 %, 7.1 % and 27.3 % of total *Chrysosporium*, respectively.

*Penicillium* occupied the third place in the number of cases of isolation and was recovered from 36.1 % samples. From this genus 4 species were isolated of which *P. chrysogenum*, *P. citrinum*, *P. funiculosum* and *P. oxalicum* were recovered in 19.4 %, 16.6 % and 5.5 % and 2.7 % of samples matching 53.8 %, 46.2 %, 15.4 % and 7.7 % of total *Penicillium*, respectively. *Geotrichum candidum* was present in moderate frequency and recovered from 30.5 % of samples (Table 4.10 and Figure 4.5).

*Trichophyton* was isolated in low frequency and recovered from 22.2 % of samples. Two species were isolated from *Trichophyton* of which *T. ajelloi* var. *ajelloi* and *T. terrestre* were recovered in 11.1 % and 8.3 % of samples matching 50.0 % and 37.5 % of total *Trichophyton*, respectively. *Alternaria* was present in low frequency and recovered from 16.6 % of samples. Three species were isolated from *Alternaria* of which *A. alternata* and *A. brassicae* were recovered in 8.3 % and 11.1 % of samples matching 50.0 % and 66.6 % of total *Alternaria*, respectively (Table 4.10 and Figure 4.5).

Also, the data in Table 4.10 reveals *Microsporum* was present in moderate frequency and recovered from 13.8 % of all samples. Three species were isolated from *Microsporum* of which *M. cookei*, *M. ferrugineum* and *M. gypsum* were recovered in 5.5 %, 8.3 %, and 8.3 % of samples matching 40.0 %, 60.0 %, and 60.0 % of total *Microsporum*.

*Candida albicans* and *Fusarium (*represented by *F. dimerum* and *F. solani)* were present in low frequency and recovered from 13.8 % of all anoxic samples. *Paecilomyces variotii*, *Sporothrix schenkii* were present in low frequency and recovered from 11.1 % of the samples. *Chaetomum cochliodes* and unidentified *yeasts* were present in low frequency and recovered from 8.3 % of samples.

*Acremonium curvulum*, *Cladosporium cladosporioides*, *Gliocladium viride*, *Trichoderma koningii* and *Verticillum* [represented by *V. chlamydosporium* and *V. lecanii* (Plate A.88)] were rare frequent and recovered from 2.7-5.5 % of all anoxic samples, respectively (Table 4.10 and Figure 4.5).

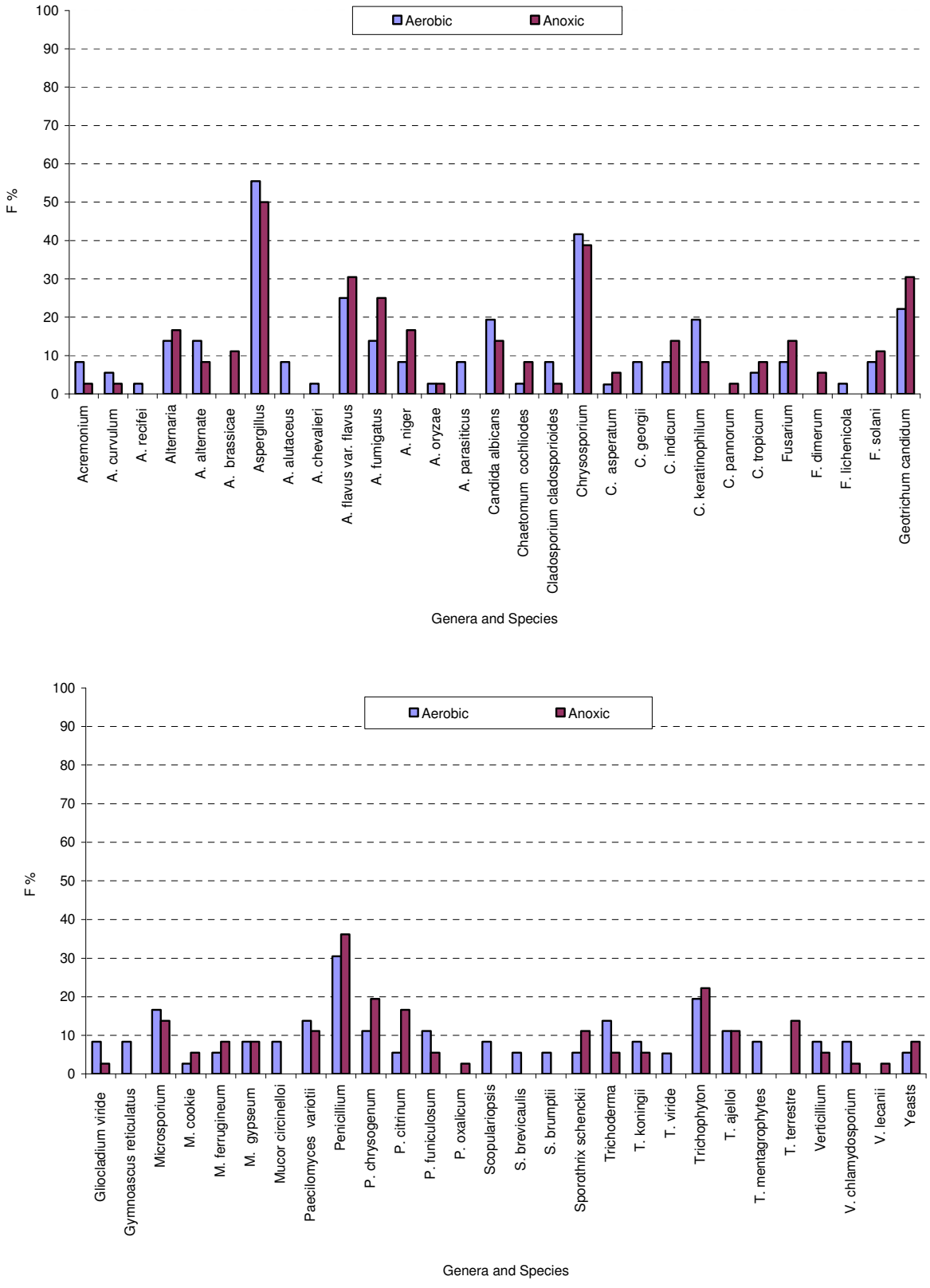
**Table 4.10:** Numbers of cases of isolation (NCI) out of 36, percentage frequency and occurrence remarks (OR) of fungal genera and species recovered from each aerobic and anoxic activated sludge samples from MBRs with hair-baiting technique on Sabouraoud's dextrose agar media with cycloheximide and chloramphenicol at 30 °C for 1-2 weeks

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | NCI | %F | OR | NCI | %F | OR |
| Dermatophytes and closely related fungi | | | | | | |
| *Chrysosporium* | 15 | 41.6 | M | 14 | 38.8 | M |
| *C. asperatum* J.W. Carmich | 1 | 2.5 | R | 2 | 5.5 | R |
| *C. georgii* (Vasravsky & Ajello) Van Oorschot | 3 | 8.3 | R | - | - | - |
| *C. indicum* (Randhawa & Sandhu) Garg | 3 | 8.3 | R | 5 | 13.8 | L |
| *C. keratinophilum* D. Frey ex Carmichael | 7 | 19.4 | L | 3 | 8.3 | R |
| *C. pannorum* (Link) Hughes | - | - | - | 1 | 2.7 | R |
| *C. tropicum* Carmichael | 2 | 5.5 | R | 3 | 8.3 | R |
| *Microsporum* | 6 | 16.6 | L | 5 | 13.8 | L |
| *M. cookie Ajello* | 1 | 2.7 | R | 2 | 5.5 | R |
| *M. ferrugineum* M. Ota | 2 | 5.5 | R | 3 | 8.3 | R |
| *M. gypseum (Bodin) Guiart & Grigoraks* | 3 | 8.3 | R | 3 | 8.3 | R |
| *Trichophyton* | 7 | 19.4 | L | 8 | 22.2 | L |
| *T. ajelloi* (Vanbreuseghem) Ajelo var. *ajelloi* | 4 | 11.1 | R | 4 | 11.1 | R |
| *T. mentagrophytes* (C. P. Robin) Sabour. var. *interdigitale* | 3 | 8.3 | R | - | - | - |
| *T. terrestre* Durie & Frey | - | - | - | 5 | 13.8 | L |
| Other fungi | | | | | | |
| *Acremonium* | 3 | 8.3 | R | 1 | 2.7 | R |
| *A. curvulum* W. Gams | 2 | 5.5 | R | 1 | 2.7 | R |
| *A. recifei* (Leão & Lôbo) W. Gams | 1 | 2.7 | R | - | - | - |
| *Alternaria* | 5 | 13.8 | L | 6 | 16.6 | L |
| *A. alternate* (Fries) Keissler | 5 | 13.8 | L | 3 | 8.3 | R |
| *A. brassicae* Sacc | - | - | - | 4 | 11.1 | R |
| *Aspergillus* | 20 | 55.5 | H | 18 | 50 | M |
| *A. alutaceus* Berkeley & Curtis var. *alutaceus* | 3 | 8.3 | R | - | - | - |
| *A. chevalieri (Eurotium chevalieri)* (L. Mangin) Thom & Church | 1 | 2.7 | R | - | - | - |
| *A. flavus* Link var. *flavus* | 9 | 25 | M | 11 | 30.5 | M |
| *A. fumigatus* Fresenius | 5 | 13.8 | L | 9 | 25.0 | M |
| *A. niger* Tiegh. | 3 | 8.3 | R | 6 | 16.6 | L |
| *A. oryzae* (ahlburg) Cohn | 1 | 2.7 | R | 1 | 2.7 | R |
| *A. parasiticus* Speare | 1 | 8.3 | R | - | - | - |
| *Candida albicans* (Robin) Berkh | 7 | 19.4 | L | 5 | 13.8 | L |
| *Chaetomum cochliodes* Palliser | 1 | 2.7 | R | 3 | 8.3 | R |
| *Cladosporium cladosporioides* (Fresenius) de Vries | 3 | 8.3 | R | 1 | 2.7 | R |
| *Fusarium* | 3 | 8.3 | R | 5 | 13.8 | L |
| *F. dimerum* Penzig | - | - | - | 2 | 5.5 | R |
| *F. lichenicola* C. Massal | 1 | 2.7 | R | - | - | - |
| *F. solani* (Mart.) Saccardo | 3 | 8.3 | R | 4 | 11.1 | R |
| *Geotrichum candidum* Link | 8 | 22.2 | L | 11 | 30.5 | M |
| *Gliocladium viride* Matr | 3 | 8.3 | R | 1 | 2.7 | R |
| *Gymnoascus reticulatus* Zukal | 3 | 8.3 | R | - | - | - |
| *Mucor circinelloides* Tiegh. | 3 | 8.3 | R | - | - | - |
| *Paecilomyces variotii* Bainier | 5 | 13.8 | L | 4 | 11.1 | R |
| *Penicillium* | 11 | 30.5 | M | 13 | 36.1 | M |
| *P. chrysogenum* Thom | 4 | 11.1 | R | 7 | 19.4 | L |
| *P. citrinum* Thom | 2 | 5.5 | R | 6 | 16.6 | L |
| *P. funiculosum* Thom | 4 | 11.1 | R | 2 | 5.5 | R |
| *P. oxalicum* Currie & Thom | - | - | - | 1 | 2.7 | R |
| *Scopulariopsis* | 3 | 8.3 | R | - | - | - |

**Table 4.10:** Continued

| Genera and Species | Aerobic activated sludge | | | Anoxic activated sludge | | |
|---|---|---|---|---|---|---|
| | **NCI** | **%F** | **OR** | **NCI** | **%F** | **OR** |
| *S. brevicaulis* (saccardo) Bainier | 2 | 5.5 | R | - | - | - |
| *S. brumptii* Salvent-Duval | 2 | 5.5 | R | - | - | - |
| *Sporothrix schenckii* Hektoen & Perkins | 2 | 5.5 | R | 4 | 11.1 | R |
| *Trichoderma* | 5 | 13.8 | L | 2 | 5.5 | R |
| *T. koningii* Oudemans | 3 | 8.3 | R | 2 | 5.5 | R |
| *T. viride* Persoon | 2 | 5.3 | R | - | - | - |
| *Verticillium* | 3 | 8.3 | R | 2 | 5.5 | R |
| *V. chlamydosporium* Goddard | 3 | 8.3 | R | 1 | 2.7 | R |
| *V. lecanii* (Zimm.) Viegas | - | - | - | 1 | 2.7 | R |
| *Yeasts* | 2 | 5.5 | R | 3 | 8.3 | R |
| Number of genera = 21 | 21 | | | 18 | | |
| Number of species = 46 | 40 | | | 34 | | |

NCI = Number of cases of isolation (out of 36)
% F = Percentage frequency of occurrence (calculated per 36 samples)
OR = Occurrence remarks: [H= High occurrence, isolated more than 18 cases (out of 36 samples)
M= Moderate occurrence, from 9 to 18 cases
L = Low occurrence, from 5 to 8 cases
R = Rare occurrence, less than 5 cases




% F = Percentage frequency of occurrence (calculated per 36 samples)

**Fig. 4.5:** The comparison between percentage frequencies of fungal genera occurrence in aerobic and anoxic activated sludge on Sabouraoud's dextrose agar media with cycloheximide and chloramphenicol

## 4.3. Interpretation of fungal occurrence in aerobic and anoxic activated sludge on different selected media

The results indicate that activated sludge is a suitable habitat for the growth and sporulation of different groups of fungi, both saprophytic and pathogenic. A variety of types of filamentous fungi and unidentified yeasts were obtained from both aerobic and anoxic activated sludge. It is clear that the wastewater treatment in MBRs has an effect on the numbers and diversity of fungal colonies existing in both aerobic and anoxic activated sludge.

*Aspergillus* (18 species), *Geotrichum candidum, Penicillium* (15 species) and yeasts contributed the broadest spectra of species in all samples tested on various types of media used. Other species were represented by 74 species belonging to 37 genera.

The fungal population in activated sludge from Amedeus pilot plant was more than which have been isolated from Margaretenhöhe plant. These results may be due to the differences in soils, wastewater and environmental or work conditions of each plant. Some species were isolated only from activated sludge of Amedeus wastewater plant (*Acremonium rutilum*, *Aspergillus nidulans* (*Emericella nidulans*), *Fusarium lichenicola* (*Cylindrocarpon lichenicola*), *Chrysosporium pannorum*, *Penicillium verrucosum var.verrucosum*, *Trichophyton mentagrophytes* and *Verticillium chlamydosprium*. While *Aspergillus fischerianus*, *Epicoccum nigrum, Paecilomyces marquandii, Penicillium islandicum*, and *Trichosporon pullulans*, were encountered only from Margaretenhöhe wastewater plant.

The obtained data showed some differences for fungal species among the aerobic and anoxic conditions. The fungal diversity present in both types of activated sludge has been similar, with different spore population. The chance of the presence of fungal spore in aerobic activated sludge was better than that in anoxic activated sludge. This trend could be explained by the continuous turning process of sludge and transfer of the same amount from the aerobic to anoxic tank.

Some fungi were recovered only from aerobic activated sludge (*Acremonium recifei*, *A. rutilum*, *Aspergillus chevalieri*, *A. nidulans (Emericella nidulans), A. parasiticus*, *A. terreus* var. *aureus*, *A. versicolor*, *Aurobasidium pullulans*, *Cladosporium herbarum*, *Epicoccum nigrum*, *Fusarium lichenicola* (*Cylindrocarpon lichenicola*), *F. roseum*, *Gibberella avenacea,, Gymnoascus reticulatus*, *Mucor hiemalis*, *Myrothesium cinctrum*, *Penicillium verrucosum var. verrucosum, Scopulariopsis brumptii, Stemphylim vesicarium, Trichophyton equinum, T. mentagrophytes* and *Ulocladium microsporum*). Also*, Alternaria brassicae, Aspergillus fischerianus, Chrysosporium pannorum, Paecilomyces marquandii, Penicillium expansum, P. islandicum, P. janczewskii,*

*P. purberulum*, *Rhinocladiella atrovirens*, *Trichophyton terrestre*, *Trichosporon pullulans,* and *Verticillium chlamydosprium* were encountered only from anoxic activated sludge (Table 4.6 to 4.10).

Some fungi were recovered only on 50 % Sucrose Czapek-Dox agar such as *Aspergillus carneus, A. fischerianus, Botryodiplodia theobromae, cladosporium herbarium, Cochliobolus lunatus, Fusarium roseum, Geosmithia lavendula* and *Paecilomyces marquandii,* Malt extract agar (*Aspergillus terreus* var. *aureus, Epicoccum nigrum, Mucor hiemalis, Penicillium janczewskii* and *P. purpurogenum*), Rose bengal cloramphenicol agar (*Gibberella avenacea*, *Myrothesium cinctrum*, *Penicillium expansum, P. purberulum, P. verrucosum* var. *verrucosum, P. islandicum, Rhinocladiella atrovirens*, *Trichophyton equinum, Trichosporon pullulans* and *Ulocladium microsporum*), Sabouraud's dextrose agar (*Acremonium rutilum* and *Stemphylium vesicarium*) and Sabouraud's dextrose agar media with Cycloheximide and Chloramphenicol (*Acremonium recifei*, *A. rutilum*, *Aspergillus chevalieri (Eu. Chevalieri)*, *A. parasiticus, chrysosporium asperatum, C. indicum, C. keratinophilum, C. pannorum, Fusarium lichenicola, Glicocladium viride, Gymnoascus reticulatus, Microsporum cookei, M. ferrugineum, M. gypseum, Scopulariopsis brumptii, Sporothrix schenckii, Trichophyton mentagrophytes* var. *interdigitals, Verticillium chlamydosporium* and *V. lecanii)* (Table 4.6 to 4.10).

These results almost agree to some extent with the finding reported by (Cooke, 1977; Cooke and Pipes, 1970; Häuslerova, 1976; Hiremath *et al*., 1985a; Abdel-Hafez and Elsharouny 1987; 1990; Bux and Kasan, 1994; Bien and Nowak, 1995; Hashem, 1995; Ali-Shtayeh *et al*., 1999; Ali-Shtayeh and Jamous 2000; Molla *et al*., 2002; Ulfig, 2003; Al-Zubeiry, 2005; Kasprzak *et al*., 2005; Soomro *et al*., 2007; Hedayati and Mirzachani, 2009; Shah *et al*., 2009; Wemedo *et al*., 2009). They indicated that the majority of moulds isolated from activated or sewage sludge and soils receiving wastewater consisted of *Aspergillus, Penicillium*, *Fusarium*, *yeasts* and *yeasts* like fungi.

The results were almost in harmony with the findings of Abdel-Hafez and El-Sharouny (1987, 1990). They isolated different *Aspergillus* species from soil in Assiut area (Egypt) receiving city sewage effluents and sewage sludge and these species were *A. flavus, A. fluvus* var. *columnaris, A. fumigatus, A. nidulans, A. niger, A. ochraceus, A. sydowii, A. terreus*, *A. versicolor* and *Emericella nidulans*. Also, the presented results were similar to the results of Bien and Nowak (1995). The authors reported that in stabilized sewage sludge from municipal wastewater treatment plant in Czestochowa the following fungi were identified: *Aspergillus sp*., *Fusarium sp*., *Penicillium sp*., *Geotrichum candidum, Candida sp*., *Rhodotorula rubra* and *Microsporum sp*. Hashem (1995) mentioned that the nineteen species belonging to 16 genera were isolated from 25 sewage sludge samples on Czapeks agar at 27 $^{o}$C for one week. *Alternaria alternate* and *Aspergillus flavus* were

recovered with 80 % frequency. Ali-Shtayeh *et al*. (1999) isolated different *Aspergillus* species from soils receiving raw city wastewater. These were *Aspergillus Candidus, A. flavipes, A. ochraceus*, *A. terrus* var. *aureus* and *A. versicolor*.

Most of the above species were isolated from the sludge in different parts of the globe. The data obtained here confirm the findings from some researchers. Ali-Shtayeh and Jamous (2000) isolated 55 species belonging to 21 genera from raw city sewage and reported that the species most commonly found in raw city sewage include *Alternaria alternata*, *Aspergillus candidus*, *Geotrichum candidum* and *Paecilomyces lilacinus*. Molla *et al*. (2002) reported that the twenty seven filamentous fungal strains representing 5 genera *Aspergillus, Penicillium, Trichoderma, Myriodontium* and *Pleurotus* were isolated from domestic wastewater sludge cake from Indah Water Konsortium.

Ulfig (2003) mentioned that the 343 keratinophilic fungal strains from 9 species were isolated from sewage sludge of wastewater treatment plant (activated sludge after prolonged aeration). The total isolation frequency was 94.4 %. *Fusarium solani*, *Phialophora melinii*, *Aspergillus versicolor* and *Fusarium oxysporum.* Al-Zubeiry (2005) indicated that the most important microorganisms involved in raw or dewatered sewage sludge were *Penicillium chrysogenum, P. citrinum* and *P. funiculosum* and *P. janczewskii*.

Also Kasprzak *et al*. (2005) encountered *Penicillium commumae, P. lividum, P. janczewskii, P. spinulosum* and *P. vulpinum* from sewage sludge selected from wastewater treatment plants. Soomro *et al*. (2007) reported that fifteen species of fungi were isolated from sludge in Pakistan, *Aspergillus niger* 19.78 % *A. flavus* 14.97 % *A. candidus* 6.95 %, *A. wentii* 6.04%, *A. fumigatus* 18.71 % *Alternaria alternate* 10.69 % *A. tenuis* 8.02 % *Drechslera spicifera* 9.02 %, *Chochliobolus lunatus* 9.62 %, *Penicillium sp*. 1.6 %, *Chaetomium crispatum* 1.06 % and *Acrimonium sp*. 2.13 %. Hedayati and Mirzakhani (2009) mentioned that from the 35 sludge samples cultured on Sabouraud's agar with cycloheximide and chloramphenicol, 326 fungal colonies belonging to 7 species were isolated. *Geotrichum* 59.5 %, *Cladosporium* 13.8 %, *Alternaria* 11.3 %, and *Penicillium* 10.7 % species were the most prevalent.

Häuslerova (1976) observed that 95 % of mould isolated from activated sludge media of *Geotrichum candidum.* Also many researchers encountered *Geotrichum candidum*, but with variable frequencies in activated or sewage sludge and soil receiving sewage effluents (Abdel-Hafez and Elsharouny, 1990; Bux and Kasan, 1994; Ali-Shtayeh *et al*., 1999; Al-Zubeiry, 2005; Kacprzak *et al*., 2005; Hedayati and Mirzakhani, 2009).

In the study 13 species of Keratinophilic fungi and dermatophytes belonging to 3 genera were isolated from both aerobic and anoxic activated sludge (*Chrsosporium asperatum, C. georgii, C. indicum, C. keratinopilum, C. pannorum, C. tropicum, Microsporum cookie, M. ferrugineum, M. gypseum* and *Trichophyton ajelloi* var. *ajelloi, T. aquinum, T. mentagrophytes* var. *interdigitale, T. terrestre*). The above species were isolated, with different numbers and frequency of occurrence, from soil or soils receiving raw city wastewater, sewage and activated sludge in different parts of the globe (Ulfig and Korcz, 1983, 1991, 1994; Filipello Marchisio, 1986; Calvo *et al*., 1984; Ali-Shtayeh, 1988; Abdel-Hafez *et al*., 1989; 1990; Abdel-Mallek *et al*., 1989; Abdel-Hafez *et al*., 1990; Ulfig and Ulfig, 1990; Filipello Marchisio *et al*., 1991; Abdullah and Hassan, 1995, Agut *et al*., 1995; Ulfig *et al*., 1996, 1997, 2007; Zarei Mahmoudabadi 1997; Jamous, 1998; Papini *et al*., 1998; Ali-Shtayeh and Jamous, 2000; Ali-Shtayeh *et al.*, 2002; Al-Sane, 2002; Khanam, and Jain, 2002; Ulfig, 2000, 2003, 2005, 2006; Oyeka, and Okoli, 2003; Hedayati *et al*., 2004; Saxena, *et al*., 2004; Al-Zubeiry, 2005; Soomro *et al*., 2007; Shrivastava *et al*., 2008; Zarei Mahmoudabadi and Zarrin, 2008; Hedayati and Mirzachani, 2009; Sharma and Meenakshi, 2010; Sharma *et al*., 2011).

Abdel-Hafez and Elsharouny (1990) reported that the *Chrysosporium tropicum, C. keratinophilum, C. asperaturn, C. indicum, C.* state of *Arthrodenna tuberculatum, C.* state of *Thielavia sepedonium, C. georgii, C. pseudomerdarium*, and *C. queenslandicum* were encountered from sewage sludge. *Chrysosporium gypseum*, *C. keratinophilum*, *C. pannorum*, and *C. tropicum* were isolated from soils receiving raw city wastewater (Ali-Shtayeh *et al*., 1999). Several species from this genus were isolated but with variable frequencies from activated and sewage sludge such as *Chrysosporium* anamorph *Aphanoascus clathratus*, C. anamorph *Aphanoascus risticulisporus/flavescens*; *C. asperatum*, C. europae, *C. gypseum,* C. *indicum, C. keratinophilum,* C. *pannorum*, C. *pruinosum, C. tropicum*, and *C. zonatum* (Ulfig and Korcz, 1983; Ulfig *et al*., 1996; Ulfig, 2003, 2006; Muhsin and Hadi, 2001; Soomro *et al*., 2007; Hedayati and Mirzachani, 2009).

Al-Zubeiry (2005) encountered three *Microsporum* species *M. canis*, *M. gypseum* and *M. manginii* from dewatered sewage. *Trichophyton ajelloi* var. *ajelloi, T. aquinum, T. mentagrophytes* var. *interdigitale* and *T. terrestre* were also isolated from sewage or activated sludge and soil receiving city wastewater (Ulfig and Korcz, 1983; Abdel-Hafez and EL-Sharouny, 1990; Ulfig *et al*., 1996; Ali-Shtayeh *et al*., 1999; Ulfig, 2003, 2006). *Trichophyton equinunm* and *T. mentagrophytes* var. *erinacei* were isolated from sewage or activated sludge (Muhsin and Hadi, 2001).

Numerous fungi are almost present in large numbers in sewage sludge or soil amended with activated sludge. Several fungi previously isolated from these substrates are known to be pathogenic

to plants, animals and humans. Several species of Keratinophilic fungi are well known animal and human mycotic agents or have been encountered frequently from animal and human dermal lesions. Most of the fungal isolates in this study are well known pathogens, e.g. *Aspergillus* some species from this genus were previously reported as causal agent from some diseases such as aspergillosis (Frey *et al*., 1979) and onychomycosis (Velez and Diaz, 1985). *Fusarium spp.* are potentially pathogenic fungi (Collins and Rinaldim 1977; Ali-Shtayeh, 1988). *Scopulariopsis sp*. are known agent of onychomycosis (Fragner and Belsan, 1975; Filipello Marchisio and Fusconi, 2001).

*Chrysosporium* species are occasionally isolated from skin and nail scrapings, especially from feet, but because they are common soil saprophytes they are usually considered as contaminants (Kane *et al*., 1997). *Microsporum gypseum* have been reported to cause human and animal infections (Ali-Shtayeh and Arda, 1986; Connole, 1990; Filipello Marchisio *et al*., 1996), *Paecilomyces lilacinus* can induce keratitis (Forster and Rebell, 1975; Agrawal *et al*., 1979; Rippon, 1988), *Alternaria alternata* has frequently been reported from infected or previously injured human skin (Rippon, 1988), *Geotrichum candidum* was reported from human dermal lesions (Restrepo and de Uribe, 1976; Rippon, 1988; Thomas, 2003 ), and *Trichophyton ajelloi* has been found to cause skin lesions in animals (Monga and Mohapatra, 1980; Rippon, 1988; Filipello Marchisio *et al*., 1995), *Trichophyton terrestre* has world wide distribution in soil and found as saprophytes on man and animals (Frey *et al*., 1979; Rippon, 1988), *Trichophyton mentagrophytes* was known to be major causative agents of human dermatophytoses (Zarei Mahmoudabadi, 1997; Shadzi *et al*., 2002), and have been described as pathogens or potential pathogens.

*Microsporum cookei* and *M. gypseum* are a geophilic fungus with a world-wide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather and *M. ferrugineum* is an anthropophilic fungus causing epidemic juvenile tinea capitis in humans. Usually produces a single inflammatory skin or scalp lesion (Onsberg, 1978; Reppon, 1988; Larone, 1995; Filipello Marchisio, 2000). *Mycrosprium cookie, Trichophyton ajelloi* var. *ajelloi, Sporothrix schenckii*, and *Scopulariopsis brevicaulis,* can be classified as etiological agents of opportunistic mycoses. This group of organisms plays a significant role in the natural degradation of keratinized residues. Keratin is the major constituent of hair, skin, and feathers, and it can find its way into soil (Mercantini *et al.,* 1986; Rajak *et al*., 1991) which may also form a link in the complex epidemiological chain that relates in both the evolutionary and developmental sense, of geophilic, zoophilic, and anthropophilic dermatophytes (Rippon, 1988).

*Sporothrix schenckii* has a world wide distribution, particularly in tropical and temperate regions. It is commonly found in soil and on decaying vegetation and is a well known pathogen of humans and animals (Domsch *et al*., 1995). *Sporothrix schenckii* causes mycotic infection of the

cutaneous or subcutaneous tissues and adjacent lymphatics characterized by nodular lesions which may suppurate and ulcerate infections caused by the traumatic implantation of the fungus into the skin, or very rarely, by inhalation into the lungs. Secondary spread to articular surfaces, bone and muscle is not infrequent, and the infection may also occasionally involve the central nervous system, lungs or genitourinary tract (Rippon, 1988).

## 4.4. Screening the fungal isolates for elimination of nitrogen, phosphorous and COD from raw wastewater

Twenty-one isolates belonging to 21 species representing 12 genera isolated from aerobic and anoxic activated sludge from MBRs were screened for elimination of nitrogen, phosphorus and COD from raw wastewater (Table 4.11 and Figures. 4.6, 4.7). The data indicated that, after 15 days incubation period two fungal isolates were the best for growth parameters and reduction of TN, $PO_4$, $NH_4$, $NO_3$ and COD of raw wastewater. These were *Aspergillus niger* (isolated from aerobic) and *Trichoderma viride* (from anoxic activated sludge) of BWB plant in Margaretenhöhe. The reduction values of nitrogen (86.3 % and 88.5 %) and phosphorous (95.0 % and 96.3 %) for *Aspergillus niger* and *Trichoderma viride*, respectively. While the ability of other isolates for elimination of nitrogen and phosphorus ranged between 34-85.3 % of nitrogen and 61-92 % of phosphorous. The lowest reduction (34.0 %) of TN appeared with *Penicillium citrinum*, while the *Aspergillus terreus* var. *terreus* recorded the low fungal activity in reducing $PO_4$ (61.0 %). These could be explained by the high rate at which N and P compounds were used up. However, some fungi straines did not use efficiently the organic compounds exited in raw wastewater. These results almost agree to some extent with the findings reported by Thanh and Simard (1973a), Akhtar and Ghaffar (1986), Hamdi (1991), Hamdi and Ellouz (1993), Hamdi *et al.* (1991), Hamdi *et al*. (1992).

Concerning the effect of the examined fungi on the elimination of $NH_4$ and $NO_3$ data in Table 3.11 and Figure 3.6, show the high differences between these isolates. The highest reduction value for either $NH_4$ (92.0 %) or $NO_3$ (87.2 %) attained with *Aspergillus niger.*

The second reduction was occupied by *Trichoderma viride* (92.5 % and 88.8 % for $NH_4$ and $NO_3$, respectively). The lowest fungal reduction of both $NH_4$ and $NO_3$ accompanied the *Doratomyces stemonitis* and *Penicillium oxalicum* respectively. This trend may be due to high activity and ability of *A. niger* and *T. viride* to uptake these nutrients from wastewater. Also, this might be related to the fungal requirements for these compounds for nutrition and building their cell wall. Results were almost in harmony with the findings of Thanh and Simard, (1973a), Akhtar and Ghaffar (1986), Jin *et al*. (1999b) and Guest and Smith (2007).

The reduction degree of COD in Table 4.11 and Figure 4.6 shows high differences among the examined isolates. Also, *A. niger* showed the highest reduction degree (75.1 %) of COD, while *T. viride* comes in the second order with 74.1 % reduction value. *Ulocladium chartarum* recorded the lowest reduction value (10.7 %) of COD. This may reflect the variations between the examined isolates in consumption of oxygen. Also, indirectly this demonstrates the effect of fungi in removing organic compounds from wastewater. These results almost agree to some extent with the finding reported by Thanh and Simard (1973a), Coulibaly (2002), Coulibaly *et al*. (2002), and Coulibaly and Agathos (2003).

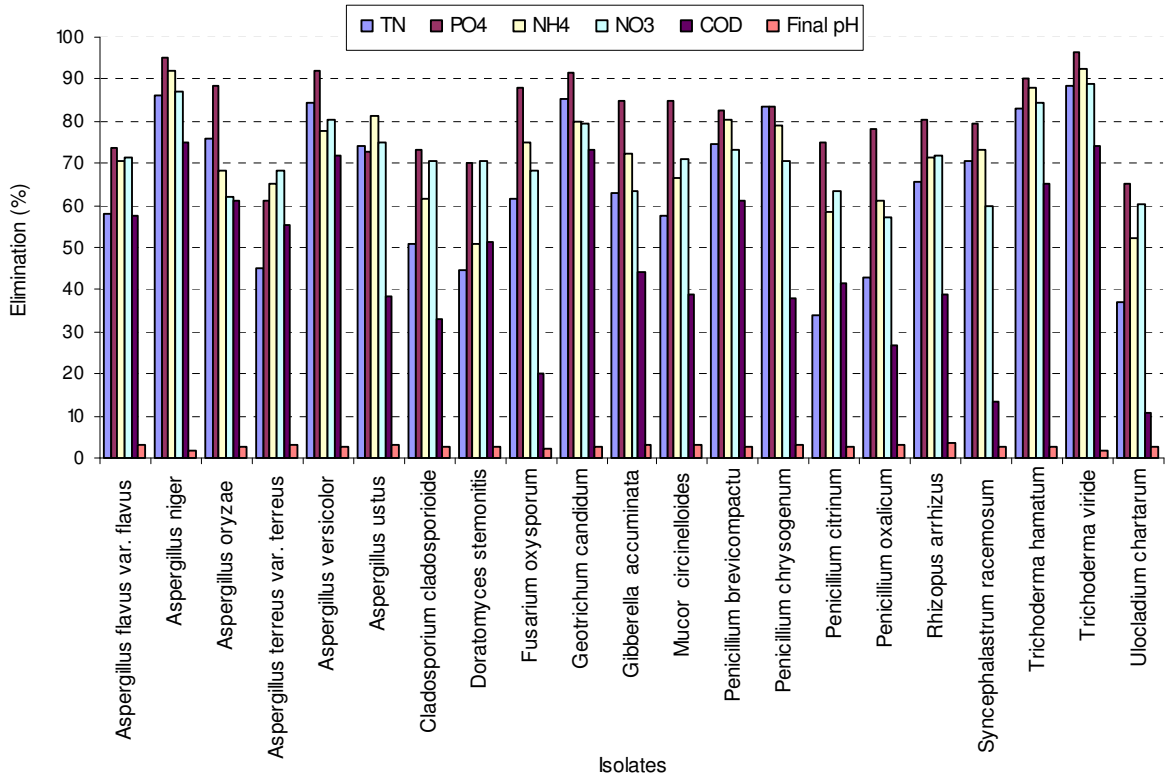
The resultes of mycellium dry matter (DM) as an indicator of fungal growth was recorded in Table 4.11 and Figure. 4.7. The highest DM value (841.0 mg/L) was found with *Aspergillus niger*, wherease the lowest (300.2 mg/L) was found in *Syncephalastrum racemosum* raw wastewater media. Also, production of protein in different isolates was a good indicator of fungal metabolism process. *Penicillium chrysogenum* produced the highest amount (207.0 mg/L) of protein, while *Rhizopus arrhizus* recorded the lowest amount (61.0 mg/L). Dry matter and protein content were used as an indicator of fungal growth rate. The differences found among the tested fungi in DM and PC might be due to the variation in biochemical and physiological processes in the investigated fungi. This trend supported by the reduction degree of N and P, which were compounds in forming amino acids and in sequentially metabolisms of fungal protein. (Thanh and Simard, 1973a; Hamdi *et al*., 1991; Fujita *et al*., 1993; Murado *et al*., 1993; Jin *et al*., 1999b; Coulibaly, 2002; Coulibaly *et al*., 2002; Coulibaly and Agathos, 2003).

Also, data in Table 4.11 and Figures 4.6, 4.7 shows that, with different studied isolates the final pH value of raw wastewater was lower than the intial pH value 4.5. After 15 days incubation period the lowest pH value (2.0) appeared with *Aspergillus niger* and *Trichoderma viride*. The highest pH value (3.4) appeared by *Rhizopus arrhizus*. Decreasing pH values in the final supernatant after 15 days may be due to forming organic acids, rearrangements of organic compounds and the production of $H^+$ protons. The other reasons may be transformation of ammonia and phosphate to nitrate/nitrite and orthophosphate, respectively. Moreover, the pH value was affected by the fungal treatment and during the population growth of fungi in treatment; the pH level changed because the fungal growth may have led to excretion of acidic metabolites. This might suppress or reduce the intensity of the growth in alkline pH of wastewater (Fakhru'l-Razi *et al*., 2002).

**Table 4.11:** Analysis of the supernatant residue as performed by various fungi grown on raw wastewater at pH 4.5 for 15 days

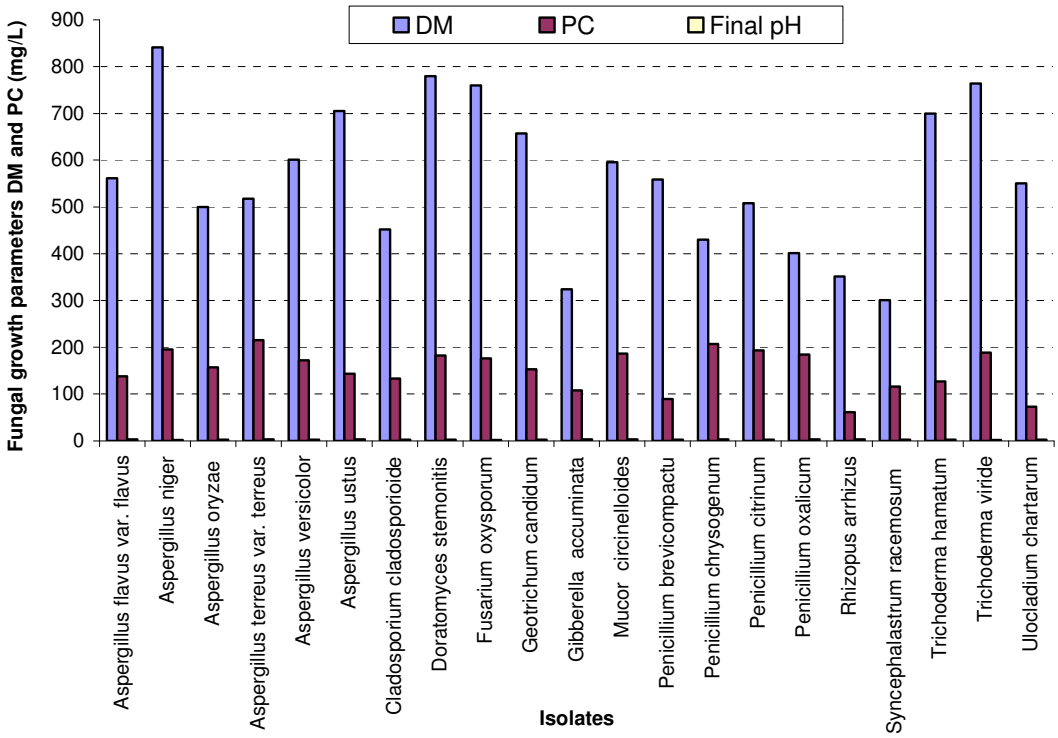
| Isolates | Supernatant % Reduction | | | | | Residue | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| | (%) | | | | | (mg/L) | | |
| *Aspergillus flavus var. flavus* | 58.0 | 73.5 | 70.5 | 71.5 | 57.4 | 562.0 | 138.0 | 3.0 |
| *Aspergillus niger* | 86.3 | 95.0 | 92.0 | 87.2 | 75.1 | 841.0 | 195.0 | 2.0 |
| *Aspergillus oryzae* | 75.8 | 88.5 | 68.3 | 62.0 | 61.0 | 500.0 | 157.0 | 2.8 |
| *Aspergillus terreus var. terreus* | 45.0 | 61.0 | 65.0 | 68.2 | 55.5 | 517.2 | 215.0 | 3.2 |
| *Aspergillus versicolor* | 84.2 | 92.0 | 77.5 | 80.4 | 71.9 | 601.0 | 172.0 | 2.6 |
| *Aspergillus ustus* | 74.0 | 72.9 | 81.3 | 75 | 38.2 | 705.0 | 143.0 | 3.1 |
| *Cladosporium cladosporioide* | 50.7 | 73.1 | 61.7 | 70.5 | 33.0 | 452.0 | 133.0 | 2.8 |
| *Doratomyces stemonitis* | 44.5 | 70.2 | 51.0 | 70.6 | 51.3 | 780.0 | 182.0 | 2.6 |
| *Fusarium oxysporum* | 61.6 | 88.0 | 75.2 | 68.5 | 20.3 | 760.0 | 176.0 | 2.2 |
| *Geotrichum candidum* | 85.3 | 91.4 | 80.0 | 79.4 | 73.0 | 657.5 | 153.0 | 2.6 |
| *Gibberella accuminata* | 63.0 | 85.0 | 72.3 | 63.3 | 44.1 | 324.4 | 108.0 | 3.2 |
| *Mucor circinelloides* | 57.5 | 85.0 | 66.3 | 71.0 | 39.0 | 596.0 | 186.0 | 3.0 |
| *Penicillium brevicompactu* | 74.6 | 82.6 | 80.5 | 73.1 | 61.0 | 558.3 | 89.0 | 2.5 |
| *Penicillium chrysogenum* | 83.6 | 83.5 | 79.2 | 70.7 | 38.0 | 430.0 | 207.0 | 3.0 |
| *Penicillium citrinum* | 34.0 | 75.0 | 58.5 | 63.3 | 41.4 | 508.0 | 193.0 | 2.5 |
| *Penicillium oxalicum* | 42.7 | 78.1 | 61.0 | 57.0 | 26.7 | 401.0 | 184.0 | 3.0 |
| *Rhizopus arrhizus* | 65.5 | 80.3 | 71.6 | 72.0 | 39.0 | 351.0 | 61.0 | 3.4 |
| *Syncephalastrum racemosum* | 70.4 | 79.3 | 73.3 | 60.0 | 13.3 | 300.2 | 116.0 | 2.8 |
| *Trichoderma hamatum* | 83.0 | 90.0 | 88.0 | 84.5 | 65.0 | 700.0 | 127.0 | 2.5 |
| *Trichoderma viride* | 88.5 | 96.3 | 92.5 | 88.8 | 74.1 | 764.2 | 189.0 | 2.0 |
| *Ulocladium chartarum* | 37.2 | 65.2 | 52.2 | 60.1 | 10.7 | 550.0 | 73.0 | 2.6 |

TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.6:** Screening the fungal isolates for elimination of nitrogen, phosphorous and COD from raw wastewater




DM= Dry matter, PC= Protein content

**Fig. 4.7:** Growth of fungal isolates and protein content in raw wastewatrer

## 4.5. Environmental factors affecting on the biological activity of *Aspergillus niger* and *Trichoderma viride* for nitrogen, phosphorus and COD elimination from raw wastewater

From the above results it can be demonstrated that, *Aspergillus niger* and *Trichoderma viride* were the best fungal isolates for elimination of TN, NH4, NO3, PO4 and COD. Also, these two fungi produced the highest growth of biomass (dry matter and protein content). Thus in the following section, these isolates were selected to examine their activity in elimination and production of fungal biomass in raw wastewater under different conditions. The factors taken into consideration were: pH values, incubation temperature and incubation period.

### 4.5.1. Effect of different pH values

The data illustrated by Figures. 4.8, 4.9, 4.10 and 4.11 (Table A.1 and A.2, respectively) show the reduction of the studied compounds as affected by the change of raw wastewater pH during incubation period of 15 days at 30 $^{o}$C temperature degree. Results reflect the reduction degree as a percentage of the initial concentration. Generally, under different examined pH values as pH increases the reduction of the studied compounds increases up to pH (4.5), while they

decreased after that by increasing the pH value (from 5.0 to 9.0). This trend was similar either inoculated raw wastewater with *A. niger* or *T. viride*. Church and Nash (1970) reported that, the optimum pH value for fungal activites lies between 4.0 and 5.0 pH.

The highest (87.9 % and 85.6 %) and lowest reduction (0.7 % and 0.5 %) of raw wastewater total nitrogen appeared with *A. niger* and *T. viride*, respectively. The maximum reduction of $NH_4$ (91.4 % and 93.8 %) and $NO_3$ (78.5 % and 85.5 %) were observed for *A. niger* and *T. viride*, respectively. On the other hand the minimum reduction degree of $NH_4$ (1.9 % and 3.2 %) and $NO_3$ (0.6 % and 1.0 %) were recorded to *A. niger* and *T. viride*, respectively. The high elimination (94.3 %) of phosphate was found in raw wastewater inoculated with *A. niger* and (93.5 %) with *T. viride*. While at pH 9.0 the reduction degree of $PO_4$ was 1.3 % and 1.8 % for *A. niger* and *T. viride*, respectively (Figures 4.8-4.11). These trends show that pH increases the elimination of N and P increased up to 4.5 which were decreased after that till the pH 9.0. Results reflect also that pH 4.5 was the best due to strong acidity and/or alkalinity affected the absorbation rates by fungi.

The pH of growth media could have an effect on cell-fungal metabolisms.Thanh and Simard (1973a) observed optimum pH between 3.5-4.0 in the removal of nitrogen and phosphates from domestic wastewater by *Trichoderma roseum*. In contrast, Akhtar and Ghaffar (1986) suggested the highest removal of $NH_4$ by *Aspergillus flavus* from raw wastewater was at pH 6.0.

Concerning the ability of the studied fungi to eliminate chemical oxygen demand (COD, %) data in Figures 4.8, 4.9, 4.10 and 4.11 show the high differences of reduction degree as a function of pH value. The results indicate under different tested pH the highest reduction was accompanied by *Aspergillus niger* while the lowest elimination was found in the treatment infected by *Trichoderma viride*. Modifying the wastewater to pH 4.5 recorded the highest reduction degree (87.6 % and 72.8 %) for *A. niger* and *T. viride*, respectively. These results almost agree to some extent with the finding reported by Church and Nash (1970), Thanh and Simard (1973a), Mannan *et al*. (2005), and Hanafi *et al*. (2010). *Aspergillus niger* was more efficient to reduce at least 60 % COD of Olive mill wastewater adjusted at pH 4.57 after 7 days of growth (Hanafi *et al*., 2010).

Mannan *et al*. (2005) reported that the *penicillium corylophilum* was capable of removing 94.40 % of COD from domestic wastewater treatment after 2 days of treatment whereas *Aspergillus niger* was capable of removing 93.20 %. The pH level was lower (acidic condition) in the fungal treatment and maximum reduction of COD was observed at 5.5 pH. Also, Jin *et al* (1998) showed that under an initial pH of 4.5-5.5 in the wastewater medium, *Aspergillus oryzae* demonstrated optimal microbial activity in the COD reduction. The optimum initial pH could be 5.0.

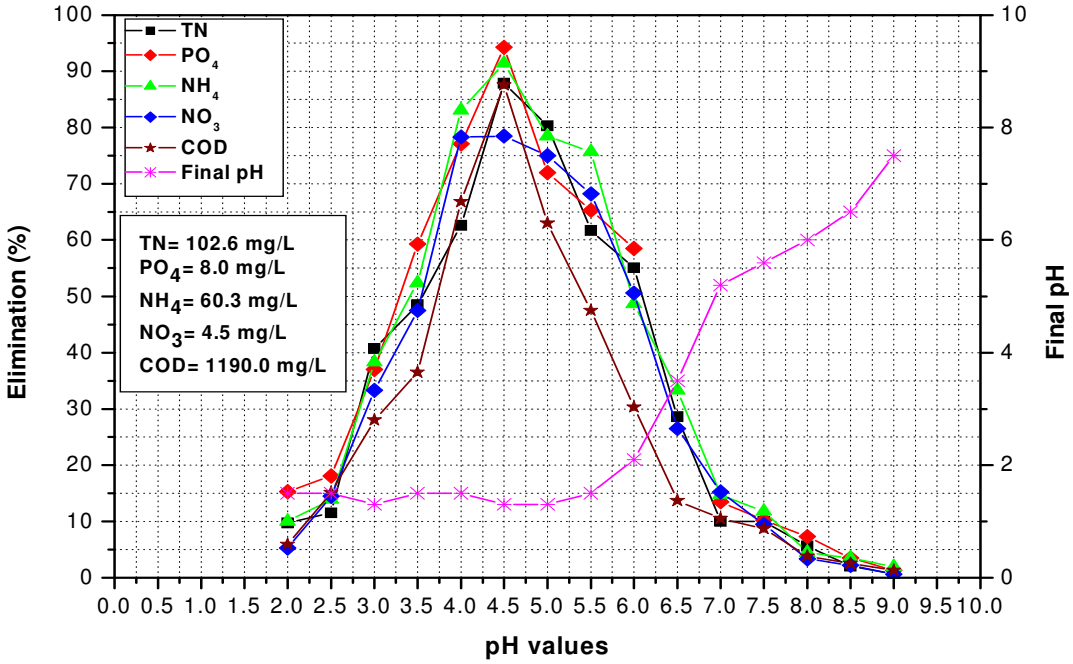
Also, the illustrated data in Figures 4.8, 4.9, 4.10, and 4.11 and (Table A.1, A.2) indicated

the presence of high relationship between wastewater pH and the dry matter (DM) of the tested fungi. The data revealed that, the DM value (529.0 and 484.6 mg/L) of fungi grown in the treatment acidified to pH 4.5 was higher than that inoculated at pH 9.0 (28.6 and 10.8 mg/L) for *A. niger* and *T. viride*, respectively. Unlike the DM value and with the exception of the fungi protein content contrary to pH 2.0 and 2.5, the PC of *T. viride* was higher than *A. niger*. Extreme alkalinity leads to the lowest content of protein for both tested fungi.

The highest value (263.5 mg/L) of PC attained with *T. viride* while the lowest forming rate found with *A. niger* (25.4 mg/L). These trends of results could be due to the pH of tested wastewater affecting the fungal cabability to absorp their requirements of necessary nutrients. Thus, consequently might influence the biochemical and physiological processes in fungi. These results were almost in harmony with the findings of Jin *et al*. (1999a, b), Robles *et al*. (2000), and Coulibaly (2002).

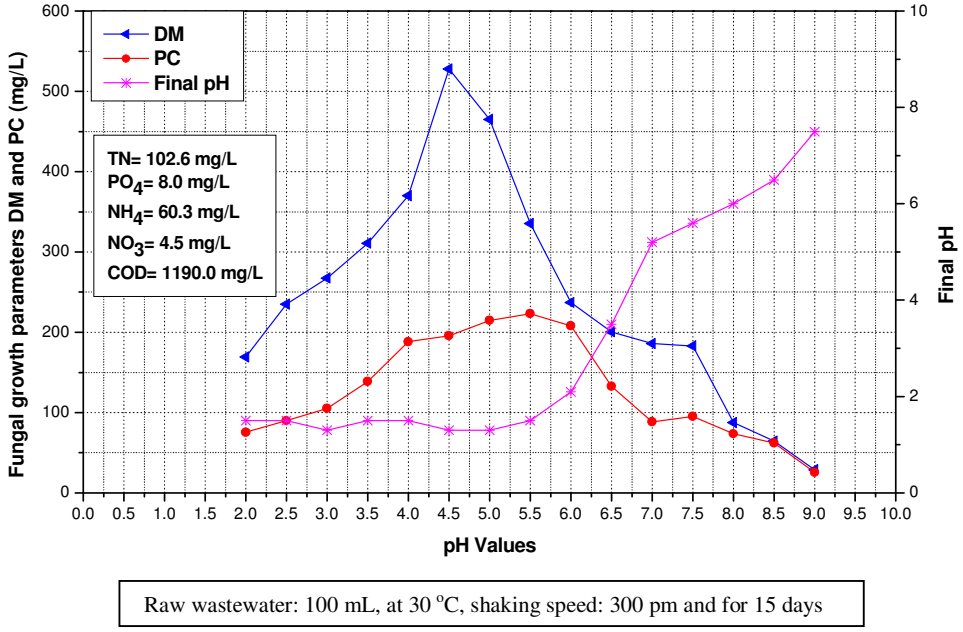
Jin *et al.* (1998) reported that under an initial pH of 4.5-5.5 in the wastewater medium, *Aspergillus oryzae* demonstrated optimal microbial activity in the production of biomass and protein content, the optimum initial pH could be 5.0. Jin *et al.* (1999b) demonstrated the maximum biomass yield and COD reduction by *Rhizopus oligosporus* grown in starch processing wastewater were achieved at initial pH of 4.0.

Jin *et al.* (1999c) screened thirty strains of microfungi for production of biomass proteins from starch processing wastewater. Four species *Aspergillus oryzae*, *Rhizopus oligosporus*, *R. arrhizus*, and *Trichoderma viride* were higher species for fungal dry biomass (5.62, 4.79, 4.47, and 3.87g $L^{-1}$) and biomass proteins content (41.5 %, 46.8 %, 48.8 5 % and 47.2 %), respectively at optimum pH values ranging from 3.5 to 5.5.



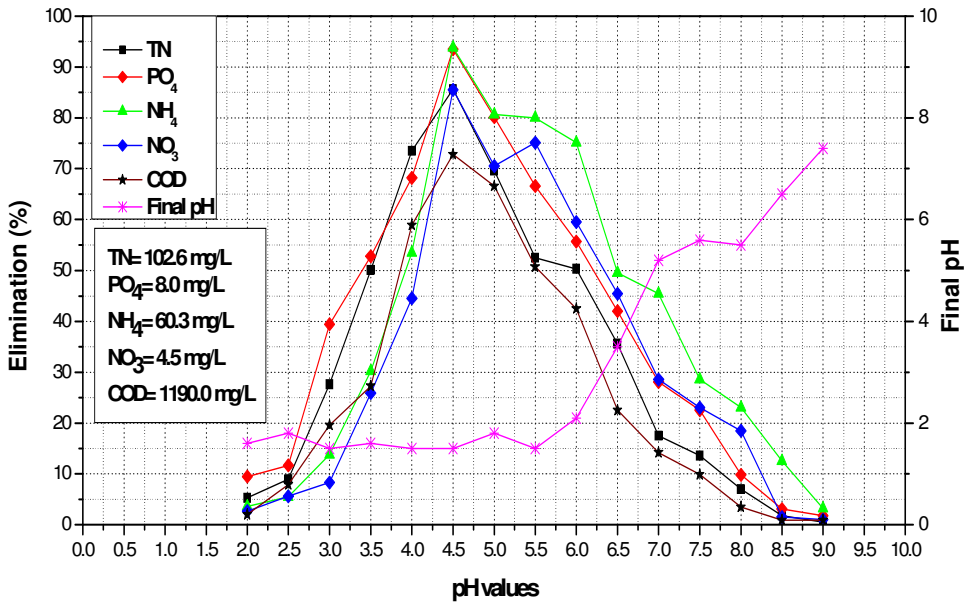

TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.8:** Impact of pH values on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater



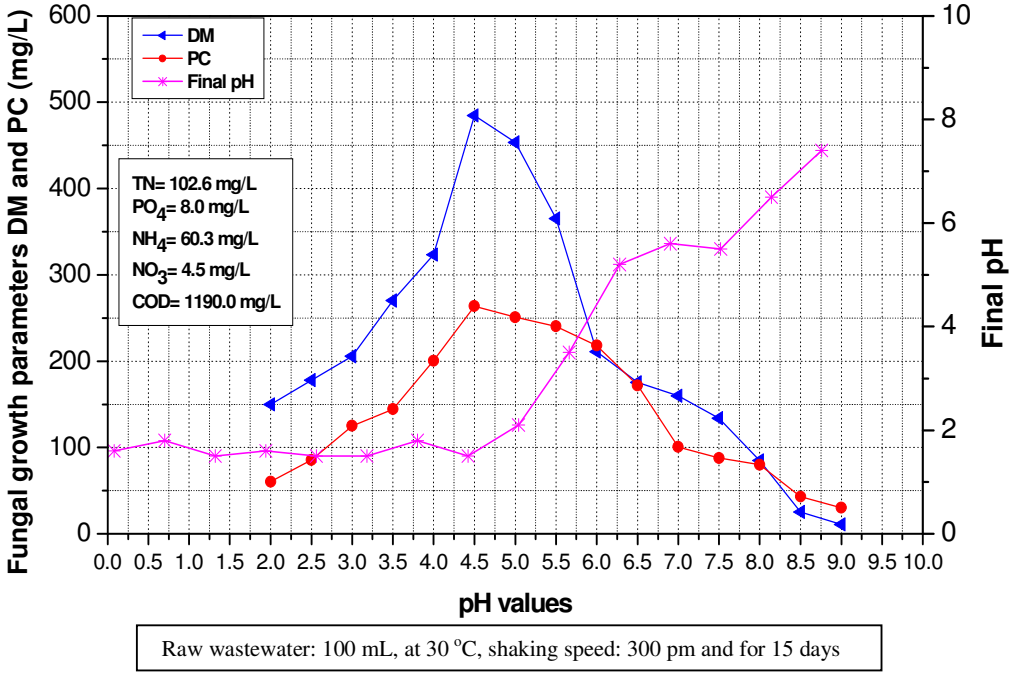

DM= Dry matter, PC= Protein content

**Fig. 4.9:** Impact of pH values on the growth of *Aspergillus niger* in raw wastewatrer




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.10:** Impact of pH values on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater




DM= Dry matter, PC= Protein content

**Fig. 4.11:** Impact of pH values on the growth of *Trichoderma viride* in raw wastewater

### 4.5.2. Effect of incubation temperature

Data in Figures 4.12, 4.13, 4.14, 4.15 and (Table A.3, A.4) show the relationships between temperature and the ability of *Aspergillus niger* and *Trichoderma viride* to eliminate the studied nutrients (N, P and COD) from raw wastewater. Also, the effect of temperature on the production of mycelium biomass was evaluated. Clearly, the elimination degree and fungal growth of *A. niger* was better than those of *T. viride*. The data indicate the highest reduction percentage and the best fungal growth were found at 30 °C temperature for either *A. niger* or *T. viride*. The studied fungi were mesophilic and their optimum temperature located between 25 to 35 °C. Temperature less or higher than 30 °C inhibits fungal growth and activities (Raper and Fennell, 1965; Pitt and Hocking, 2009).

The *A. niger* incubated at 30 °C attained the highest values of TN (80.1 %), $PO_4$ (93.4 %), $NH_4$ (96.0 %), $NO_3$ (88.5 %), COD (80.3 %), DM (633.5 mg/L) and PC (233.1 mg/L). Whereas the lowest values of TN (1.5 %), $PO_4$ (3.3 %), $NH_4$ (12.0 %), $NO_3$ (5.2 %), COD (4.7 %), DM (16.3 mg/L) and PC (5.0 mg/L) observed at 5.0 °C (Figures 3.12 and 3.13). Also the data proved that, the activity of *A. niger* was decreased at above and below of 30 °C temperature. In other words, temperature higher and lower than 30 °C led to decreased fungal activity and consequently decreased the values of tested compounds. These results almost agree to some extent with the finding reported by Fujita *et al*. (1993), Murado *et al*. (1993), Jin *et al*. (1999a), Garcia *et al*. (2000), Coulibaly (2002), and Öngen *et al*. (2007).

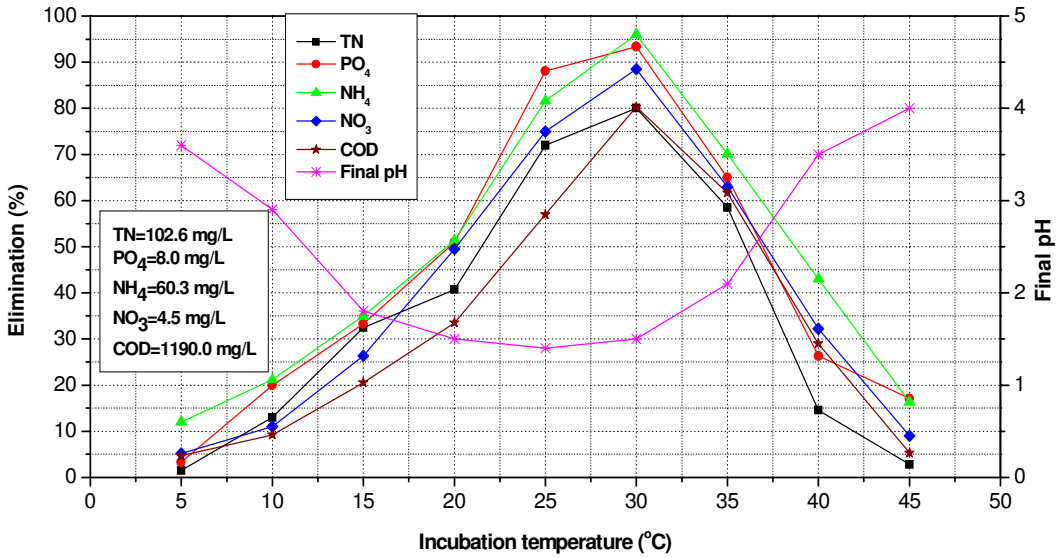
Robles *et al.* (2000) reported that the maximum fungal dry weight of *Penicillium sp.* (strain *Penicillium* 4) grown in undiluted wastewater was occured at 28 °C. Kissi *et al*. (2001) reported that the highest decrease in COD was observed at 28 °C after 6 days incubation with *Phanerochaete chrysosporium*. In contrast, Akhtar and Ghaffar (1986) reported the optimum removal of $NH_4$ by *Aspergillus flavus* from domestic wastewater was observed at 20 °C. Also, Khanongnuch *et al.* (2006) reported that the white-rot fungus *Coriolus versicolo* reduced 67 % of COD from textile wastewater at 37 °C.

Concerning the effect of temperature on the activity of *Trichoderma viride*, the data in Figures 4.14 and 4.15 shows the high variation of fungal response. The 25 °C corresponded to the highest elimination rate of TN (69.4 %), $PO_4$ (80.3 %) and $NO_3$ (71.0 %), while the highest reduction percentage of $NH_4$ (93.0 %) and COD (68.3 %) were found at 30 °C.

Also, data in Fig 3.4 indicate the great influence of examined temperature on the values of DM and PC of *T. viride*. At 30 °C the fungus produced the highest values of mycelium DM and PC (511.7 mg/L and 203.5 mg/L, respectively). On the other hand, *T. viride* grown in wastewater at

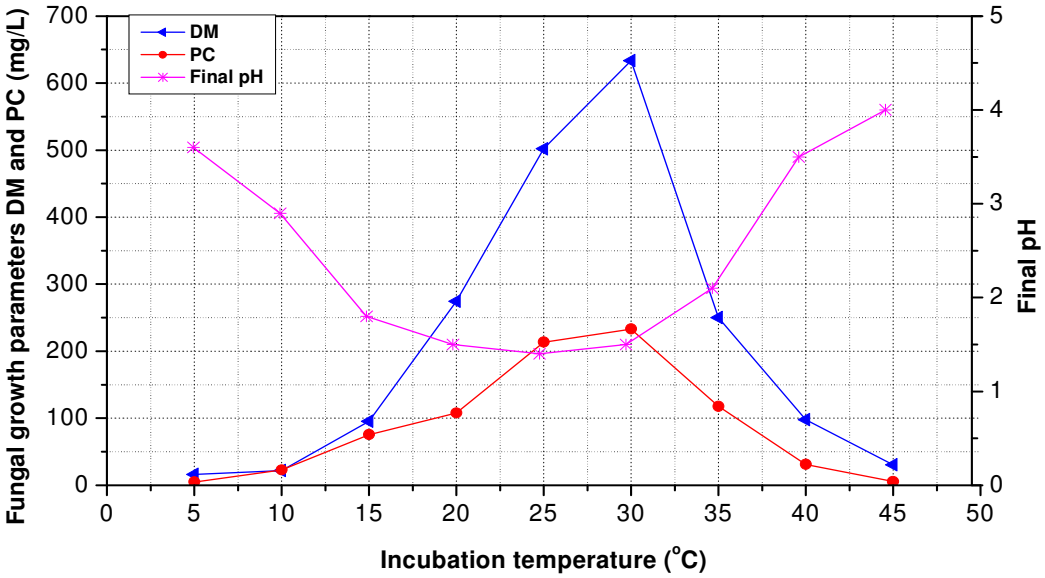
5 °C temperature recorded the lowest values of $PO_4$ (5.2 %), $NO_3$ (2.5 %), and DM (18.0 mg/L), while the lowest values of TN (1.5 %), COD (1.8 %), and PC (5.1 mg/L) were found at 45 °C. The lowest elimination rate (5.8 %) of $NH_4$ created by *T. viride* was found at 5 and/or 45 °C temperature degree.

The results were almost in harmony with the findings of Thanh and Simard (1973a), Abdul Karim and Kamil (1989), Guest and Smith (2007), D'Urso *et al.* (2008), and Gonçalves *et al.* (2009). Thanh and Sumard (1973a) mentioned that temperature ranged from 15 to 30 °C was the optimum temperature for protein content, mycelium yield and removal of nitrogen and phosphorus compounds from demostic wastewater by *Trichoderma roseum*. Also, Abdul Karim and Kamil (1989) mentioned the highest reduction of COD and the maximum dry weight of mycelium and protein content by *Trichoderma viride* cultivated in oil mill wastewater were at 28 ± 2 °C. Guest and Smith (2007) found that the maximum removal of $NH_4$ and $PO_4$ from wastewater by *Phome sp.* and *Geotrichum sp.*, respectively at 21 °C. Jin *et al.* (1999b) reported that the maximum biomass yield and COD reduction by *Rhizopus oligosporus* in wastewater occurred at 30-37 °C. Also the author mentioned that, *R. oligosporus* grow poorly at temperature below 28 °C and above 45 °C.



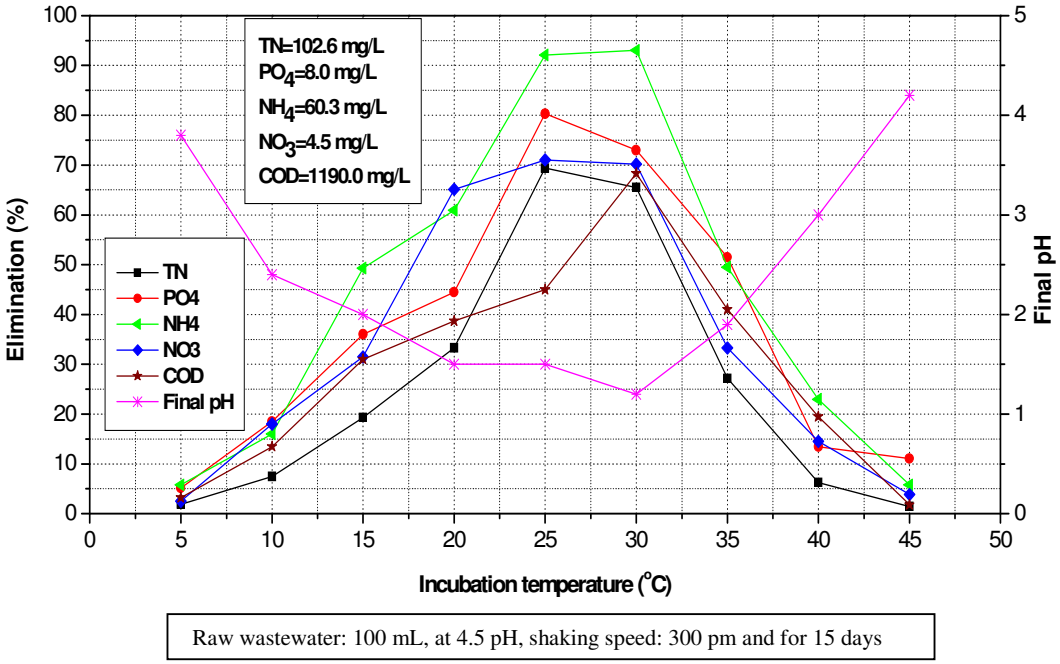

TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.12:** Impact of incubation temperature (°C) on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater



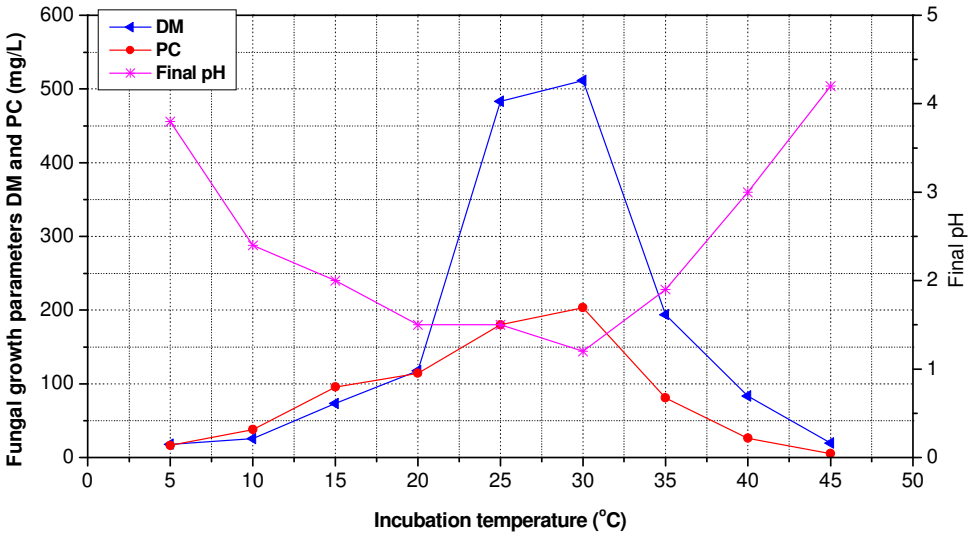

DM= Dry matter, PC= Protein content

**Fig. 4.13:** Impact of incubation temperature (°C) on the growth of *Aspergillus niger* in raw wastewater




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.14:** Impact of incubation temperature (°C) on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater




Raw wastewater: 100 mL, at 4.5 pH, shaking speed: 300 pm and for 15 days

DM= Dry matter, PC= Protein content

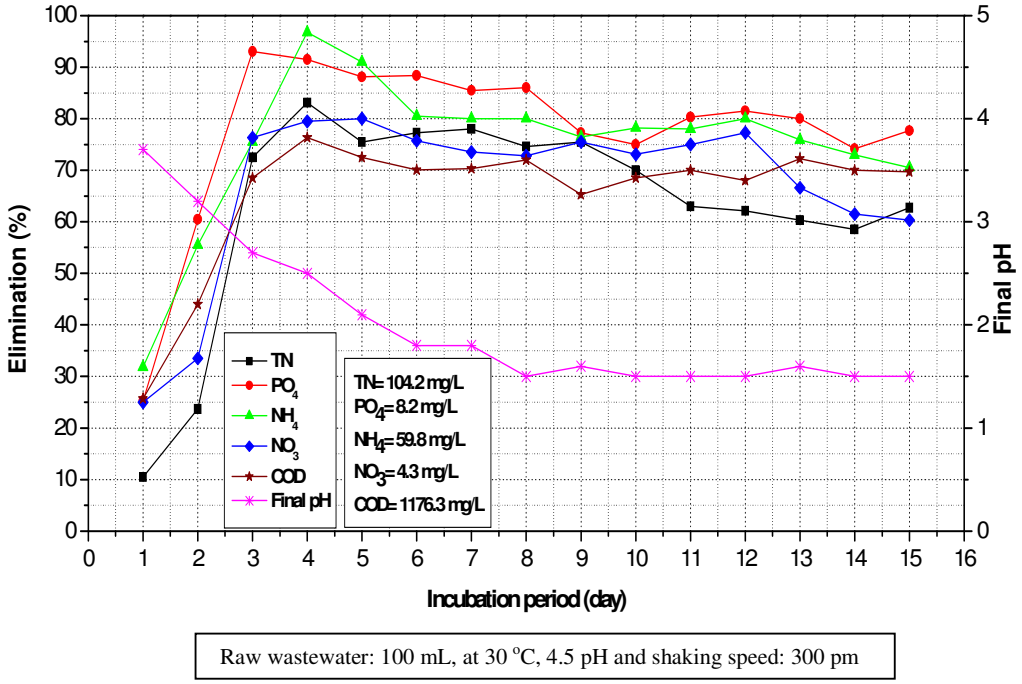
**Fig. 4.15:** Impact of incubation temperature (°C) on the growth of *Trichoderma viride* in raw wastewater

### 4.5.3. Effect of incubation period

Several previous studies demonstrated the growth of fungi in batch cultures arranged to four distinct growth phases (i.e. lag, exponential, stationary and declining phase) (Singh, 2006). The data in Figures 4.16, 4.17, 4.20, 4.21 and (Table A.5, A.6) showed the variations of the studied compounds (TN, $PO_4$, $NH_4$, $NO_3$, COD, DM and PC) as a function of incubation period. The data reveal that, *T. viride* recorded the highest influence of all examined properties compared to *Aspergillus niger* (except DM). This trend may show that the metabolic activities of *Trichoderma viride* are higher than those of *Aspergillus niger*.

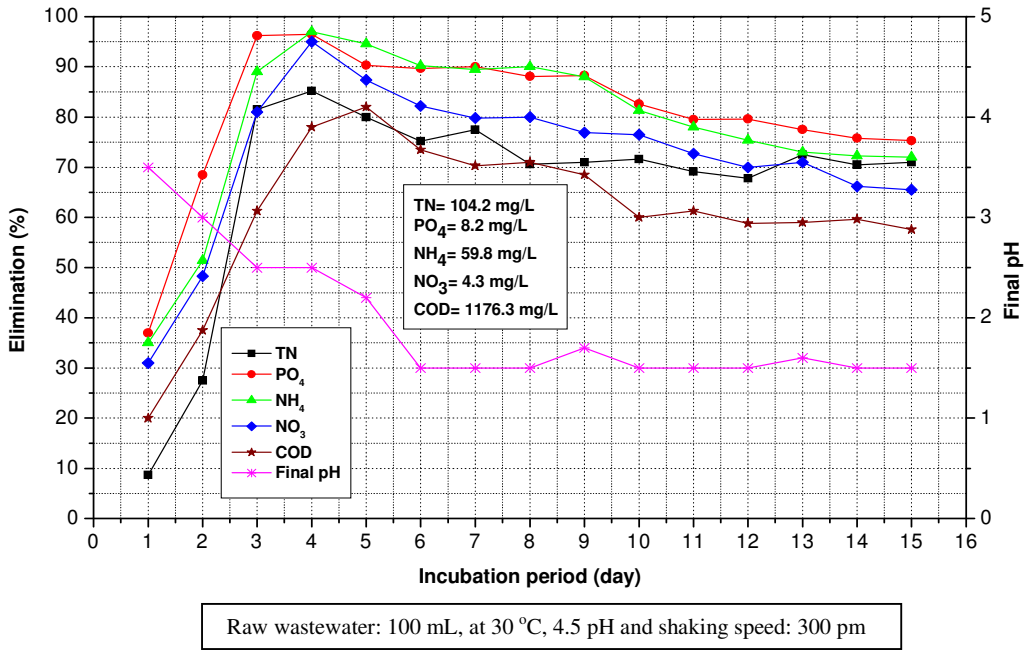
Data in Figures 4.16, 4.20 and Table A.5 show that *A. niger* attained with the highest elimination of TN (83.1 %), $NH_4$ (96.7 %) and COD (76.3 %) after four days, while the greater reduction degree (80.0 %) of $NO_3$ appeared at day five of incubation time. The 3 days of incubation was the best period for reduction of $PO_4$ noticed for *A. niger*. The maximum percentage of the reduction of phosphate was 93.0 %. On the other hand, the low reduction rates of TN (10.5 %), $PO_4$ (25.3 %), $NH_4$ (31.8 %), $NO_3$ (25.0 %) and COD (25.7 %) by *A. niger* were observed at the first day. The fungal grown in raw wastewater gave the best dry matter yield (DM, 698.0 mg/L) at 8 days from the beginning, while the low mycelia growth (36.0 mg/L) after one day. Also, Fig 4.5 indicates that *A. niger* at day seven recorded the highest protein concentration (187.0 mg/L), whereas the lowest values (35.0 mg/L) found after1 day.

Data in Figures 4.17, 4.21 and Table A.6 show the high elimination of the tested parameters in inoculated raw wastewater by *Trichoderma viride*. The best reduction rate appeared after 4 days of TN (85.2 %), $PO_4$ (96.5 %), $NH_4$ (97.0 %), and $NO_3$ (95.0 %), while the highest elimination (82.0 %) of COD was found at day five. Similarly as noticed with *A. niger* the data showed the lowest reduction attained by *T. viride* were TN (8.7 %), $PO_4$ (37.0 %), $NH_4$ (35.0 %), $NO_3$ (31.0 %), and COD (20.0 %) after 24 h. Also, the results in Fig 3.6 indicate that *T. viride* recorded the highest DM (602.2 mg/L) found after 10 days, while the highest (256.0 mg/L) of PC was measured after 8 days. However, the low formation rate of either DM (22.5 mg/L) or PC (15.0 mg/L) of *T. viride* appeared at first day.




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.16:** Impact of incubation period (day) on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater




TN= Total nitrogen, COD= Chemical oxygen demand

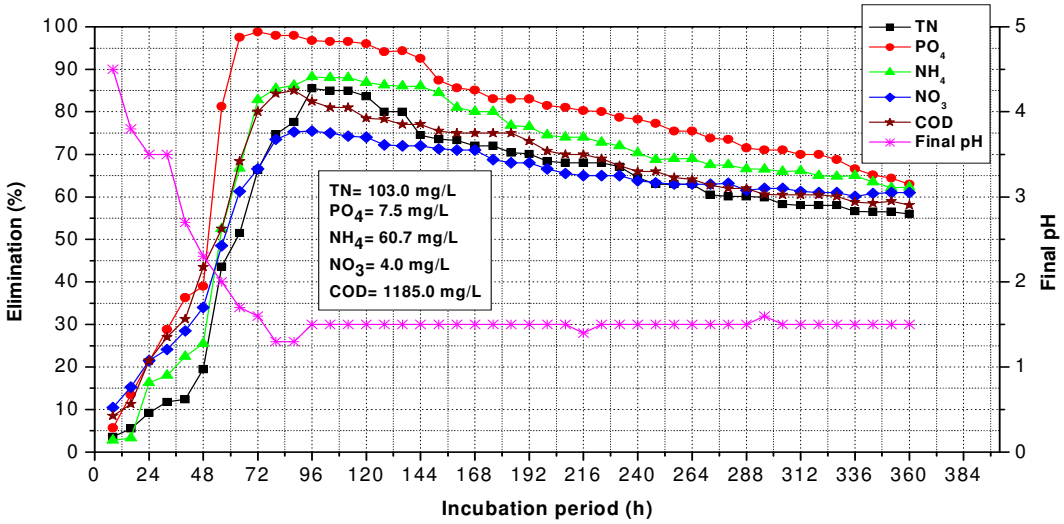
**Fig. 4.17:** Impact of incubation period (day) on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater

Data in Figure 4.18 and Table A.7 show that *A. niger* attained with the highest elimination of TN (85.5 %), $NH_4$ (88.2 %), and $NO_3$ (75.5 %) after 96 h, while the greater reduction value (85.0 %) of COD appeared at the 88 h of incubation time. The 72 h of incubation was the best period for reduction of $PO_4$ noticed for *A. niger*. The maximum percentage of the reduction of phosphate was 98.8 %. On the other hand, the low elimination rates of TN (3.6 %), $PO_4$ (5.7 %), $NH_4$ (2.8 %), $NO_3$ (10.5 %), and COD (8.5 %) by *A. niger* were observed after 8 h from incubation time.

Data in Figure 4.20 and Table A.7 explain the best dry matter yield and protein content of *Aspergillus niger* (713.5 mg/L and 172.5 mg/L) were obtained after 160 h from incubation time, respectively. While the lowest dry matter yield and protein content (7.0 mg/L and 35.0 mg/L) were found after 8 h, respectively.

Data in Figure 4.19 and Table A.8 show the high elimination of the tested parameters in raw wastewater accompanied with *Trichoderma viride*. The higher elimination appeared after 88 h of TN (86.4 %) and $PO_4$ (92.5 %), 64 h of $NH_4$ (97.0 %) and 72 h of $NO_3$ (88.5 %), while the highest elimination (80.0 %) of COD was found at 96 h from incubation time. Similarly as noticed with *A. niger* the data showed the lowest reduction degree attained by *T. viride* were TN (1.5 %), $PO_4$ (2.3 %), $NH_4$ (4.1 %), $NO_3$ (2.8 %), and COD (5.5 %) after 8 h.

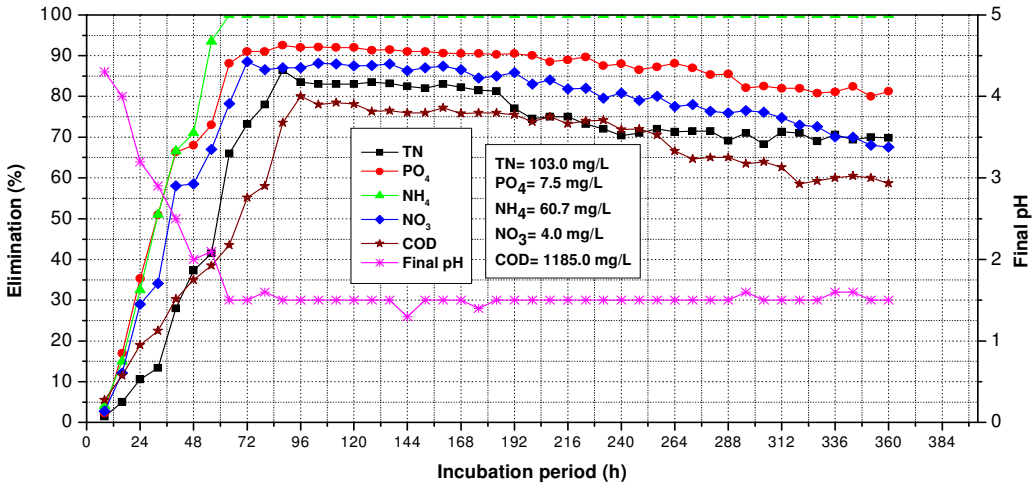
The results in Figure 4.21 and Table A. 8 indicated the *T. viride* recorded the highest dry matter (683.9 mg/L) founded after 184 h, while the highest concentration (247.5 mg/L) of protein content was measured at 168 h of incubation period. However, the low formation rate of either DM (22.5 mg/L) or PC (15.0 mg/L) of *T. viride* was appeared at 8 h.




Raw wastewater: 100 mL, at 30 °C, 4.5 pH and shaking speed: 300 pm

TN= Total nitrogen, COD= Chemical oxygen demand

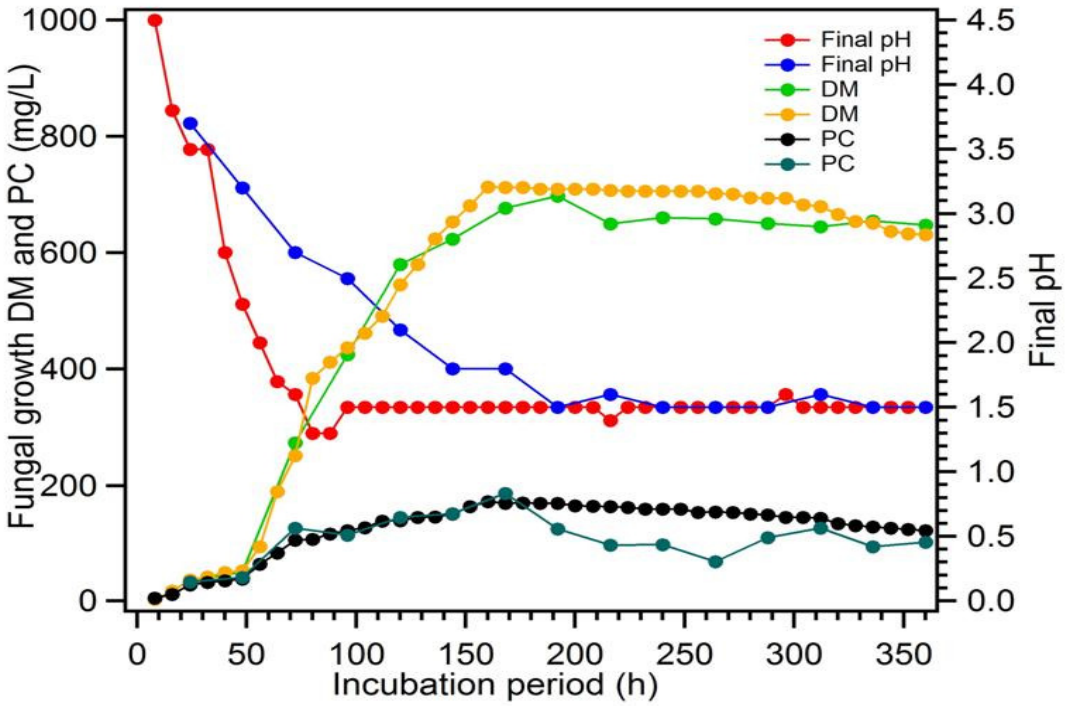
**Fig. 4.18:** Impact of incubation period (h) on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater




Raw wastewater: 100 mL, at 30 °C, 4.5 pH and shaking speed: 300 pm

TN= Total nitrogen, COD= Chemical oxygen demand

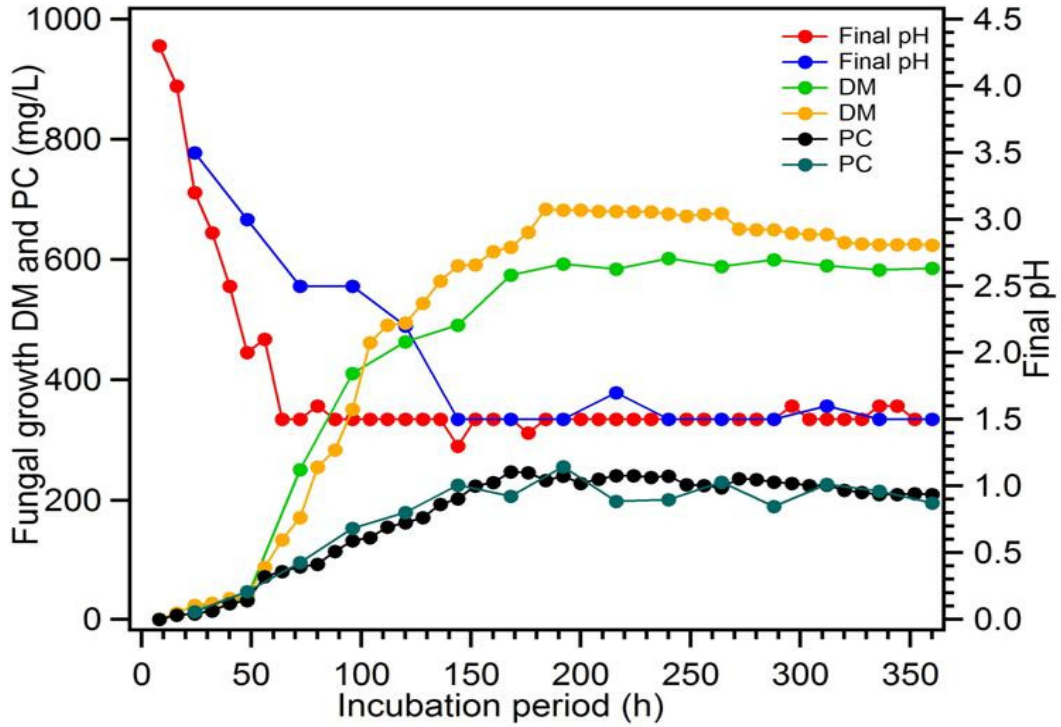
**Fig. 4.19:** Impact of incubation period (h) on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater




Raw wastewater: 100 mL, at 30 °C, 4.5 pH and shaking speed: 300 pm

DM= Dry matter, PC= Protein content

**Fig. 4.20:** Impact of incubation period on the growth of *Aspergillus niger* in raw wastewatrer




Raw wastewater: 100 mL, at 30 °C, 4.5 pH and shaking speed: 300 pm

DM= Dry matter, PC= Protein content

**Fig. 4.21:** Impact of incubation periods hes on the growth of *Trichoderma viride* in raw wastewatrer

The results were almost in harmony with the findings of (Thanh and Simard, 1973a; Hiremath *et al*. (1985b); Fujita *et al*., 1993; Murado *et al*., 1993; Jin *et al*., 1999a, b, c; Yesilada *et al*., 1999; Couilibaly, 2002; Selvam *et al*., 2002; Alam *et al*., 2003; 2004; Jaouani *et al*., 2003; 2005; Zahng *et al*., 2008).

Akhtar and Ghaffar (1986) study is the removal of $NH_4$ from domestic wastewater by several species of fungi found that the higher reduction degree (52.3 %) with *Aspergillus flavus* attained after 5 days. Hamdi *et al*. (1991) demonstrated that after 72 h the COD removal from Olive mill wastewaters in flasks by *Aspergillus niger* was 61.6 % and 3075.0 mg/L soluble protein content. Moreover, Jin *et al*. (1998) reported that the best *Aspergillus oryzae* biomass yield (5098.0 g/L), protein content (3728.0 mg/L and COD reduction (9420.0 mg/L) from starch processing wastewater at 30 and 37 °C, respectively after 24 h incubation.

Abdul Karim and Kamil (1989) investigated the treatment of Palm oil mill effluent using *Trichoderma viride* and found that, more than 95 % reduction in COD was achieved after 240 to 336 h of fermentation. Also, the fungal biomass was 1037.0-1042.0 mg/L of mycelium with a protein content of 37.6 - 40.7 %. In shake fermentation using *T. viride* to treat winery wastewater.

Zahng *et al*. (2008) demonestrated that, more than 5000.0 mg/L of fungal biomass was produced, 84.0 % to 90.0 % COD reduction, fungal biomass contained approximately 19.8 % protein and maximum fungal cell growth could be achieved in 24 h incubation.

Alam *et al*. (2004) reported that, the maximum COD removal (93.0 %) was obtained by *Penicillium sp* incubated in sludge wastewater at 150 r/min and 33 $^{o}$C after 3 days. In another study Garcia *et al*. (2000) reported that *Geotrichum candidum* removed 25-38 % COD from wastewater after 20 days in shaker flasks. Mannan *et al*. (2005) found the maximum removal of COD from wastewater activated sludge was recorded as 94.40 % after 2 days of treatment by *Penicillium corylophilum*, the COD removal efficiency was slightly decreased after 3–5 days.

Hiremath *et al*. (1985b) performed a similar study, they tested seven fungal species isolated from a wastewater stabilization pond. The major goal of the study was to maximize biomass production of fungi as a food source for animal or human consumption. The trials were conducted in 2 L conical flasks containing 1.5 L of sterile fresh wastewater. Flasks were inoculated with pure cultures of fungi and incubated at room temperature for 10 days. The culture flasks were gently agitated twice a day. The study reported BOD5 removal between 53.0 % and 72.0 %, phosphate removal from 34.0 % to 77.0 %, and ammonia nitrogen removal between 49.0 % and 77.0 %. Due to the experimental design, the cultures were most likely completely anaerobic for the entire 10 days incubation.

Robles *et al.* (2000) screened seven strains of *Penicillium spp.* for biomass production of Olive oil industry wastewater and found that, after 20 days of cultivation, most of the strains produced a considerable amount of biomass. The best reduction of COD (74.75 % and 60.75 %) was obtained by *Penicillium* 1 and *Penicillium* 4, respectively. Yesilada *et al.* (1999) screened seven fungal isolates for biological treatment of Olive oil mill wastewater at pH 4.9 and 30 $^{o}$C for several incubation periods and reported that the best reduction of COD was obtained with *Coriolus versicolor*, *Funalina trogii* and *Pleurotus sajorcaju* (63-70 %) after 3 days of growth.

## 4.6. Elimination of nitrogen, phosphorous and COD from raw wastewater nutrients in Batch reactor systems by *Aspergillus niger* and *Trichoderma viride*

### 4.6.1. Aerobic batch reactor (day)

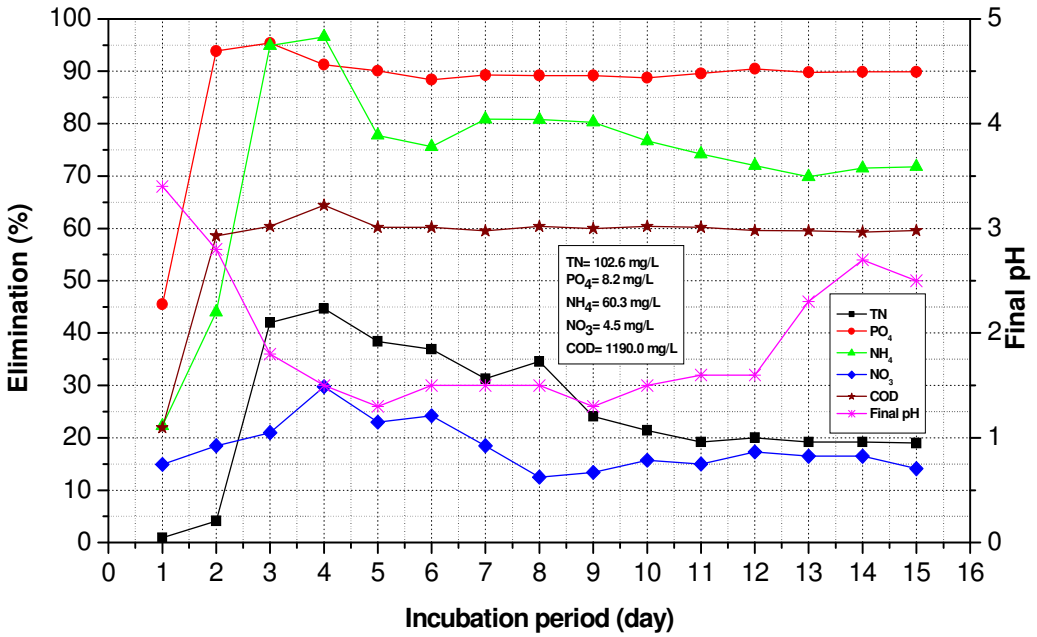
Elimination of the studied compounds under stable source of oxygen flow rate (68 L/h) by *Aspergillus niger* and *Trichoderma viride* in a 2L batch reactor for 15 days has been illustrated in Figures 4.22, 4.23 and tabulated in Table A.9, A10. Generally, both examined fungi the relationships between experimental period and reduction was positive, with the highest reduction degree at 4 and 5 days for *A. niger* and *T. viride* respectively. After that the elimination of all compounds slightly decreased till the end of incubation period. With the exception of $NO_3$ and COD the data reveal that the ability of *T. viride* for biological wastewater treatment was better than *A. niger*.

Data in Figure 4.22 and (Table A.9) show the variation rate of eliminated nutrients by *A. niger* inoculated in raw wastewater. Throughout incubation period the highest reduction was recorded for $PO_4$ followed by $NH_4$, COD, $NO_3$ and finally TN. Total nitrogen appeared with the highest reduction (44.7 %) at day 4, while the lowest rate (0.9 %) found after 24 h. TN was increased with increasing time up to the day 4, after that it was slightly decreased until the end of incubation period. The highest reduction rate (95.4 %) of $PO_4$ attained after 3 days, whereas the lowest value (45.5 %) was recorded on the first day. The 4 day of incubation was the best period for reduction of $NH_4$ and $NO_3$. The maximum percentage of $NH_4$ and $NO_3$ reduction were 96.9 % and 29.8 %, respectively. While the minimum reduction (22.3 % and 14.9 %) recorded to $NH_4$ and $NO_3$, respectively. Also, treated raw wastewater by *A. niger* observed the highest elimination (64.4 %) of chemical oxygen demand at day 4. Similarly to TN, $PO_4$, $NH_4$ and $NO_3$ the lowest reduction rate (21.9 %) of COD was found after 24 h.

Regarding to the elimination as affected by *T. viride*, the data in Figure 4.23 and (Table A.10) show the high differences among the studied compounds during incubation period. In

general, during incubation period the highest reduction value recorded to $NH_4$ followed by $PO_4$, COD, TN and finally $NO_3$. The data reveal that all the tested attributes found with the highest reduction after 5 days (except $NO_3$).

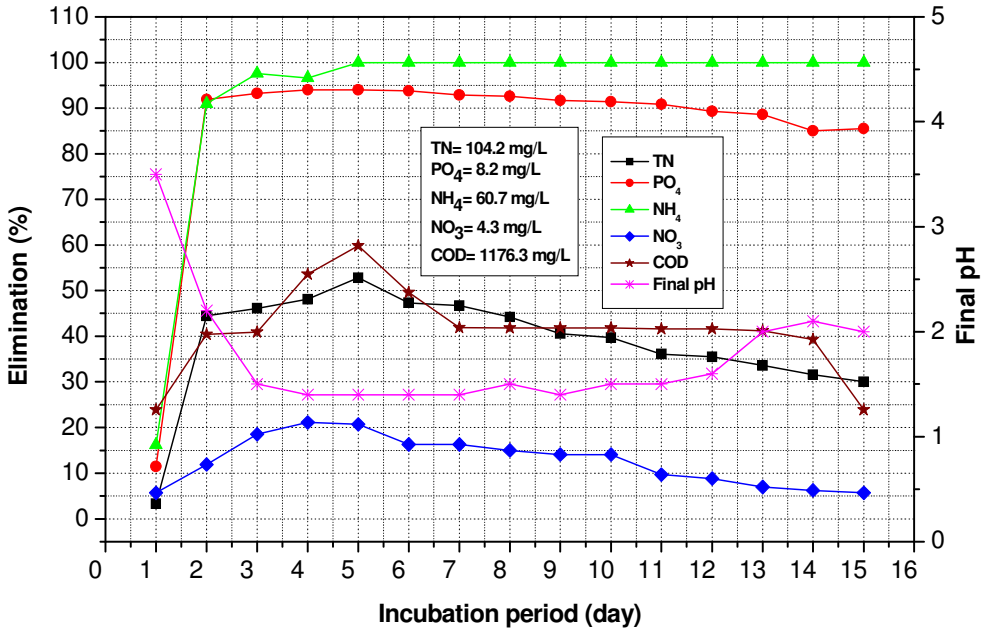
The maximum percentage of eliminated TN was 52.8 % while the minimum was 3.3 % found after 24 h. Raw wastewater infected by *T. viride* showed the highest reduction (94.0 %) of $PO_4$ on day 4. The lowest reduction (11.5 %) of $PO_4$ was attained on the first day of incubation period. The 5 days of incubation showed the maximum reduction (100 %) of $NH_4$ while $NO_3$ recorded the highest elimination rate (21.1 %) on day 4. Both $NH_4$ and $NO_3$ recorded the lowest reduction values (16.2 % and 5.7 %, respectively) in the first day of incubation period. Also, *T. viride* recorded the best elimination of COD after 5 days, with 59.8 % maximum reduction value. Finally, on first day of incubation the examined fungus grown in aerobic batch reactor recorded the lowest reduction value (23.9 %) of COD (Figure 4.23).




Batch: 2L, stirring speed: 300 rpm, air flow rate: 68 L/h, at 30°C, 4.5pH and for 15 days

TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.22:** Impact of incubation period (day) on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater in (aerobic batch)




TN= Total nitrogen, COD= Chemical oxygen demand

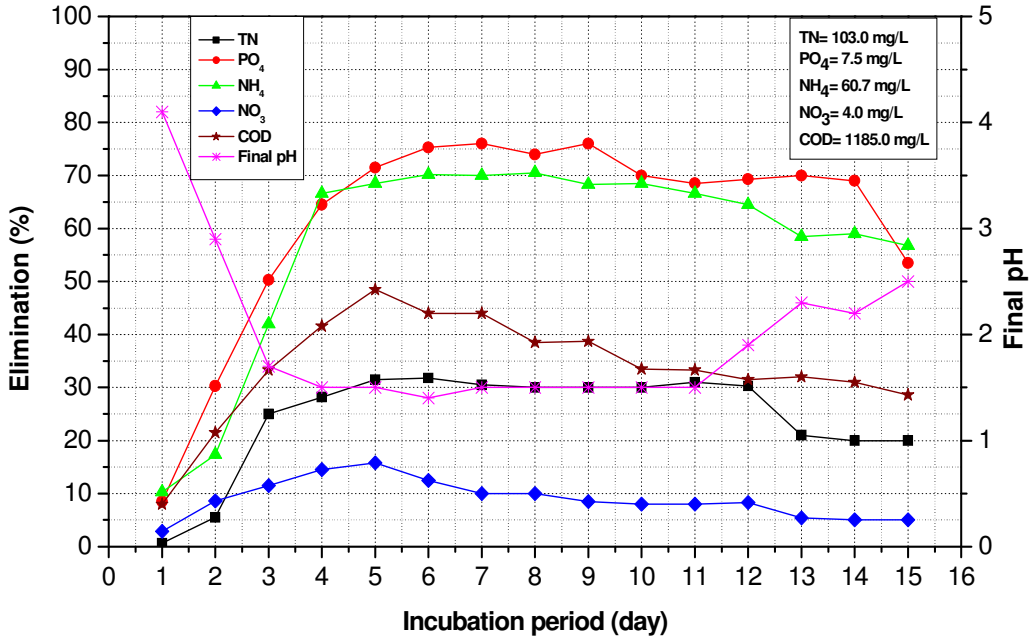
**Fig. 4.23:** Impact of incubation period (day) on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater in (aerobic batch)

### 4.6.2. Anaerobic batch reactor (day)

Data in Figure 4.24 and Table A.11 showed the relationships between incubation period and elimination of the tested compounds as affected by *Aspergillus niger*. Generally, during the 15 days incubation the highest reduction was recorded for $PO_4$ followed by $NH_4$, COD, TN and finally $NO_3$. Total nitrogen appeared with the maximum reduction (31.8 %) after 6 days, while it recorded the lowest value (0.6 %) on the first day. Eliminated $PO_4$ from raw wastewater by *A. niger* attained with the highest value (76.0 %) at day 7 and lowest value (8.5 %) after 24 h. 8 and 5 days of incubation was the best period for reduction of $NH_4$ and $NO_3$, respectively . The maximum reduction of $NH_4$ (70.5 %) and $NO_3$ (15.8 %), wherease the minimum reduction was 10.3 % ($NH_4$) and 2.9 % ($NO_3$). Also, results in Fig 3.26 indicate the best reduction (48.5 %) of chemical oxygen demand (COD) observed at day 5, while the lowest value (8.0 %) was found at the first day.

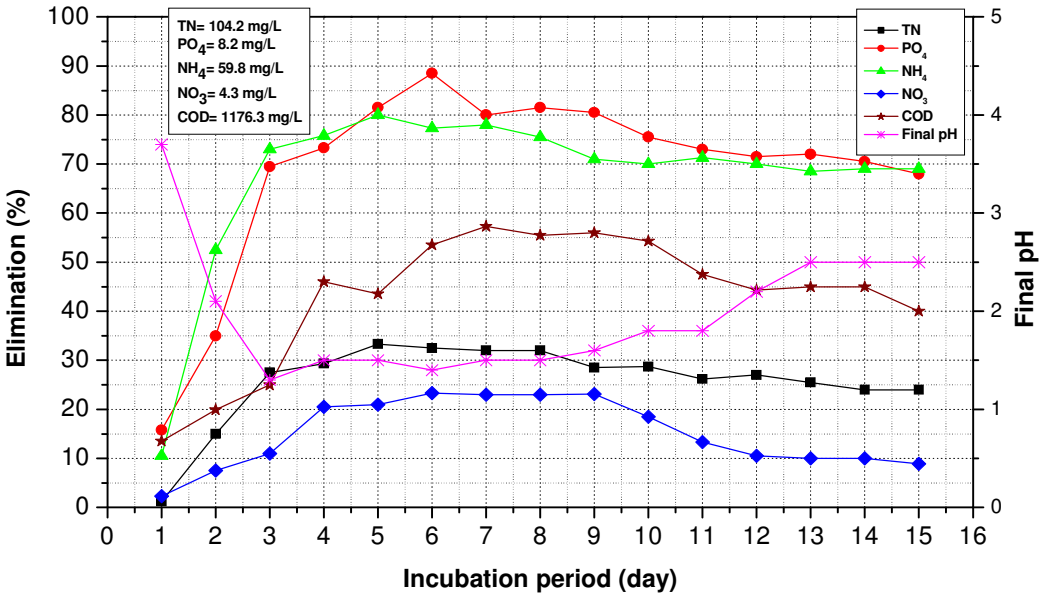
As for the effect of *Trichoderma viride* on the elimination of the examined compounds, data in Fig 4.25 and Table A12 show the high variations during incubation period. In general, during incubation time the highest reduction was recorded for $PO_4$, followed by $NH_4$, COD, TN and finally $NO_3$. TN observed with the maximum reduction (33.3 %) on day 5, while the minimum elimination (1.2 %) was found after 24 h. Wastewater inoculated by *T. viride* observed with the highest

reduction (88.5 %) of $PO_4$ after 6 days. The minimum reduction of $PO_4$ was 15.8 % which resulted in the first day. Also, the results in Fig 3.27 show the maximum reduction (80.0 %) of $NH_4$ while $NO_3$ appeared with the highest elimination (23.3 %) on day 6. Either $NH_4$ or $NO_3$ attained with the minimum reduction (10.5 and 2.3 %, respectively) in the first day. Also, *Trichoderma viride* observed with the best reduction rate of COD after 7 days from the beginning, with 57.3 % maximum reduction value. Finally, the examined fungus grown in anaerobic batch reactor recorded the lowest reduction value (13.5 %) of COD after 24 h of incubation (Fig. 4.25).




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.24:** Impact of incubation period (day) on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater in (anaerobic batch)




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.25:** Impact of incubation period (day) on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater in (anaerobic batch)

### 4.6.3. The growth parameters of the examined fungi incubated in batch reactor

The growth parameters of mycelium dry matter (DM) and protein content (PC) of *Aspergillus niger* and *Trichoderma viride* were measured at the end of incubation period. Data in Table 3.12 showed the DM and PC of the studied fungi incubated in the batch reactor under aerobic and anaerobic conditions. Clearly, the results indicate that, both *A. niger* and *T. viride* appeared with the highest values of either DM or PC in the batch cultured under aerobic condition compared with those in anaerobic batch reactor. Also, under different examined conditions the highest values of both DM and PC were recorded for *T. viride*, while the lowest contents were found in *A. niger* (Table 4.12).

**Table 4.12:** Mycelium dry weight and protein content of *Aspergillus niger* and *Trichoderma viride* under different aeration conditions at the end of incubation period (15 days)

| Isolates | Aerobic batch reactor (15 day) | | Anaerobic batch reactor (15 day) | |
|---|---|---|---|---|
| | DM (mg/L) | PC (mg/L) | DM (mg/L) | PC (mg/L) |
| ***A. niger*** | 5030.0 | 253.0 | 3040.0 | 217.5 |
| ***T. viride*** | 7010.0 | 311.5 | 4080.0 | 263.8 |

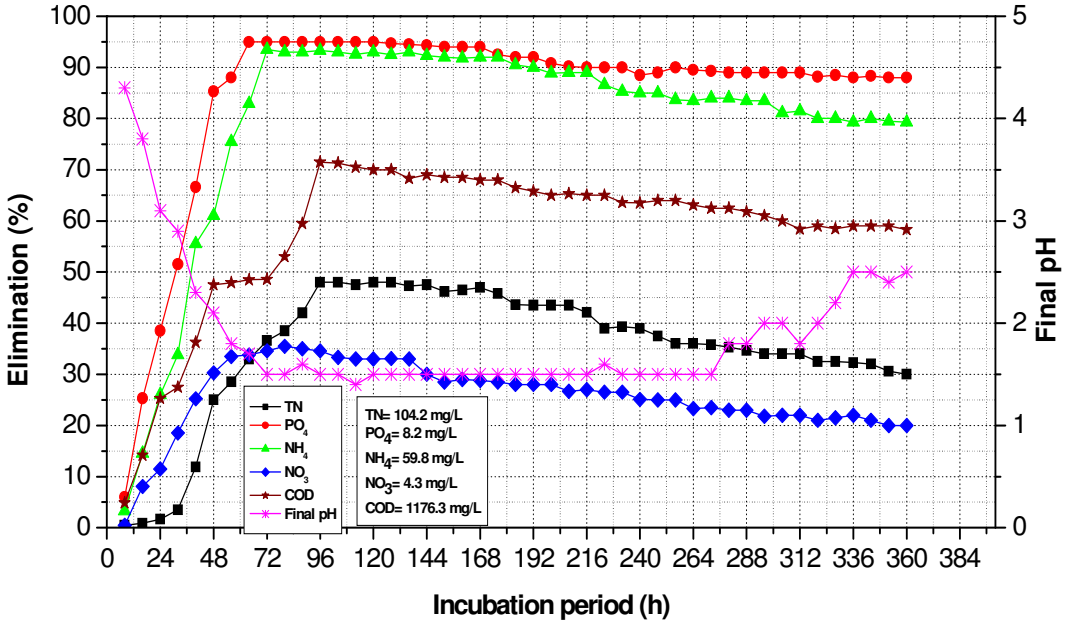
DM= mycelium dry weight, PC= protein content

### 4.6.4. Aerobic batch reactor (h)

Data in Figures 4.26, 4.27 and Tables A.13, A14 show the relationships between incubation period (h) and elimination of the tested compounds as affected by *Aspergillus niger* and *Trichoderma viride* under stable air flow rate (68 L/h) in 2 L batch reactor for 15 days. The highest elimination between 64 to 96 h, and 72 to 120 h was obtained for *A. niger* and *T. viride*, respectively. After that the elimination of all compounds was slightly decreased till the end of incubation period. With the exception of COD the data reveals that the ability of *T. viride* for biological wastewater treatment was better than *A. niger*.

Data in Figure 4.26 and Table A.13 show the variation of eliminated nutrients by *A. niger* inoculated in raw wastewater. Throughout incubation period the highest reduction rate recorded to $PO_4$ followed by $NH_4$, COD, $NO_3$ and finally TN. Total nitrogen and COD appeared with the highest elimination (48.0 % and 71.5 %) at 96 h, respectively. While the lowest rate (0.5 % and 4.9 %) was found after 8 h incubation, respectively. The eliminations of TN and COD were increased with increasing time up to the 96 h, after that it was slightly decreased until the end of incubation period. The highest elimination rate (95.0 %) of $PO_4$ attained after 64 h, whereas the lowest value (6.0 %) recorded after 8 h incubation. The 72 h of incubation was the best period for elimination of $NH_4$. The maximum percentage of $NH_4$ reduction was 93.5 % and the lowest value (3.3 %) recorded after 8 h incubation. While the highest elimination (35.5 %) of $NO_3$ recorded at 80 h from incubation time. Similarly to TN, $PO_4$, $NH_4$ and COD the lowest reduction (0.5 %) of $NO_3$ was found after 8 h.

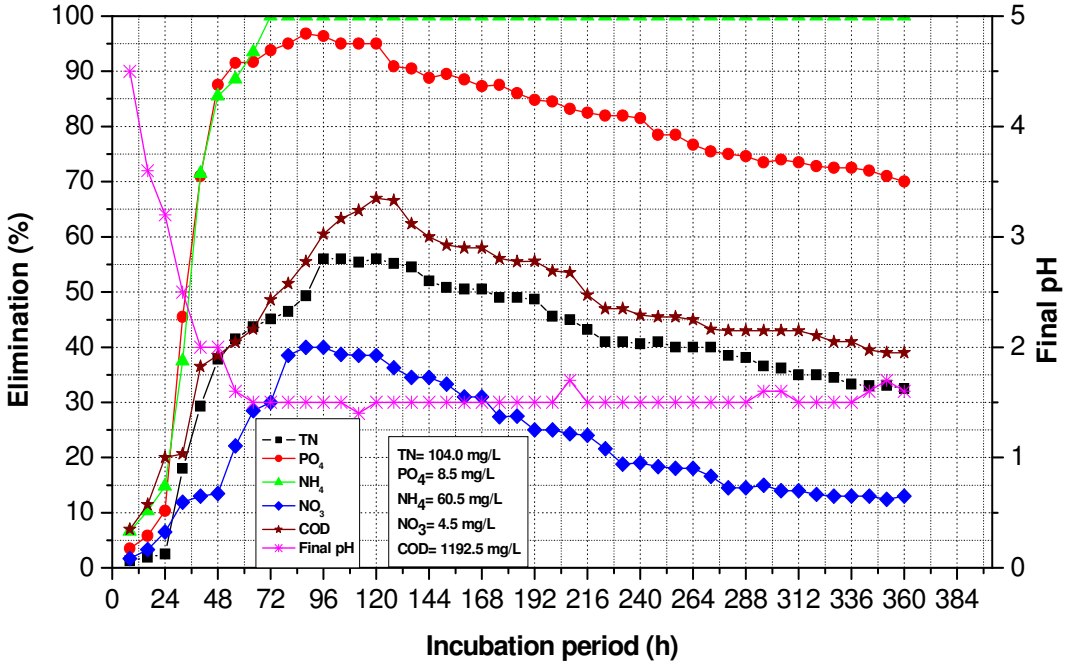
Regarding to the elimination rate (%) as affected by *T. viride*, the data in Figure 4.27 and Table A.14 show the high differences among the studied compounds during incubation period. In general, during incubation period the highest reduction value was recorded for $NH_4$ followed by $PO_4$, COD, TN and finally $NO_3$. The data reveal that the highest reduction of TN (56.0 %) was recorded after 96 h from the beginning, while the lowest elimination (1.3 %) of TN found at 8 h. The maximum percentage of eliminated $PO_4$ was 96.8 % after 88 h, whilst the minimum was 3.5 % found after 8 h. Raw wastewater inoculated by *T. viride* observed with the highest reduction (100 %) of $NH_4$ at 72 h. The lowest reduction (6.6 %) of $NH_4$ attained at 8 h of incubation period. The 88 h of incubation showed the maximum reduction (40.0 %) of $NO_3$ while COD appeared with the highest elimination (67.0 %) at 120 h. Also both $NO_3$ and COD recorded the lowest elimination values (1.7 % and 7.0 %, respectively) at 8 h of incubation period (Figure 4.27).




Batch: 2L, stirring speed: 300 rpm, air flow rate: 68 L/h, at 30°C, 4.5pH and for 15 days (each 8h)

TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.26:** Impact of incubation period (h) on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater (anaerobic batch)




Batch: 2L, stirring speed: 300 rpm, air flow rate: 68 L/h, at 30°C, 4.5pH and for 15 days (each 8h)

TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.27:** Impact of incubation periods (h) on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater (aerobic batch)

**Table 4.13:** Mycelium dry weight and protein content of *Aspergillus niger* and *Trichoderma viride* under aerobic conditions at the end of incubation period (15 days)

| Isolates | Aerobic batch reactor (15 day) | |
|---|---|---|
| | DM (mg/L) | PC (mg/L) |
| ***A. niger*** | 5080.0 | 265.0 |
| ***T. viride*** | 7050.0 | 280.0 |

DM= mycelium dry weight, PC= protein content

The previous results of batch reactors incubated under arobic and anaerobic condition were supported by several researchers. The obtained clear results demonstrated the maximum reduction of the examined compounds accompanied with *Aspergillus niger* and *Trichoderma viride* were observed in aerobic condition. Also, the data reveal that, the fungal growth parameters (DM and PC) attained with the highest yields for the fungi grown in the wastewater media treated with oxygen. These trends could be related to the oxygen is very important and necessary for fungal growth and metabolisms processes, while the existence of $N_2$ in the media may inhibit the fungal activities. Oxygen is used in respiration in most organisms. The fungi include species that are obligately aerobic or obligately anaerobic. However many fungi are in between, with the capacity to function facultatively in aerobic and anaerobic conditions. Oxygen is used for oxidative metabolism, to generate energy. Moreover, the low elimination and fungal growth with the wastewater provided with nitrogen may be due to the predicted toxic effect of $N_2$ (Tabak and Cooke, 1968; Yang and Lucas, 1970; Pitt and Hocking 2009).

The results were almost in harmony with the findings of (Church *et al*. 1973; Ek and Eriksson, 1980; Hamdi *et al*. 1992; Hamdi and Ellouz, 1993; Hamdi and Garcia, 1993; Borja *et al*. 1993; 1995a; b; 1998; Blanquez *et al*. 2002; and others). Church *et al*. (1973) have reported the successful use of the fungus *Trichoderma viride* in aerated lagoon and oxidation ditch to treat corn and pea canning wastes and greater than 95.0 % COD removal of the wastes was achieved. They demonstrated that, the fungi have the ability to convert dissolved and suspended organic matter into a mycelium that is high in protein content. *Sporotichum pulverulentum* can reduce the COD up to 52.0 %, with 5.7 g/L fungal biomass and 42.0 % protein content after inoculated in wastewater lined in 25 $m^3$ batch reactor (Ek and Eriksson, 1980). Blanquez *et al*. (2002) mentioned that, the white rot fungus *Phanerochaete flavido-alba* removed 75.0 % COD from Olive mill wastewater after 6 days in aerobic batch fermenter.

Also, Hamdi *et al*. (1992) reported that, *Aspergillus niger* grown in Olive mill wastewater for 72 h at pH 5.6 and under aerobic condition, removed 58.0 % COD accompanied with increasing

production of biomass and 30.0 % fungal protein content. While with the same fungus and under anaerobic condition the reduction of COD was 60.4 %. In other studies, anaerobic digestion after pretreatment in batch reactor with *A. niger* removed over 60 % of COD (Hamdi and Ellouz, 1993; Hamdi and Garcia, 1993).

Borja *et al.* (1998) compared anaerobic digestion of OMW pretreated by two different fungi and a bacterium: *Geotrichum candidum*, *Aspergillus terreus* and *Azotobacter chroococcum*. These organisms decreased the COD concentration of Olive mill wastewater by 59 %, 87 % and 79 %, respectively.

Garrido Hoyos *et al.* (2002) used *Aspergillus terreus* for aerobic treatment of Olive mill wastewater in 5 L reactor at 200 rpm, a temperature of 30 °C and 5.26 pH. The mean values for chemical oxygen demand (COD) elimination rate were 126.3 mg $L^{-1}$ $h^{-1}$ in the first 24 h and 77.3 mg $L^{-1}$ $h^{-1}$ at 72 h. An increase in airflow allowed higher degradation percentages in less time (COD: 65.77 % and BOD: 85.41 %). Gonçalves *et al.* (2009) screened 5 strains from *yeasts* for biological treatment of Olive mill wastewater in aerobic 2 L bioreactor (Biolab, B, Braun), and demonstrated that both strains were able to grow on wastewater without dilution, but *candida cylindracea* was the best strain reduction of 70.2 % COD at 27 $^{o}$C after 22 h incubation.

Guest and Smith (2007) determined the fungi potential for ammonium and orthophosphate reduction in wastewater and demonstrated under aerobic condition in a 2 L batch reactor (3 days incubation), *penicillium sp* can eliminate up to 37.0 and 8.0 % for $NH_4$ and $PO_4$, respectively. The authors reported that under anaerobic condition *Geutrichum sp.* decreased the concentration of $NH_4$ and o-$PO_4$ to 56.0 and 53.0 %, respectively. Moreover, reduction degree of $NH_4$ (59.0 %) and o-$PO_4$ (39.0 %) accompanied with *Phoma sp.* and *Mucor sp.*, respectively. Jasti *et al.* (2006) reported that the maximum chemical oxygen demand (COD) removal of 78.0 % from corn processing wastewater was achieved at a 5 h with a biomass yield of 0.44 g/L by *Rhizopus oligosporus* in biofilm reactor. Jimenez *et al.* (2003) screened *Penicillium sp.*, *P. decumbens*, *P. lignorum* and *Aspergillus niger* for aerobic and anaerobic biotreatment of beet molasses wastewater. Average COD removals were similar in the four species, achieving maximum values of 52.1 % and 50.7 %, respectively, on the fifth day of fermentation with *Penicillium sp.* and P. decumbens in aerobic fermentation. While the highest COD removal of 93 % COD was found in the anaerobic digestion with *Penicillium decumbens*.

Jin *et al.* (2001) mentioned that a fungal biomass productivity in a range of 0.85-0.92 g $m^{-3}/h^{-1}$ and removals of 95.0 % COD were achieved for *Aspergillus oryzae* and *Rhizopus arrhizus* grown in raw starch processing wastewater at 35 $^{o}$C after 12 h cultivation in

external air-lift bioreactor. The growth of *Aspergillus niger* in undiluted liquid effluent from a palm oil mill at 28 °C for 24 h and at an aeration rate of 3.0 l/min resulted in the chemical oxidation demand (COD) of the effluent being reduced by 66 % (Neo, 1979). Malandra *et al.* (2003) studied the microorganisms associated with a rotating biological contactor treating winery wastewater. One of the yeasts isolates was able to reduce the COD of synthetic wastewater by 95.0 % and 46.0 % within 24 h under aerated and non-aerated conditions, respectively. Martinez-Garcia *et al.* (2007) used the *yeasts Candida tropicalis* to aerobically pretreat Olive mill wastewater prior to anaerobic digestion. The combined system resulted in a 93.0 % reduction in COD of wastewater.

Mishra *et al.* (2004) reported that the *Aspergillus foetidus* and *A. niger* were able to grow in Potato chips industry wastewater under aerobic condition at optimum pH 6.0 within 60 h of incubation. *A. foetidus* and *A. niger* were able to reduce COD by about 60.0 % and produce 2.4 and 2.85 $g/L^{-1}$ biomass, respectively. Amendment of the wastewater with different N and P sources increased the biomass production and COD reduction substantially. Vikineswary *et al.* (1997) demonstrated that, *Myceliophthora thermophila* and *Trichoderma harzianum* grown in Palm oil sludge, yielded 28.6 and 24.4 g/L of mycelial biomass with chemical oxidation demand (COD) reductions of 74 % and 68 %, respectively after 24 h growth in batch fermentor at pH 4.0 and 30 °C for *T. harzianum* and pH 5.0 and 45 °C for *M. thermophila*. *Penicillium sp.* and *Aspergillus niger* cultivated on undiluted wastewater, removed respectively 65 and 78% of the COD after 15 days cultivation. Respective values of 45 and 51 % were obtained for the yeasts *Candida boidinii* and *Geotrichum candidum*. Maximal removal of the COD was attained after about 6–7 days cultivation.

## 4.7. Effect of pH 7.5 on the growth and elimination activites of *Aspergillus niger* and *Trichoderma viride* in raw wastewater (aerobic batch)

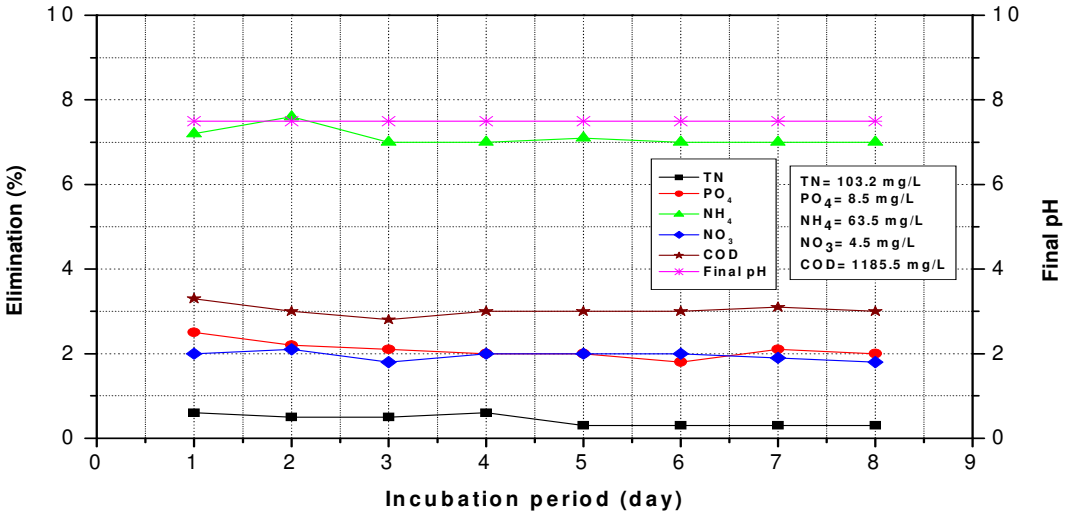
Data in Figures 4.28, 4.29 and (Tables A.15, A.16) showed that *Aspergillus niger* and *Trichoderma viride* did not grow during incubation period in raw wastewater and thus the ability of these fungus for elimination of nutrients was very weak or may be non-existent at pH 7.5. Throughout incubation period the highest elimination of TN, $PO_4$, $NH_4$, $NO_3$ and COD ranged between (0.6 to 7.2 %) and (1.3 to 11.0 %) from initial concentrations of these nutrients on first day by *A. niger* and *T. viride*, respectively. This is due to the inhibitory effects resulting from the change in pH during the incubation period by adding NaOH (1 N), which did not allow to growth of fungus and perform normal metabolic activity. From the above results, it is clear, that pH of the medium had a marked effect on the growth and sporulation of these fungi. Generaly *Aspergillus niger* and *Trichoderma viride* were affected by high alkaline conditions

Researchers have reported that the pH values play a important role for growth of fungi. Dix

and Webster (1994) pointed out that environmental $H^+$ concentration has direct effect on fungal metabolism due to the buffering system in hyphae but may influence the ionization of salts in solution and the permeability of the plasmalemma of the hyphae. Furthermore, fungal metabolic activity is affected by $H^+$ concentration. Fungi usually grow best in environments that are slightly acidic (a pH of 5 or so) but some species can also do well in higher pH levels. Hydrogen ion concentration in a medium could affect growth either indirectly by its effect on the availability of nutrients or directly by action on the cell surfaces. The acid/alkaline requirement for growth of all fungi is quite broad, ranging from pH 3 to above pH 8, with optimum around pH 5, if nutrient requirements are satisfied. In general, *Aspergillus* species are more tolerant to acidic pH while *Penicillium* species appear to be more tolerant to alkaline pH (Wheeler *et al*., 1991).

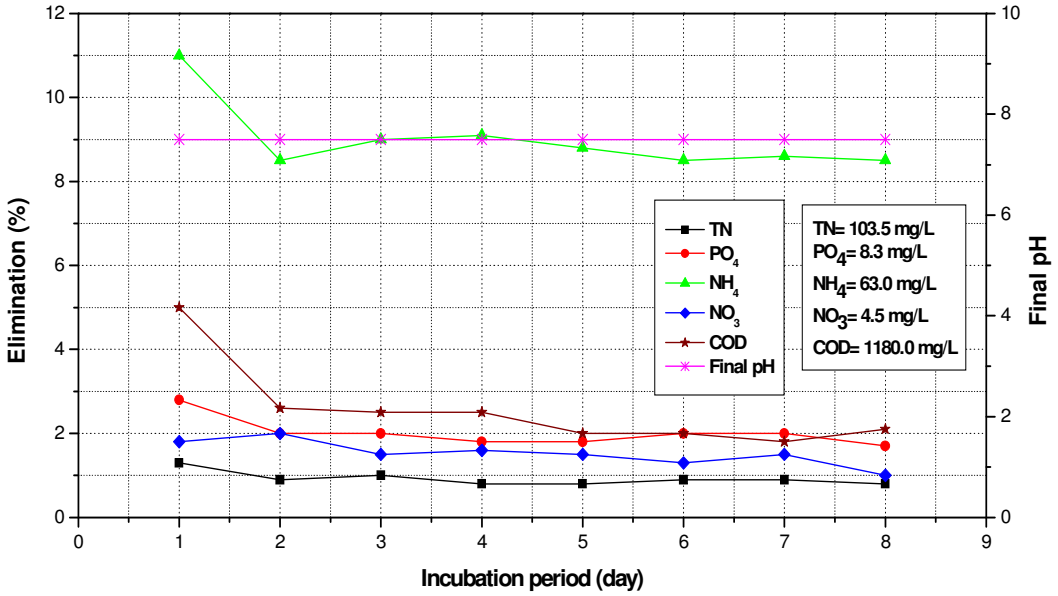
Most fungi are little affected by pH over a broad range, commonly 3–8. Some conidial fungi are capable of growth down to pH 2 and yeasts down to pH 1.5. However, as pH moves away from the optimum, usually about pH 5, the effect of other growth limiting factors may become apparent when superimposed on pH. *A. niger* is able to grow down to pH 2.0 (Pitt, 1981). Only slight differences in growth rates were observed on media based on pH 4.0 and up to 6.5, at various water activities (Avari and Allsopp, 1983).

Abdel-Rahim and Arbab (1985) reported that conidiospores of *Aspergillus niger* were very sensitive to change in the hydrogen ion concentration (pH). The germination and the length of formed germ tubes increased with pH reach their maximum rates at pH 4.5. Also, Holmquist *et al.* (1983) reported maximal growth of several species from *Aspergillus* at pH 5.0.




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.28:** Impact of pH 7.5 value on the activity of *Aspergillus niger* for nutrients elimination from raw wastewater (aerobic batch)




TN= Total nitrogen, COD= Chemical oxygen demand

**Fig. 4.29:** Impact of pH 7.5 value on the activity of *Trichoderma viride* for nutrients elimination from raw wastewater (aerobic batch)

# 5. SUMMARY AND CONCLUSIONS

## I- Occurrence of fungi in activated sludge from MBRs

- One hundred-eight species belonging to 40 genera were collected from 36 samples of each aerobic and anoxic activated sludge from wastewater treatment plants with MBRs from two places in Berlin (Amedeus pilot plant in Berlin/Wedding and BWB plant in Berlin/Margaretenhöhe) on 50 % Sucrose Czapek-Dox (29 genera and 62 species), Malt extract agar (26 and 60), Rose bengal chloramphenicol agar (27 and 60), Sabouraud's dextrose agar (29 and 61) and Sabouraud's dextrose agar with cycloheximide and chloramphenicol (21 and 46) at 30 $^{o}$C for 8 to 15 days.
- *Aspergillus* (18 species), *Penicillium* (15 species) and *Geotrichum candidum* contributed the broadest spectrum of fungal species.
- The best isolation media for fungi were 50 % Sucrose Czapek's Dox agar from aerobic activated sludge (28 genera and 58 species) and Malt extract agar from anoxic activated sludge samples (25 genera and 53 species).
- The fungal diversity present in aerobic and anoxic activated sludge from both Amedeus and Margaretenhöhe plants has been similar, with different spore population. The chance of the presence of fungal spore in aerobic activated sludge was better than that in anoxic activated sludge. This trend could be explained by the continuous turning process of sludge from the aerobic into anoxic tank, the differences in soils, wastewater and environmental or work conditions of each plant Some species were isolated only from activated sludge of Amedeus wastewater plant (*Acremonium rutilum*, *Aspergillus nidulans* (*Emericella nidulans*), *Fusarium lichenicola* (*Cylindrocarpon lichenicola*), *Chrysosporium pannorum*, *Penicillium verrucosum var. verrucosum*, *Trichophyton mentagrophytes* and *Verticillium chlamydosprium*). While *Aspergillus fischerianus*, *Epicoccum nigrum, Paecilomyces marquandii, Penicillium islandicum*, and *Trichosporon pullulans*, were encountered only from Margaretenhöhe wastewater plant.
- Some fungi were recovered only from aerobic activated sludge (22 species), Also, (12 species) were encountered only from anoxic activated sludge.
- Thirteen species of Keratinophilic fungi and dermatophytes belonging to 3 genera were isolated from both aerobic and anoxic activated sludge samples.

**II- The ability of fungal isolates for elimination of nutrients from raw wastewater**

- Twenty-one isolates were screened for elimination of TN, $NH_4$, $NO_3$, $PO_4$ and COD from raw wastewater. *Aspergillus niger* and *Trichoderma viride* were the best fungi for growth activities and elimination of compounds from raw wastewater.

- The best environmental conditions for dry matter, protein content and elimination of TN, $NH_4$, $NO_3$, $PO_4$ and COD from raw wastewater by *Aspergillus niger* in shaked flasks were as follows: optimum pH 4.5 for dry matter and compounds, while pH 6.0 for protein content. Temperature 30 $^oC$ for all studied attributes and incubation period, 6 days for dry matter, 7 day for protein content, 3-4 days for elimination of $PO_4$, 5 days for $NH_3$ and 4 days for elimination of TN, $NO_4$ and COD.

- The best environmental conditions for dry matter, protein content and elimination of compounds from raw wastewater by *Trichoderma viride* in shaked flasks were as follows: optimum pH, 4.5 for all examined parameters; 25 $^oC$ for elimination of TN, $PO_4$ and $NO_3$, while 30 $^oC$ for dry matter, protein content and elimination of $NH_4$ and COD, and incubation period, 3-4 days for elimination of TN, $PO_4$, $NH_4$ and $NO_3$, 4-5 days for COD and dry matter and 6-7 days for protein content.

- The highest elimination of TN, $NH_4$, $NO_3$, and COD from raw wastewater by incubated *Aspergillus niger* in batch reactor under aerobic condation was obtained at day 4. The highest reduction of $PO_4$ was attained after 3 day. Dry matter of fungal mycelium and protein content after 15 days incubation were 5030.0 mg/L and 253.0 mg/L, respectively.

- *Aspergillus niger* eliminated 48.0 % and 71.5 % after 96 h of total nitrogen and COD respectively, from wastewater in aerobic batch. The highest reduction degree (95.0 %) of $PO_4$ was attained after 64 h and 72 h of incubation was the best period for elimination of $NH_4$. While the highest elimination (35.5 %) of $NO_3$ was recorded after 80 h. Dry matter of fungal mycelium and protein content after 15 days incubation were 5080.0 mg/L and 265.0 mg/L, respectively.

- The best elimination of TN, $NH_4$, $PO_4$, and COD from raw wastewater by *Trichoderma viride* under aerobic condition was attained after 5 days. The highest reduction degree of $NO_3$ was attained after 3 days. The dry matter of *Trichoderma viride* and protein content after 15 days incubation were 7010.0 mg/L and 311.5 mg/L, respectively.

- *Trichoderma viride* eliminate 56.0 % from TN of raw wastewater after 96 h of incubation. The maximum elimination (96.8 % and 40.0%) of $PO_4$ and $NO_3$ were recorded after 88 h, respectively. The highest reduction (100 %) of $NH_4$ was observed after 72 h, while the best

elimination rate (67.0 %) of COD appeared after 120 h incubation period. The dry matter of *Trichoderma viride* and protein content after 15 days incubation in aerobic batch were 7050.0 mg/L and 280.0 mg/L, respectively.

- The highest elimination rate of TN, $PO_4$, $NH_4$, $NO_3$, and COD from raw wastewater by grown *Aspergillus niger* in batch reactor under anaerobic condition was attained after 6, 7, 8, and 5 days incubation, respectively. While the lowest rate was found after 1 day. The dry matter of *Aspergillus niger* and protein content after 15 days incubation were 3040.0 mg/L and 217.5 mg/L, respectively.
- The best elimination of TN, $NH_4$, $PO_4$, and COD from raw wastewater by *Trichoderma viride* under anaerobic condition was attained at day 5. The highest reduction rate of $NO_3$ was attained after 3 days, whereas the lowest value was recorded in the first day. Dry matter of *Trichoderma viride* and protien content after 15 days incubation were 4080.0 mg/L and 263.8 mg/L, respectively.
- Stabilize the pH value of raw wastewater at 7.5 by adding NaOH inhibit the growth and ability of *Aspergillus niger* and *Trichoderma viride* for elimination of nutrients through incubation periods.

**To conclude**, activated sludge produced from MBRs is rich in Cycloheximide-resistant keratinophilic fungi, including the dermatophytes and related species. Most fungi were recovered in the present investigation can be considered as potential pathogens and some of these fungi also produce mycotoxins.

Therefore, all workers in the field of activated sludge process, wastewater treatment and farm operation should be careful to avoid mycotic infections and the productions must be adapted to control the spread of pathogenic fungi in the environment. The workers in wastewater treatment plants, especially in activated sludge facilities have a health risk during the treatment processes. Health risks are associated with the pathogens, which may spread through being directly, or indirectly ingested into the human body. Pathogens and toxic compounds may be disseminated through sludge and sewage, as well as through aerosols. Perhaps the most important single factor is to make sure that sewage workers know how to avoid infection and that they are aware of and use protective measures in their daily work.

The one of most important questions is the position of wastewater treatment plants. They must be far away from cities and human communities and must be built in the suitable place from a public health point of view.

Fungi are heterotrophy and were able to grow and eliminate phosphorus, nitrogen, COD and other compounds from raw wastewater under specific conditions such as low pH, temperature, lower oxygen and carbon source concentration.

Several explanations can be offered on the ability of fungi to resist inhibitory compounds in wastewater. First, mycelial growth may provide greater protection to sensitive organelles of fungi. The larger surface area would act in the same manner as the extra polysaccharide matrix of a biofilm; a type of adsorption matrix. Second, fungi are eukaryotic cells, which contain significantly genes providing other methods for dealing with inhibitory compounds.

The biomass produced during fungal wastewater treatment has contained a higher value of protein. The fungi can be used to derive valuable biochemicals and can also be used as a protein source. Various high-value biochemicals are produced by commercial cultivation of fungi under aseptic conditions using expensive substrates. The fungal biomass produced from wastewater treatment could be used as a source of food for animal or human consumption on a cheap available medium (wastewater).

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# APPENDIX

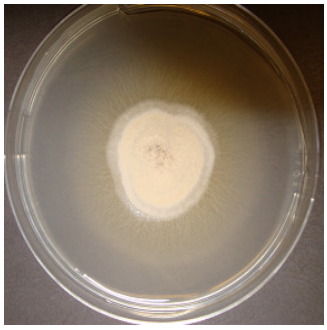


Plate A.1: *Acremonium curvulum*, 7-day-old, colony on MEA

Plate A.2: *Acremonium recifei*, 7-day-old, colony on SDA

Plate A.3: *Acremonium strictum*, 7-day-old, colony on MEA

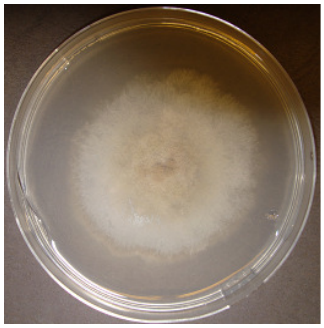


Plate A.4: *Alternaria alternata*, 7-day-old , colony on MEA

Plate A.5: *Alternaria chlamydospora*, 7-day-old, colony on MEA

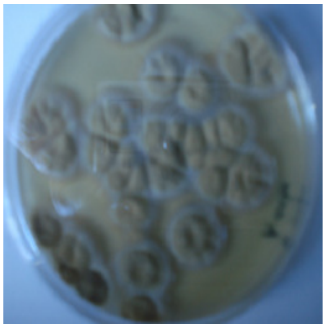


Plate A.6: *Aspergillus alutaceus*, 7-day-old , colony on MEA

Plate A.7: *Aspergillus candidus*, 7-day-old , colony on MEA

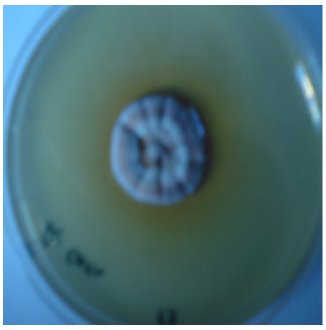


Plate A.8: *Aspergillus carneus*, 7-day-old, colony on MEA

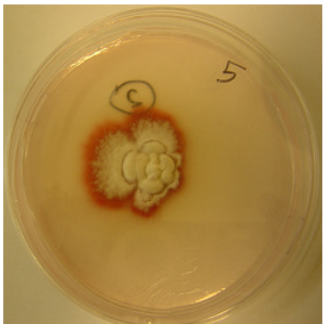


Plate A.9: *Aspergillus chevalieri* (*Eurotium chevalieri*), 7-day-old , colony on CZ

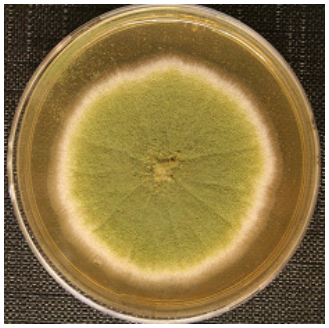


Plate A.10: *Aspergillus flavus*, 7-day-old , colony on MEA

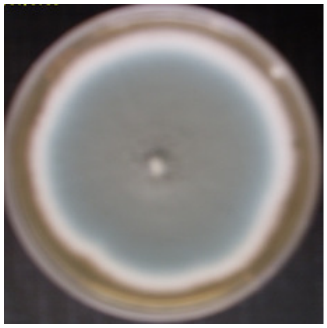


Plate A.11: *Aspergillus fumigatus*, 7-day-old, colony on MEA

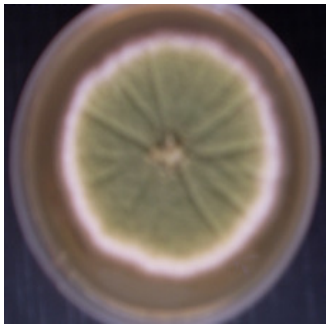


Plate A.12: *Aspergillus nidulans* (*Emericella nidulans*), 7-day-old, colony on MEA

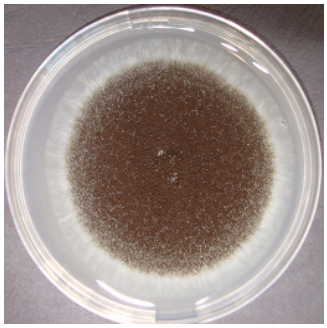


Plate A.13: *Aspergillus niger*, 7-day-old, colony on CZ

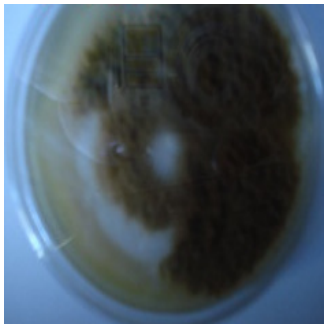


Plate A.14: *Aspergillus oryzae*, 7-day-old, colony on MEA

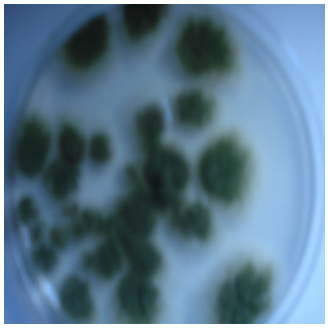


Plate A.15: *Aspergillus parasiticus*, 7-day-old, colony on MEA

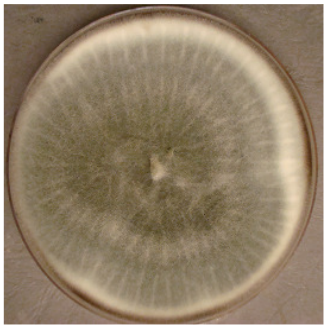


Plate A.16: *Aspergillus sydowii*, 7-day-old, colony on MEA

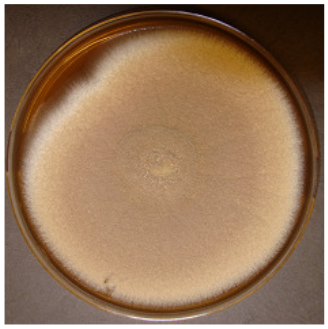


Plate A.17: *Aspergillus terreus* var. *terreus*, 7-day-old, colony on MEA

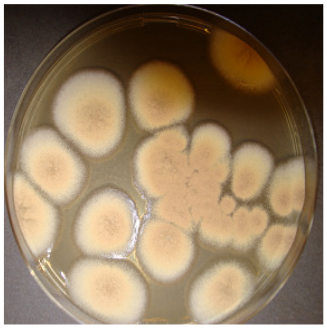


Plate A.18: *Aspergillus terreus* var. *africanus,* 7-day-old, colony on MEA

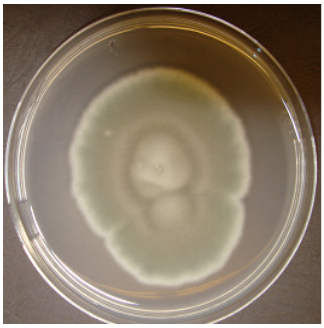


Plate A.19: *Aspergillus ustus,* 7-day-old , colony on CZ

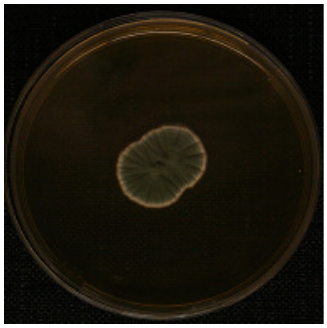


Plate A.20: *Aspergillus virsicolor*, 7-day-old , colony on MEA

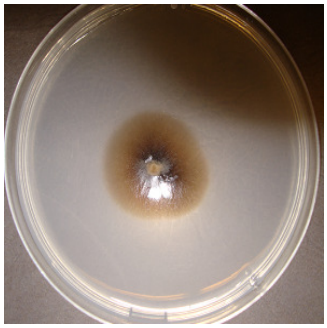


Plate A.21: *Aurobasidium pullulans,* 7-day-old, colony on CZ

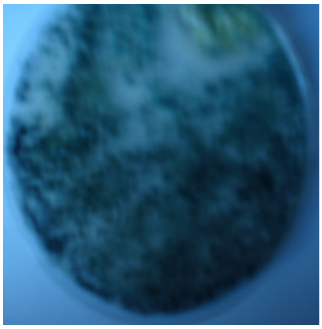


Plate A.22: *Botryodiplodia theobronae,* 7- day-old, colony on MEA

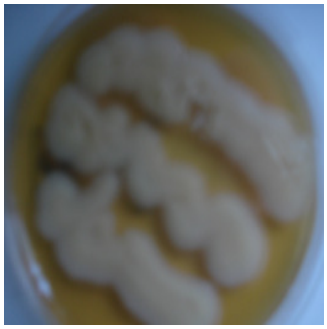


Plate A.23: *Candida albicans,* 3-day-old, colony on SDA

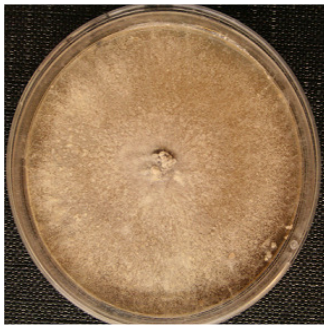


Plate A.24*: Chaetomum cochliodes*, 7- day old, colony on MEA

Plate A.25: *Chaetomum globosum*, 7-day-old, colony on MEA

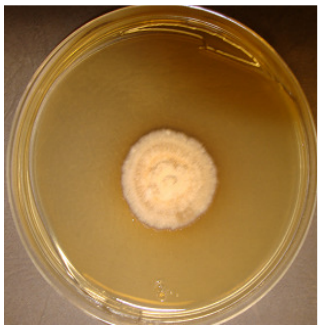


Plate A.26: *Chrysosporium georgii*, 7- day old, colony on SDA

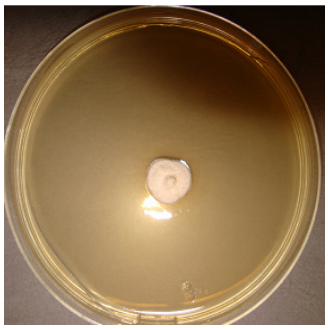


Plate A.27: *Chrysosporium indicum*,7- day old, colony on SDA

Plate A.28: *Chrysosporium keratinophilum,*7- day old, colony on SDA

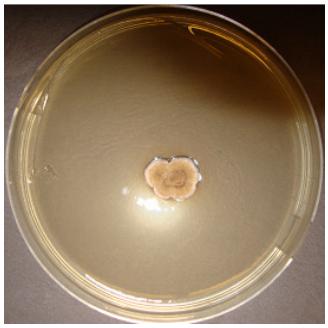


Plate A.29*: Chrysosporium tropicum*, 7- day old, colony on SDA

Plate A.30: *Cladosporium cladosporioides,* 7-day old, colony on CZ

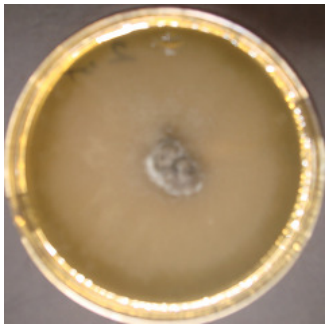


Plate A.31: *Cladosporium oxysporum*,7- day old, colony on MEA

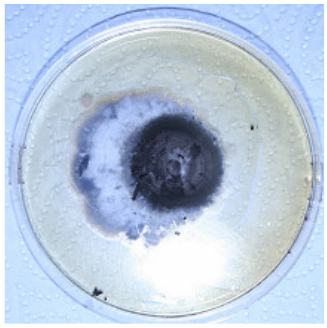


Plate A.32: *Cochliobolus lunatus*, 7- day old, colony on CZ

Plate A.33: *Doratomyces stemonitis*, 7- day old, colony on MEA

Plate A.34: *Epicoccum nigrum,*7- day old, colony on MEA

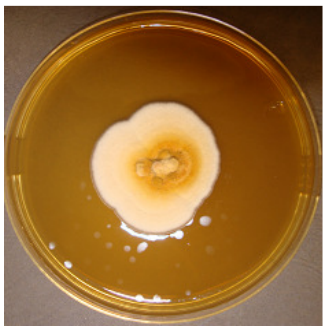


Plate A.35: *Fusarium dimerum,* 7- day old, colony on SDA

Plate A.36: *Fusarium. lichenicola*,7- day old, colony on SDA

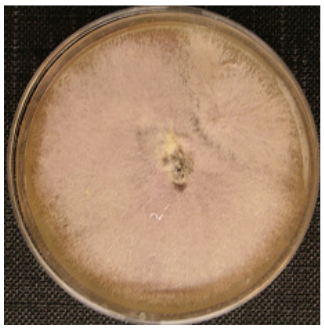


Plate A.37: *Fusarium oxysporum*,7- day old, colony on SDA

Plate A.38: *Fusarium solani* 7- day old, colony on SDA

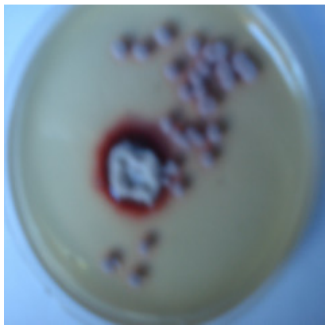


Plate A.39: *Geosmithia lavendula,* 7- day old, colony on CZ

Plate A.40: *Geotrichum candidum,* 7- day old, colony on SDA

Plate A.41: *Gibberella accuminata,* 7- day old, colony on CZ

Plate A.42: *Gibberella avenacea,* 7- day old, colony on SDA

Plate A.43: *Gibberella fujikuroi* var *fujikuroi*, 7- day old, colony on MEA

Plate A.44*: Gliocladium roseum*,7- day old colony on MEA

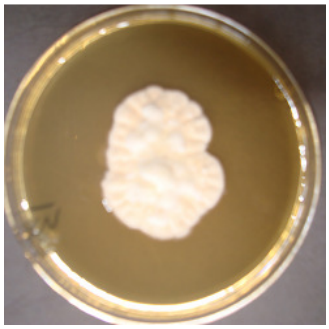


Plate A.45: *Gliocladium viride,* 7- day old, colony on SDA

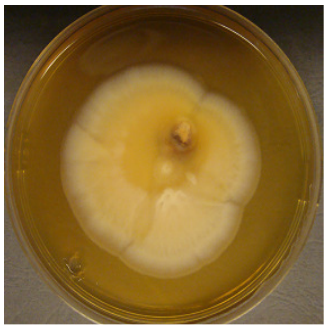


Plate A.46: *Gymnoascus reesii*, 7- day old, colony on SDA

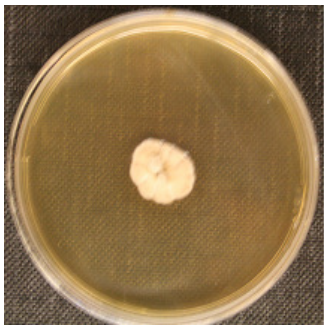


Plate A.47: *Microsporum ferrugineum*, 7- day old, colony on SDA

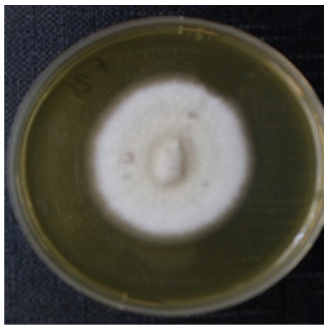


Plate A.48: *Microsporum gypseum*, 7- day old, colony on SDA

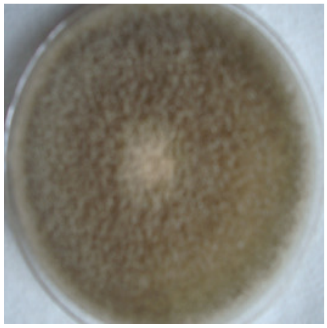


Plate A.49: *Mucor circinelloides*, 7- day old, colony on CZ

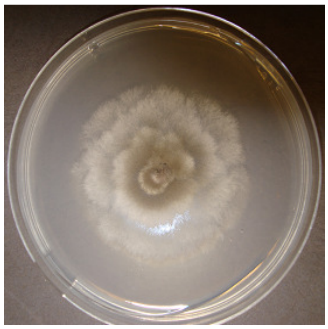


Plate A.50: *Oidiodendron griseum,* 7- day old, colony on CZ

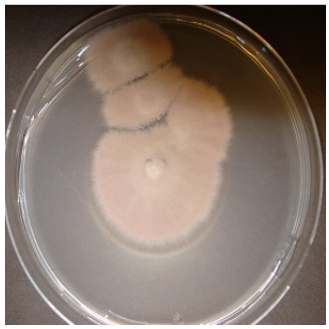


Plate A.51: *Paecilomyces lilacinus,* 7- day old, colony on CZ

Plate A.52*: Paecilomyces marquandii*, 7- day old, colony on CZ

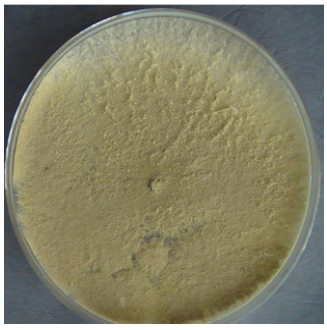


Plate A.53: *Paecilomyces variotii,* 7- day old, colony on CZ

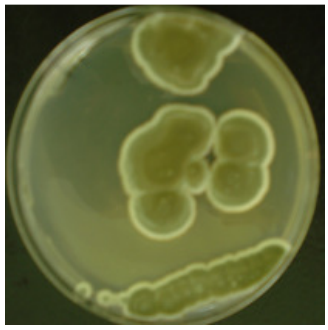


Plate A.54: *Penicillium brevicompactum*, 7- day old, colony on MEA

Plate A.55*: Penicillium chrysogenum*, 7- day old colony on MEA

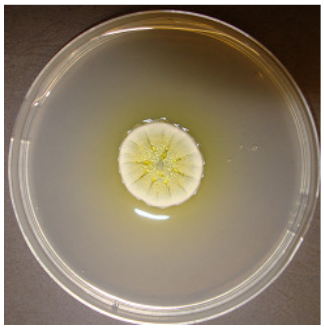


Plate A.56: *Penicillium citrinum*, 7- day old , colony on MEA

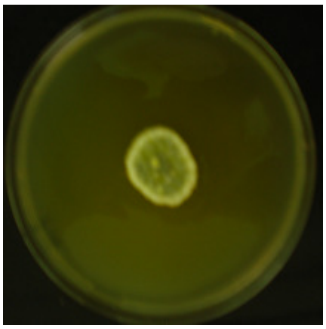


Plate A.57: *Penicillium corylophilum*, 7- day old, colony on MEA

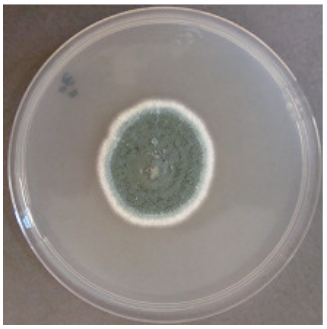


Plate A.58: *Penicillium expansum*, 7- day old, colony on MEA

Plate A.59: *Penicillium funiculosum*, 7- day old, colony on MEA

Plate A.60: *Penicillium glabrum,* 7- day old, colony on MEA

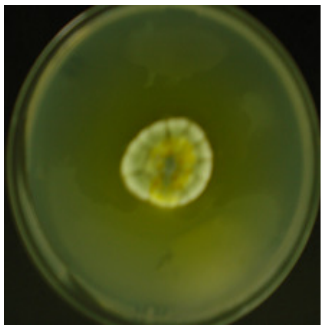


Plate A.61: *Penicillium islandicum,* 7- day old, colony on MEA

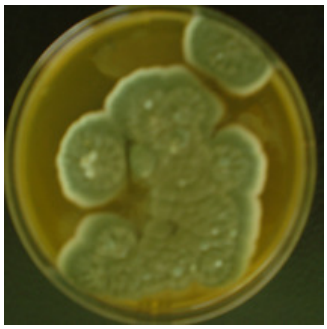


Plate A.62: *Penicillium janczewskii*, 7- day old, colony on MEA

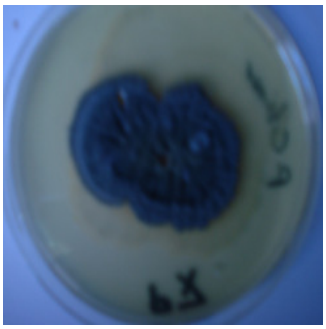


Plate A.63: *Penicillium oxalicum*, 7- day old, colony on MEA

Plate A.64: *Penicillium puberulum*, 7- day old, colony on CZ

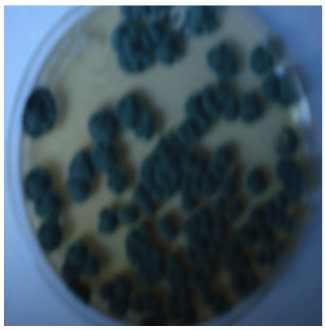


Plate A.65: *Penicillium roquefortii*, 7- day old, colony on MEA

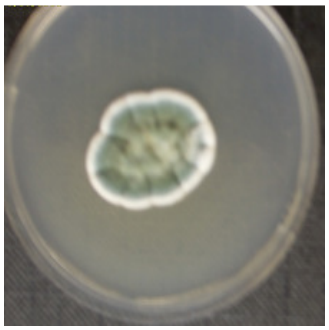


Plate A.66: *Penicillium verrucosum,* 7- day old, colony on CZ

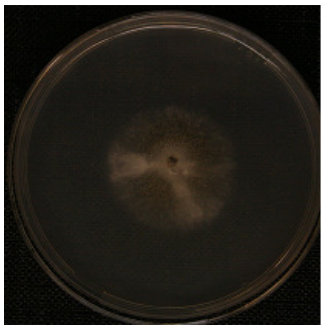


Plate A.67: *Phialophora verrucosa*, 7- day old, colony on CZ

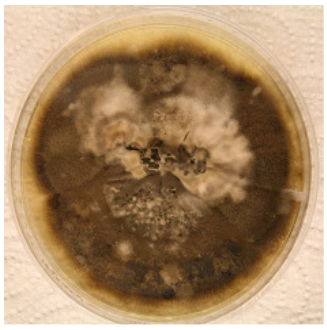


Plate A.68: *Rhinocladiella atrovirens,* 7- day old, colony on CZ

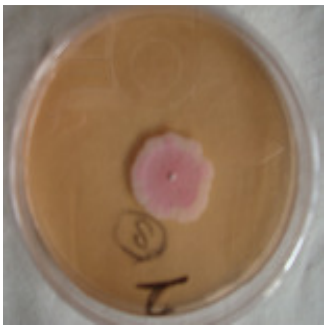


Plate A.69*: Rhodotorula rubra,* 3- day old, colony on SDA

Plate A.70: *Scopulariopsis brevicaulis*, 7- day old, colony on CZ

Plate A.71: *Scopulariopsis brumptii,* 7- day old, colony on MEA

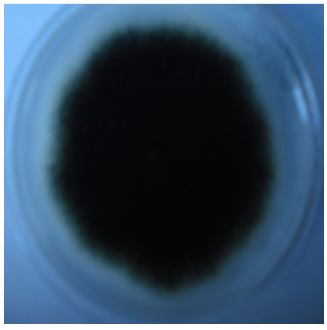


Plate A.72: *Setosphora rostrata*, 7- day old, colony on CZ

Plate A.73: *Sporothrix schenkii*, 7- day old, colony on SDA

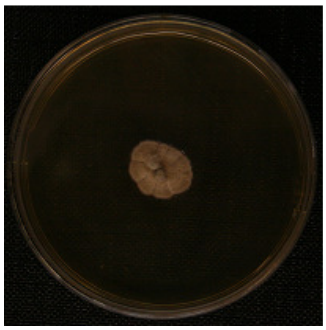


Plate A.74: *Stachybotrys chartarum*, 7- day old, colony on MEA

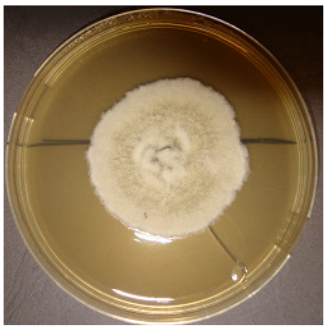


Plate A.75: *Stemphylium vesicarium*, 7- day old, colony on MEA

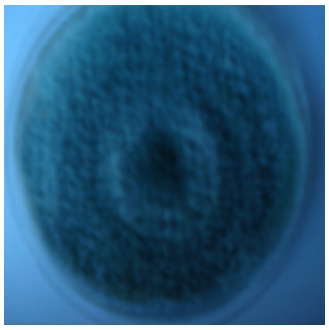


Plate A.76: *Syncephalastrum racemosum,* 7-day old, colony colony on CZ

Plate A.77: *Trichoderma hamatum*, 7- day old, colony on CZ

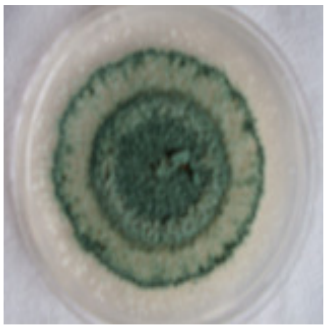


Plate A.78: *Trichoderma viride*, *koningii*, 7- day old, colony on CZ

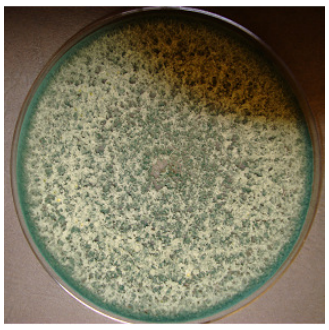


Plate A.79: *Trichoderma viride*, 7- day old, colony on CZ

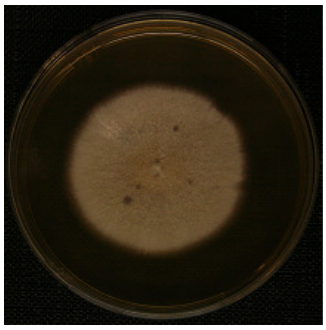


Plate A.80: *Trichophyton ajelloi* var. *ajelloi*, 7- day old, colony on SDA

Plate A.81: *Trichophyton equinunm*, 7- day old, colony on SAD

Plate A.82: *Trichophyton mentagrophytes* var. *interdigitale* ,7- day old, colony on SDA

Plate A.83: *Trichophyton terrestre,*7- day old, colony on SDA

Plate A.84: *Trichospoon pullulans*,7- day old, colony on SDA

Plate A.85: *Ulocladium chartarum*,7- day old, colony on CZ

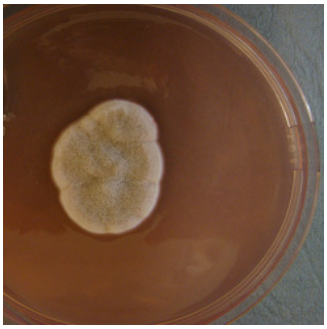


Plate A.86: *Ulocladium microsporum*,7- day old, colony on MEA

Plate A.87: *Verticillum chlamydosporium,*7- day old, colony on MEA

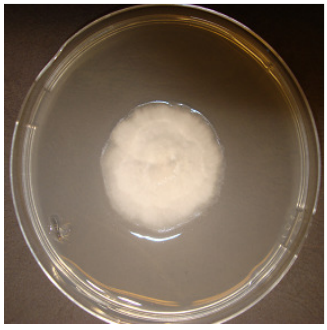


Plate A.88*: Verticillum lecanii*, 7- day old, colony on CZ

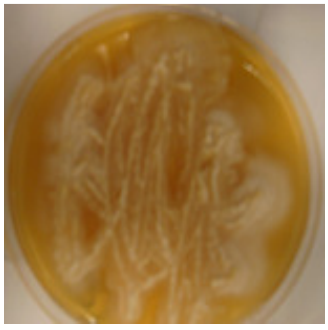


Plate A.89: *Yeast*, 3- day old, colony on PDA

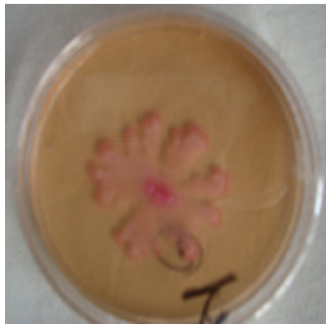


Plate A.90: *Yeast*, 3- day old, colony on RBA

Plate A.91: Fungal colonies, 7- day old, on CZ

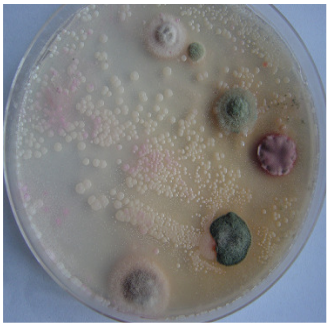


Plate A.92: Fungal colonies, 7- day old, on CZ

Plate A.93: Fungal colonies, 7- day old, on MEA

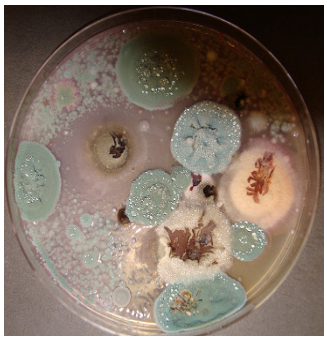


Plate A.94: Fungal colonies, 7- day old, on MEA

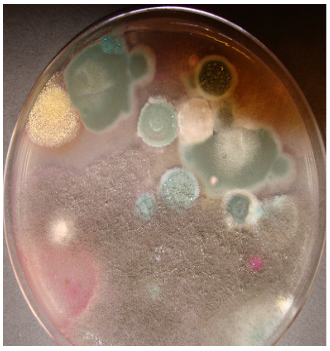


Plate A.95: Fungal colonies, 7- day old, on RBA

Plate A.96: Fungal colonies, 7- day old, on RBA

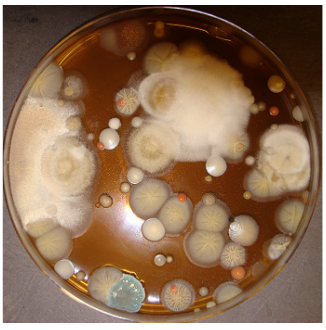


Plate A.97: Fungal colonies, 7- day old, on SDA

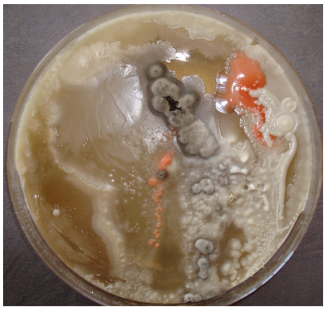
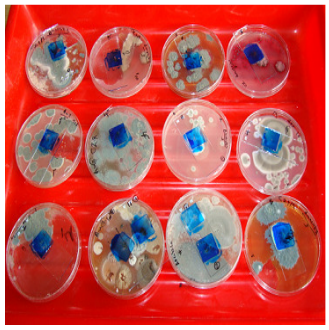


Plate A.98: Fungal colonies, 7- day old, on SDA



A. 99: Identification of fungal species by staining

A. 100: Static incubator for agar cultures

A. 101: Shaker incubator for raw wastewater cultures

A. 102: Culture of *Aspergillus niger* in shaker flask

A. 103: Culture of *Trichoderma viride* in shaker flask

A. 104: Batch reactor of *Aspergillus niger*

A. 105: Batch reactor of *Trichoderma viride*

**Table A.1:** Impact of different pH values on the nutrients elimination and fungal growths parameters of *Aspergillus niger* inoculated in raw wastewater

| pH | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 2.0 | 9.7 | 15.3 | 10.0 | 5.3 | 5.9 | 169.0 | 75.5 | 1.5 |
| 2.5 | 11.5 | 18.1 | 13.9 | 14.5 | 15.1 | 235.0 | 90.0 | 1.5 |
| 3.0 | 40.7 | 37.0 | 38.3 | 33.3 | 28.0 | 267.5 | 105.2 | 1.3 |
| 3.5 | 48.5 | 59.3 | 52.4 | 47.5 | 36.5 | 310.8 | 138.5 | 1.5 |
| 4.0 | 62.6 | 77.1 | 83.1 | 78.3 | 66.8 | 370.0 | 188.3 | 1.5 |
| 4.5 | 87.9 | 94.3 | 91.4 | 78.5 | 87.6 | 528.0 | 195.7 | 1.3 |
| 5.0 | 80.3 | 72.0 | 78.5 | 75.0 | 63.0 | 465.3 | 215.0 | 1.3 |
| 5.5 | 61.7 | 65.3 | 75.7 | 68.2 | 47.5 | 335.5 | 223.2 | 1.5 |
| 6.0 | 55.1 | 58.5 | 48.7 | 50.6 | 30.3 | 237.0 | 208.0 | 2.1 |
| 6.5 | 28.6 | 31/0 | 33.3 | 26.5 | 13.7 | 200.5 | 132.6 | 3.5 |
| 7.0 | 10.0 | 13.5 | 14.7 | 15.2 | 10.5 | 185.8 | 88.5 | 5.2 |
| 7.5 | 10.0 | 10.2 | 11.8 | 9.5 | 8.7 | 183.0 | 95.3 | 5.6 |
| 8.0 | 5.6 | 7.3 | 4.5 | 3.3 | 3.8 | 87.6 | 73.7 | 6.0 |
| 8.5 | 2.0 | 3.5 | 3.5 | 2.2 | 2.5 | 64.5 | 62.0 | 6.5 |
| 9.0 | 0.7 | 1.3 | 1.9 | 0.6 | 1.3 | 28.6 | 25.4 | 7.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.2:** Impact of different pH values on the nutrients elimination and fungal growths parameters of *Trichoderma viride* inoculated in raw wastewater

| pH | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 2.0 | 5.3 | 9.5 | 3.6 | 2.7 | 2.0 | 150.0 | 60.3 | 1.6 |
| 2.5 | 8.9 | 11.7 | 5.5 | 5.6 | 7.9 | 178.0 | 85.5 | 1.8 |
| 3.0 | 27.6 | 39.4 | 13.8 | 8.3 | 19.6 | 205.5 | 125.0 | 1.5 |
| 3.5 | 50.1 | 52.8 | 30.2 | 25.9 | 27.3 | 270.2 | 144.2 | 1.6 |
| 4.0 | 73.5 | 68.2 | 53.5 | 44.5 | 58.9 | 323.4 | 200.5 | 1.5 |
| 4.5 | 85.6 | 93.5 | 93.8 | 85.5 | 72.8 | 484.6 | 263.5 | 1.5 |
| 5.0 | 69.6 | 80.1 | 80.7 | 70.5 | 66.6 | 453.5 | 251.0 | 1.8 |
| 5.5 | 52.5 | 66.6 | 80.0 | 75.1 | 50.7 | 365.2 | 240.2 | 1.5 |
| 6.0 | 50.3 | 55.7 | 75.1 | 59.5 | 42.5 | 211.0 | 218.0 | 2.1 |
| 6.5 | 35.6 | 42.0 | 49.5 | 45.4 | 22.5 | 175.3 | 172.0 | 3.5 |
| 7.0 | 17.5 | 28.1 | 45.4 | 28.6 | 14.2 | 159.8 | 100.5 | 5.2 |
| 7.5 | 13.6 | 22.5 | 28.6 | 23.0 | 9.9 | 134.0 | 87.7 | 5.6 |
| 8.0 | 7.0 | 9.8 | 23.0 | 18.5 | 3.5 | 85.0 | 80.0 | 5.5 |
| 8.5 | 1.8 | 3.1 | 12.5 | 1.5 | 0.9 | 25.5 | 43.0 | 6.5 |
| 9.0 | 0.5 | 1.8 | 3.2 | 1.0 | 0.9 | 10.8 | 30.0 | 7.4 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.3:** Impact of incubation temperature (°C) on the nutrients elimination and fungal growths parameters of *Aspergillus niger* inoculated in raw wastewater

| Temperature (°C) | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 5 | 1.5 | 3.3 | 12.0 | 5.2 | 4.7 | 16.3 | 5.0 | 3.6 |
| 10 | 13.0 | 20.0 | 21.2 | 11.0 | 9.2 | 21.8 | 22.8 | 2.9 |
| 15 | 32.5 | 33.3 | 35.0 | 26.3 | 20.5 | 95.3 | 75.5 | 1.8 |
| 20 | 40.7 | 51.0 | 51.3 | 49.5 | 33.5 | 274.5 | 108.0 | 1.5 |
| 25 | 72.0 | 88.1 | 81.6 | 75.0 | 57.0 | 501.8 | 213.5 | 1.4 |
| 30 | 80.1 | 93.4 | 96.0 | 88.5 | 80.3 | 633.5 | 233.1 | 1.5 |
| 35 | 58.5 | 65.0 | 70.1 | 63.0 | 61.7 | 250.2 | 117.5 | 2.1 |
| 40 | 14.6 | 26.3 | 43.0 | 32.2 | 29.0 | 98.0 | 31.0 | 3.5 |
| 45 | 2.8 | 17.1 | 16.3 | 9.0 | 5.3 | 30.3 | 5.5 | 4.0 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.4:** Impact of incubation temperature (°C) on the nutrients elimination and fungal growths parameters of *Trichoderma viride* inoculated in raw wastewater

| Temperature (°C) | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 5 | 1.9 | 5.2 | 5.8 | 2.5 | 3.2 | 18.0 | 16.3 | 3.8 |
| 10 | 7.5 | 18.5 | 16.0 | 18.0 | 13.5 | 25.5 | 38.0 | 2.4 |
| 15 | 19.3 | 36.0 | 49.3 | 31.5 | 31.0 | 73.3 | 95.6 | 2.0 |
| 20 | 33.3 | 44.5 | 60.9 | 65.1 | 38.7 | 118.0 | 114.2 | 1.5 |
| 25 | 69.4 | 80.3 | 92.1 | 71.0 | 45.0 | 483.3 | 180.0 | 1.5 |
| 30 | 65.5 | 73.0 | 93.0 | 70.2 | 68.3 | 511.7 | 203.5 | 1.2 |
| 35 | 27.2 | 51.5 | 49.5 | 33.3 | 41.0 | 194.0 | 81.0 | 1.9 |
| 40 | 6.3 | 13.5 | 23.0 | 14.5 | 19.5 | 83.5 | 26.2 | 3.0 |
| 45 | 1.5 | 11.1 | 5.8 | 3.9 | 1.8 | 19.7 | 5.1 | 4.2 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.5:** Impact of incubation period (day) on the nutrients elimination and fungal growths parameters of *Aspergillus niger* inoculated in raw wastewater

| Incubation period (days) | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 1 | 10.5 | 25.3 | 31.8 | 25.0 | 25.7 | 36.0 | 35.0 | 3.7 |
| 2 | 23.7 | 60.5 | 55.5 | 33.5 | 44.0 | 51.5 | 42.5 | 3.2 |
| 3 | 72.5 | 93.0 | 75.5 | 76.3 | 68.5 | 274.0 | 127.5 | 2.7 |
| 4 | 83.1 | 91.5 | 96.7 | 79.5 | 76.3 | 425.0 | 115.1 | 2.5 |
| 5 | 75.5 | 88.1 | 91.0 | 80.0 | 72.5 | 580.5 | 146.0 | 2.1 |
| 6 | 77.3 | 88.4 | 80.5 | 75.7 | 70.1 | 623.6 | 152.3 | 1.8 |
| 7 | 78.0 | 85.5 | 80.0 | 73.5 | 70.3 | 677.0 | 187.0 | 1.8 |
| 8 | 74.6 | 86.0 | 80.0 | 72.8 | 72.0 | 698.0 | 126.0 | 1.5 |
| 9 | 75.5 | 77.3 | 76.5 | 75.5 | 65.3 | 650.0 | 98.0 | 1.6 |
| 10 | 70.0 | 75.0 | 78.2 | 73.1 | 68.5 | 661.0 | 98.6 | 1.5 |
| 11 | 63.0 | 80.3 | 78.0 | 75.0 | 70.0 | 658.5 | 69.4 | 1.5 |
| 12 | 62.1 | 81.5 | 80.0 | 77.3 | 68.0 | 651.0 | 110.5 | 1.5 |
| 13 | 60.3 | 80.0 | 75.9 | 66.6 | 72.2 | 645.0 | 127.1 | 1.6 |
| 14 | 58.5 | 74.2 | 73.0 | 61.5 | 70.0 | 655.0 | 95.5 | 1.5 |
| 15 | 62.7 | 77.7 | 70.5 | 60.3 | 69.7 | 648.0 | 103.2 | 1.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.6:** Impact of incubation period (day) on the nutrients elimination and fungal growths parameters of *Trichoderma viride* inoculated in raw wastewater

| Incubation period (days) | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 1 | 8.7 | 37.0 | 35.0 | 31.0 | 20.0 | 22.5 | 15.0 | 3.5 |
| 2 | 27.5 | 68.5 | 51.4 | 48.3 | 37.5 | 38.0 | 48.3 | 3.0 |
| 3 | 81.5 | 96.2 | 89.0 | 81.0 | 61.3 | 251.2 | 96.6 | 2.5 |
| 4 | 85.2 | 96.5 | 97.0 | 95.0 | 78.0 | 411.0 | 153.3 | 2.5 |
| 5 | 80.0 | 90.3 | 94.6 | 87.4 | 82.0 | 463.5 | 180.0 | 2.2 |
| 6 | 75.2 | 89.7 | 90.2 | 82.2 | 73.5 | 491.7 | 225.5 | 1.5 |
| 7 | 77.5 | 90.0 | 89.5 | 79.8 | 70.3 | 574.5 | 207.0 | 1.5 |
| 8 | 70.6 | 88.1 | 90.0 | 80.0 | 71.0 | 593.3 | 256.0 | 1.5 |
| 9 | 71.0 | 88.3 | 88.0 | 76.9 | 68.5 | 584.8 | 198.5 | 1.7 |
| 10 | 71.6 | 82.6 | 81.3 | 76.5 | 60.0 | 602.2 | 201.3 | 1.5 |
| 11 | 69.1 | 79.5 | 78.0 | 72.7 | 61.3 | 588.8 | 230.5 | 1.5 |
| 12 | 67.8 | 79.6 | 75.4 | 70.0 | 58.8 | 600.0 | 189.8 | 1.5 |
| 13 | 72.5 | 77.5 | 73.0 | 71.0 | 59.0 | 590.1 | 227.1 | 1.6 |
| 14 | 70.5 | 75.8 | 72.3 | 66.2 | 59.6 | 583.5 | 215.5 | 1.5 |
| 15 | 71.0 | 75.3 | 72.0 | 65.5 | 57.6 | 586.0 | 195.4 | 1.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.7:** Impact of incubation period (h) on the nutrients elimination and fungal growths parameters of *Aspergillus niger* inoculated in raw wastewater

| Incubation period (h) | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 8 | 3.6 | 5.7 | 2.8 | 10.5 | 8.5 | 5.1 | 7.0 | 4.5 |
| 16 | 5.5 | 13.5 | 3.3 | 15.3 | 11.3 | 18.9 | 13.3 | 3.8 |
| 24 | 9.2 | 21.3 | 16.3 | 21.5 | 21.5 | 38.5 | 29.5 | 3.5 |
| 32 | 11.8 | 28.8 | 18.0 | 24.1 | 27.0 | 43.5 | 34.0 | 3.5 |
| 40 | 12.4 | 36.3 | 22.5 | 28.5 | 31.3 | 51.0 | 37.1 | 2.7 |
| 48 | 19.5 | 39.0 | 25.5 | 34.0 | 43.5 | 53.6 | 39.5 | 2.3 |
| 56 | 43.6 | 81.3 | 52.5 | 48.5 | 52.5 | 95.3 | 65.0 | 2.0 |
| 64 | 51.5 | 97.5 | 66.7 | 61.3 | 68.4 | 189.5 | 84.4 | 1.7 |
| 72 | 66.3 | 98.8 | 82.8 | 66.6 | 80.0 | 251.5 | 106.5 | 1.6 |
| 80 | 74.7 | 98.0 | 85.5 | 73.5 | 84.3 | 385.0 | 108.0 | 1.3 |
| 88 | 77.6 | 98.0 | 86.2 | 75.3 | 85.0 | 412.5 | 117.5 | 1.3 |
| 96 | 85.5 | 96.7 | 88.2 | 75.5 | 82.4 | 437.1 | 123.0 | 1.5 |
| 104 | 85.0 | 96.5 | 88.0 | 75.0 | 81.0 | 462.5 | 128.0 | 1.5 |
| 112 | 85.0 | 96.5 | 88.0 | 74.3 | 81.0 | 491.3 | 139.5 | 1.5 |
| 120 | 83.6 | 96.0 | 86.9 | 74.0 | 78.5 | 545.5 | 140.3 | 1.5 |
| 128 | 80.0 | 94.1 | 86.3 | 72.2 | 78.3 | 580.0 | 146.0 | 1.5 |
| 136 | 80.0 | 94.3 | 86.0 | 72.0 | 77.0 | 624.1 | 146.5 | 1.5 |
| 144 | 74.5 | 92.5 | 86.0 | 72.0 | 77.1 | 653.5 | 151.5 | 1.5 |
| 152 | 73.6 | 87.4 | 84.5 | 71.3 | 75.5 | 681.0 | 164.0 | 1.5 |
| 160 | 73.3 | 85.6 | 81.0 | 71.0 | 75.0 | 713.5 | 172.5 | 1.5 |
| 168 | 72.0 | 85.1 | 80.0 | 71.0 | 75.0 | 712.5 | 170.0 | 1.5 |
| 176 | 72.0 | 83.1 | 80.0 | 68.8 | 75.0 | 712.5 | 170.3 | 1.5 |
| 184 | 70.4 | 83.0 | 76.8 | 68.0 | 75.0 | 710.0 | 169.8 | 1.5 |
| 192 | 70.1 | 83.0 | 76.5 | 68.0 | 73.1 | 710.0 | 170.0 | 1.5 |
| 200 | 68.4 | 81.5 | 74.5 | 66.6 | 70.8 | 710.0 | 165.5 | 1.5 |
| 208 | 68.0 | 81.0 | 74.0 | 65.5 | 70.0 | 710.0 | 164.7 | 1.5 |
| 216 | 68.0 | 80.3 | 74.0 | 65.0 | 70.0 | 707.3 | 164.5 | 1.4 |
| 224 | 68.0 | 80.1 | 72.8 | 65.0 | 69.0 | 706.0 | 163.0 | 1.5 |
| 232 | 67.1 | 78.7 | 72.0 | 65.0 | 67.3 | 706.0 | 160.0 | 1.5 |
| 240 | 64.5 | 78.2 | 70.3 | 63.8 | 66.0 | 706.0 | 160.0 | 1.5 |
| 248 | 63.0 | 77.3 | 68.8 | 63.3 | 66.0 | 706.0 | 160.0 | 1.5 |
| 256 | 63.0 | 75.5 | 69.0 | 63.0 | 64.5 | 706.0 | 154.0 | 1.5 |
| 264 | 63.0 | 75.5 | 69.0 | 63.0 | 64.1 | 701.8 | 155.0 | 1.5 |
| 272 | 60.5 | 73.8 | 67.5 | 63.0 | 62.7 | 701.5 | 154.1 | 1.5 |
| 280 | 60.1 | 73.5 | 67.5 | 63.2 | 62.0 | 694.5 | 151.5 | 1.5 |
| 288 | 60.1 | 71.5 | 66.6 | 61.5 | 62.0 | 694.1 | 150.0 | 1.5 |
| 296 | 59.9 | 71.0 | 66.5 | 62.0 | 60.6 | 694.3 | 146.0 | 1.6 |
| 304 | 58.3 | 71.0 | 66.0 | 62.0 | 60.5 | 682.5 | 146.0 | 1.5 |
| 312 | 58.0 | 70.0 | 66.1 | 61.3 | 60.5 | 680.0 | 144.2 | 1.5 |
| 320 | 58.0 | 70.0 | 65.0 | 61.0 | 60.5 | 666.6 | 135.0 | 1.5 |
| 328 | 58.0 | 68.8 | 64.8 | 61.0 | 60.1 | 654.5 | 131.5 | 1.5 |
| 336 | 56.6 | 66.6 | 65.0 | 60.1 | 58.8 | 651.5 | 129.4 | 1.5 |
| 344 | 56.5 | 65.1 | 63.5 | 60.8 | 58.5 | 637.0 | 127.1 | 1.5 |
| 352 | 56.5 | 64.4 | 62.1 | 61.0 | 59.0 | 633.3 | 125.3 | 1.5 |
| 360 | 56.0 | 63.0 | 62.3 | 61.0 | 58.1 | 631.5 | 123.0 | 1.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.8:** Impact of incubation period (h) on the nutrients elimination and fungal growths parameters of *Trichoderma viride* inoculated in raw wastewater

| Incubation period (h) | Supernatant % elimination | | | | | Residue (mg/L) | | Final pH |
|---|---|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | DM | PC | |
| 8 | 1.5 | 2.3 | 4.1 | 2.8 | 5.5 | 2.5 | 2.0 | 4.3 |
| 16 | 5.0 | 16.9 | 15.0 | 12.1 | 11.5 | 13.0 | 9.2 | 4.0 |
| 24 | 10.6 | 35.3 | 32.5 | 29.0 | 19.0 | 25.7 | 11.5 | 3.2 |
| 32 | 13.4 | 51.0 | 51.0 | 34.1 | 22.5 | 29.3 | 16.5 | 2.9 |
| 40 | 28.0 | 66.3 | 66.6 | 58.0 | 30.3 | 36.5 | 28.0 | 2.5 |
| 48 | 37.3 | 68.0 | 71.0 | 58.5 | 35.0 | 41.3 | 33.3 | 2.0 |
| 56 | 41.5 | 73.0 | 93.5 | 67.0 | 38.5 | 88.5 | 73.0 | 2.1 |
| 64 | 66.0 | 88.1 | 100.0 | 78.2 | 43.5 | 134.0 | 81.5 | 1.5 |
| 72 | 73.2 | 91.0 | 100.0 | 88.5 | 55.2 | 171.5 | 89.5 | 1.5 |
| 80 | 78.0 | 91.0 | 100.0 | 86.5 | 58.0 | 255.0 | 94.0 | 1.6 |
| 88 | 86.4 | 92.5 | 100.0 | 87.0 | 73.5 | 283.5 | 115.2 | 1.5 |
| 96 | 83.5 | 92.0 | 100.0 | 87.0 | 80.0 | 351.6 | 133.0 | 1.5 |
| 104 | 83.0 | 92.1 | 100.0 | 88.1 | 78.0 | 462.5 | 138.0 | 1.5 |
| 112 | 83.0 | 92.0 | 100.0 | 88.0 | 78.5 | 491.3 | 155.5 | 1.5 |
| 120 | 83.0 | 92.0 | 100.0 | 87.5 | 78.1 | 495.0 | 163.0 | 1.5 |
| 128 | 83.5 | 91.3 | 100.0 | 87.6 | 76.3 | 528.0 | 171.0 | 1.5 |
| 136 | 83.2 | 91.5 | 100.0 | 88.0 | 76.5 | 564.7 | 193.5 | 1.5 |
| 144 | 82.5 | 91.0 | 100.0 | 86.3 | 76.0 | 590.0 | 202.5 | 1.3 |
| 152 | 82.0 | 91.0 | 100.0 | 87.0 | 76.0 | 592.0 | 224.0 | 1.5 |
| 160 | 83.0 | 90.6 | 100.0 | 87.4 | 77.2 | 613.5 | 229.3 | 1.5 |
| 168 | 82.2 | 90.5 | 100.0 | 86.5 | 75.8 | 621.0 | 247.5 | 1.5 |
| 176 | 81.5 | 90.5 | 100.0 | 84.5 | 76.0 | 645.5 | 246.3 | 1.4 |
| 184 | 81.3 | 90.3 | 100.0 | 85.0 | 76.0 | 683.9 | 233.5 | 1.5 |
| 192 | 77.1 | 90.5 | 100.0 | 85.8 | 75.5 | 682.7 | 240.0 | 1.5 |
| 200 | 74.5 | 90.0 | 100.0 | 83.0 | 73.8 | 683.0 | 228.5 | 1.5 |
| 208 | 75.0 | 88.5 | 100.0 | 84.0 | 75.0 | 680.5 | 235.0 | 1.5 |
| 216 | 75.0 | 89.0 | 100.0 | 81.9 | 73.3 | 680.3 | 241.0 | 1.5 |
| 224 | 73.2 | 89.7 | 100.0 | 82.0 | 74.0 | 680.0 | 241.2 | 1.5 |
| 232 | 72.0 | 87.5 | 100.0 | 79.6 | 74.2 | 680.0 | 238.0 | 1.5 |
| 240 | 70.4 | 88.0 | 100.0 | 80.8 | 71.9 | 676.2 | 240.0 | 1.5 |
| 248 | 71.0 | 86.5 | 100.0 | 79.0 | 72.0 | 673.1 | 226.0 | 1.5 |
| 256 | 72.0 | 87.2 | 100.0 | 80.0 | 70.5 | 675.3 | 224.5 | 1.5 |
| 264 | 71.3 | 88.1 | 100.0 | 77.5 | 66.6 | 677.0 | 221.0 | 1.5 |
| 272 | 71.5 | 87.0 | 100.0 | 78.0 | 64.7 | 651.2 | 236.0 | 1.5 |
| 280 | 71.5 | 85.3 | 100.0 | 76.3 | 65.0 | 650.0 | 234.5 | 1.5 |
| 288 | 69.1 | 85.5 | 100.0 | 76.0 | 65.0 | 650.0 | 230.0 | 1.5 |
| 296 | 71.0 | 82.1 | 100.0 | 76.5 | 63.5 | 644.5 | 228.5 | 1.6 |
| 304 | 68.3 | 82.5 | 100.0 | 76.1 | 64.0 | 642.5 | 225.0 | 1.5 |
| 312 | 71.3 | 82.0 | 100.0 | 74.8 | 62.6 | 642.0 | 225.5 | 1.5 |
| 320 | 71.0 | 82.0 | 100.0 | 73.0 | 58.5 | 628.6 | 217.0 | 1.5 |
| 328 | 69.0 | 80.8 | 100.0 | 72.6 | 59.3 | 626.9 | 213.5 | 1.5 |
| 336 | 70.6 | 81.1 | 100 | 70.1 | 60.0 | 625.0 | 210 | 1.6 |
| 344 | 69.4 | 82.4 | 100 | 70.0 | 60.5 | 625.0 | 210 | 1.6 |
| 352 | 70.0 | 80.0 | 100 | 68.0 | 60.0 | 625.0 | 211 | 1.5 |
| 360 | 69.8 | 81.2 | 100 | 67.5 | 58.7 | 624.6 | 210 | 1.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand, DM= Dry matter, PC= Protein content

**Table A.9:** Impact of incubation period (day) on the nutrients elimination of *Aspergillus niger* inoculated in raw wastewater (aerobic Batch)

| Incubation period (days) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 1 | 0.9 | 45.5 | 22.3 | 14.9 | 21.9 | 3.4 |
| 2 | 4.1 | 93.9 | 44.0 | 18.5 | 58.6 | 2.8 |
| 3 | 42.0 | 95.4 | 94.9 | 21.0 | 60.4 | 1.8 |
| 4 | 44.7 | 91.3 | 96.6 | 29.8 | 64.4 | 1.5 |
| 5 | 38.4 | 90.1 | 77.8 | 23.0 | 60.2 | 1.3 |
| 6 | 36.9 | 88.4 | 75.6 | 24.2 | 60.2 | 1.5 |
| 7 | 31.3 | 89.3 | 80.9 | 18.5 | 59.5 | 1.5 |
| 8 | 34.6 | 89.2 | 80.8 | 12.5 | 60.4 | 1.5 |
| 9 | 24.1 | 89.2 | 80.3 | 13.4 | 60.0 | 1.3 |
| 10 | 21.4 | 88.8 | 76.7 | 15.7 | 60.4 | 1.5 |
| 11 | 19.2 | 89.6 | 74.2 | 15.0 | 60.2 | 1.6 |
| 12 | 20.0 | 90.5 | 72.0 | 17.3 | 59.6 | 1.6 |
| 13 | 19.2 | 89.8 | 69.9 | 16.5 | 59.5 | 2.3 |
| 14 | 19.2 | 89.9 | 71.5 | 16.5 | 59.3 | 2.7 |
| 15 | 19.0 | 89.9 | 71.8 | 14.1 | 59.6 | 2.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

**Table A.10:** Impact of incubation period (day) on the nutrients elimination of *Trichoderma viride* inoculated in raw wastewater (aerobic Batch)

| Incubation period (days) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 1 | 3.3 | 11.5 | 16.2 | 5.7 | 23.9 | 3.5 |
| 2 | 44.5 | 91.9 | 91.0 | 11.9 | 40.4 | 2.2 |
| 3 | 46.1 | 93.2 | 97.6 | 18.5 | 40.9 | 1.5 |
| 4 | 48.1 | 94.0 | 96.6 | 21.1 | 53.6 | 1.4 |
| 5 | 52.8 | 94.0 | 100.0 | 20.7 | 59.8 | 1.4 |
| 6 | 47.3 | 93.8 | 100.0 | 16.3 | 49.6 | 1.4 |
| 7 | 46.7 | 92.9 | 100.0 | 16.3 | 41.9 | 1.4 |
| 8 | 44.2 | 92.6 | 100.0 | 15.0 | 41.8 | 1.5 |
| 9 | 40.6 | 91.7 | 100.0 | 14.1 | 41.8 | 1.4 |
| 10 | 39.7 | 91.4 | 100.0 | 14.1 | 41.8 | 1.5 |
| 11 | 36.1 | 90.8 | 100.0 | 9.7 | 41.6 | 1.5 |
| 12 | 35.5 | 89.3 | 100.0 | 8.8 | 41.6 | 1.6 |
| 13 | 33.6 | 88.6 | 100.0 | 7.0 | 41.2 | 2.0 |
| 14 | 31.6 | 85.0 | 100.0 | 6.2 | 39.3 | 2.1 |
| 15 | 30.0 | 85.5 | 100.0 | 5.7 | 23.9 | 2.0 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

**Table A.11:** Impact of incubation period (day) on the nutrients elimination of *Aspergillus niger* inoculated in raw wastewater (anaerobic Batch)

| Incubation period (days) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 1 | 0.6 | 8.5 | 10.3 | 2.9 | 8.0 | 4.1 |
| 2 | 5.5 | 30.3 | 17.3 | 8.6 | 21.5 | 2.9 |
| 3 | 25.0 | 50.3 | 42.0 | 11.5 | 33.3 | 1.7 |
| 4 | 28.2 | 64.5 | 66.6 | 14.5 | 41.6 | 1.5 |
| 5 | 31.5 | 71.5 | 68.5 | 15.8 | 48.5 | 1.5 |
| 6 | 31.8 | 75.3 | 70.2 | 12.5 | 44.0 | 1.4 |
| 7 | 30.5 | 76.0 | 70.0 | 10.0 | 44.0 | 1.5 |
| 8 | 30.0 | 74.0 | 70.5 | 10.0 | 38.5 | 1.5 |
| 9 | 30.0 | 76.0 | 68.3 | 8.5 | 38.7 | 1.5 |
| 10 | 30.0 | 70.0 | 68.5 | 8.0 | 33.5 | 1.5 |
| 11 | 31.0 | 68.5 | 66.6 | 8.0 | 33.3 | 1.5 |
| 12 | 30.3 | 69.3 | 64.5 | 8.3 | 31.5 | 1.9 |
| 13 | 21.0 | 70.0 | 58.5 | 5.4 | 32.0 | 2.3 |
| 14 | 20.0 | 69.0 | 59.0 | 5.0 | 31.0 | 2.2 |
| 15 | 20.0 | 53.5 | 56.8 | 5.0 | 28.6 | 2.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

**Table A.12:** Impact of incubation period (day) on the nutrients elimination of *Trichoderma viride* inoculated in raw wastewater (anaerobic Batch)

| Incubation period (days) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 1 | 1.2 | 15.8 | 10.5 | 2.3 | 13.5 | 3.7 |
| 2 | 15.0 | 35.0 | 52.5 | 7.5 | 19.9 | 2.1 |
| 3 | 27.5 | 69.5 | 73.0 | 11.0 | 25.0 | 1.3 |
| 4 | 29.3 | 73.3 | 75.8 | 20.5 | 46.0 | 1.5 |
| 5 | 33.3 | 81.5 | 80.0 | 21.0 | 43.5 | 1.5 |
| 6 | 32.5 | 88.5 | 77.3 | 23.3 | 53.5 | 1.4 |
| 7 | 32.0 | 80.0 | 78.0 | 23.0 | 57.3 | 1.5 |
| 8 | 32.0 | 81.5 | 75.5 | 23.0 | 55.5 | 1.5 |
| 9 | 28.5 | 80.5 | 71.0 | 23.1 | 56.0 | 1.6 |
| 10 | 28.7 | 75.5 | 70.0 | 18.5 | 54.3 | 1.8 |
| 11 | 26.2 | 73.0 | 71.3 | 13.3 | 47.5 | 1.8 |
| 12 | 27.0 | 71.5 | 70.0 | 10.5 | 44.3 | 2.2 |
| 13 | 25.5 | 72.0 | 68.5 | 10.0 | 45.0 | 2.5 |
| 14 | 24.0 | 70.5 | 69.0 | 10.0 | 45.0 | 2.5 |
| 15 | 24.0 | 68.0 | 69.0 | 8.9 | 40.0 | 2.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

**Table A.13:** Impact of incubation period (h) on the nutrients elimination of *Aspergillus niger* inoculated in raw wastewater (aerobic Batch)

| Incubation period (h) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 8 | 0.5 | 6.0 | 3.3 | 0.5 | 4.9 | 4.3 |
| 16 | 0.9 | 25.3 | 14.5 | 8.1 | 14.2 | 3.8 |
| 24 | 1.7 | 38.5 | 26.0 | 11.5 | 25.3 | 3.1 |
| 32 | 3.5 | 51.5 | 33.8 | 18.5 | 27.5 | 2.9 |
| 40 | 11.9 | 66.6 | 55.5 | 25.2 | 36.3 | 2.3 |
| 48 | 25.0 | 85.3 | 61.0 | 30.3 | 47.5 | 2.1 |
| 56 | 28.5 | 88.0 | 75.5 | 33.5 | 47.9 | 1.8 |
| 64 | 33.0 | 95.0 | 82.9 | 33.8 | 48.5 | 1.7 |
| 72 | 36.6 | 95.0 | 93.5 | 34.6 | 48.6 | 1.5 |
| 80 | 38.5 | 95.0 | 93.0 | 35.5 | 53.0 | 1.5 |
| 88 | 42.0 | 95.0 | 93.0 | 35.0 | 59.5 | 1.6 |
| 96 | 48.0 | 95.0 | 93.3 | 34.6 | 71.5 | 1.5 |
| 104 | 48.0 | 95.0 | 93.0 | 33.3 | 71.3 | 1.5 |
| 112 | 47.5 | 95.0 | 92.6 | 33.0 | 70.5 | 1.4 |
| 120 | 48.0 | 95.0 | 93.0 | 33.0 | 70.0 | 1.5 |
| 128 | 48.0 | 94.7 | 92.5 | 33.1 | 70.0 | 1.5 |
| 136 | 47.3 | 94.5 | 93.0 | 33.0 | 68.3 | 1.5 |
| 144 | 47.5 | 94.3 | 92.3 | 30.0 | 69.0 | 1.5 |
| 152 | 46.2 | 94.0 | 92.0 | 28.5 | 68.5 | 1.5 |
| 160 | 46.5 | 94.0 | 91.8 | 29.0 | 68.5 | 1.5 |
| 168 | 47.0 | 94.0 | 92.0 | 28.8 | 68.0 | 1.5 |
| 176 | 45.8 | 92.5 | 92.0 | 28.5 | 68.0 | 1.5 |
| 184 | 43.6 | 92.0 | 90.5 | 28.0 | 66.5 | 1.5 |
| 192 | 43.5 | 92.0 | 90.0 | 28.0 | 65.8 | 1.5 |
| 200 | 43.5 | 90.8 | 88.9 | 28.0 | 65.0 | 1.5 |
| 208 | 43.5 | 90.2 | 89.0 | 26.7 | 65.3 | 1.5 |
| 216 | 42.1 | 90.0 | 89.0 | 27.0 | 65.0 | 1.5 |
| 224 | 39.0 | 90.0 | 86.6 | 26.5 | 65.0 | 1.6 |
| 232 | 39.3 | 90.0 | 85.3 | 26.5 | 63.6 | 1.5 |
| 240 | 39.0 | 88.5 | 85.0 | 25.1 | 63.5 | 1.5 |
| 248 | 37.5 | 89.0 | 85.0 | 25.0 | 64.0 | 1.5 |
| 256 | 36.0 | 90.0 | 83.7 | 25.0 | 64.0 | 1.5 |
| 264 | 36.0 | 89.5 | 83.5 | 23.3 | 63.1 | 1.5 |
| 272 | 35.8 | 89.3 | 84.0 | 23.5 | 62.5 | 1.5 |
| 280 | 35.3 | 89.0 | 84.0 | 23.0 | 62.5 | 1.8 |
| 288 | 34.7 | 89.0 | 83.5 | 23.0 | 61.8 | 1.8 |
| 296 | 34.0 | 89.0 | 83.5 | 21.8 | 61.0 | 2.0 |
| 304 | 34.0 | 89.0 | 81.1 | 22.0 | 60.0 | 2.0 |
| 312 | 34.0 | 89.0 | 81.5 | 22.0 | 58.4 | 1.8 |
| 320 | 32.5 | 88.2 | 80.0 | 21.0 | 59.0 | 2.0 |
| 328 | 32.5 | 88.5 | 80.0 | 21.5 | 58.5 | 2.2 |
| 336 | 32.3 | 88.0 | 79.3 | 22.0 | 59.0 | 2.5 |
| 344 | 32.0 | 88.3 | 80.0 | 21.0 | 59.0 | 2.5 |
| 352 | 30.6 | 88.0 | 79.5 | 20.0 | 59.0 | 2.4 |
| 360 | 30.0 | 88.0 | 79.3 | 20.0 | 58.3 | 2.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

**Table A.14:** Impact of incubation period (h) on the nutrients elimination of *Trichoderma viride* inoculated in raw wastewater (aerobic Batch)

| Incubation period (h) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 8 | 1.3 | 3.5 | 6.6 | 1.7 | 7.0 | 4.5 |
| 16 | 1.9 | 5.8 | 10.3 | 3.3 | 11.5 | 3.6 |
| 24 | 2.5 | 10.3 | 14.8 | 6.5 | 20.0 | 3.2 |
| 32 | 18.0 | 45.5 | 37.5 | 11.9 | 20.7 | 2.5 |
| 40 | 29.3 | 71.0 | 71.5 | 13.0 | 36.5 | 2.0 |
| 48 | 37.8 | 87.5 | 85.5 | 13.5 | 38.5 | 2.0 |
| 56 | 41.5 | 91.5 | 88.6 | 22.1 | 40.9 | 1.6 |
| 64 | 43.7 | 91.7 | 93.5 | 28.5 | 43.3 | 1.5 |
| 72 | 45.1 | 93.8 | 100.0 | 30.0 | 48.6 | 1.5 |
| 80 | 46.5 | 95.0 | 100.0 | 38.5 | 51.5 | 1.5 |
| 88 | 49.3 | 96.8 | 100.0 | 40.0 | 55.5 | 1.5 |
| 96 | 56.0 | 96.4 | 100.0 | 40.0 | 60.5 | 1.5 |
| 104 | 56.0 | 95.0 | 100.0 | 38.7 | 63.3 | 1.5 |
| 112 | 55.4 | 95.0 | 100.0 | 38.5 | 64.8 | 1.4 |
| 120 | 56.0 | 95.0 | 100.0 | 38.5 | 67.0 | 1.5 |
| 128 | 55.2 | 90.9 | 100.0 | 36.3 | 66.6 | 1.5 |
| 136 | 54.5 | 90.5 | 100.0 | 34.5 | 62.4 | 1.5 |
| 144 | 52.0 | 88.8 | 100.0 | 34.5 | 60.0 | 1.5 |
| 152 | 50.8 | 89.5 | 100.0 | 33.3 | 58.5 | 1.5 |
| 160 | 50.5 | 88.5 | 100.0 | 31.0 | 58.0 | 1.5 |
| 168 | 50.5 | 87.3 | 100.0 | 31.0 | 58.0 | 1.5 |
| 176 | 49.0 | 87.5 | 100.0 | 27.4 | 56.1 | 1.5 |
| 184 | 49.0 | 86.0 | 100.0 | 27.5 | 55.5 | 1.5 |
| 192 | 48.7 | 84.8 | 100.0 | 25.0 | 55.6 | 1.5 |
| 200 | 45.6 | 84.5 | 100.0 | 25.0 | 53.8 | 1.5 |
| 208 | 45.0 | 83.2 | 100.0 | 24.3 | 53.5 | 1.7 |
| 216 | 43.2 | 82.5 | 100.0 | 24.0 | 49.5 | 1.5 |
| 224 | 41.0 | 82.0 | 100.0 | 21.6 | 47.0 | 1.5 |
| 232 | 41.0 | 82.0 | 100.0 | 18.8 | 47.0 | 1.5 |
| 240 | 40.6 | 81.5 | 100.0 | 19.0 | 45.8 | 1.5 |
| 248 | 41.0 | 78.5 | 100.0 | 18.3 | 45.5 | 1.5 |
| 256 | 40.0 | 78.5 | 100.0 | 18.0 | 45.5 | 1.5 |
| 264 | 40.0 | 76.7 | 100.0 | 18.0 | 45.0 | 1.5 |
| 272 | 40.0 | 75.5 | 100.0 | 16.6 | 43.3 | 1.5 |
| 280 | 38.5 | 75.0 | 100.0 | 14.5 | 43.0 | 1.5 |
| 288 | 38.1 | 74.6 | 100.0 | 14.5 | 43.0 | 1.5 |
| 296 | 36.6 | 73.5 | 100.0 | 15.0 | 43.0 | 1.6 |
| 304 | 36.2 | 74.0 | 100.0 | 14.0 | 43.0 | 1.6 |
| 312 | 35.0 | 73.5 | 100.0 | 14.0 | 43.0 | 1.5 |
| 320 | 35.0 | 72.8 | 100.0 | 13.3 | 42.1 | 1.5 |
| 328 | 34.5 | 72.5 | 100.0 | 13.0 | 41.0 | 1.5 |
| 336 | 33.3 | 72.5 | 100.0 | 13.0 | 41.0 | 1.5 |
| 344 | 33.0 | 72.0 | 100.0 | 13.0 | 39.5 | 1.6 |
| 352 | 33.1 | 71.0 | 100.0 | 12.4 | 39.0 | 1.7 |
| 360 | 32.5 | 70.0 | 100.0 | 13.0 | 39.0 | 1.6 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

**Table A.15:** Impact of incubation period (day) on the nutrients elimination of *Aspergillus niger* inoculated in raw wastewater (aerobic Batch) at pH 7.5

| Incubation period (days) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 1 | 0.6 | 2.5 | 7.2 | 2.0 | 3.3 | 7.5 |
| 2 | 0.5 | 2.2 | 7.6 | 2.1 | 3.0 | 7.5 |
| 3 | 0.5 | 2.1 | 7.0 | 1.8 | 2.8 | 7.5 |
| 4 | 0.6 | 2.0 | 7.0 | 2.0 | 3.0 | 7.5 |
| 5 | 0.3 | 2.0 | 7.1 | 2.0 | 3.0 | 7.5 |
| 6 | 0.3 | 1.8 | 7.0 | 2.0 | 3.0 | 7.5 |
| 7 | 0.3 | 2.1 | 7.0 | 1.9 | 3.1 | 7.5 |
| 8 | 0.3 | 2.0 | 7.0 | 1.8 | 3.0 | 7.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

**Table A.16:** Impact of incubation period (day) on the nutrients elimination of *Trichoderma viride* inoculated in raw wastewater (aerobic Batch) at pH 7.5

| Incubation period (days) | Supernatant % elimination | | | | | Final pH |
|---|---|---|---|---|---|---|
| | TN | $PO_4$ | $NH_4$ | $NO_3$ | COD | |
| 1 | 1.3 | 2.8 | 11.0 | 1.8 | 5.0 | 7.5 |
| 2 | 0.9 | 2.0 | 8.5 | 2.0 | 2.6 | 7.5 |
| 3 | 1.0 | 2.0 | 9.0 | 1.5 | 2.5 | 7.5 |
| 4 | 0.8 | 1.8 | 9.1 | 1.6 | 2.5 | 7.5 |
| 5 | 0.8 | 1.8 | 8.8 | 1.5 | 2.0 | 7.5 |
| 6 | 0.9 | 2.0 | 8.5 | 1.3 | 2.0 | 7.5 |
| 7 | 0.9 | 2.0 | 8.6 | 1.5 | 1.8 | 7.5 |
| 8 | 0.8 | 1.7 | 8.5 | 1.0 | 2.1 | 7.5 |

Percentages were calculated taking the values of untreated raw wastewater as 100%.
TN= Total nitrogen, COD= Chemical oxygen demand

## List of publications launched in frame of this study

1- **Awad, M. F. and M. Kraume (2010):** The Occurrence of Fungi in Activated Sludge from MBRs. *International Journal of Chemical and Biological Engineering* 3-4: 180-183.

2- **Awad, M. F. and M. Kraume (2011):** Fungal diversity in activated sludge from membrane bioreactors in Berlin. *Canadian Journal of Microbiology*, 2011, 57:(8) 693-698.

3- **Awad, M. F. and M. Kraume (2011):** Mycological survey of activated sludge in MBRs, *Mycoses*. 54: (5), 229-235.

4- **Awad, M. F. and M. Kraume (2011):** Keratinophilic fungi in activated sludge of wastewater treatment plants in Berlin, Germany. *Mycology: An International Journal of Fungal Biology*. DOI: 10.1080/21501203.2011.603103.

5- **Awad, M. F. and M. Kraume (2011):** Mycoflora of activated sludge with MBRs in Berlin, Germany. *International Journal of Biological* and *Life Sciences*. In press.

6- **Awad, M. F and. M. Kraume (2010):** Occurrence of fungi in activated sludge from MBRs. Fourth Saudi Science Conference Contribution of Science Faculties in the Development Process of KSA , March 21-24$^{th}$, Al-Madinah Al-Munawwarah, KSA. Proceding book. POS-MIC-67, 93.

7- **Awad, M. F. and M. Kraume (2010):** The Occurrence of fungi in activated sludge from MBRs. International Conference on Environmental Sciences and Engineering WASET**,** November 24-26, 2010, Venice, Italy. Issue 71. ISSN: 1307-6892. Proceeding book 813-816.

8- **Awad, M. F. and M. Kraume (2011):** Mycoflora of activated sludge with MBRs in Berlin, Germany. International Conference on Agricultural and Biosystems Engineering WASET, July 13-15, 2011, Amsterdam, Netherlands. Issue 78. ISSN: 376x-3778. Proceeding book 773-778.