Universidade de Aveiro Departamento de Química
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Estudo e optimização do funcionamento de uma ETAR industrial

Study and optimization of the operation of an industrial WWTP

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, realizada sob a orientação científica da Doutora Ana Maria Rebelo Barreto Xavier, Professora Auxiliar do Departamento de Química da Universidade de Aveiro e da Engenheira Anabela Ferreira Antunes, Diretora de Produção da Prio Biocombustíveis, S.A..

Aos meus pais

o júri

presidente

Prof. Doutora Ivonne Delgadillo Giraldo
Professora associada com agregação da Universidade de Aveiro

Prof. Doutora Ana Maria Rebelo Barreto Xavier
Professora auxiliar da Universidade de Aveiro

Doutor Paulo Alexandre da Costa Lemos
Investigador Auxiliar, REQUIMTE/CQFB, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

Engenheira Anabela Ferreira Antunes
Diretora de Produção da Prio Biocombustíveis, S.A.

agradecimentos

Agradeço à Prof. Doutora Ana Xavier, do Departamento de Química da Universidade de Aveiro, por ter aceite a orientação deste trabalho, ajuda e constante disponibilidade.

Agradeço à Engª. Anabela Antunes e Doutora Rita Manso da Prio Biocombustíveis pela proposta do tema de investigação, ajuda, orientação e incentivo.

Agradeço ao Jaime Jerónimo e Lídia Pascoal da Prio Biocombustiveis pelos conhecimentos transmitidos relativamente ao funcionamento da ETAR e de equipamentos de laboratório.

Agradeço a todos os chefes de turno e operários da Prio Biocombustíveis pela recolha de amostras e simpatia.

Agradeço a todos os colegas de curso pelo apoio e alegre convivência ao longo destes anos.

Agradeço ao Nuno Silva pelo incentivo e apoio ao longo deste trabalho.

Agradeço de forma especial aos meus pais pelo apoio constante ao longo da minha vida e pelos valores transmitidos.

palavras-chave

ETAR industrial, águas residuais, lamas ativadas, DAF, análise da microfauna, indicadores biológicos de poluição

resumo

O presente trabalho tem como objetivo a otimização do funcionamento de uma ETAR industrial, para tratamento de águas residuais resultantes da produção de biodiesel. A ETAR industrial em estudo engloba dois processos de tratamento: tratamento físico-químico através da utilização de uma unidade DAF e, tratamento biológico aeróbio por lamas ativadas.

Ao longo deste estudo vários parâmetros físico-químicos foram analisados em particular sólidos totais, sólidos suspensos voláteis, carência química de oxigénio, teor em azoto amoniacal e fósforo total para além do pH e temperatura. Conseguiu-se optimizar a metodologia de análise dos sólidos totais reduzindo o tempo total da análise. Implementou-se a técnica de monitorização microbiológica das lamas activadas tendo-se identificado e quantificado alguns grupos de microrganismos característicos dos processos de lamas ativadas: ciliados nadadores, ciliados sésseis, ciliados móveis de fundo, amebas com teca, pequenos flagelados, ciliados carnívoros, *Opercularia* sp. e pequenos metazoários. Após a recolha destes dados físico-químicos e biológicos, diários e/ou periódicos, fez-se a avaliação do desempenho da ETAR. Foram identificadas as variáveis de controlo físico-químico que apresentavam maior variabilidade, nomeadamente o rácio alimentação/microrganismo (F/M) e o tempo de retenção de sólidos (SRT). Verificou-se que a instabilidade do F/M tem origem no facto da ETAR ter sido projetada para tratamento de um maior caudal de efluente ou de um efluente mais poluído. No caso do SRT a causa é o ineficiente sistema de purga de lamas. Concluiu-se que determinados parâmetros operacionais devem ser alterados por forma a otimizar-se o processo: o volume útil dos bioreactores deve ser reduzido de 110 m^3 para 80 m^3, a concentração de sólidos suspensos presente nos bioreactores deve reduzir-se em 20% e a purga de lamas deve ser cerca de 2 m^3 por dia controlada de modo a poder manter a concentração de biomassa constante nos reatores.

Relacionou-se ainda o efeito da alteração dos parâmetros físico-químicos na estrutura da comunidade da microfauna, através do teste de correlação não paramétrico de Spearman'rho. Esta análise estatística multivariada permitiu a identificação de bioindicadores do desempenho do sistema de lamas ativadas, e a criação de grupos de controlo positivo e negativo que tornam possível avaliar rapidamente o desempenho da ETAR através da monitorização das suas lamas.Com este estudo a empresa ficou dotada de mecanismos rápidos de avaliação do desempenho da ETAR de modo a que o seu funcionamento possa ser muito mais controlado.

keywords

Industrial WWTP, wastewater, activated sludge, DAF, microfauna analysis, biological indicators of pollution

abstract

The main goal of the present work is the optimization of the operation of an industrial wastewater treatment plant to treat biodiesel wastewater. The industrial WWTP in study comprises two treatment processes: physicochemical treatment through the use of a DAF unit and, biological treatment by activated sludge.

During the study several physicochemical parameters were analyzed, namely total solids, volatile suspended solids, chemical oxygen demand, ammoniacal nitrogen and phosphorus content, besides pH and temperature. The methodology of total solids determination was optimized, reducing the analysis time. Microbiologic motorization of the activated sludge was implemented, with the identification and quantification of groups of microorganisms characteristics of the activated sludge process: free-swimming ciliates, sessile ciliates, crawling ciliates, testate amoebae, small flagellates, carnivorous ciliates, *Opercularia* sp. and small metazoan. After collecting these physicochemical and biological data, daily or periodically, the performance of the plant was evaluated. Therefore the physicochemical control variables that presents greatest variation were identified, namely food to microorganism ratio (F/M) and solids retention time (SRT). It was verified that the F/M instability is originated by the fact that the WWTP was designed to treat a larger amount of effluent or a more polluted one. On the other hand, SRT variability is caused by the inefficient sludge removal system. It was concluded that certain operational parameters should be changed in order to optimize the process: the useful volume of each bioreactor must be reduce from 110 m^3 to 80 m^3, the amount of suspended solids present in the bioreactors must be reduced in 20% and the amount of sludge purged from the system must be around 2 m^3 per day, in order to maintain the biomass concentration constant in the reactors.

It was also investigated the effect of the modification of the physicochemical parameters on the microfauna community, through the nonparametric correlation test of Spearman'rho. This multivariate statistical analysis allowed the identification of biological biomarkers of the activated sludge system performance, and the establishment of groups of positive and negative control, that make possible to quickly evaluate the performance of the WWTP by monitoring their sludge. With this study the company was provided with methodologies of rapid assessment of the performance of the WWTP so that its operation may be much more controlled.

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Abbreviations

AE	Aeration tank
AN	Ammoniacal nitrogen
BOD	Biological oxygen demand
BOD_5	5-days biological oxygen demand
C:N:P	Carbon, nitrogen and phosphorus ratio
COD	Chemical oxygen demand
DAF	Dissolved air flotation
DGGE	Denaturing gradient gel electrophoresis
DO	Dissolved oxygen
EPS	Extracellular polymeric substances
FAME	Fatty acid methyl esters
FAS	Ferrous ammonium sulphate
FISH	Fluorescent in situ hybridization
F/M	Food to microorganism ratio
FS	Fixed solids
GC	Gas chromatograph
HPLC	High-performance liquid chromatograph
HRT	Hydraulic residence time
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
N	Sample size
O&G	Oil and grease
PAO	Polyphosphate accumulating organisms
PCA	Principal component analysis
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SBI	Sludge biotic index
SD	Standard deviation
SRT	Solids retention time
SS	Settleable solids
SVI	Sludge volume index

TDS	Total dissolved solids
TOC	Total organic carbon
TP	Total phosphorus
TS	Total solids
TSS	Total suspended solids
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant
iWWTP	Industrial wastewater treatment plant
p	*p*-value
ρ	Spearman correlation coefficient

1. INTRODUCTION

1.1. Wastewater

Wastewater can be defined as the flow of water that has been used by a community and is discharged into a receiving water body with altered physical and/or chemical parameters (Jördening and Winter, 2005). Wastewater are mainly constituted by water (about 99,94%) and a small fraction of dissolved or suspended materials (0,006%) (Lin and Lee, 2007). Wastewater is typically classified according to their origin in domestic when derived from residential areas, industrial when resulting from human activities associated with the processing and manufacturing of raw material, and commercial when originated from offices, shops, hotels and others. In large urban areas is inevitable the combination between the three types of wastewater, appearing the concept of municipal or urban wastewater (Gray, 2004; Jern, 2006; Wiesmann *et al.*, 2007; Lofrano and Brown, 2010).

Industrial wastewater, in focus in this work, has characteristics slightly different from domestic wastewater and thus is important a brief definition of the two types. Domestic wastewater is a complex mixture of water (approximately 99%) and organic and inorganic constituents. The inorganic components include chlorides and sulfates, nitrogen in various forms and phosphorus, as well as carbonates and bicarbonates. Proteins and carbohydrates constitute about 90% of the organic matter present in domestic sewage. Since domestic wastewater include human wastes, also contains large number of microorganisms and some of them can be pathogenic, causing diseases such as cholera, typhoid and tuberculosis. On the other hand, industrial wastewaters have very diverse compositions depending on the type of industry and materials processed. Some industrial wastewaters can present values of total suspended solids (TSS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) around thousands of mg/L (Jern, 2006). Due to the high organic concentration, industrial wastewater may be also nutrient deficient, namely in terms of nitrogen and phosphorus that are used by microorganism in biological wastewater treatment systems (e.g., activated sludge) (Gerardi, 2006; Jern, 2006). In contrast to domestic wastewater, pH values well beyond the range of 6-7 are usually found. Industrial wastewaters can have different characteristics even from the same type of industry, but in different geographical locations. The cause of these differences is related to the operating procedures adopted and the raw materials used, starting with the origin of the water used.

Moreover, the characteristics of the wastewater can vary within the same industry over time, for instance due to unusual discharges (Jern, 2006).

1.2. Wastewater characteristics

An understanding of wastewater characteristics is very important in design, operation and management of collection and treatment of wastewater. Since there are legal requirements restricting the discharge of wastewater, it is also fundamental to know the water characteristics after treatment. Wastewater is characterized in terms of its physical, chemical and biological composition (Tchobanoglous *et al.*, 2003; Jern, 2006; Lin and Lee, 2007).

1.2.1. Physical characteristics

Physical characteristics include solids content, temperature, particle size distribution, turbidity, color, odor, transmittance, gravity and specific weight (Tchobanoglous *et al.*, 2003).

Temperature and solids content are the most important factors in the treatment of sewage. Temperature affects the chemical reactions, biological activity and the concentration of dissolved gases. In turn, solids, that comprise matter suspended or dissolved, affect the operation and design of treatment units. They are divided into various fractions (Figure 1.1) and their concentration provides useful information for characterization and control of wastewater treatment process (Tchobanoglous *et al.*, 2003).

The total solids (TS) are quantified by evaporating a water sample to dryness and measuring the mass of the residue left in a vessel. The division of these pollutants into dissolved (TDS) and suspended solids (TSS) is essential as many of the treatment processes are only effective against one of these. As can be observed in Figure 1.1, this division is achieved by filtration, thus is not well defined since the pore size of the filter used is not universal (0,45 – 2,0 μm) (Henze *et al.*, 1997; Tchobanoglous *et al.*, 2003; Jördening and Winter, 2005). When the evaporation residue obtained in total, suspended or

dissolved solids determination is subjected to ignition at 550 °C, fixed and volatile solids are quantified. The weigh lost in ignition is reported as volatile solids (VS) and corresponds to the organic fraction present in a water sample. Volatile solids test indicates the amount of the total solids that can potentially be destroyed chemically or biologically, volatilized or adsorbed. On the other hand, fixed solids (FS) are indicators of the inorganic fraction of the sample as they relate to the residue that remains after ignition. This residue cannot be destroyed and these solids must be converted or removed by some physical or chemical method. Finally, the standard test for quantifying the settleable solids (SS) consists of placing a sample in a 1 liter Imhoff cone and verifying the amount that settles (ml) after a specific period of time (American Public Health Association, 1999; Tchobanoglous *et al.*, 2003; Alley, 2007).

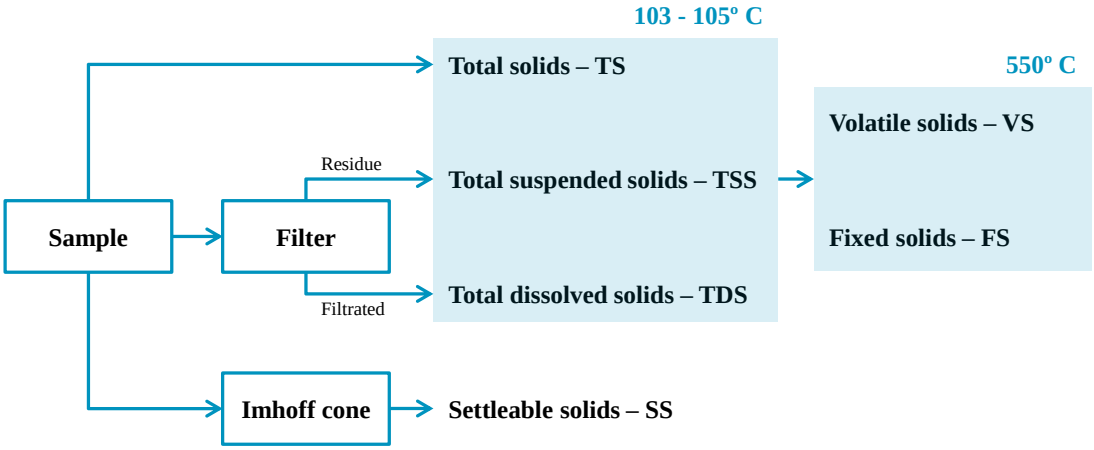


Figure 1.1: Relation between the different solids found in water and wastewater, adapted from Henze *et al.*(1997) and Tchobanoglous *et al.*(2003).

1.2.2. Chemical characteristics

The chemical constituents of wastewater are typically classified as inorganic and organic (Tchobanoglous et al., 2003). Organic matter may include carbohydrates, oil, grease, surfactants, proteins, pesticides, volatile organic compounds and toxic chemicals. Inorganics include heavy metals, nutrients (nitrogen and phosphorus), chlorides, sulfur and gases (Lin and Lee, 2007).

Since wastewater contains a large variety of organic constituents, the individual determination of each one requires the use of sophisticated instrumentation capable of

measuring trace concentrations, as gas chromatograph (GC) and high-performance liquid chromatograph (HPLC) (American Public Health Association, 1999; Tchobanoglous *et al.*, 2003). Thus, collective analysis that measures aggregate organic matter comprising a number of organic constituents with common characteristics is used, and can be represented by the equation of oxidation of the organic matter:

$$C_{18}H_{19}O_9N + 17{,}5O_2 + H^+ \rightarrow 18CO_2 + 8H_2O + NH_4^+ \quad (1.1)$$

Collective analyses relies on the base that if organic matter is oxidized, the amount of oxygen consumed (BOD, COD) or the amount of carbon dioxide produced (total organic carbon –TOC) can be measured (Henze *et al.*, 1997). So, the organic content of wastewater is usually measured as a 5-days biochemical oxygen demand (BOD_5), chemical oxygen demand (COD) and total organic carbon (TOC). The BOD_5 test measures the amount of oxygen required to a biological oxidation of the organic matter in the sample during 5 days at 20ºC. Since the BOD_5 test is time consuming, COD is routinely performed after establishing the relationship between BOD_5 and COD for a specific wastewater treatment plant (Lin and Lee, 2007).

The chemical oxygen demand indicates the organic content present in a sample that is susceptible of being oxidised by a strong chemical oxidant, under controlled conditions (Wiesmann *et al.*, 2007). The quantity of oxidant consumed is expressed in terms of its oxygen equivalent. So, COD represents the amount of oxygen needed for the complete chemical oxidation of both organic and inorganic compounds, susceptible to oxidation, presents in water (Cheremisinoff, 2006; Amjad, 2010). COD is often used as a measure of pollutants in wastewater and natural waters, and expresses the organic charge of the effluent (Tchobanoglous *et al.*, 2003).

Besides organic content, is also given special attention to nitrogen and phosphorus concentration (Wiesmann *et al.*, 2007). These are naturally present in the receiving water bodies and are essential to the life cycle of the microorganisms. However, the discharge of nitrogenous and phosphorous compounds into receiving water bodies may alter their fertility that lead to excessive plant growth, phenomenon known as eutrophication. The subsequent impacts of such growth include increased turbidity, oxygen depletion and toxicity issues. Thus, the wastewater treatment must comprise the nutrient removal to

ensure that the nutrient limiting conditions is maintained in the receiving water body (Ribeiro *et al.*, 2005; Jern, 2006). Nitrogen has several oxidation states, but the more common forms in wastewater are ammonia (NH_3), ammonium (NH_4^+), nitrogen gas (N_2), nitrite ion (NO_2^-) and nitrate ion (NO_3). The usual forms of phosphorus, found in aqueous solutions include orthophosphate (e.g., PO_4^{3-}, HPO_4^{2-} and $H_2PO_4^-$), polyphosphate and organic phosphate (Tchobanoglous *et al.*, 2003).

1.2.3. Biological characteristics

The biological characteristics of wastewater are fundamental and very important in the control of diseases caused by pathogenic organisms, and because of the essential role played by bacteria and other microorganisms in the decomposition and stabilization of organic matter (Tchobanoglous *et al.*, 2003; Vesilind, 2003)

Organisms found in wastewater include bacteria, fungi, algae, protozoa, plants, animals and viruses (Tchobanoglous *et al.*, 2003). Many microorganisms, namely bacteria and protozoa, are responsible and beneficial for biological treatment processes of wastewater. However, some pathogenic bacteria, fungi, protozoa and viruses found in wastewater can be the cause of public contamination (Gerardi, 2006; Lin and Lee, 2007). Thus, there are legal requirements that define the maximum value acceptable to various microbiological parameters, in order to not endanger the public health. In Portugal, this information is present in the Decree-Law no. 236/98 of August 1st that establishes water quality standards in order to protect, preserve and improve water resources.

1.3. Wastewater treatment

Wastewater treatment plants (WWTP) consist of a combination of unit process arranged in a sequence, such that each would support the performance of the downstream unit process or processes as wastewater progresses through the plant. The amount of treatments, and thus the complexity of the plant, is dependent on the treated effluent quality objectives and the nature of the raw wastewater. However, the unit processes can be classified into five

groups: preliminary treatment, primary treatment, secondary treatment, tertiary treatment and sludge treatment, Figure 1.2 (Jern, 2006; Shon *et al.*, 2006).

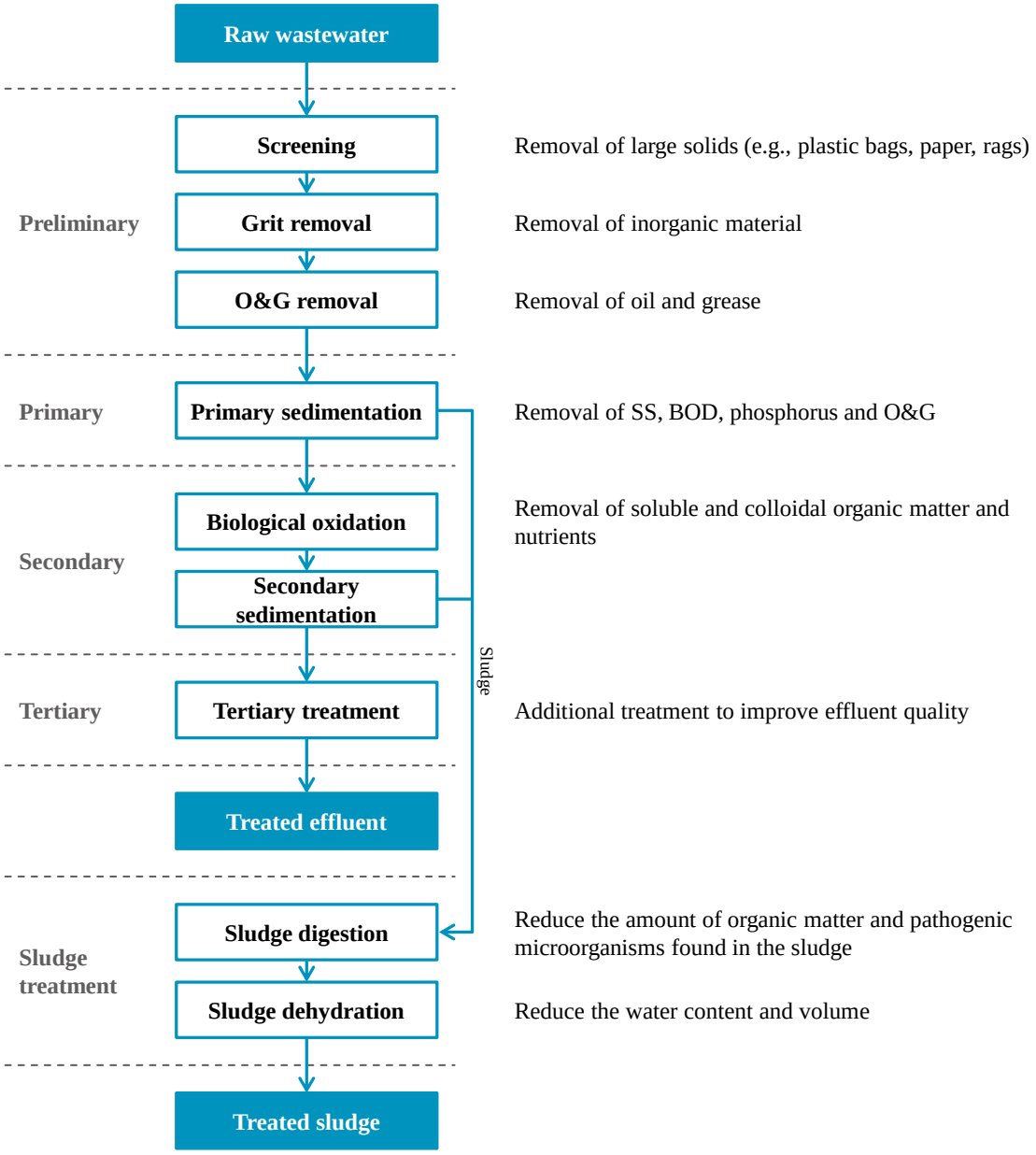


Figure 1.2: Typical stages in the conventional treatment of wastewater, adapted from Lin and Lee (2007).

1.3.1. Preliminary treatment

Preliminary systems are designed to physically remove larger suspended and floating material, heavy inorganic solids and excessive amounts of oil and grease. The aim of this treatment is to improve the performance of the downstream processes through protection of

equipment and flow equalization (Lin and Lee, 2007). Preliminary treatment operations typically include coarse screening to remove large materials, and grit removal. Grit is inert inorganic material such as sand particles, eggshells or metal fragments, and their removal process consists in reducing the flow rate of the effluent to permit the deposition of the particles. In some larger plants with effluents rich in oil and grease, these are likewise removed avoiding interference with the oxygen transfer in the biological treatment process. Usually they rise to the surface during the retention time of the effluent in the tank, being then removed manually or by mechanical skimmers (Ebel, 1994; Jern, 2006).

1.3.2. Primary treatment

The purpose of primary treatment is to reduce the flow velocity of the wastewater sufficiently to permit suspended solids to settle, but floating materials are also removed by skimming (Lin and Lee, 2007). Usually, in clarifiers, quiescent conditions, combined with processes such as flocculation and physical adsorption, allows the solids to settle in the bottom of the tank, by gravity, forming a sludge layer (Gray, 2005; Jern, 2006). In large clarifiers a scrapper located near the base of the clarifier moves the sludge into a collector from where is pumped into the sludge treatment stage (Chatzakis *et al.*, 2006; Jern, 2006).

This treatment removes approximately 50% to 70% of suspended solids and consequently 30-40% of BOD_5 is also eliminated, since organic matter settles with suspended solids (Tchobanoglous *et al.*, 2003; Jern, 2006; Shon *et al.*, 2006; Eurostat, 2010). The use of a coagulant (chemical, organic or semi-organic), in this treatment stage, allows increasing the removal of suspended solids and BOD_5 to values of 90% and 70%, respectively (Chatzakis *et al.*, 2006; Jern, 2006). Approximately 10% of the phosphorus is also removed by primary sedimentation, and oil and grease along with other floating matter are skimmed off from the basin surface (Lin and Lee, 2007).

1.3.3. Secondary treatment – Biological treatment

The objective of secondary treatment is the removal of soluble and colloidal organic matter remaining after the first stages of treatment. Thus, wastewater is exposed to a microbial

population, essentially bacteria, which convert the organic matter in new cells, energy, and also carbon dioxide and water (Gray, 2005; Jern, 2006; Shon *et al.*, 2006). The biological treatment removes more than 90% of suspended solids and a considerable part of the nutrients (Eurostat, 2010).

The biological treatment involves the transformation (i.e., oxidation) of dissolved and particulate biodegradable constituents into acceptable end products, capture and incorporation of suspended and colloidal solids into a biological floc or biofilm, transformation or removal of nutrients such as nitrogen and phosphorus, and in some cases, removal of specific trace organic constituents and compounds. In particular, for industrial wastewater the objective is to remove or reduce the concentration of organic and inorganic compounds. The biological processes used for wastewater treatment can be divided into two main categories: suspended growth and immobilised growth processes. In suspended growth processes, the microorganisms responsible for treatment are maintained in liquid suspension by appropriate mixing methods, and can operate in aerobic or anaerobic conditions. The most common suspended growth process used is the activated sludge system. On the other hand, in attached growth processes the microorganism are adsorbed to an inert material, such as rock, gravel, sand, plastic and other synthetic materials. Adsorbed growth processes can also be operated as aerobic or anaerobic processes (Tchobanoglous *et al.*, 2003).

1.3.4. Tertiary treatment

After the secondary treatment, the effluent only contains about 5-20% of the initial amount of organic matter. However, this effluent may still be rich in phosphates and nitrates, responsible for the phenomenon of eutrophication discussed above. Tertiary treatment is an expensive process, which involves physical, chemical and biological methods. The processes used include chemical coagulation, granular media filters, diatomaceous earth filters, and ultra- and nanofiltration. For nitrogen control, techniques such as biological assimilation, nitrification and denitrification, besides ion exchange are used. Soluble phosphorus may be removed by chemical precipitation or biological uptake for cell growth. Membrane physical processes such as reverse osmosis and ultrafiltration also help to achieve phosphorus decrease, but these are primarily employed for decrease of dissolved

inorganic solids. Finally, chlorine compounds are used for disinfection or destruction of organisms. However, the effluents containing chlorine when released into rivers and lakes can react, producing carcinogenic compounds which in turn may enter the food chain or be directly ingested by human beings. So, prior removal of chlorine compounds is necessary. As an alternative to chlorine, ultraviolet light or ozone treatment can be used to destroy the microorganisms without the addition of carcinogenic compounds (Black, 2002; Lin and Lee, 2007; Eurostat, 2010).

1.3.5. Sludge treatment

The by-product of treating wastewater is sludge: accumulated settled solids either humid or mixed with a liquid component as a result of natural or artificial processes. This sludge cannot be simply disposed due to its microbiological and chemical characteristics. In fact, according to their origin, they tend to concentrate heavy metals and poorly biodegradable organic compounds as well as potentially pathogenic organisms (e.g., viruses and bacteria). According to the Decree-Law no. 73/2011 of July 17th , they are classified as infectious waste and must be forwarded to a licensed operator for appropriate treatment in order to protect public health and the environment, as established in article 6 of the referred Decree-Law. The characteristics of the sludge depend on the source, i.e., the sector that produced it. For example, industrial sludge will be more contaminated by non-biodegradable compounds while agricultural sludge can contain more potentially pathogenic organisms. Sludge composition determines the type of treatment required and defines disposal options (Eurostat, 2010).

Anaerobic digesters are usually used to reduce the amount of solids. The products of this reaction are simple organic molecules and gases such as carbon dioxide and methane. Methane can be used for heating the anaerobic reactor and as energy supply to the WWTP (Black, 2002). The digested sludge is then dehydrated to reduce water content and therefore their volume. Methods used include drying beds, filter presses, and centrifuges. Finally, sludge is normally used in agriculture as a fertilizer, since they contain organic matter and nutrients. However, if the sludge is severely contaminated, for instance with heavy metals, has to be incinerated (Jern, 2006; Eurostat, 2010).

1.4. Activated sludge

The most frequently used process for the purification of wastewater through aerobic biodegradation is the activated sludge system. This is a suspended growth system that appeared in the early twentieth century in England, and has since been globally adopted as a secondarybiological treatment in wastewater treatment processes. This process consists essentially in oxidizing the organic matter to CO_2, H_2O, NH_3, and new cells by aerobic treatment. An important feature of this process is the ability of the microbial cells to form flocs, which allows their sedimentation in a solid/liquid separator, and subsequent separation from the treated effluent (Eckenfelder and Grau, 1992; Tchobanoglous *et al.*, 2003; Gray, 2004; Bitton, 2005; Ratledge and Kristiansen, 2006). The biochemical processes occur in two stages: the first corresponds to the synthesis of cellular material from the initial organic fraction, where nutrients as nitrogen and phosphorus are used, equation 1.2. At this stage the energy needed is obtained by the oxidation of organic matter. The second phase relates to the endogenous respiration/metabolism of the cellular material in accordance with equation 1.3.

$$\mathrm{COHNS} + \mathrm{O_2} + \mathrm{Nutrients} \xrightarrow{\text{Bacteria}} \mathrm{CO_2} + \mathrm{NH_3} + \mathrm{C_5H_7NO_2} + \begin{matrix}\text{Other}\\ \text{Products}\end{matrix} \tag{1.2}$$

$$\mathrm{C_5H_7NO_2} + 5\mathrm{O_2} \xrightarrow{\text{Bacteria}} 5\mathrm{CO_2} + 2\mathrm{H_2O} + \mathrm{NH_3} + \mathrm{Energy} \tag{1.3}$$

The organic matter in wastewater is represented by COHNS, which serves as the electron donor while oxygen serves as the electron acceptor. The endogenous respiration reaction (equation 1.3) only shows relatively simple end products and energy, since it represents a complete oxidation. Sometimes the oxidation of organic matter is not complete and stable organic products are also produced (Tchobanoglous *et al.*, 2003).

The activated sludge process comprises two basic treatment steps: the first occurs in the aeration tank and the second in a secondary sedimentation tank, Figure 1.3. In the aeration tank, contact time is provided for mixing and aerating influent wastewater with the suspension of microorganisms, typically referred as the mixed liquor suspended solids (MLSS) or mixed liquor volatile suspended solids (MLVSS). Mechanical equipment is used to keep the flocs, particles and microorganisms in suspension in the liquid phase to avoid the formation of anaerobic zones in the deeper layers of the aeration tank, and to ensure the necessary oxygen transfer. The mixed liquor then flows to a clarifier where the

microbial suspension settles and is separated from the treated effluent. The settled biomass, described as activated sludge because of the presence of active microorganisms, is reintroduced in the aeration tank to continue the biodegradation of the influent organic material. However, a portion of biomass is removed from the system daily or periodically, since the process produces excess biomass that would accumulate along with the non-biodegradable solids present in the influent wastewater (Ganczarczyk, 1983; Tchobanoglous *et al.*, 2003). The recirculation of a large portion of biomass is another important feature of the activated sludge process. This allows the mean cell residence time (sludge age) to be much greater than the hydraulic retention time. In practice, helps to maintain a large number of microorganisms in the system that efficiently oxidizes organic compounds in a relatively short time (Bitton, 2005).

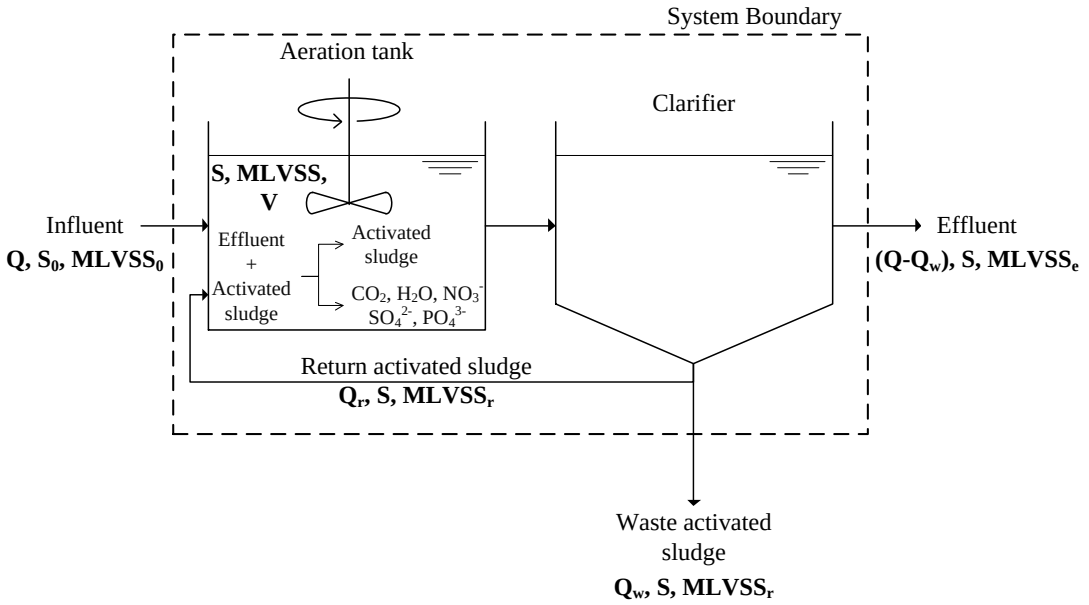


Figure 1.3: Schematic diagram of the activated sludge process, adapted from Tchobanoglous *et al.* (2003).

Legend of Figure 1.3:

- **$MLVSS_0$ –**Volatile suspended solids in influent (mg/L)
- **MLVSS –**Volatile suspended solids in aeration tank (mg/L)
- **$MLVSS_e$ –** Volatile suspended solids in effluent (mg/L)
- **$MLVSS_r$ –** Volatile suspended solids in the return line from clarifier (mg/L)
- **Q –** Influent flowrate (m^3/d)
- **Q_w –**Waste sludge flowrate (m^3/d)
- **Q_r –**Recirculation flowrate (m^3/d)
- **S_0 –**Influent substrate concentration, BOD or COD (mg/L)

- **S** –Effluent substrate concentration, BOD or COD (mg/L)
- **V** – Aeration tank volume (m^3)

1.4.1. Operational parameters of the process

Certain operational parameters must be monitored periodically for the correct operation of the activated sludge process (Ouano, 1983).

1.4.1.1. Dissolved Oxygen

Since the wastewater treatment by activated sludge is an aerobic process it is necessary to ensure that dissolved oxygen is always present in the aeration tank. The amount of oxygen required depends on the organic load of the influent and the degree of treatment desired (Cheremisinoff, 2006). Taking into account the equation 1.3is possible to conclude that if all the cells were completely oxidized, the COD produced would be equivalent to 1,42 times the concentration of cells as MLVSS:

$$\frac{5 \times \mathrm{M}(\mathrm{O_2})}{\mathrm{M}(\mathrm{C_5H_7NO_2})} = \frac{5 \times 32\ \mathrm{g/mol}}{113\mathrm{g/mol}} = 1{,}42\ \mathrm{g\ O_2/g\ cells} \tag{1.4}$$

Thus, the theoretical oxygen requirement can be calculated by the following equation, where R_0 is the amount of oxygen needed, and P_x the amount of biomass in excess as MLVSS as explained in section 1.4.1.9.

$$\mathrm{R_0(g/d)} = \mathrm{Q(m^3/d)} \times \left(\mathrm{S_0(mg/L)} - \mathrm{S(mg/L)}\right) - 1{,}42\mathrm{P_x(g)} \tag{1.5}$$

When the process includes a nitrification step is also necessary to consider the oxygen required for oxidation of ammonia to nitrate and nitrite. N_0 and N represent the total concentration of nitrogen in the influent and effluent, respectively (Lin and Lee, 2007).

$$\mathrm{R_0(g/d)} = \mathrm{Q(m^3/d)} \times \left(\mathrm{S_0(mg/L)} - \mathrm{S(mg/L)}\right) - 1{,}42\mathrm{P_x(g)} + 4{,}33\mathrm{Q(m^3/d)} \times (\mathrm{N_0} - \mathrm{N})\mathrm{(mg/L)} \tag{1.6}$$

1.4.1.2. pH

Most microorganisms grow at a pH range close to neutrality (Csuros and Csuros, 1999). Thus, pH of the mixed liquor should be maintained within the range of 6.5 to 9.0 (ideally 6.0 to 8.0). Gradual fluctuations within this range will normally not upset the process. But rapid fluctuations or fluctuations outside this range can reduce organism activity (Spellman, 2003). Consequently, this is an important parameter that must be controlled in order to avoid a decrease in cell viability (Cheremisinoff, 2006).

1.4.1.3. Mixed liquor suspended solids

As mentioned above, MLSS concentration can be used to express, roughly, the amount of biomass present in the aeration tank (Cheremisinoff, 2006). In theory, the higher the MLSS concentration the greater the efficiency of the process, however high values of MLSS are limited by the availability of oxygen in the aeration tank, and by the capacity of the sedimentation unit. Once the MLSS measurement does not discriminate between organic and inorganic matter, the organic fraction may be estimated by the determination of mixed liquor volatile suspended solids. However MLVSS also does not distinguish between the biochemically active and the inert material. Therefore, a more complex technique must be employed to measure the biochemical sludge activity (Gray, 2004), like the measurement of the protein content of the biomass (Yücesoy *et al.*, 2012). For daily operational control of the system, MLSS is adequate. The MLVSS and other measures of sludge activity are mainly used in research and development work (Gray, 2004).

1.4.1.4. Temperature

Microorganisms can be classified into three groups according to their response to temperature: thermophiles, mesophiles and psychrophiles.

Since ambient temperature usually ranges from 15 to 37°C, mesophilic microorganisms are the most important group in the wastewater treatment. This group grows when temperature ranges from 20 to 40 °C, and shows a peak of growth at 35-37 °C (Henze *et al.*, 1997).

The biological process can also occur in thermophilic range, 50-60 °C. The psychrophilic microorganisms group, with optimal growth below 20 °C, is only important in the treatment plants in cold climates (Ouano, 1983; Henze *et al.*, 1997).

1.4.1.5. Food to microorganism ratio – F/M

The F/M ratio is defined as the rate of BOD or COD applied per unit volume of mixed liquor (Tchobanoglous *et al.*, 2003). This means that the F/M ratio represents the amount of substrate available for the biomass in the aeration tank. F/M can be calculated from the following expression (Lin and Lee, 2007).

$$\mathrm{F/M} = \frac{S_0\ (\mathrm{mg/L}) \times \mathrm{Q(m^3/d)}}{\mathrm{MLVSS\ (mg/L)} \times \mathrm{V(m^3)}} \tag{1.7}$$

When the F/M ratio is high, the microorganisms are in the exponential growth phase. With excess food, the rate of metabolism is at a maximum with large BOD removals achieved. However, under these conditions the microorganisms do not form flocs and are generally dispersed, making the sedimentation process less efficient. In contrast, a low F/M ratio put the microorganisms into a food limited environment, which causes a rapid decrease in metabolic rate until the endogenous respiration phase starts, with cell lysis and resynthesis taking place (Gray, 2004). This parameter can be controlled by the adjustment of the waste activated sludge (WAS) flowrate, i.e., amount of sludge periodically removed from the system, since it is related to the concentration of suspended solids in the aeration tank (Bitton, 2005).

1.4.1.6. Organic load

Organic load is the amount of organic matter entering the treatment plant. As referred above is usually measured as BOD, COD or TOC. Considering the influent flowrate is possible to determine the organic load entering the plant, daily (Spellman, 2003).

$$\mathrm{Organic\ load\ (g/d)} = \mathrm{S_0(mg/L)} \times \mathrm{Q(m^3/d)} \tag{1.8}$$

1.4.1.7. Solids retention time

Solids retention time (SRT) can be defined as the average time that the sludge remains in the system, i.e., sludge age. By definition the SRT is the ratio between solids in the system and the mass of solids removed daily (Tchobanoglous *et al.*, 2003).

$$\mathrm{SRT(d)} = \frac{\mathrm{MLVSS\ (mg/L)} \times \mathrm{V(m^3)}}{\mathrm{Q_w(m^3/d)} \times \mathrm{MLVSS_r(mg/L)} + \mathrm{(Q - Q_w)(m^3/d)} \times \mathrm{MLVSS_e(mg/L)}} \quad (1.9)$$

An activated sludge system usually has a SRT in the range of 15 to 20 days, and like the F/M ratio this parameter is also controlled by the WAS flowrate (Tchobanoglous *et al.*, 2003; Lin and Lee, 2007). When the solids retention time is too long biological deterioration of the flocs may occur, providing very small flocs and imposing a higher turbidity to the effluent. This phenomenon is known as pinpoint floc (Tchobanoglous *et al.*, 2003).

1.4.1.8. Sludge volume index

The sludge volume index is a measure of the activated sludge settling ability, and is expressed as the volume in milliliters occupied by one gram of suspended solids that settles after 30 minutes.

$$\mathrm{SVI\ (mL/g)} = \frac{\mathrm{Volume\ of\ settled\ sludge\ (mL/L)} \times 1000}{\mathrm{MLVSS\ (mg/L)}} \quad (1.10)$$

A high SVI value indicates a poor settleability. Usually a SVI > 120 indicates ineffective sedimentation properties, and a SVI < 80 is characteristic of a good sludge (Gray, 2004).

1.4.1.9. Solids production

This parameter represents the amount of sludge produced per day, i.e., the amount of solids that must be removed daily to maintain a constant biomass concentration in the bioreactor. It can be calculated through a mass balance of the process, considering the amount of suspended solids in the influent wastewater, the amount of suspended solids wasted daily and the contribution of the substrate for the biomass growth. In equation 1.11, Y is a

kinetic constant that represents the amount of organic material that is used for biomass growth; the theoretical value is between 40-80% (Tchobanoglous *et al.*, 2003).

$$P_x(g/d) = S_0(mg/L) \times Q(m^3) \times Y + MLVSS_0(mg/L) \times Q(m^3/d) - MLVSS_e(mg/L) \times Q(m^3) \quad (1.11)$$

1.4.1.10. Nutrients

In addition to carbon compounds, the microorganisms involved in the activated sludge process need nitrogen, phosphorus and trace amounts of iron, calcium, sodium and other minerals. These nutrients can be supplied to the system in the form of salts and minerals, and not necessarily as proteins or organometallic compounds. Thus, commercial fertilizers such as urea, ammonium phosphate, ammonium sulfate and ammonium nitrate are commonly used additives to suppress the nitrogen requirements. Ammonium phosphate can also be used to eliminate the phosphorus lack. Elements that are needed in trace amounts occur naturally in effluents, and thus it is not necessary to add additives. Cells are composed, approximately, of 50% carbon, 10% nitrogen and 2% of phosphorus, so the carbon, nitrogen and phosphorus ratio (C:N:P) usually acceptable for biological treatment is 100:5:1 (Ouano, 1983; Bitton, 2005). However, in literature other relations are found: 100:20:1, 100:10:1 and 250:7:1 (Jefferson *et al.*, 2001)

1.4.2. Microfauna in activated sludge

Activated sludge systems form an ecosystem with a complex trophic web where the different populations establish relations of competition, predation and even cannibalism, Figure 1.4.

Biotic components are represented by decomposers or primary producers (bacteria and fungi) that utilize the dissolved organic matter in the wastewater, and by consumers or predators (protozoa and small metazoan) that feed on dispersed bacteria and other organisms (Madoni, 1994; Lee *et al.*, 2004; Ginoris *et al.*, 2007a).

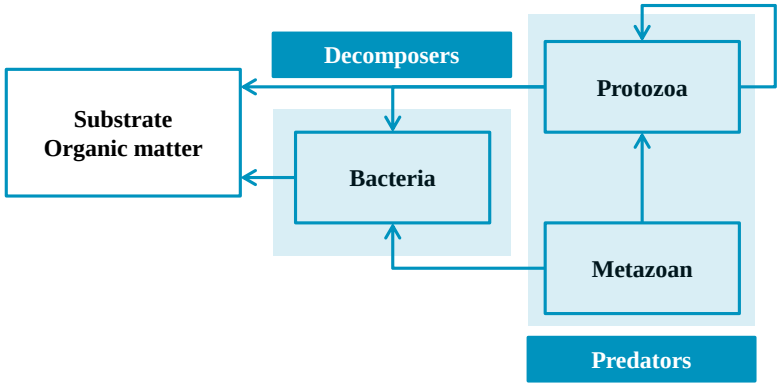


Figure 1.4: Trophic network present in the aeration tank, adapted from Gray (2004).

Yiannakopoulou (2010) also identified three trophic levels: the first consists of bacteria and accounts for practically 90% of the total community population, the second level includes bacterivorous protozoa and metazoan (rotifers and nematodes) and the third comprises carnivorous protozoa, Figure 1.5.

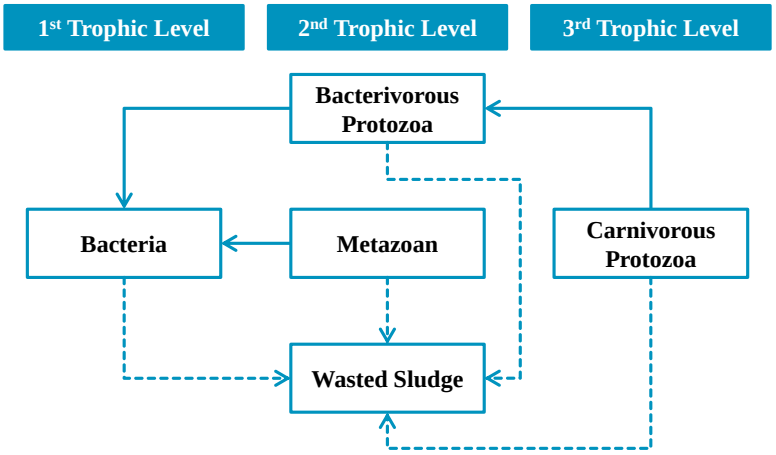


Figure 1.5: Ecological trophic network of a microbial community developed in an activated sludge process, adapted from Yiannakopoulou (2010).

Bacteria dominate over all groups in number and biomass, and clearly play a vital role in the conversion of the wide diversity of organic compounds and in the biological removal of nitrogen and phosphorus (Bento *et al.*, 2005; Moussa *et al.*, 2005; Madoni, 2011).

1.4.2.1. Bacteria

The most important microorganisms in a biological treatment process are bacteria, since they are responsible for the degradation of the organic matter and the structuring of the

flocs (Tchobanoglous *et al.*, 2003; Bitton, 2005). Bacteria are prokaryotic unicellular microorganisms that occur in three basic shapes: spheres (*coccus*), rods (*bacillus*), and spiral forms (*spirillum*) (Csuros and Csuros, 1999; Bitton, 2005).

Only a minor fraction (5-20%) of the organic matter in the sludge floc is made up of bacteria. The rest consists mainly of gelatinous extracellular material that is segregated by bloc-forming bacteria and surrounds the outer membrane: extracellular polymeric substances (EPS). Only a small number of bacteria are floc formers, namely *Achromobacter*, *Aerobacter*, *Citromonas*, *Flavobacterium*, *Pseudomonas*, and *Zoogloea* (Gerardi, 2006; Wilén *et al.*, 2008). EPS are defined as extracellular polymeric substances produced by some fungi and bacteria, with a diversified chemical composition. EPS compounds belong to different classes of macromolecules and not only to carbohydrates (Czaczyk and Myszka, 2007). These organic polymers form a cell protective layer for against the harmful external environment, for instance biocides and sudden changes of pH (Liu and Fang, 2003; Li *et al.*, 2011). On the other hand, such molecules can be the support of the flocs, since bioflocculation can be defined as the interaction, of the exopolymer of individual floc-forming cells, to form a three-dimensional matrix. The EPS holds the various microorganisms together in a matrix onto which organic fibers, organic and inorganic particles as well as various colloids can be adsorbed. So it is a micro-environment that allows a close proximity between microorganisms, and where organic material can be trapped and digested by extracellular enzymes. EPS segregation is an important process since they are essential for the floc structure and stability, as well as for determination of their physico-chemical and biological properties. The use electron microscopy confirmed that microbial cells inside the flocs are cross-linked by EPS, forming a polymeric network with pores and channels. Such a polymeric network has a vast surface area, capable of adsorbing organic and inorganic particles, facilitating their removal from the system and increasing the weight of the flocs, which improves the sedimentation process (Gray, 2004; Bitton, 2005; Shon *et al.*, 2006; Wilén *et al.*, 2008). Thus an effective bioflocculation is the key for an efficient solid-liquid separation of activated sludge from the treated water (Wilén *et al.*, 2008).

a) b)

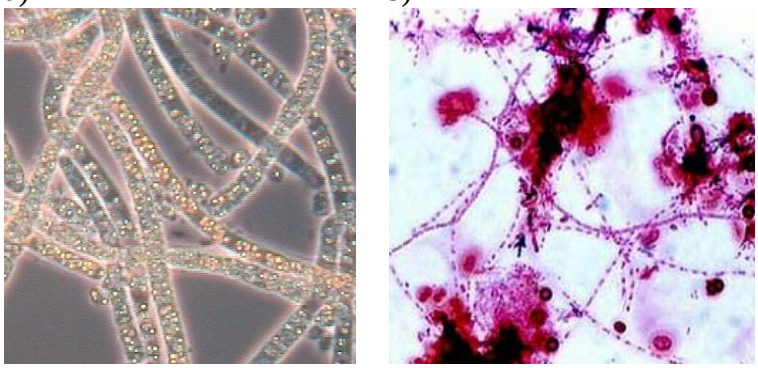


Figure 1.6: Filamentous bacteria that can be found in the mixed liquor a) *Beggiatoa* e b) *Nocardia sp*., Black (2002).

Filamentous bacteria are resistant to grazing and multiply quickly (Yiannakopoulou, 2010). When they occur in excess in the treatment process do not allow the sedimentation of the sludge in the secondary sedimentation tank, resulting in a phenomenon known as bulking. Bulking results in the contamination of the treated effluent with floating matter, namely biomass that should have settled (Black, 2002). Excessive filaments result in flocs with entrapped air, and, these less dense flocs rise to the surface of the tank causing foaming problems (Chua *et al.*, 2000). The presence and dominance of filamentous bacteria may be due to the presence of particular substrates that favor their growth (Yiannakopoulou, 2010).

1.4.2.2. Protozoa

In modern systems, with low loading and high sludge retention time the presence of protozoa such as ciliates, flagellates and amoebae is very common. Protozoa are unicellular eukaryotic organisms that have a crucial role in obtaining a good effluent quality with low suspended solids (Moussa *et al.*, 2005; Ginoris *et al.*, 2007b; Madoni, 2011).

Protozoans comprise a large diverse assortment of microscopic organisms that live as single cells or in colonies (Papadimitriou *et al.*, 2010). Lists of protozoa species found in activated sludge plants have been reported by several authors and a complete list of 228 species has been published by Curds (1975). Of the 228 protozoa species listed for the activated sludge plants about 160 belong to the phylum Ciliophora, and only a limited number of these has been observed frequently (Madoni, 2011). Most protozoa are strict

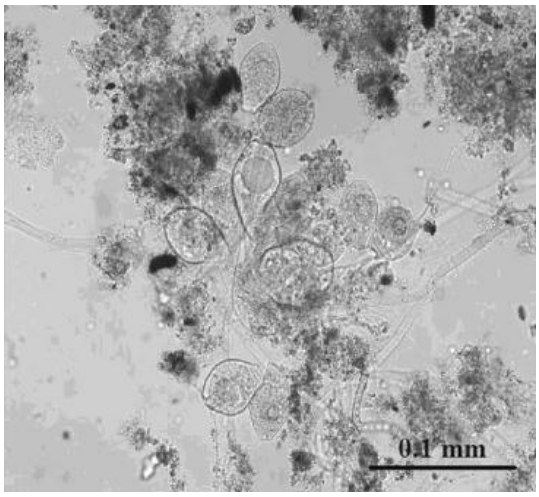


Figure 1.7: A common protozoa found in activated sludge, *Vorticella*, Ni *et al*. (2010).

aerobes, but some including amoebae and flagellates can survive in anaerobic conditions (Gerardi, 2006). It has been estimated that the protozoa biomass can reach values of 250 mg/L (dry weigh), constituting over 9% of the volatile solids (Madoni, 2011).

Protozoan predation, via phagocytosis, of both dispersed bacteria and peripheral cells of the flocs improves sludge sedimentation and effluent quality by reducing effluent turbidity, biological oxygen demand and suspended solids, and decreases the risk of potential bacterial pathogens as they contribute to the reduction of coliforms (Miller and Miller, 2000; Papadimitriou *et al.*, 2010; Ntougias *et al.*, 2011). Even though some protozoa can eat flocculated bacteria, most protozoa can only graze on suspended bacteria and particles. In this way they have a significant effect on the effluent quality, being generally assumed that the primary role of protozoa in wastewater treatment is the clarification of the effluent (Madoni, 2011).

Protozoa also release inorganic and organic products into their surroundings. These products are mainly recycled nutrients, such as nitrogen and phosphorus, and organic carbon, but might also include stimulatory compounds that contribute to the dissolved organic carbon pool and affect the growth of bacteria. Thus, among indirect effects of protozoa on bacteria can be pointed out the excretion of mineral nutrients that result in an accelerated usage of the carbon source, and the excretion of growth-stimulating compounds that can enhance bacterial activity. Nevertheless, these indirect effects cannot increase carbon mineralization under carbon limitation conditions. Therefore, in wastewater systems with low substrate concentration, this process is of little importance (Madoni, 2011).

As previously described, protozoa are usually aerobic and bacterivorous, but carnivorous protozoa, which feed on other protozoa, are also observed (Jenkins, 1993; Ginoris *et al.*, 2007b). The bacterivorous ciliates in activated sludge can be subdivide into three groups on the basis of their behavior: 1) free swimmers, swim in the liquor fraction and remain

suspended in the sedimentation tank; 2) crawlers, move on the surface of the sludge flocs; and 3) sessile or attached that are firmly attached by a stalk to the flocs and precipitate with them during sedimentation. All bacterivorous ciliates depend on ciliary currents to force suspended bacteria to enter the oral cavity. So, while free-swimming and attached ciliates are in competition for the bacteria dispersed in the liquid phase, crawling forms feed upon particles that only lightly adhere to the sludge and that are dislodged by the feeding currents easily. Therefore these biological systems consist of populations in constant competition with each other for foodstuff (Madoni, 1994).

1.4.2.3. Metazoan

Metazoans are multicellular organisms that may be microscopic or macroscopic in size (Gerardi, 2006). They have a slow growth rate and most of them are predators that feed upon bacteria and protozoa (Bento *et al.*, 2005). The most commonly observed metazoan in the activated sludge process include free-living nematodes and rotifers (Gerardi, 2006).

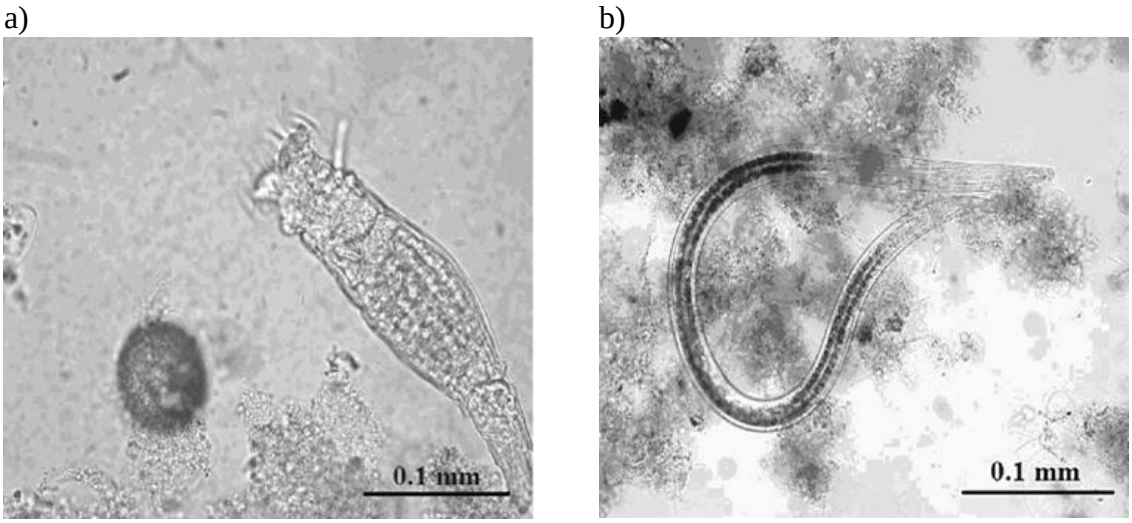


Figure 1.8: Metazoans found in activated sludge, a) rotifer and b) nematode, Ni *et al.* (2010).

Metazoans usually are present in highly variable numbers. Unless the SRT of the activated sludge process is > 20 days, most metazoan are not provided with sufficient time to reproduce and usually are present in relatively small numbers (< 200 individuals per milliliter) (Gerardi, 2006). Rotifers were shown to have two distinct effects on suspended particles: consumption of dispersed bacteria and improvement of settling and aggregation

of the flocs. Nematode abundance in activated sludge systems generally represents less than 1% of the microfauna and their presence is limited by the short residence time of the biomass in the system. Tardigrades, gastrotrichs and oligochaetes were rarely recorded in daily microscopic examinations of the activated sludge (Zhou *et al.*, 2008).

1.4.3. Protozoa as indicators of activated sludge performance

Studies on the dynamics and succession of protozoa in activated sludge have suggested that flagellates predominate in the early stages only because of their low energy requirements. As the flagellates decrease, they are replaced by free-swimming ciliates and then by crawling and attached ciliates. Three stages in the time span can be identified from the beginning to the stabilization of the system. The plant start-up is characterized by the presence of species typical of raw sewage: free swimming bacterivorous ciliates and small heterotrophic flagellates. With the stabilization of the sludge they are replaced by other functional groups. The second phase is characterized by the proliferation of ciliates typical of the activated sludge habitat: crawling and attached ciliates. In this stage the species structure changes with the progressive formation of activated sludge. The third phase, stabilization, is characterized by a protozoa community whose structure reflects the stable condition of the aeration tank environment, with a balance between the organic loading and the sludge that is produced, removed and recycled (Madoni, 2011). Food availability is decisive on the species succession during these stages. Small flagellates and small free-swimming ciliates require a higher amount of bacteria due to their inefficient food capture ability. Thus during the star-up, when there is a low hydraulic residence time (HRT) and a high F/M ratio, these protozoa dominate. On the opposite, sessile ciliates and metazoan increase when HRT is higher and F/M ratio is lower due to their ability of floc adhesion or more efficient food capture mechanism (Ginoris *et al.*, 2007b). In the third stage species characteristic of the colonization phases are not observed, unless any dysfunction causes a regression in the environmental conditions. Protozoa are sensitive to environmental change caused by variations in influent quality or operating conditions, for instance sludge age, organic loading rate, F/M ratio and aeration intensity (Dubber and Gray, 2009). In particular, ciliates are proposed to be representative indicators of the operation of the

wastewater treatment plants because of their rapid response to changes in wastewater composition and plant operating conditions (Ntougias *et al.*, 2011).

The main factors influencing the biotic community of activated sludge are organic sludge loading and sludge retention time. These parameters determine both the time required for the growth of organisms and the amount of food available to them. Low organic loading rate is associated with long sludge retention time, stable aerobic conditions and poor feeding substrate. These factors result in a smaller number of dispersed bacteria, a high abundance of species with a small diversity, predominance of a group of protozoa consisting of testate amoebae, crawling and attached ciliates and the presence of small metazoan. Because of the lower abundance of dispersed bacteria, these groups are able to obtain enough food to prosper through high efficiency wastewater clarification (attached ciliates with a wide peristome and rotifers), feeding within sludge flocs (rotifers, nematodes and amoebae) and feeding on the lightly adherent bacteria on the flocs surface (crawling ciliates). Long sludge retention time also provides adequate time for the growth of the organisms. On the other hand, the increase of organic load improves feeding condition but is associated with the reduction of sludge retention time and often with obstacles related to aerobic conditions. It is also observed a faster growth of dispersed bacteria, increases in the overall size of the microfauna, the decline of species diversity and the domination of taxa characterized by low feeding efficiency: small flagellates, free swimming ciliates and/or attached ciliates with a narrow peristome. These organisms require a high concentration of dispersed bacteria and are able to tolerate oxygen deficiency. A high rate of proliferation (flagellates and swimming ciliates) and a sedentary way of living (attached ciliates) protect them against leaching from the system, which would be expected due to the reduced sludge retention time (Drzewicki and Kulikowska, 2011).

Since the species and functional groups of protozoa depend on the environmental conditions and most of the protozoa found in activated sludge systems have ubiquitous distribution in all continents, the structure of the protozoa community can be considered a valid indicator of the purification plant performance. Any major variations in the plant performance are thus indicated by the dominant group of protozoa. So, the routine analysis of these eukaryotic microorganisms community is becoming increasingly common to

determine activated sludge plant performance (Madoni, 1994; Zhou *et al.*, 2008; Madoni, 2011).

Curds and Cockburn (1970) were probably the first to use protozoa as indicators. They carried out a comprehensive study of the protozoa population in activated sludge plants in the UK, and found that a rich protozoa community was related to effluents of high quality while a community with only few species in small number was associated with low quality effluent. Since then a series of plant studies has been conducted to further explore the relationship between the protozoa community structure, effluent quality and plant operation conditions (Esteban *et al.*, 1991; Madoni, 1994; Dubber and Gray, 2011). Several researchers developed methods for biological monitoring of the process. Performance indexes have also been developed based on ciliate diversity and abundance as indicators of activated sludge performance (Drzewicki and Kulikowska, 2011; Dubber and Gray, 2011). In 1994, Madoni summarized the knowledge on the ecology of activated sludge grouping microfauna organisms into positive and negative keygroups. The positive keygroups consist of testate amoebae and crawling and attached ciliates; negative keygroups comprises small flagellates, swimming bacterivorous ciliates and the peritrichs ciliates *Vorticella microstoma* and *Opercularia* sp.. In addition, this study revealed that in order to take place an efficient treatment a high protozoa density (> 10^3 organisms/mL) with a well-diversified community, where no overwhelming predomination species or group of species are observed, should be present in the aeration tank. When such is not the case, the identification of the dominant group(s) allows diagnosis of the particular state of functionality of the plant. Madoni also developed the sludge biotic index (SBI) to display the results of the microscopic analysis of the activated sludge into numerical values that translate the biological quality of the sludge. The method proposed is based on the assumption that the dominance of key groups, and the abundance and number of microfauna species in activated sludge vary depending on the physicochemical parameters and on the efficiency of the treatment process (Madoni, 1994). Although the SBI method may not be used for all systems because has limitations. For instance, Drzewicki and Kulikowska (2011) conclude that the method does not apply in WWTP working with shock loadings of organic substances and nitrogen. Pérez-Uz *et al.* (2010) also stated that protist communities are different in N-removal systems and, pointed out that bioindicator indexes must be adapted to the type of treatment process. Furthermore some correlations

between protozoa populations and specific physicochemical characteristics, reported in several studies, are often influenced by the plant type examined and by the operating conditions used, so examination of protozoa microfauna in other types of WWTP may reveal new associations (Ntougias *et al.*, 2011). In recent years the improvement of the biotechnology of the activated sludge processes, with important innovations (i.e., tertiary treatment or advanced process such as biological nutrient removal), also affects the use of protozoa as indicators of the performance of the system (Madoni, 2011). So, these findings support that results from previous studies cannot be directly extrapolated to new wastewater treatment plant, and therefore each case must be studied individually, since species and abundance of protozoa vary specifically with the type of process used (Yiannakopoulou, 2010; Dubber and Gray, 2011).

Table 1.1 resumes the indicator value of each group of protozoa as described in literature, adapted from Jenkins (1993), Madoni (1994), Bento *et al*. (2005), Serrano *et al*. (2008) and Madoni (2011).

Table 1.1: Relation between the dominant protozoa group and the process characteristics.

	Dominant group	Performance	Indicator value
Protozoa	**Flagellates**		
	Small	Low	➢ Poorly aerated sludge ➢ Overloading ➢ Fermenting substances involved ➢ Low SRT ➢ Very high F/M (> 0,9) ➢ High bacteria concentration ➢ Presents during start-up or recovery from upset conditions ➢ May occur bulking ➢ Sludge with low sedimentation characteristics (dispersed biomass)
	Large		➢ Rarely observed ➢ Very diluted organic matter
	Amoebae		
	(Overall)		➢ Presents during start-up or recovery from upset conditions ➢ High numbers may indicate low DO
	Small naked amoebae	Mediocre	➢ Transient phenomena (discontinuous load, recent sludge extraction) ➢ High load
	Large naked amoebae	Low	➢ Transient phenomena (discontinuous load, recent sludge extraction) ➢ Low effluent quality

Protozoa	**Testate amoebae**	High	➢ Low and/or diluted loading ➢ High SRT ➢ High DO ➢ Associated to biological removal of nitrogen ➢ Low ammoniacal-N concentrations ➢ Low SVI values ➢ Excellent effluent quality
	Ciliates		
	(Overall)	Good	➢ Moderate to low organic load ➢ High F/M ➢ High bacteria concentration ➢ Low hydraulic retention time
	Free Swimming		
	(Overall)		➢ Absence of other ciliates: aeration rate too high (not the dissolved oxygen concentration) ➢ Presence of other ciliates: mature or stabilized sludge
	Small	Mediocre	➢ Low SRT ➢ High F/M (0,6-0,9) ➢ Poorly aerated sludge
	Large	Mediocre	➢ Overloading ➢ Poorly aerated sludge
	Crawling	Moderate to good	➢ Moderate to low organic load ➢ Decrease with increasing organic load (not observed in sludge above 0,6 kgBOD/kgMLSS.d) ➢ High hydraulic retention time ➢ Low F/M (< 0,6) ➢ High numbers may indicate good oxygen conditions ➢ Inversely related to SVI: high numbers associated to SVI values smaller than 200
	Sessile	In decline	➢ Low organic load ➢ Transient phenomena (discontinuous load, recent sludge extraction) ➢ Washout conditions – rapid increase of F/M ➢ Mature or stabilized sludge ➢ High numbers may indicate good oxygen conditions ➢ 0,3 < F/M < 0,6
	***Vorticella microstoma*(1)**	Low	➢ Present during the first phase of colonization ➢ Poor oxygen conditions ➢ High F/M ➢ Low values of MLSS ➢ High SVI
	***Operculária spp.* (1)**	Low	➢ High final effluent BOD concentration ➢ High loading ➢ Presence of toxic substances ➢ High ammoniacal-N concentration ➢ Lack of oxygen
	Suctorids(2)	Good	➢ High quality effluent ➢ Lightly loaded
	Sessile and crawling	Good	
Rotifers			➢ Only presents when DO is at least several mg/L ➢ Increasing stabilization of organic wastes

(1) When this group is present in high numbers it must be considered as separate keygroup
(2) Carnivorous ciliates

1.4.4. Sampling and counting of microfauna populations

Protozoa abundance in the biological treatment is normally over 10^4 cells/mL, and ranges from 10^5-10^6 cells/mL depending on the type of process. In consequence, sample volume does not need to be large and 25-100 mL would be enough to carry out the study of the protozoa populations. To preserve the populations in a similar state to the natural conditions, containers should be large enough to keep an air chamber on the top of the sample and samples should be kept cool and away from direct sunlight until processing in the laboratory (Serrano *et al.*, 2008). Samples must be kept in living conditions and should be processed as soon as possible, according to literature within 3-5 h of collection, or within 8 h as proposed by Dubber and Gray (2009) who found that significant changes in community structure occur beyond this period (Madoni, 1994; Dubber and Gray, 2009; Pérez-Uz *et al.*, 2010). Oxygen depletions can be avoid, once in the lab, using mechanical agitation with a magnetic stirrer, a shaker or an aquarium air pump, this will avoid the death of those most oxygen sensitive protozoa (Serrano *et al.*, 2008).

Activated sludge population composition and diversity have been investigated by several new molecular techniques including fluorescent in situ hybridization (FISH), restriction fragment length polymorphism (RFLP), microautoradiography and polymerase chain reaction (PCR) Denaturing gradient gel electrophoresis (DGGE), that permit direct visualization and rapid comparison of the structure of bacterial communities, has also been used (Burgess *et al.*, 2002; Tchobanoglous *et al.*, 2003; Lopez *et al.*, 2005; Wilén *et al.*, 2008; Abdi and Williams, 2010; Li *et al.*, 2011). These molecular techniques can provide an accurate and detailed identification of protozoan diversity in sewage wastewater treatment (Ntougias *et al.*, 2011). However, as the information regarding population composition and diversity is a good indicator of the process performance, and can be used to change operating parameters for the maintenance of process performance, it has to be quick, cheap and easy to acquire. So far, such molecular techniques are rarely available on wastewater treatment plant sites (Burgess *et al.*, 2002). Therefore, the methodology most used is the direct counting. Enumeration of living cells is done by direct microscopy on aliquots of unfixed samples. Since ciliate numbers are usually not very large in the biological process, counting chambers are generally not necessary, and small aliquots taken with a calibrated pipette are mounted and counted on a slide under a coverslip

(Serrano *et al.*, 2008). Estimation of small flagellate population, however, is normally done in Fuchs Rosenthal chambers due to their reduced size and high density, often reaching 10^7-10^8 individual/L (Madoni, 1994; Dubber and Gray, 2009). When the number of ciliates is quite large and counting time is foreseen to take longer, a chamber can be prepared sealing the borders of the coverslip with a fine line of vaseline before mounting it on the calibrated sub-sample volume. This would avoid drying out of the sample, result of the heating caused by longer observation time under the microscope. The counting area is defined by the coverslip and counting should be done with 100x or 200x microscope magnifications. Differences in the sub-sample volume to be counted can be found in literature (Table 1.2), so decisions on volume and replicates should be a balance between degree of precision and time spending (Serrano *et al.*, 2008). Nevertheless, a recent study conclude that the analysis of six replicate of 25 µL sub-samples provide good species recovery and estimation of abundance of protozoa community (Dubber and Gray, 2009). Live observation is important because some characteristics are only observed in this state, although staining is necessary to identify ciliates to species level. The classic techniques involve the use of different silver salts that generally precipitate specifically on microtubular structures. Live cell observation is important in the identification of ciliates to genus level, since characteristics such as shape, movement, color or certain structures (e.g., nuclei, oral area and contractile vacuoles) are only visible when the organism is alive (Serrano *et al.*, 2008).

Table 1.2: Sub-sample counting methodology used by previous authors in wastewater studies on activated sludge.

Volume (µL)	Replicates	Reference
50	4-5	Jenkins (1993)
25	1-2	Madoni (1994)
5	2	Lee *et al.* (2004)
25	3	Zhou *et al.* (2008)
25	6	Dubber and Gray (2009)
25	2	Pérez-Uz *et al.* (2010)
25	6	Ntougias *et al.* (2011)
25	2	Tocchi *et al.* (2012)

1.5. Case study: IWWTP PrioBiocombustíveis

1.5.1. Biodiesel production

PrioBiocombustíveis is a subholding of Martifer group that operates in the biofuels sector. The biodiesel plant located in Gafanha da Narazé uses as raw material vegetable oils, mainly soybean, rapeseed and palm oil. The biodiesel production (Figure 1.9) initiates with the neutralization/degumming of the crude oil that comprises two stages: treatment and neutralization followed by washing. The aim of this process is the neutralization of free fatty acids, and soapstock (sodium soaps of the free fatty acids) is obtained as a by-product (Martifer and Prio, 2008a). The next stage is the transesterification that implies the chemical reaction between triglycerides and methanol to produce fatty acid methylesters (FAME) and glycerin, in the presence of the catalyst, sodium methoxide. The products are then separated and the methylester phase is submitted to a washing and drying process, to reduce the water and methanol content to the European standard values for biodiesel. Glycerin and wash water are forwarded to a distillation column in order to recuperate the methanol excess. After dehydration, glycerin is also commercialized (Martifer and Prio, 2008b).

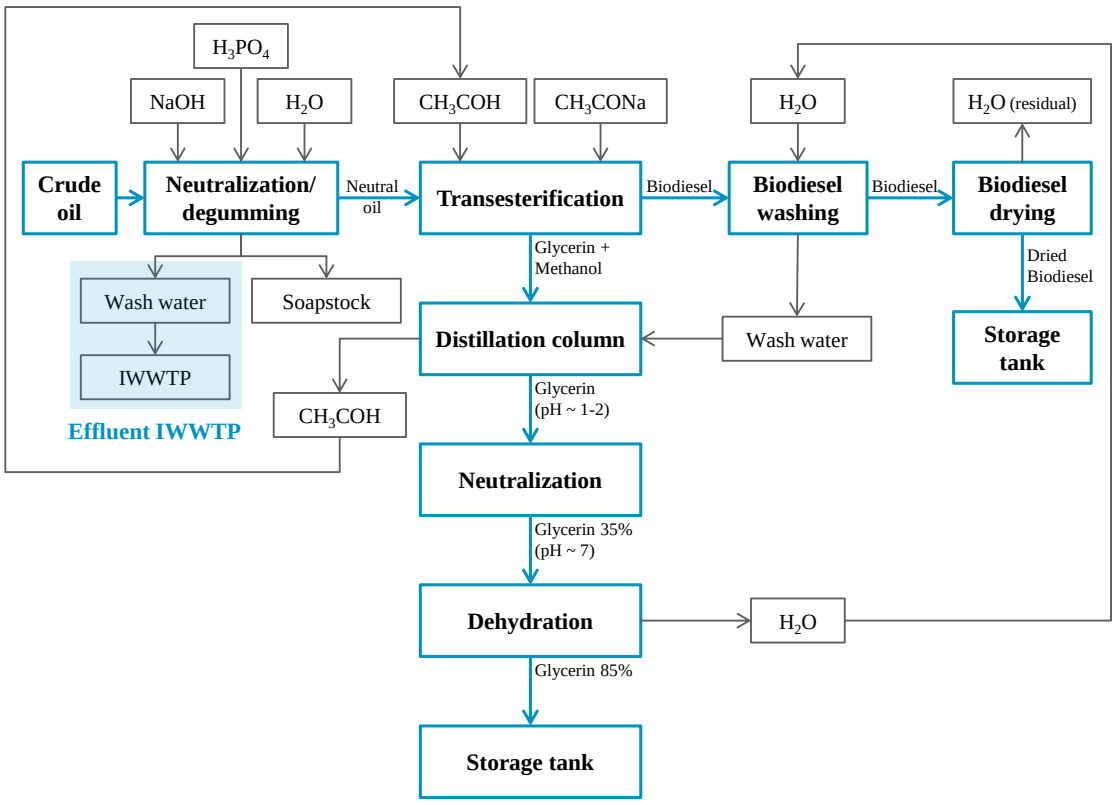


Figure 1.9: Schematic representation of the biodiesel production at PrioBiocombustíveis.

1.5.2. Operation of the industrial wastewater treatment plant

The schematic representation of the industrial wastewater treatment plant (IWWTP) operation is present in Figure 1.10. The process is divided in different stages; in general four steps are identified.

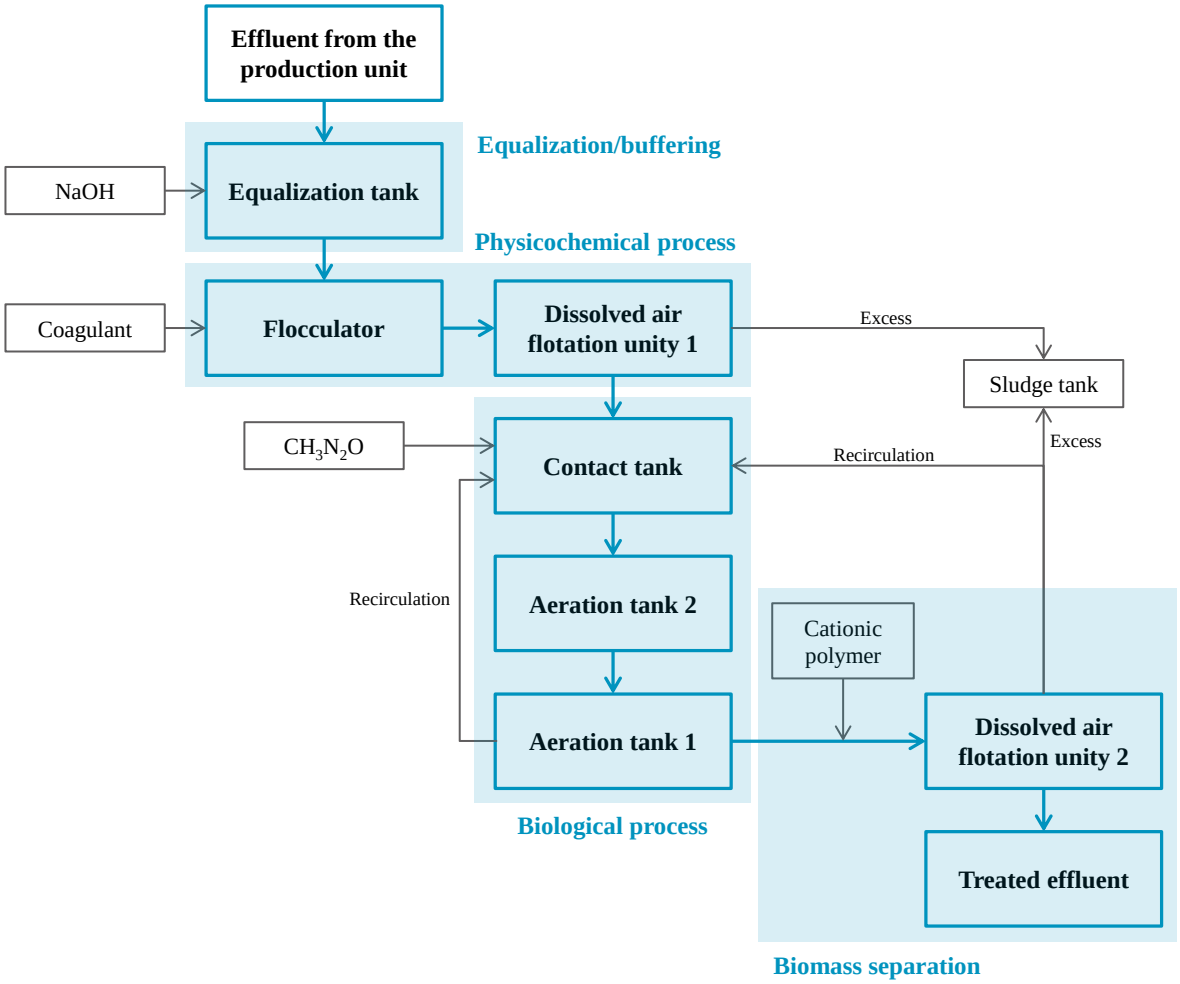


Figure 1.10: Schematic representation of the operation of the IWWTP, course of the wastewater in bold.

1.5.2.1. Buffering/equalization

The effluent produced by the factory, mainly from the neutralization/degumming of the neutral oil, is collected in an equalization tank. The aim of the equalization/buffer tank is the reduction of costs by stabilization of peaks in pollution, pH and flow, creating an effluent with quite constant flow and composition for the wastewater treatment system. This tank is equipped with a mixer and a pH measurement. The first must be always

submersed and can operate continuously or in a pulse/pause mode, to limit the accumulation of sediment on the bottom and create a homogenous mixture. The pH controller sends a signal to the NaOH dosing pump to automatic adjustment of pH, which must be kept around 5 to an efficient coagulation step (Redox, 2010).

1.5.2.2. Physicochemical treatment

The objective of the physicochemical treatment is the removal of most of the suspended and emulsified solids and insoluble COD, mainly residual oil. Wastewater is pumped from the equalization tank to a DAF unit (dissolved air flotation) with an adjustable flow. In the effluent compartment of the DAF unit the amount of TSS of the effluent is measured. When the amount of TSS is too high indicates that the DAF unit is not working correctly and too much polluted water can be sent to the biological treatment system. Thus, when the effluent turbidity is higher than aloud, the wastewater is sent back to the buffer tank, to be reprocessed. The sludge produced in this treatment stage, which can be denominated as chemical sludge, is directed to a sludge tank and later to a licensed operator (Redox, 2010).

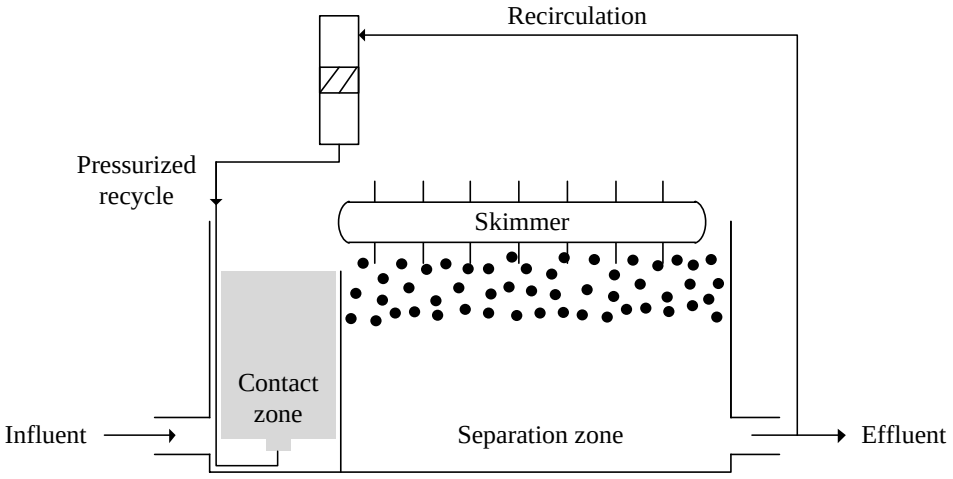


Figure 1.11: Schematic representation of a DAF unit where it is observed the contact zone and separation zone, adapted from Edzwald *et al.*(2010).

DAF is a clarification process that can be used to remove low density particles in suspension. DAF functioning is based on the natural fluctuation tendency of some particles. This means that after a chemical pretreatment where flocs are formed, micro air bubbles attach to flocs causing them to float to the surface, and making clarification of the

water below the flotation zone (Al-Shamrani *et al.*, 2002a; Crossley and Valade, 2006; Han *et al.*, 2007; Edzwald, 2010; Edzwald and W.W.A.A., 2011; Rattanapan *et al.*, 2011).

The chemical pretreatment in PRIO is a coagulation process. This involves the addition of chemicals that cause aggregation of particles not settleable to form large masses of solid material, flocs, which are easily removed (Spellman, 2003; Cheremisinoff, 2006; Adlan *et al.*, 2011). The addition of a coagulating agent is important since most of the small suspended and colloidal material in wastewater have a negative electrostatic charge. Thus in stable conditions the Brownian motion (random movement) keeps the particles disperse almost indefinitely as a result of the natural repulsion of similar charges, and due to small particle sizes (0,01 to 1,0 μm). The attractive forces of Van der Waals become considerably small when compared with the electric charge repulsion. This means that the negative electrostatic charge has to be neutralized by the action of the coagulant to allow agglomeration of the particles (Tchobanoglous *et al.*, 2003; Cheremisinoff, 2006; Lin and Lee, 2007). There are several coagulants on the market that can be grouped into three main categories: chemical, semi-organic and organic. In this IWWTP is used a semi-organic coagulant, Ambifloc BIO 865 F, a polymer with high cationic density and molecular weight, with chemical formula $(C_8H_{16}ClN)_n$ (SNF Ambientagua, 2008).

The coagulating agent, which is prepared in a separate unit from a powder or concentrated liquid, is added to the flow of water passing through the flocculator in the influent side, point B from Figure 1.12. There are two sampling sites in the flocculator: point A1 for sampling of the effluent prior to the addition of chemical additives and point A2 for sampling wastewater after the addition of chemicals, to check whether the coagulant is in the optimal dose (Redox, 2010).

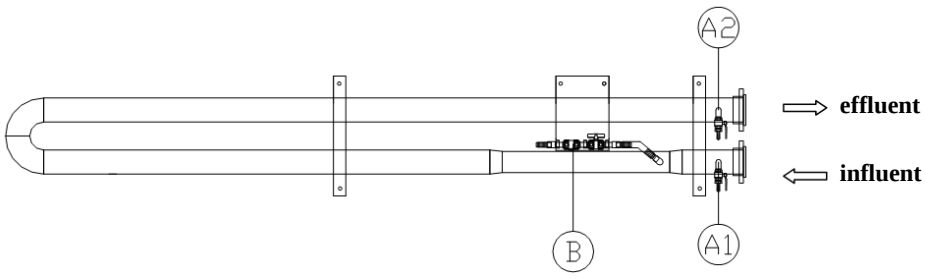


Figure 1.12: Representation of the flocculator, adapted from the manual of operation of the FBR, Redox (2010).

After the coagulation step the effluent enters the DAF 1 unit, where two zones are observed, Figure 1.11. The former corresponds to the contact zone that is delimited from the separation zone (Edzwald, 2010). In the contact zone, the water is exposed to micro-bubbles. The micro-bubbles are produced by the saturation of air in a pressurizing device, of a portion of the clarified or filtered water (recirculation of DAF) and sudden pressure reduction, followed by injection in the contact zone (Crossley and Valade, 2006). The bubbles attach to the flocs increasing its buoyancy, allowing them to rise to the surface. This can occur by trapping or chemical adsorption of air bubbles in the insoluble solids or inside the structure of the flocs (Al-Shamrani *et al.*, 2002b; Hanafy and Nabih, 2007). The water carrying the suspension of floc–bubble aggregates, free bubbles, and unattached floc particles flows to the second part of the tank, the separation zone. Here free bubbles and floc–bubble-aggregates rise to the surface of the tank forming a floating sludge layer. Periodically, the sludge is removed by a skimming or hydraulic flooding. Clarified or treated water exits the separation zone by a collection manifolds or, more simply, by an opening at the end of the tank (Crossley and Valade, 2006; Edzwald, 2010). The purpose of the recirculation system is the minimization of the energy used, maximization of the air content in the recirculation flow and the creation of micro bubbles with suitable size and distribution. The diameter of the bubbles are typically between 10 and 120 μm (Al-Shamrani *et al.*, 2002b; Rubio *et al.*, 2002; Crossley and Valade, 2006; Dafnopatidou and Lazaridis, 2008). The recirculation flow is saturated at pressures of 1,70 to 4,80 atm by a pressurizing pump. This flow is maintained under pressure for about 0,5 to 3,0 min to ensure dissolution of the air in water, then the pressure is released and returns to 1,0 atm. Since, according to Henry's law, the solubility of air in an aqueous solution increases with increasing pressure, likewise the pressure reduction causes a decrease in solubility of the air in solution, and consequently the formation of air bubbles of microscopic dimension (Rubio *et al.*, 2002; Hanafy and Nabih, 2007). Saturated water is normally introduced in the main flow through a series of manifolds located in the contact zone. The injection of the recycle flow must be diffused to minimize floc damage caused by excessive shear forces in the contact zone. Thus, the pressure reduction device is a key component for achieving an efficient performance of the DAF process (Crossley and Valade, 2006).

1.5.2.3. Biological treatment

The biological treatment is an activated sludge system that removes the dissolved pollution from the DAF 1 effluent.

1.5.2.3.1. Aeration tanks

The water flows out of the DAF 1 to the contact tank (Figure 1.11), where it is mixed with the biomass and then pumped to the aeration tank 2. The contact tank has a blower that should work continuously to provide air for the mixture of biomass and effluent and the pH should be maintained neutral. When needed, urea (CH_4N_2O) is added as a nutrient source of nitrogen for biomass growth. In the aeration tanks biomass metabolizes the biodegradable pollution that is the organic carbon. The aeration tank 2 has four mixer aerator pumps for mixing and aerating the water. Two of these mixer aerators get water from aeration tank 2 and the other two get water from aeration tank 1. This creates a difference in levels between the two aeration tanks and an open connection between these tanks cause a gravity flow to aeration tank 1. In aeration tank 1 a mixer is installed in the bottom part that must be always submerged and work continuously. On the top of the tank a dissolved air measurement device is responsible for the star and stop of the aerating system (Redox, 2010).

1.5.2.3.2. Biomass separation

Another DAF unit is used to separate the effluent water from the biomass. The water mixed with biomass flows from the aeration tank 1 to DAF 2 and is mixed with another cationic polymer to create stable flocs. In the flotation unit small air bubbles give buoyancy to the flocs. These flocs from a sludge layer on the top of the unit from where it will be skimmed of the water into the sludge compartment. After this separation, cleaned water leaves the flotation unit and the biomass is partially reintroduced in the system, in the contact tank, while the remaining is removed out of the system.

Taking into account Figure 1.3 and the information derived from the literature, in Figure 1.13 is present a schematic representation of the activated sludge system in study, that enables a mass balance of the process. The two aeration tanks are considered as one since there are no significant differences between them. And the contact tank volume is neglected since the useful volume of the aeration tanks is not 100% as considered in calculations.

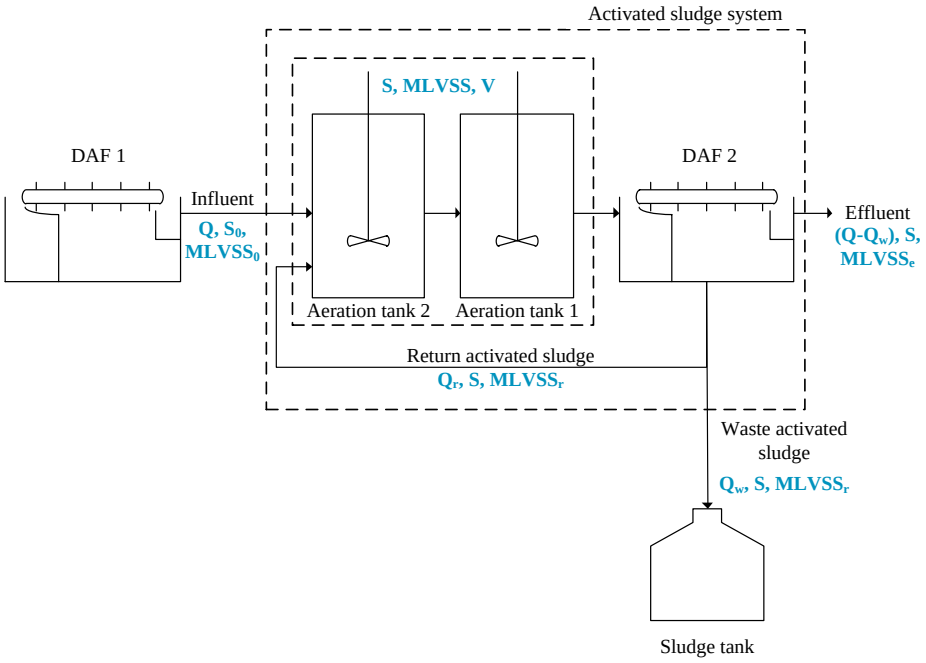


Figure 1.13: Schematic representation of the activated sludge system in study with identification of the main flows and control variables.

1.6. Objectives of the study

1.6.1. Main objective

Considering the information provided, the work developed at PrioBiocombustíveis during the internship had as main objective the optimization of the operation of the IWWTP.

1.6.2. Specific objectives

1. Understanding the processes and knowing the performance of each unitary process;
2. Evaluation and optimization of the daily control analysis;

3. Physiochemical treatment:
   3.1. Evaluation of the total solids and COD removal efficiency;
   3.2. Evaluation of the operation conditions, i.e., pH and coagulant in use;
4. Biological treatment:
   4.1. Evaluation of the COD removal efficiency;
   4.2. Study of control parameters to identify those with high variations over time in order to be optimized;
   4.3. Establishment of a microbiological monitoring protocol;
   4.4. Evaluation of the influence of physicochemical parameters changes in microfauna community;
   4.5. Establishment of microbial positive and negative control groups.

2. EXPERIMENTAL SECTION

2.1. Sampling procedures

Samples were collected daily during the study period by operators of PrioBiocombustíveis in 1,0 L plastic vessels. Activated sludge samples for microscopic examination were collected from the biological reactors immediately before microscopic observation, and containers with these mixed liquor samples were allowed half empty to avoid oxygen limitation. Samples for analysis of physicochemical parameters were also taken at five different treatment stages: before and after physicochemical treatment (S1 and S2), aeration tank 1 and 2 (S3 and S4), and at the end of the entire treatment process (S5), Figure 2.1.

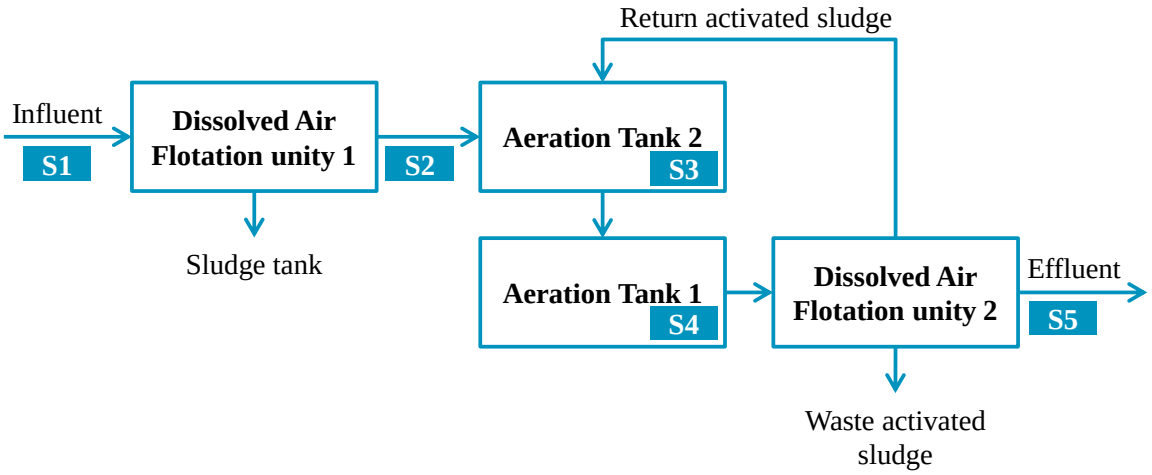


Figure 2.1: Sampling sites.

2.2. Physicochemical analysis

Physicochemical analyses of the samples collected at the five treatment stages were performed daily, with exception for the phosphorus analysis that were performed weekly.

2.2.1. Total solids

Total solids were determined according to the Standard Methods, section 2540 B. Initially clean Petri dishes were prepared according to the following procedure: Petri dishes were heated to 103-105 ºC for 1 hour in a drying oven (MMM Medcenter, Munich, Germany), and cooled in a desiccator. The weight of a prepared Petri dish was determined in an

analytical balance (Sartorius AG, Göttingen, Germany), and 5000 µL of well-mixed sample was pipetted to the dish. The sample was then evaporated to dryness in the oven for 9 hours at 103-105 ºC and cooled in a desiccator. Finally the dried sample was weighted and total solids calculated according to equation 2.1, were A represents the weight of dried residue and Petri dish (g) and B the weight of Petri dish (g). All samples were analysed in duplicate.

$$\text{Total Solids (g/L)} = \frac{(\text{A} - \text{B})}{5{,}000 \times 10^{-3}\text{L}} \tag{2.1}$$

2.2.2. Volatile suspended solids

Volatile suspended solids of mixed liquor samples (S3 and S4) were also determined according to the Standard Methods, sections 2540 D and E. For this analysis porcelain dishes containing glass fiber filter disks of porosity of 1,2 µm (Whatman, Kent, United Kingdom) were ignited at 550 ºC for 1 hour in a muffle (Nabertherm, Lilienthal, Germany), and cooled in a desiccator. After assembly of the filtration apparatus and filter, a homogeneous sample volume of 5000 µL was pipetted onto the glass fiber filter with applied vacuum. The filter was removed from the filtration apparatus, transferred to the porcelain dish and dried for 2 hours at 103-105 ºC in the oven. After cooling in a desiccator the weight was determined. The residue produced was then ignited at 550 ºC for 2 hours in the muffle and transferred to a desiccator for cooling. Finally the dish and filter was weighted and volatile suspended solids were determined according to equation 2.2, where C represents the weight of residue, dish and filter before ignition (g) and D the weight of residue, dish and filter after ignition (g). All samples were analysed in duplicate.

$$\text{Volatile Suspended Solids (g/L)} = \frac{(\text{C} - \text{D})}{5{,}000 \times 10^{-3}\text{L}} \tag{2.2}$$

2.2.3. Chemical oxygen demand

Chemical oxygen demand was determined using a commercial kit (Hanna Instruments, Rhode Island, USA) with adaption of EPA method 410.4. Samples from the aeration tanks and effluent at the end of the treatment (S3, S4 and S5) were analysed at medium range

(150-1500 ± 1 mg/L) and the two remaining samples (S1 and S2) were analysed at high range (1500-15000 ± 10 mg/L).

For this analysis a reactor, HI 839800 COD Reactor 2008 Series (Hanna Instruments, Rhode Island, USA), was pre-heated to 150 ºC and the reaction time was programed to 2 hours. Samples from the two aeration tanks (S3 and S4) were first centrifuged for 5 minutes at 4000 rpm in a centrifuge angle rotor, Cencom II (JP Selecta, Barcelona, Spain). Effluent sample collect before the physicochemical treatment (S1) was diluted by an appropriate dilution factor, according to the result from the previous day, usually a 1:10 factor was applied. An appropriate volume of sample was added to a reaction vial with a micropipette: 2000 μL for mid-range and 200,0 μL for high range. The blank vial, prepared with distilled water, is stable for several months at room temperature thus it was only prepared when the lot of reagents was changed. The vials were then inserted into the reactor, where samples were digested in the presence of dichromate at 150 ºC for 2 hours, followed by cooling at room temperature. After cooling the absorbances were measured in a multiparameter photometer HI 83214 Bench Photometer for Wastewater Treatment Application (Hanna Instruments, Rhode Island, USA), after calibration with the blank vial. The instrument directly displayed the concentration in mg COD/L for mid-range and for high range the result was multiplied by a 10 factor.

Occasionally, due to a malfunction in the reactor, COD was determined according to Standard Methods, section 5220 C, closed reflux titrimetric method. Initially the reagent solutions were prepared: standard potassium dichromate digestion solution (0,01667 M), sulfuric acid-silver sulfate solution (5,5 g Ag_2SO_4/kg H_2SO_4) and standard ferrous ammonium sulphate titrant (FAS) (0,100 M). The ferroin indicator solution was acquired from Sigma-Aldrich (Madrid, Spain). FAS solution was standardized against digestion solution, since is a secondary standard: 10,00 mL of distilled water was added to 5,000 mL of digestion solution and after the addition of 1 to 2 drops of ferroin indicator the solution was titrated with FAS. The molarity of FAS solution was calculated according to the equation 2.3.

$$\text{Molarity (M)} = \frac{\text{Volume of digestion solution titrated (mL)}}{\text{Volume of FAS used in titration (mL)}} \times 0{,}1000 \qquad (2.3)$$

A sample volume of 2,500 mL, 1,500 mL of digestion solution and 3,500 mL of sulfuric acid reagent were added to culture tubes, which were closed with caps and placed in the oven at 150 ºC for 2 hours. After cooling to room temperature and the addition of 1-2 drops of ferroin indicator, the solution was titrated with standardized FAS, until a color change from blue-green to reddish brown was observed. The same protocol was applied to a blank containing the reagents and a volume of distilled water equal to the sample volume. Finally COD was calculated according to equation 2.4, where E represents the volume of FAS used for blank titration (mL), B the volume of FAS used for sample titration (mL) and M the molarity of FAS. Samples were analysed in duplicate. Since this procedure is applicable to COD values between 40 and 400 mg/L, appropriate dilution was used (American Public Health Association, 1999).

$$\text{COD (mg/L)} = \frac{(\text{E} - \text{F}) \times \text{M} \times 8000}{\text{Volume of sample (mL)}} \quad (2.4)$$

2.2.4. Ammonia nitrogen

Ammonia nitrogen of mixed liquor samples and sample at the end of the treatment system (S3, S4 and S5) were determined using a commercial kit (Hanna Instruments, Rhode Island, USA) with adaption of Nessler method. Samples were usually analysed at low range (0,00-3,00 ± 0,01 mg/L) and occasionally at high range (0-100 ± 1 mg/L).

An appropriate volume of sample was added to reaction vials, 5,000 mL for low range and 1,000 mL for high range, to make the blank solution. After calibration of the photometer with the blank, 4 drops of HI 93764-0 Nessler reagent was added to the vials and the concentration of ammonia nitrogen were measured after approximately 3 minutes of reaction. The instrument directly displayed the concentration in mg/L.

Once a multiparameter meter was purchased, MultiMeter MM 41 (Crison Instruments, Barcelona, Spain), this parameter was determined with an ammonium ion selective electrode.

2.2.5. Total phosphorus

Total phosphorus of the sample collected after the physicochemical treatment and of the samples from the aeration tanks (S2, S3 and S4) were determined using a commercial kit (Hanna Instruments, Rhode Island, USA) with an adaptation of Standard Methods, section 4500-P C, vanadomolybdophosphoric acid method. All samples were analysed at high range (0,0-100,0 ± 0,1 mg/L).

The COD Reactor was pre-heated to 150 ºC and the reaction time programed to 30 minutes. Samples were diluted in distillate water with a dilution factor of 1:10. A volume of 5,000 mL of diluted sample was added to the reaction vials, followed by the addition of one packet of potassium persulfate for phosphorus analysis to each vial. The vials were then inserted into the reactor, where samples were digested for 30 minutes at 150ºC, followed by cooling at room temperature. A volume of 2,000 mL of sodium hydroxide and 500,0 μL of molybdovanadate reagent was added to each vial. After 7 minutes of reaction the photometer was calibrated with the blank solution, and the total phosphorus concentration was measured. The instrument displayed the concentration of phosphate in mg/L, so in order to convert to total phosphorus concentration the result was multiplied by a factor of 0,326. Since the blank solution is only stable for one day at room temperature, and this analysis was only performed weekly, a new blank vial was always prepared with distillate water.

2.2.6. pH

pH of all samples collected was determined with an microprocessor pH meter (Hanna Instruments, Rhode Island, USA). Once a multiparameter meter was purchased, MultiMeter MM 41, this parameter was determined with this instrument.

2.3. Enumeration of protozoa and metazoan

Enumeration of the microfauna present in the mixed liquor of the two aeration tanks (S3 and S4) was carried out daily by microscopic examination within 1 hour of collection, in

order to avoid changes in community structure and to ensure living conditions. Microfauna abundance was determined with a sub-sampling technique: a 25,0 µL volume of the mixed liquor was taken with a micropipette and samples were examined in duplicated using an optical microscope, MBL2000S (A. Krüss Optronic, Hamburg, Germany), at 100x or 400x magnification depending on species size. Protozoa was identified to genus level and metazoan to phylum level (rotifera and nematoda) "*in vivo*" using several identification keys (Bick, 1972; Jenkins, 1993; Bento *et al.*, 2005; Serrano *et al.*, 2008; Madoni, 2011). When colonial species was observed (e.g., *Epistylis* and *Opercularia*), all individuals of the colony were counted. The flagellate abundances in the mixed liquor samples were assessed by qualitative analysis only, and were not quantified by counting using a Fuchs-Rosenthal chamber as recommend, since the counting method is time consuming and was intended to establish a method easy and fast to be used periodically by the wastewater operators. This qualitative analysis considers 5 levels of abundance, Table 2.1. The same analysis was employed to the filamentous bacteria abundance Figure 2.2.

Table 2.1: Subjective scoring system for the evaluation of the abundance of small flagellates and filamentous bacteria.

Symbol	Abundance
-	Absent
+	Present
++	Uncommon
+++	Frequent
++++	Very frequent
+++++	Highly frequent

The microorganisms identified were then grouped into functional groups according to literature and the indicator value of each group as described in Table 1.1, introduction section (Jenkins, 1993; Madoni, 1994; Bento *et al.*, 2005; Serrano *et al.*, 2008; Madoni, 2011). These groups are small flagellates, large flagellates, testate amoebae, free swimming ciliates, crawling ciliates, sessile ciliates, *Opercularia* sp. and metazoans. Since, as described by Madoni (1994) only bacterivorous ciliates belong to the three ciliate groups, carnivorous attached ciliates as the suctorians *Podophrya* and *Tokophrya* are excluded by the "sessile ciliates" group thus a "carnivorous ciliates" keygroup was created.

To avoid possible false identifications of microorganisms, substantial practice with several references was required and conducted before proceeding.

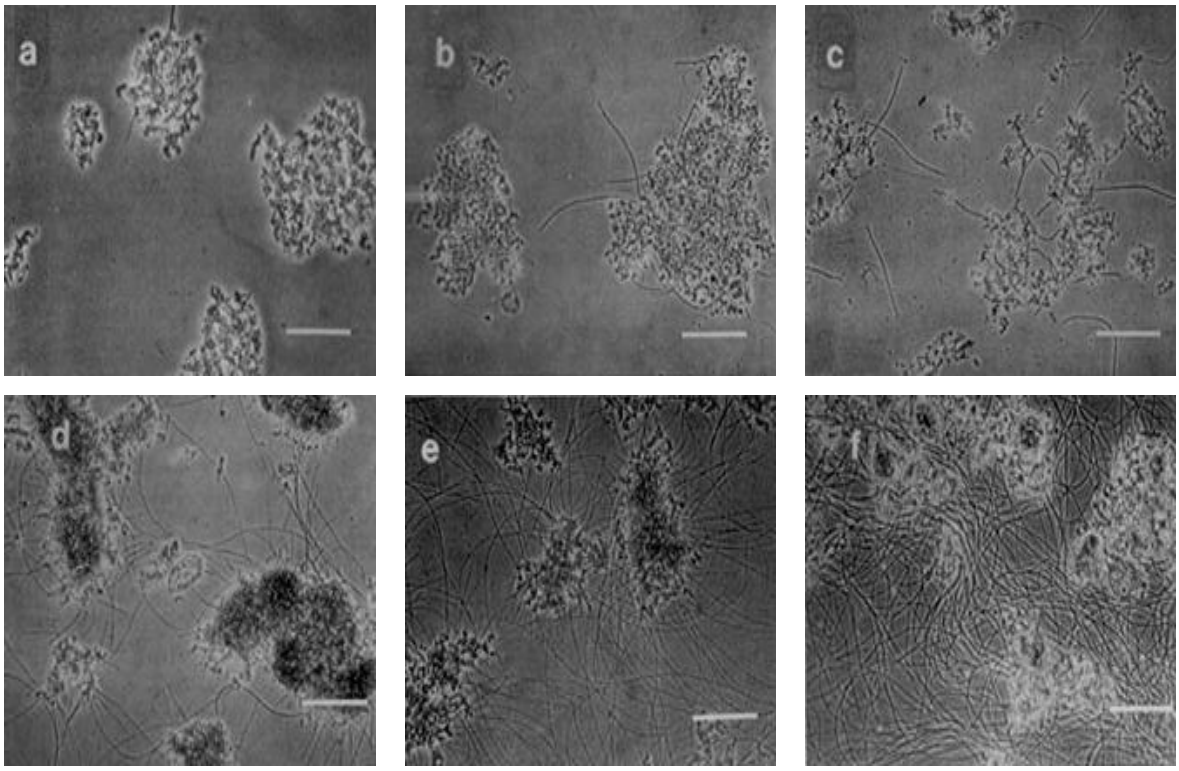


Figure 2.2: Filament abundance categories using subjective scoring system: a) absent; b) present; c) uncommon; d) frequent; e) very frequent; f) highly frequent. Photographs taken using phase contrast at 100x magnification; the bar indicates 100 μm, Jenkins (1993) and Gray (2004).

2.4. Multivariate statistical analysis

In order to show the role of the protozoa and metazoan communities in activated sludge, and which variables measured at the IWWTP can affect the structure of the microfauna communities, the results were explored with a multivariate statistical analysis.

Large magnitude differences in absolute abundances of some of the counts were solved using relative abundances as a percentage of total microorganisms present, as described in literature (Pérez-Uz *et al.*, 2010). The data analysis was performed using IBM SPSS v. 20.0 (IBM Corporation, New York, USA) and Matlab v. 7.11 (MathWorks, Massachusetts, USA). A non-parametric correlation coefficient test of Spearman's Rank was used to obtain a correlation matrix of all physicochemical parameters *versus* relative abundance of functional groups. The principal component analysis (PCA) was used to reveal clusters

among the observations, and to enable the visualization of the correlations between the parameters in study in a correlation circle.

Correlation analysis examines the strength with which two sets of measurements show positive or negative linear association, by calculating a correlation coefficient. The Spearman's Rank correlation is used when the data are not normally distributed and is not even possible to transform it to be normally distributed (Ennos, 2007).

PCA is a technique for reducing the amount of data when there is correlation present (Miller and Miller, 2000). PCA computes new variables called *principal components* that are obtained as linear combinations of the original variables. The first principal component is required to have the largest possible variance (i.e., this component will explain the largest variance of the data). The second component is computed under the constraint of being orthogonal to the first component and to have the largest possible variance, the other components are computed likewise. The values of these new variables are called *factor scores*, and a graphic representation of them indicates the pattern of similarity of the observations by displaying them as points in maps. The correlation between a component and a variable estimates the information they share. This correlation is called *loading*, and the graphic representation of the variables by their *loadings* constitute the circle of correlation (Abdi and Williams, 2010). In the correlation circle only parameters far from the biplot center are statistically well explained by the two components represented. The parameters that are close to each other are in normal correlation, those related to others by 180º rotation are inversely correlated, while the parameters related by 90º rotation are independent (Avella *et al.*, 2011).

2.5. Waste treatment

At the end of the analyses all samples from the IWWTP were collected to a common container and subsequently redirected to the IWWTP, except for the sample of the effluent at the end of the treatment process (S5) that has been eliminated by the domestic sewage system, as already obeyed the admission parameters of SIMRIA. The collection vessels were washed and reused.

Lastly, the reaction vials were collected in a suitable vessel and sent to a licensed operator in order to be safely discarded because they contained different waste pollutants.

3. RESULTS AND DISCUSSION

3.1. Preliminary studies

3.1.1. Total solids determination

Daily, total solids determination was made in Prio using 9 hours of oven evaporation. In order to optimize the time of analyses a comparative study using 9 hours and 2 hours of evaporation was carried out. The results of this study are shown in Table 3.1.

Table 3.1: Results of the TS (g/L) for 9 hours and 2 hours of oven evaporation.

Sample	Aeration tank 1		Aeration tank 2		Final treatment effluent	
	9 hours	2 hours	9 hours	2 hours	9 hours	2 hours
1	10,10 ± 0,021	11,70 ± 0,018	10,39 ± 0,022	12,02 ± 0,021	0,690 ± 0,021	1,640 ± 0,020
2	11,63 ± 0,020	12,07 ± 0,021	11,74 ± 0,020	12,28 ± 0,021	1,140 ± 0,020	0,980 ± 0,021
3	12,15 ± 0,022	11,94 ± 0,022	12,36 ± 0,021	12,18 ± 0,020	1,820 ± 0,021	1,780 ± 0,023
4	11,23 ± 0,019	11,30 ± 0,021	10,38 ± 0,021	11,52 ± 0,022	1,360 ± 0,021	1,420 ± 0,020
5	11,38 ± 0,023	10,98 ± 0,020	11,14 ± 0,022	11,34 ± 0,022	1,340 ± 0,020	1,780 ± 0,023
6	11,16 ± 0,021	11,12 ± 0,021	11,10 ± 0,019	11,18 ± 0,021	1,270 ± 0,020	1,420 ± 0,022
7	7,820 ± 0,020	7,800 ± 0,021	7,800 ± 0,020	7,780 ± 0,019	0,990 ± 0,022	1,020 ± 0,020
8	8,820 ± 0,022	8,830 ± 0,020	8,890 ± 0,021	8,930 ± 0,020	1,400 ± 0,020	1,510 ± 0,021
9	9,050 ± 0,022	9,320 ± 0,022	9,000 ± 0,022	9,240 ± 0,021	1,310 ± 0,020	1,250 ± 0,019
10	13,07 ± 0,024	12,12 ± 0,020	13,25 ± 0,020	12,16 ± 0,020	1,880 ± 0,021	1,300 ± 0,021

A two-sample Student's *t*-test showed that the mean of the total solids concentration, when two different evaporation times were used is not significantly different for a $\alpha = 0,05$ level of significance. This observation is valid since the *p*-value obtain is greater than 0,05 using a Student's t distribution with 58 degrees of freedom, $t(58) = -0,118$, $p = 0,907$. Consequently optimization was made reducing the oven evaporation time for 2 hours. This change allows the possibility of making actions in the IWWTP in the proper day, according to results obtained and during the administrative schedule of the company (9:00h to 18:30h).

3.1.2. Relation between volatile suspended solids and total solids

In literature is reported that the volatile suspended solids are about 80% of total solids, and this value is usually used in daily calculations (Lin and Lee, 2007). In order to find out if in

this IWWTP there is also a relation between these two parameters and its magnitude VSS were analysed and compared to TS. Results in Table 3.2.

Table 3.2: Results of TS (g/L) and VSS (g/L) determined for both aeration tanks and relation between TS and VSS (%).

Sample	TS (g/L)	VSS (g/L)	Relation between VSS and TS (%)
1	11,21 ± 0,020	8,600 ± 0,021	76,72 ± 0,26
2	9,460 ± 0,021	7,860 ± 0,021	83,09 ± 0,32
3	9,700 ± 0,021	8,110 ± 0,021	83,61 ± 0,31
4	7,820 ± 0,022	6,580 ± 0,020	84,14 ± 0,38
5	7,800 ± 0,020	6,520 ± 0,022	83,59 ± 0,38
6	9,050 ± 0,022	7,230 ± 0,021	79,89 ± 0,34
7	9,000 ± 0,022	7,100 ± 0,020	78,89 ± 0,33
8	7,500 ± 0,020	6,210 ± 0,020	82,80 ± 0,38
9	7,850 ± 0,021	6,240 ± 0,022	79,49 ± 0,39
10	8,260 ± 0,020	6,490 ± 0,021	78,57 ± 0,36
11	8,300 ± 0,020	6,520 ± 0,020	78,55 ± 0,34
12	8,320 ± 0,019	7,010 ± 0,020	84,26 ± 0,33
13	8,030 ± 0,020	6,570 ± 0,020	81,82 ± 0,36
		Mean (%)	**81,19**

A relation between VSS and TS was obtained around 81,19 %. A one-sample Student's *t*-test confirms that the mean relation value (mean = 81,19, SD = 2,58, N = 13) is not significantly different from the hypothesized value of 80%, $t\,(12) = -1{,}66$, $p = 0{,}12$. Thus in further analysis the 80% value was assumed and only total solids were determined, since TS determination involves less experimental work and results are provided faster.

3.1.3. Total solids concentration of WAS

Total solids concentration of waste activated sludge (WAS) is one of the variables used in the determination of the solids retention time (SRT). As this value tends to be constant (mean = 29,66), it was not determined daily. In the beginning of this study this variable was determined for a few samples collected in different days and then a medium value of 30 mg/L was considered in the calculation of SRT.

Table 3.3: Total solids concentration of waste activated sludge.

Sample	TS (g/L)
1	30,91 ± 0,02
2	28,38 ± 0,02
3	29,04 ± 0,02
4	29,08 ± 0,02
5	29,35 ± 0,02
6	28,96 ± 0,02
7	29,36 ± 0,02
8	29,42 ± 0,02
9	29,08 ± 0,02
10	29,29 ± 0,02
11	29,29 ± 0,02
12	29,25 ± 0,02
13	29,21 ± 0,02
14	32,27 ± 0,02
15	30,18 ± 0,02
16	30,41 ± 0,02
17	30,71 ± 0,02
Mean	**29,66**

3.2. Physicochemical treatment

Influent and effluent of the DAF 1 were analysed in terms of COD concentration and solids content daily during the study period. Results show that the influent COD is very variable (52126 ± 44359 mg/L). This is due to start/stop of biodiesel production and maintenance operations such as cleaning, which loads the effluent with soluble and insoluble COD, glycerine and oil respectively. These maintenance operations were performed when the biodiesel production was stopped, and correspond to the peaks found in the influent COD concentration, Figure 3.1. After this treatment the COD in DAF 1 effluent is usually below 15000 mg/L. Analysing the evolution in influent and effluent COD concentration during the experimental period, Figure 3.1, is clear that the increase in influent COD does not imposes an increase in effluent COD. This means that the DAF process is efficient in COD removal.

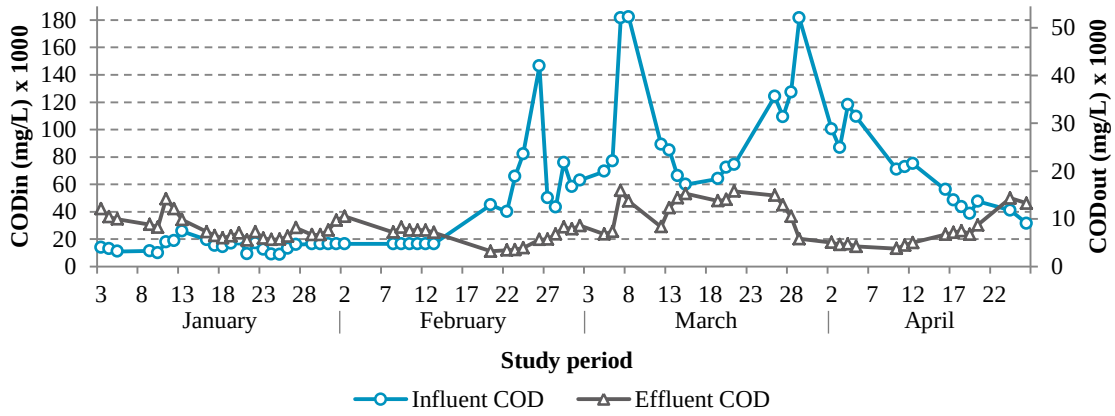
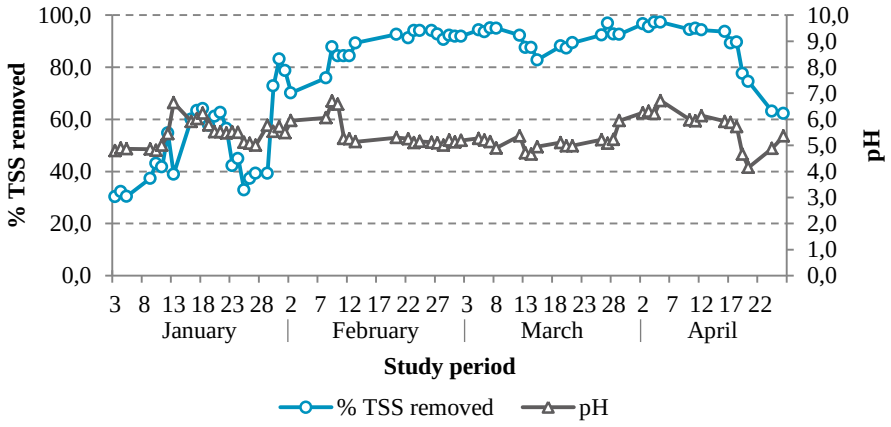


Figure 3.1: Variation of the influent and effluent COD concentration during the experimental period.

Since the dissolved air flotation is a clarification process that relies on the removal of suspend matter as solids and oil, it is very important to know the efficiency of solids removal in order to evaluate the process performance.

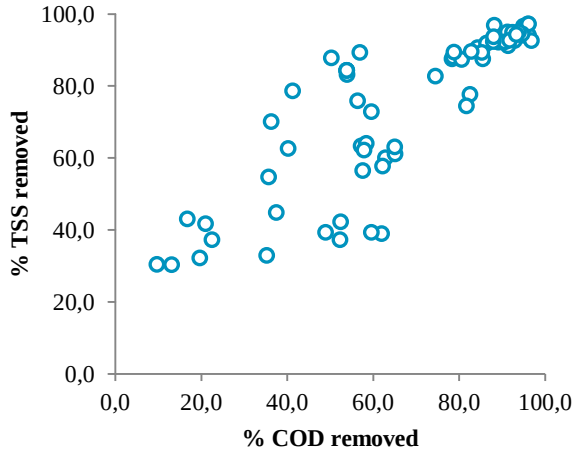


TSS removal	
Mean	75,65
Std. Deviation	21,64
Median	87,44
Minimum	30,32
Maximum	97,30

Figure 3.2: Performance of TSS removal and pH variation.

Figure 3.2 presents the performance of solids removal during the study period. As observed, the efficiency of the physicochemical treatment was not constant, with a minimum of 30,32% and a maximum of 97,30%. The results also show that the efficiency of solids removal is usually above 70%. This value agrees with the values found in literature since according to other studies over 70% of suspended solids removal is typically achieved using a DAF system (de Nardi *et al.*, 2008; Koivunen and Heinonen-Tanski, 2008; Rojas *et al.*, 2008). Thus, these results suggest that the physicochemical treatment is working correctly and the coagulant used is adequate.

The relationship between the efficiency of COD removal and the efficiency of total suspended solids removal is given in Figure 3.3. Correlation analysis shows that there is a strong association between the two variables, r(68) = 0,891, p < 0,001. Thus the COD removed in this stage of treatment is essentially insoluble COD (e.g., oil) and is strongly dependent of solids elimination. According to Rattanapan *et al.* (2011) when the wastewater contains oil, solids elimination is influenced by the pH of the medium.



Pearson correlation test	
Correlation coefficient	0,891**
Sig. (2-tailed)	0,000
N	70

** correlation is significant at the 0.01 level (2-tailed)

Figure 3.3: Graph showing the relationship between TSS and COD removal efficiency, and table with Pearson correlation analysis results.

In these conditions, Rattanapan *et al.* (2011) found that the pH is more important for the proper operation of the process than the coagulant concentration, since oil drop coalescence is greater when the medium is acidified, resulting in the formation of larger droplets (Al-Shamrani *et al.*, 2002a; Rattanapan *et al.*, 2011). A low pH value affects the carboxyl function (N-COO^-) on the surface of the oil drops allowing them to approximate and flocculate. The big oil droplets, result of flocculation, can then rise to the surface of DAF unit and are removed by the skimmer system (Rattanapan *et al.*, 2011). According to Fujii *et al.* (2007) this phenomenon is classified as demulsification. The emulsification-

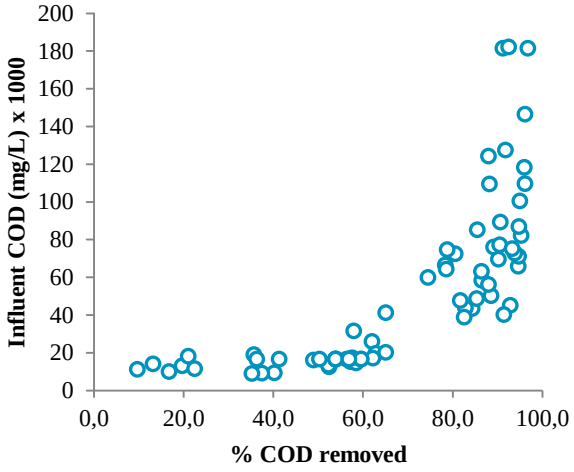


Figure 3.4: Relation between COD removal efficiency and influent COD concentration.

demulsification cycle is reversible and related to pH. This means that at pH values above 7.7 the emulsion is stable but when the pH decreases to values between 5.0 to 6.1 the emulsion destabilizes, and large drops of oil on the surface of the effluent are observed when the pH reaches a value lower than 4.0 (Fujii *et al.*, 2007).

It was also observed that the efficiency of COD removal is correlated with the influent COD, Figure 3.4. The performance of physicochemical treatment improves when the influent COD increases, even without significant variations in the pH, Figure 3.2. A possible explanation is related to the oil concentration, once when oil concentration increases in the influent the COD value is greater. The operational conditions of the DAF lead to the formation of large oil drops that arise to the surface of the unit, dragging suspended solids that are removed by the skimmer. This is observation is supported by studies that reveals that when a combination of solid particles and oil droplets are present, solid-oil attachment particles are formed (Deng *et al.*, 2009; Ali *et al.*, 2011). This means that when the unit operates correctly the presence of oil improves the solids removal and consequently the COD removal.

In short, pH control is critical to ensure a correct physicochemical treatment providing the maximum insoluble COD removal. The coagulant in use also seems to be the correct one since it seems be allowing solids flocculation. However COD removal efficiency is greater when the influent COD concentration increases.

3.3. Activated sludge process

Before presenting and explaining the results is important to refer that during the study period it was necessary to make a re-inoculation of the mixed liquor with activated sludge from a municipal WWTP. This re-inoculation was needed due to a decrease in the viable biomass concentration, confirmed by microscopic observation.

3.3.1. Operational parameters

3.3.1.1. Temperature and pH

The pH was maintained at biological level, around 7.0 with little variation during the experimental period. In other hand, temperature suffered some fluctuations, arising from the weather conditions, which had impact on the microfaunal community. At the beginning of the study period the temperature was low, about 19 ºC, resulting of both, the low environmental temperature experienced during the winter and of the low microbial activity. As the environmental temperature increased, the temperature of the bioreactors also increased, to a value closed to the growth peak of mesophilic organisms, 35 – 37ºC (Henze et al., 1997).

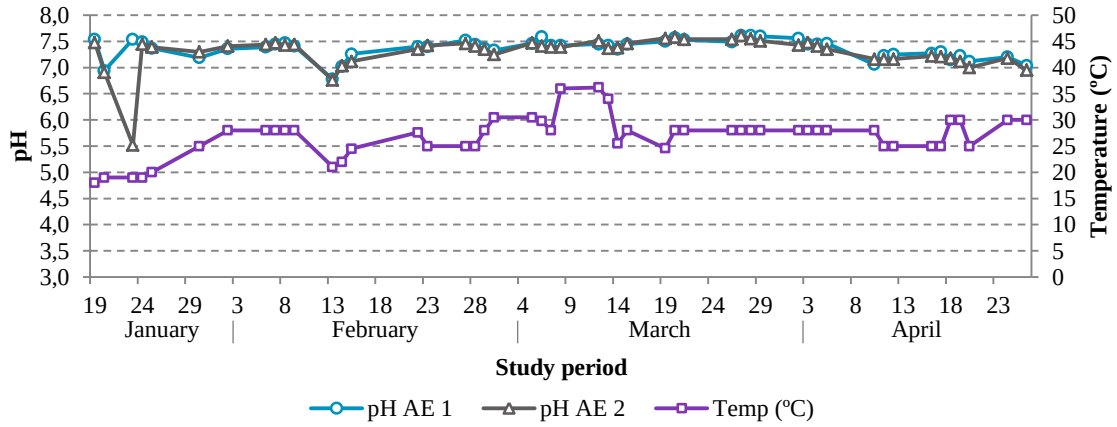


Figure 3.5: Variation of the temperature and pH in aeration tank 1 and 2 during the experimental period.

3.3.1.2. Nutrients

As previously described, the microorganisms involved in the activated sludge process need nutrients, mainly nitrogen and phosphorus. The effluent produced by the biodiesel production unit has phosphorus in excess, since phosphoric acid (H_3PO_4) is used in the degumming/neutralization of the crude oil, Figure 1.9. This explains why phosphorus is always in excess in the mixed liquor, with a concentration usually above 60 mg/L, Figure 3.6a. In relation to nitrogen, the effluent produced has no nitrogen in composition, thus urea was added in order to suppress the need of this nutrient. The ammoniacal nitrogen concentration was kept around 2,0 mg/L, Figure 3.6b. The amount of urea added was

determined considering a C:N:P ratio of 200:5:1 (design value) and a nitrogen concentration in urea of 8,4% (Foresa, 2010), according to equation 3.1 where S2 and S3 represent the sampling sites and Q the influent feed rate (m^3/d).

$$\text{Urea(kg)} = \frac{\frac{\text{COD(S2)}/1000 \times \text{Q} \times 5}{200} - \frac{\text{AN(S3)}}{1000} \times \text{Q} \times 100}{8{,}4} \quad (3.1)$$

The results also show a peak in ammoniacal nitrogen concentration that reached values around 40 mg/L, due to a urea dosage problem.

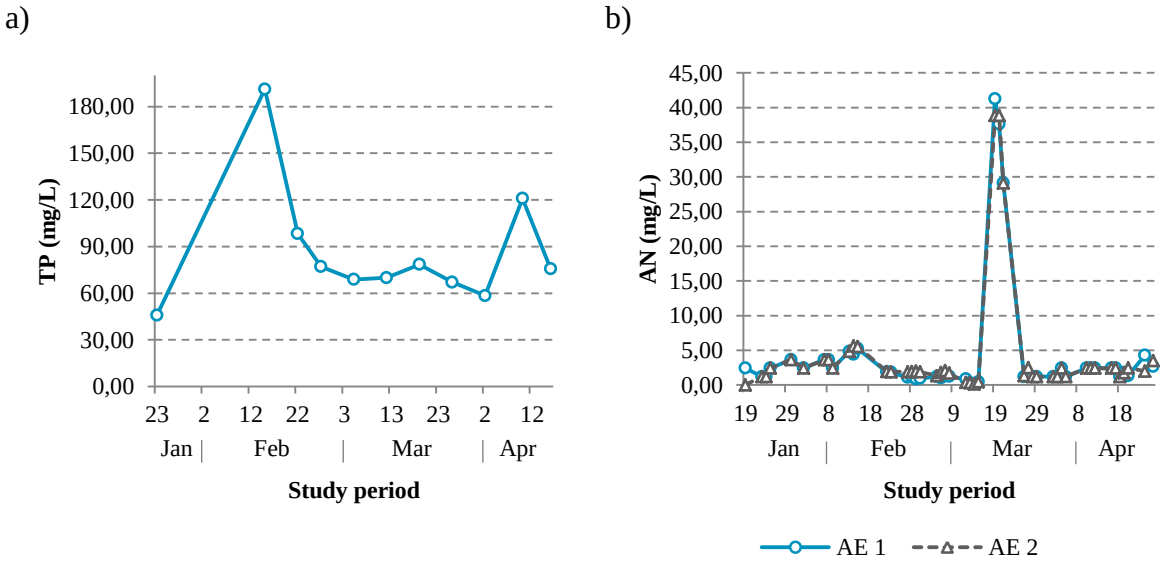


Figure 3.6: Variation of the a) total phosphorous concentration in aeration tank 1 and b) N-ammoniacal concentration in aeration tank 1 and 2, during the experimental period.

In Table 3.4 can be observed that the real C:N:P ratio is very different from the theoretical of 200:5:1, or from the others found in literature, 100:5:1 (Ouano, 1983), 100:20:1, 250:7:1 e 100:10:1 (Jefferson *et al.*, 2001; Bitton, 2005). In fact the phosphorous concentration is always in excess. The system is not prepared to biologically remove phosphorous because it involves an anaerobic stage before the activated sludge process, in order to provide competitive advantage to polyphosphate accumulating organisms (PAO) (Tchobanoglous *et al.*, 2003). In this case it would be viable to remove phosphorus chemically, by adding a coagulant (e.g., aluminium or iron salts) in DAF 1 (Henze *et al.*, 1997). Therefore the physicochemical treatment should remove phosphorus along with suspended solids, and the relation between organic matter, nitrogen and phosphorus concentration would be more adequate.

Table 3.4: C:N:P ratios observed during the experimental period.

Sample	Date	C:N:P
1	23-01-2012	825:1:23
2	15-02-2012	236:1:19
3	22-02-2012	135:1:149
4	27-02-2012	220:1:39
5	05-03-2012	205:1:34
6	12-03-2012	190:0:35
7	19-03-2012	212:21:39
8	26-03-2012	164:1:34
9	02-04-2012	122:1:29
10	10-04-2012	165:1:60
11	16-04-2012	128:1:38

3.3.1.3. COD removal

The COD removal efficiency of the activated sludge process is present in Figure 3.7. The average COD concentration of the influent was 8715 mg/L, with high variability (SD = 555,41) and a maximum of 17220 mg/L. The effluent COD concentration had a medium value of 541,69 mg/L. This results into a COD removal performance of 92,84% in average. Value that agrees with literature since COD removal efficiencies of over 80%, in activated sludge systems, are reported in other studies (Pérez-Uz *et al.*, 2010; Cui *et al.*, 2012).

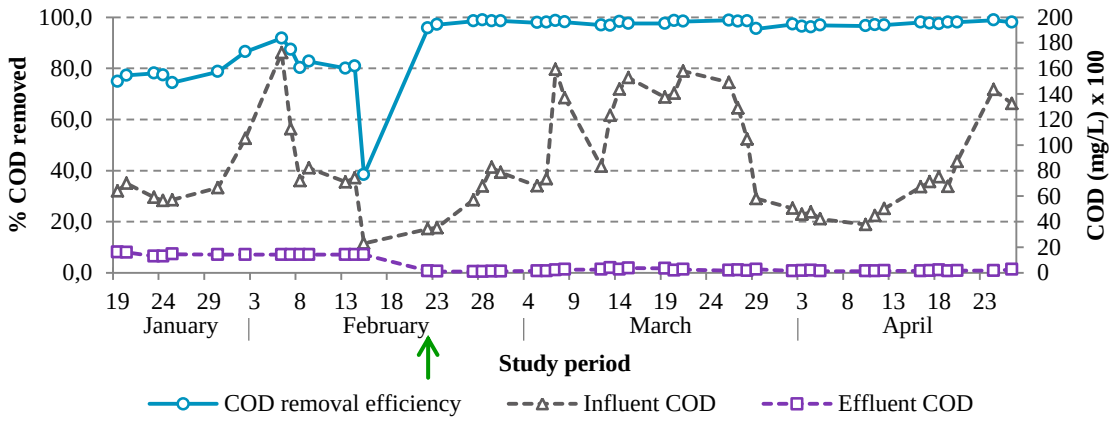


Figure 3.7: Variation of the COD removal efficiency and influent and effluent COD concentration, green arrow indicates the re-inoculation.

It can also be noticed that the COD removal efficiency improved in 16,89% after the re-inoculation, $t(47) = -19{,}828$, $p = 0{,}000$, Figure 3.8a, even with no significant change

observed in the influent COD, $t(47) = -0{,}679$, $p = 0{,}500$. This shows a COD decrease of 86,95% on the final effluent, $t(47) = -49{,}439$, $p = 0{,}000$, Figure 3.8b.

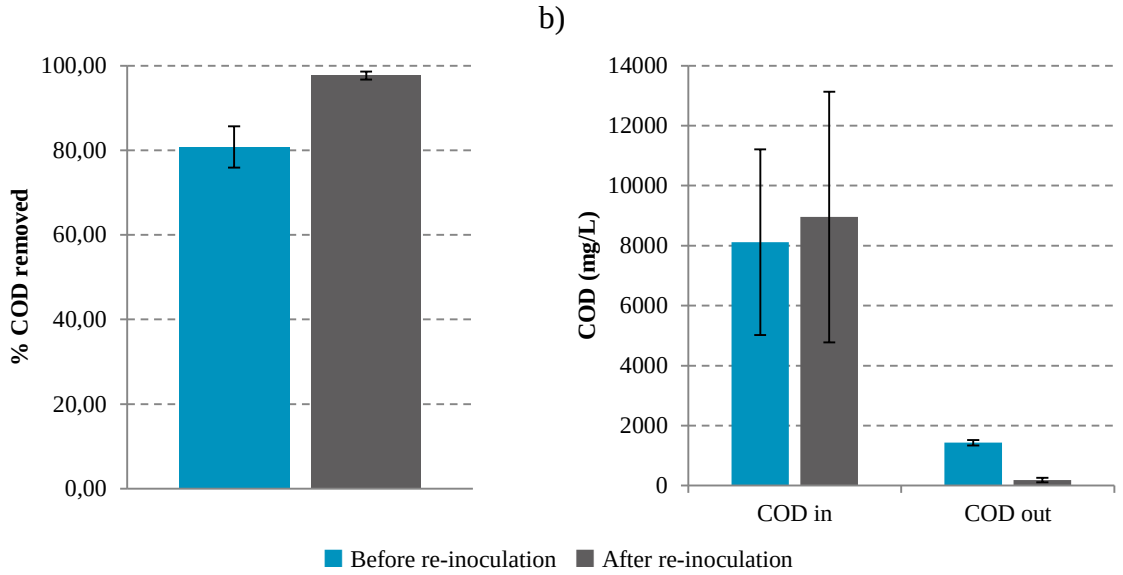


Figure 3.8: Bar chart showing the means with standard error bars of the a) COD removal efficiency and b) influent and effluent COD concentration, before and after the re-inoculation.

3.3.1.4. Mixed liquor volatile suspended solids

As described the mixed liquor volatile solids are used to estimate the microorganisms concentration. In this study the MLVSS where determined using a relation between TS and VSS.

Figure 3.9 presents the MLVSS variation during the experimental period. The MLVSS concentration was usually kept between 7,0 and 8,0 g/L, with some oscillations that can be explained by the inefficient waste activated sludge removal system. The system used for the sludge removal is not adequate and do not allow an accurate sludge extraction. This means that the operators cannot be sure of the amount of sludge removed. This creates a system destabilization since sometimes the amount of sludge removed is higher than needed, removing a large number of microorganisms, and on the other hand, occasionally the sludge amount removed is lower than needed, increasing the sludge age.

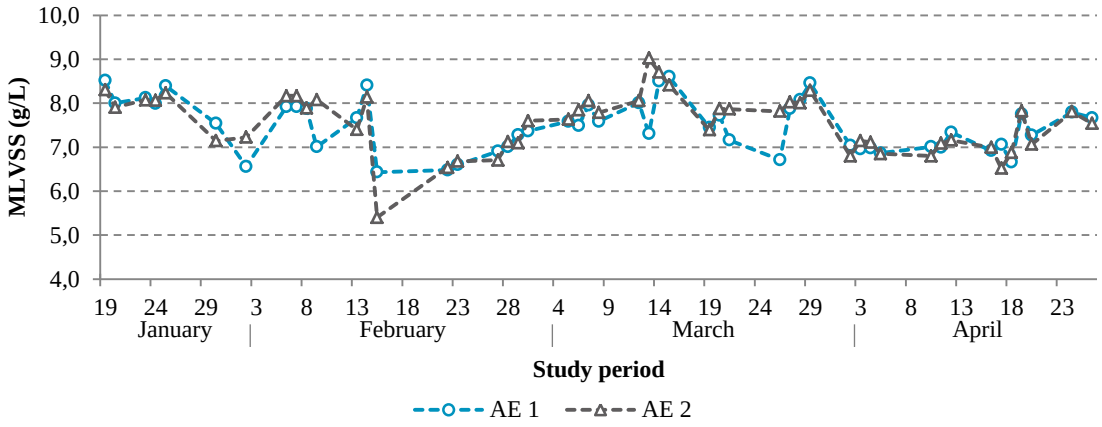


Figure 3.9: Variation in mixed liquor volatile suspended solids concentration in aeration tank 1 and 2 during the experimental period.

3.3.1.5. Solids retention time

The solids retention time, sludge age, was determined according to equation 3.2, which is the adaptation of the equation 1.9 to the system in study. In this case SRT was calculated using the medium value of the MLVSS concentration of both aeration tanks. And, the suspended solids in the waste activated sludge (WAS) flow was considered constant and 30,00 mg/L. Also, in equation 3.2, Q_w represents the WAS flowrate (m^3/d), 110 is the volume of each bioreactor (m^3) and 0,80 the relation between VSS and TS.

$$\mathrm{SRT(d)} = \frac{\frac{\mathrm{ST(S3)} + \mathrm{ST(S4)}}{2} \times 110 \times 2 \times 0{,}80}{Q_w \times 30{,}00 \times 0{,}80 + (Q - Q_w) \times \mathrm{ST(S5)} \times 0{,}80} \tag{3.2}$$

As can be observed in Figure 3.11a the SRT was not constant during the experimental period, and even not considering the outlier values the SRT is far from uniformity, Figure 3.11b. In some days the results indicate an old sludge, with a SRT higher than 20 days. However this was not coherent with the microscopic observations, since the microfauna found in these days was not indicator of an old sludge. With these SRT values it was expected the detection of plenty metazoans, as they have a long life cycle and thus only proliferate in systems with a high SRT, but this was not observed at all. Further on, the structure of the biological floc also indicated that the SRT values determined were not corresponding to reality. An old sludge is characterized by large flocs mainly composed of dead cells surrounded by a viable bacterial layer. In lightly loaded plants like this with low

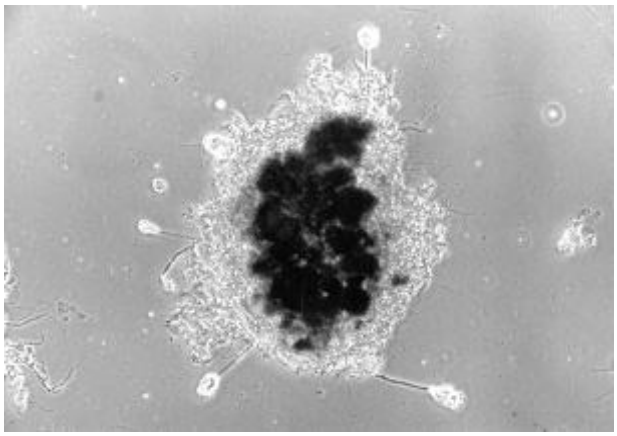


Figure 3.10: Compact floc with a dark central inclusion and secondary colonisation of sessile ciliates (x200), Gray 2004.

F/M values, old sludge presents flocs with a darker central core or inclusions, which are made up primarily of inorganic material (e.g., iron hydroxide, calcium phosphate) along with non-biodegradable organic material. The central core is surrounded by a lighter, less dense region that is composed of active microorganisms. This is effect of repeated periods of active growth and subsequent starvation undergone by the flocs, results in compact flocs with the older non-degraded material in the centre (Gray, 2004), Figure 3.10.

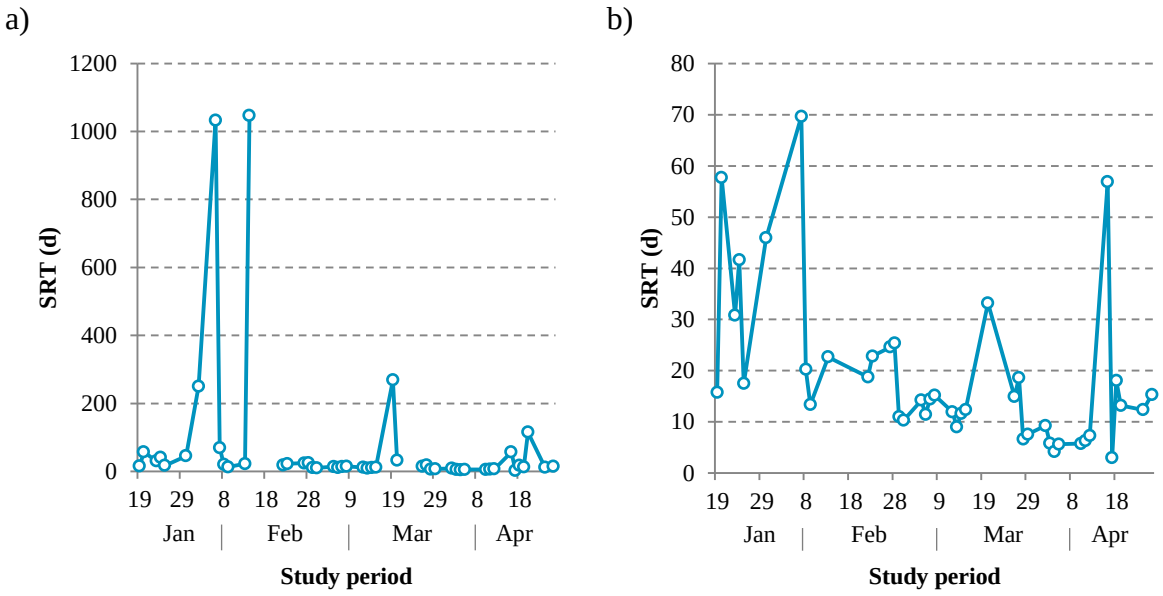


Figure 3.11: Variation observed in solids retention time, a) considering outlier values and b) excluding outlier values.

Once again these inconsistent results are consequence of the ineffective removal system and of the impossibility of purging out of the system a pre-determined amount of sludge. Improvement of the purge system would be useful in order to create a more uniform SRT and MLVSS. The amount of sludge to be removed can be determined rearranging the equation 3.2 and considering a constant value for SRT for instance 15 days.

$$Q_w(m^3/d) = \frac{\frac{(ST(S3) + ST(S4))}{2} \times 110 \times 2 \times 0{,}8}{15} - Q \times ST(S5) \times 0{,}8}{30 - ST(S5) \times 0{,}8} \quad (3.3)$$

The theoretical value calculated according to equation 3.3 revels that the amount of sludge to be removed is more uniform than the real quantity removed, and is around 2,0 m^3/day, Figure 3.12.

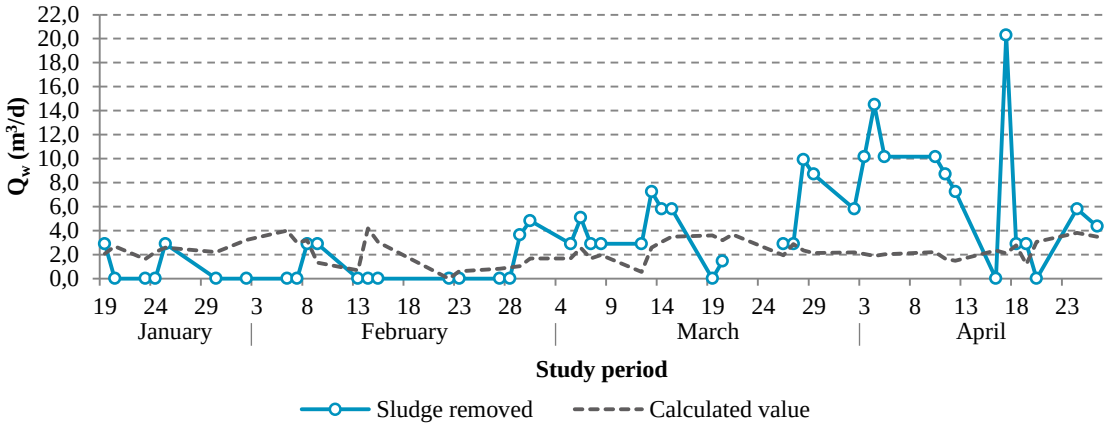


Figure 3.12: Graphical representation of the amount of sludge removed and the theoretical value calculated according to equation 3.3.

3.3.1.6. Food to microorganism ratio

The food to microorganism ratio was daily calculated according to equation 3.4, coming from equation 1.7. Once again was considered the medium value of the MLVSS concentration of both aeration tanks.

$$F/M = \frac{\frac{COD(S2)}{1000} \times Q}{\frac{TS(S3) + TS(S4)}{2} \times 110 \times 2 \times 0{,}8} \quad (3.4)$$

The results, Figure 3.13, showed that the F/M value is very variable and usually very low, below 0,25. Consequently, the microorganisms are under substrate limiting conditions, which cause a rapid decrease in metabolic rate until an endogenous respiration phase begins with cellular lysis. This is the main explanation for the decrease in microorganisms density observed in January/February that conducted to a re-inoculation of the mixed liquor.

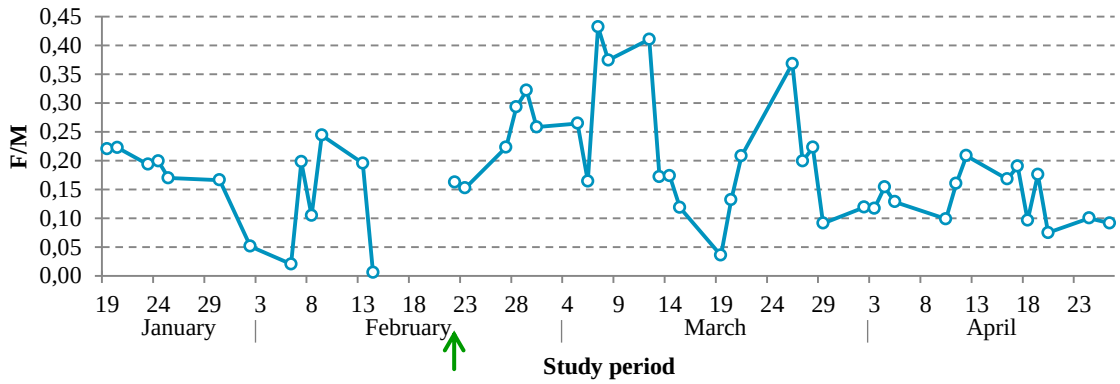
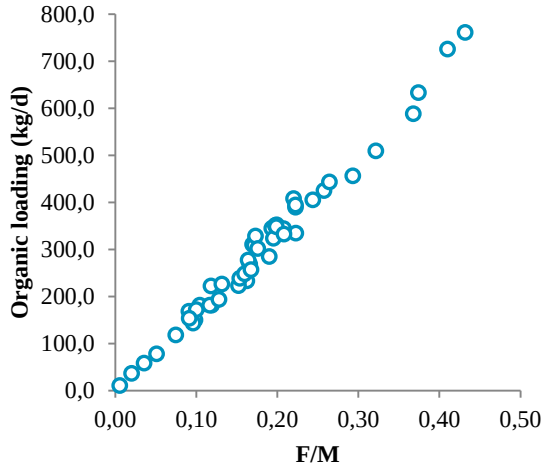


Figure 3.13: F/M values observed during the experimental period, green arrow indicates the re-inoculation.

The F/M variability is associated with the organic loading, $t(48) = 0{,}991$, $p < 0{,}001$, Figure 3.14. Thus the feed rate is vital to maintain the activated sludge system in balance, since the organic loading is defined as the organic matter per m^3 per day and is calculated according to equation 1.8, as discussed before.



Pearson correlation test	
Correlation coefficient	0,991**
Sig. (2-tailed)	0,000
N	48

** correlation is significant at the 0.01 level (2-tailed)

Figure 3.14: Graph showing the relationship between F/M and organic loading and table with Pearson correlation test results.

3.3.1.7. Feed rate

As previously identified the feed rate is an important parameter, and the optimal value can be calculated rearranging the equation 3.4:

$$Q = \frac{1000 \times F/M \times \left(\frac{TS(S3) + TS(S4)}{2}\right) \times 110 \times 2}{COD(S2)} \quad (3.5)$$

Considering a fixed value of 0,3 for F/M, since for a conventional activated sludge system the F/M ratio ranges from 0,2 to 0,5 (Spellman, 2003), a theoretical value (Value 1) was calculated. As can be observed in Figure 3.15, this value does not agree with the real feed rate value. In other words, the project design for the activated sludge system is not adequate to the amount of effluent produced daily by the unit production of biodiesel. Possibly, the project considered a larger amount of effluent produced or a more polluted one with higher COD value. Another theoretical value (Value 2) was determined reducing the useful volume of the two aeration tanks from 110 m^3 to 80 m^3 and the MLVSS value in 20%. In Figure 3.15 can be verified that this value is more close to the real feed rate.

Thus these new conditions: volume of 80 m^3 and MLVSS between 5,0 and 7,0 g/L, allow the activated sludge system to operate with a feed rate more close to a theoretical value, which enables the F/M ratio to be maintained constant, F/M = 0,3.

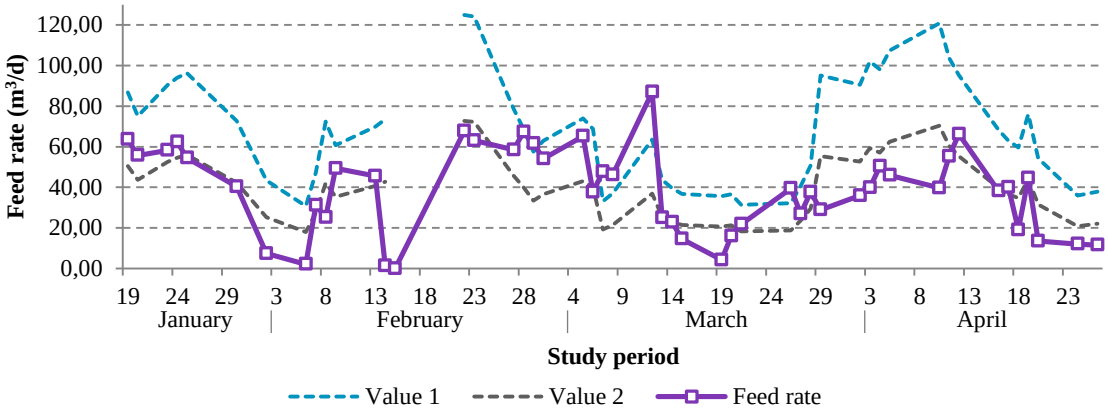


Figure 3.15: Graphical representation of the feed rate and two theoretical values calculated considering F/M=0,3, value 1 for 110 m^3 of useful volume of the aeration tanks and MLVSS concentration between 7,0 and 8,0 g/mL and value 2 for 80 m^3 of useful volume of the aeration tanks and MLVSS concentration between 5,0 and 7,0 g/mL.

3.3.2. Biological parameters

3.3.2.1. Floc structure

During the experimental period the structural characteristics of the biological flocs showed no significant variations. The flocs remained small and weak, and a dispersed growth of bacteria was also observed. Filamentous bacteria were abundant in the beginning of the study and then decreased in abundance to a negligible level.

These observations are supported by the results of two external evaluations, conducted in two different periods, 17th December 2011 and 28th May 2012. The first report indicates small (< 150 μm in diameter), irregular and weak flocs, and dispersed growth of unicellular bacteria Figure 3.16a. The filamentous bacteria population was dominated by a filament belonging to the Type 0041 morphgroup, Figure 3.16b (GE, 2011). The second report also indicates a dispersed growth of bacteria, Figure 3.16c and d. In this case the filamentous bacteria concentration was reduced, and the filaments were bellowing to Type 021N morphgroup. The report also identified a nutritional imbalance in the bioreactors (Ematsa, 2012), confirming what was previously discussed in section 3.3.1.2.

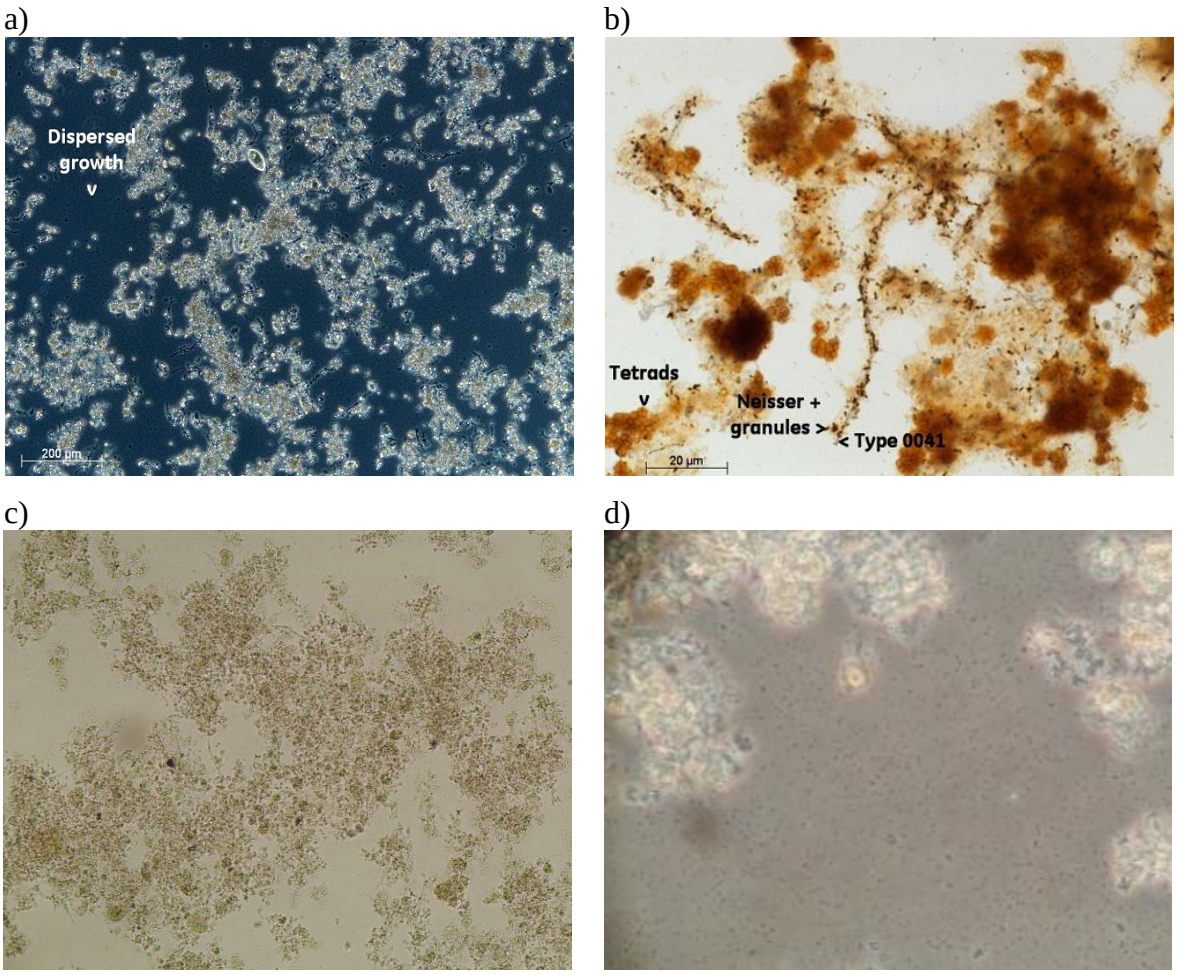


Figure 3.16: Photomicrographs of the activated sludge from the external reports, a) and b) from report 1 (Nov 2011), c) and d) from report 2 (Apr 2012). a) overview of the activated sludge sample from AE 1, 400x; b) detail of the sample from AE 1 showing Type 0041 with attached growth of unicellular bacteria, Neisser staining, 1000x; c) overview of the activated sludge sample, 100x; d) dispersed growth of bacteria, 1000x; GE (2011) and Ematsa (2012).

3.3.2.2. Microfauna community

Occurrence and abundance of each microfauna group in the activated sludge system is shown in Table A.3 (annexes). A total of 49 samples were analysed, where 9 protozoa genera and 2 metazoa phyla were identified, showing high variability in abundance during the study period. The protist community associated to the biological reactor was organized in three main groups: flagellates, amoebae and ciliates.

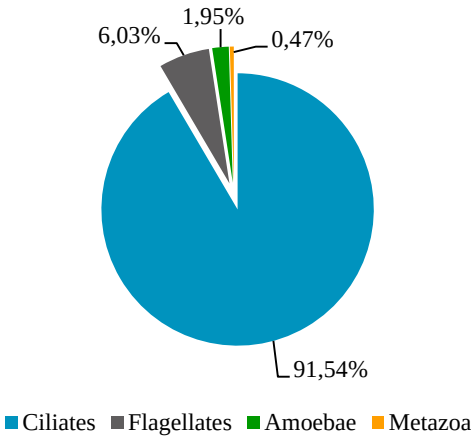


Figure 3.17: Relative abundance of microfauna groups during the experimental period.

During the study period the ciliates group was the most important part of the community, representing about 91,5% of the total microfauna abundance, followed by the flagellates group with 6,0% of relative abundance and finally amoebae and metazoan with 1,9% and 0,5% respectively, Figure 3.17.

As previously referred the ciliates group are subdivide into three groups according to their behaviour, and in this work a carnivorous ciliate group was also considered. As can be observed in Figure 3.18, the dynamics of the ciliates changed with the re-inoculation. Before the re-inoculation (Figure 3.18a) the ciliate group was dominated by free swimming ciliates and occasionally sessile ciliates were observed. After the re-inoculation (Figure 3.18b) sessile ciliates were the dominant ciliate group and the relative abundance of the free swimming decreases severely. Also, carnivorous ciliates started to occur in daily observations.

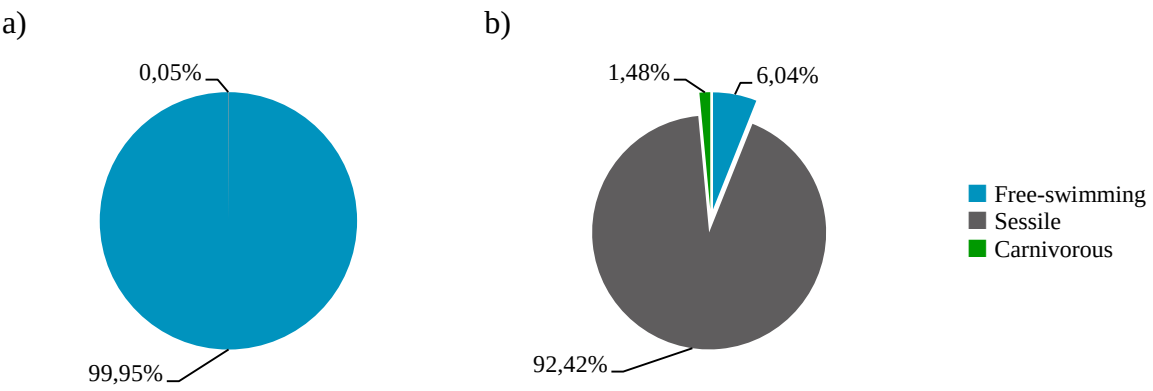


Figure 3.18: Relative abundance of the subgroups of ciliates; a) before re-inoculation and b) after re-inoculation.

In Figure 3.19, the plot of the density (microorganism/mL) of the microfauna groups during the experimental period reveals three distinct phases. In the first stage the microfauna was dominated by free-swimming ciliates. This stage corresponds with the phase where COD removal was low, Figure 3.7. Then the total density of microfauna decreased until the need for a new activated sludge has been recognised and a re-inoculation made. After the re-inoculation an adaption stage was observed, of approximately 2 weeks. Here oscillation and succession of populations, created by the relationships of competition and predation, were observed until a dynamic stability was reached in stage 3. In stage 3, the stable phase, the microfauna were dominated by sessile ciliates, with free-swimming ciliates and testate amoebae usually observed in the mixed liquor. Carnivorous ciliates and metazoan were also occasionally detected. In the last weeks of the experimental period a large flagellate, *Peranema* sp., started to be noticed.

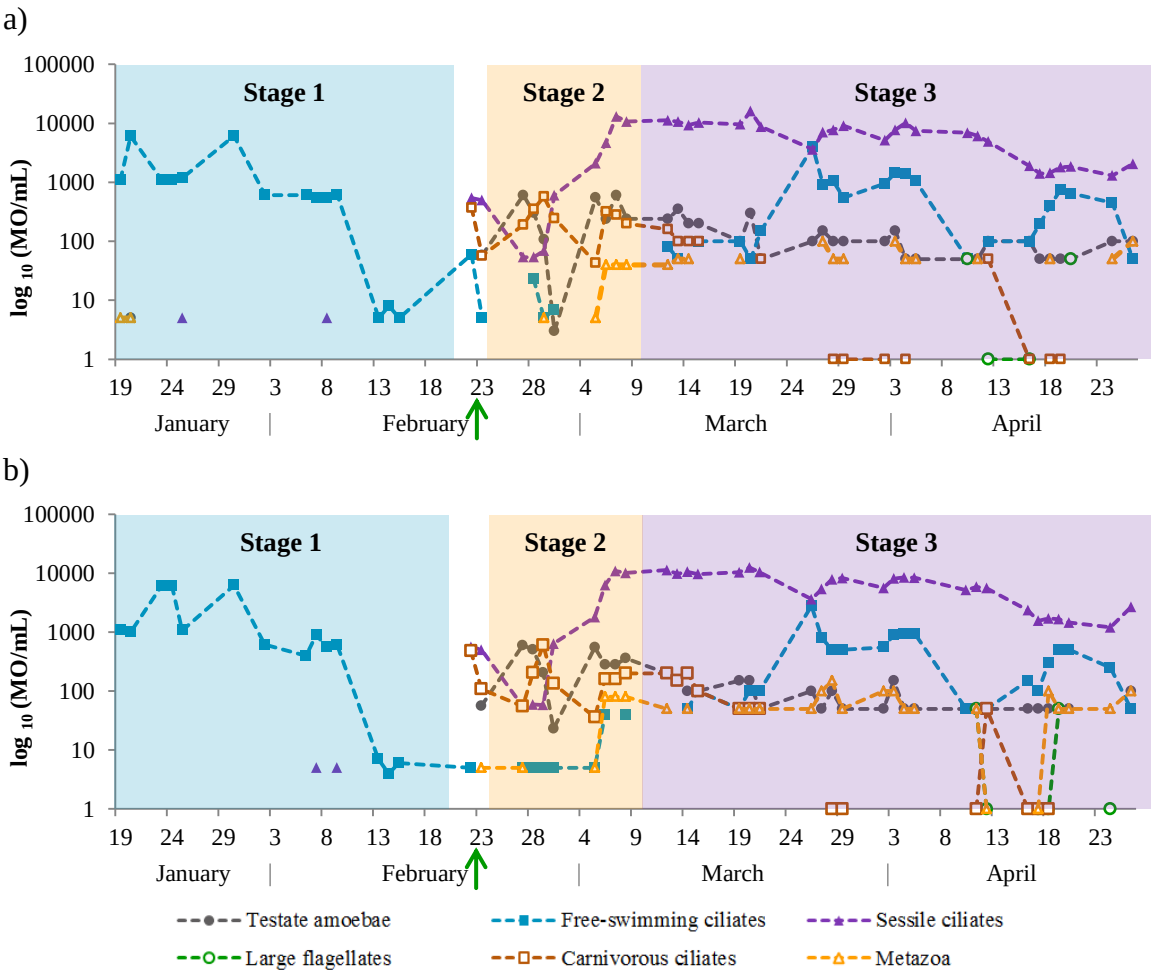
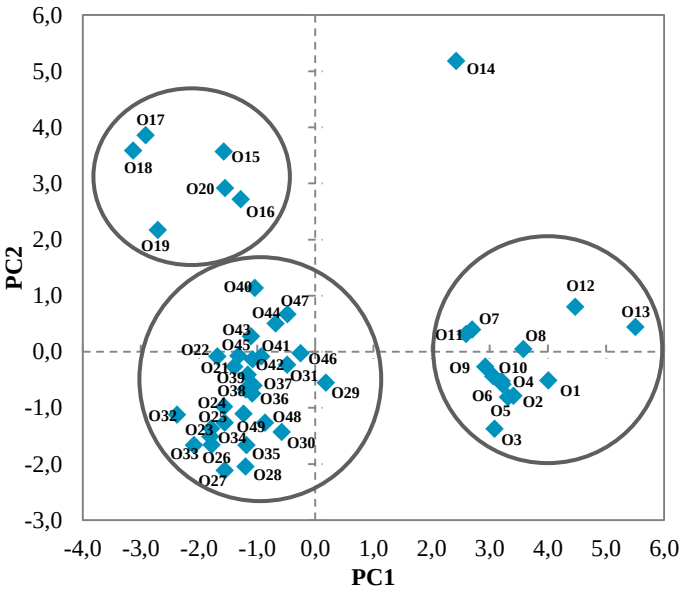


Figure 3.19: Succession of microorganism during the experimental period; a) aeration tank 1 and b) aeration tank 2, green arrow indicates the re-inoculation.

A PCA analysis was computed and the results support the existence of 3 distinct phases in the activated sludge system operation, identified in Figure 3.19. The two first principal components, which account for 48% and 49% of accumulated variance for the results from aeration tank 1 and aeration tank 2 respectively, are represented in Figure 3.20. As can be observed the PCA analysis placed the observations in three groups: a) observation 1 to observation 13; b) observation 15 to observation 20; and c) observation 21 to observation 49. The first group is equivalent to the first stage identified before and corresponds to a phase of a poor operation of the activated sludge. Observation 14 is placed alone and is representative of an extreme malfunction, since it corresponds to the day where the lower density of total microfauna and the lower COD removal (38,37%) values were noticed. The second group encloses the samples from the adaptation phase after the re-inoculation, and finally the third group represents a period of proper functioning of the activated sludge system experienced after the adaptation phase. This period is characterized by a microfauna community diversified, where sessile ciliates were the most important part of the community, total microfauna density usually around 8000 individuals/mL and excellent performance of COD removal (mean = 98,07 %).

a)



b)

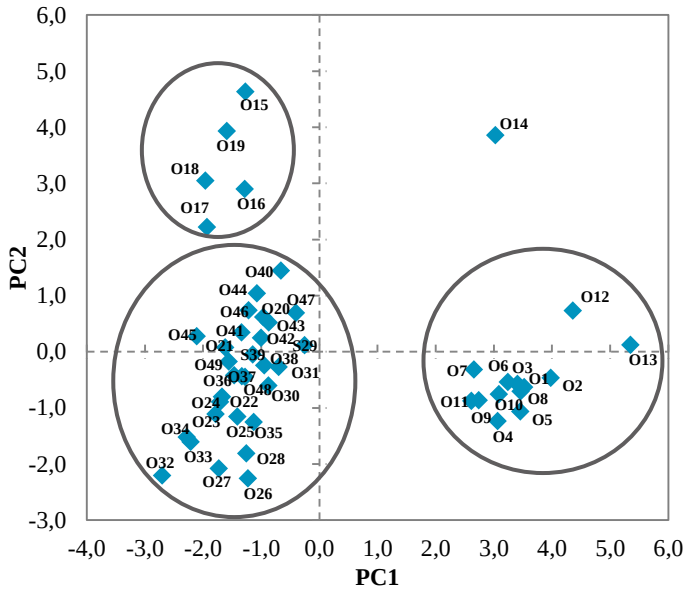


Figure 3.20: Schematic presentation of the two principal components which covered 48% and 49% of the total variability for AE 1 and AE 2, respectively; a) results obtained for samples from AE 1 and b) results obtained for samples from AE 2.

3.3.3. Correlation between physicochemical and biological parameters

To investigate the relation between biological and physicochemical parameters, the non-parametric correlation coefficient test of Spearman's rho was used to obtain a correlation matrix of all physicochemical parameters versus relative abundance of functional groups. The results are presented in Tables 3.5 and 3.6 and will be discussed in section 3.3.3.

Table 3.5: Correlation coefficients between functional groups and physiochemical parameters for aeration tank 1. ** correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed).

	% COD removed	Effluent COD	MLVSS	AN	TP	F/M	SRT	SVI	Temp (°C)
Testate amoebae	0,775**	-0,678**	-0,202	-0,396**	-0,294	0,194	-0,207	0,000	0,526**
Small flagellates	-0,568**	0,622**	0,414**	0,310*	-0,472	-0,150	0,413**	0,000	-0,482**
Large flagellates	0,055	-0,258	-0,190	0,144	0,324	-0,147	0,011	-0,612	-0,054
Free-swimming ciliates	-0,613**	0,592**	0,082	0,381**	-0,179	-0,193	0,327*	0,342	-0,675**
Sessile ciliates	0,600**	-0,622**	-0,164	-0,203	0,074	-0,121	-0,503**	-0,039	0,447**
Crawling ciliates	0,174	-0,235	-0,174	-0,175	0,100	0,125	0,098	0,204	0,152
***Opercularia* sp.**	0,701**	-0,475**	-0,111	-0,127	-0,092	-0,010	-0,192	-0,607	0,208
Carnivorous ciliates	0,489**	-0,560**	-0,132	-0,597**	0,019	0,415**	-0,184	0,414	0,371*
Metazoa	0,252	-0,139	0,106	-0,221	-0,017	-0,075	-0,370*	-0,445	0,392*
Diversity (No of genera)	0,604**	-0,550**	-0,037	-0,357*	-0,108	0,038	-0,403**	-0,869*	0,322
Total microfauna density	0,384**	-0,163	0,143	-0,228	-0,318	0,151	-0,368*	-0,500	0,570**

Table 3.6: Correlation coefficients between functional groups and physiochemical parameters for aeration tank 2. ** correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed); [a] not observed in aeration tank 2.

	% COD removed	Effluent COD	MLVSS	AN	TP	F/M	SRT	SVI	Temp (ºC)
Testate amoebae	0,735**	-0,711**	-0,326*	-0,265	-0,193	0,253	-0,198	-0,126	0,361*
Small flagellates	-0,433**	0,485**	0,218	0,229	-0,472	-0,062	0,394**	0,134	-0,464**
Large flagellates	0,164	-0,265	-0,234	-0,006	0,000	-0,093	-0,304*	0,000	-0,114
Free-swimming ciliates	-0,617**	0,582**	0,123	0,266	-0,055	-0,220	0,345*	0,143	-0,632**
Sessile ciliates	0,612**	-0,626**	-0,231	-0,266	0,074	-0,095	-0,478**	0,039	0,447**
Crawling ciliates [a]	--	--	--	--	--	--	--	--	--
***Opercularia* sp.**	0,589**	-0,331*	-0,030	-0,214	-0,146	-0,088	-0,289*	-0,559	0,463**
Carnivorous ciliates	0,523**	-0,516**	-0,143	-0,264	0,210	0,362*	-0,115	0,786*	0,349*
Metazoa	0,552**	-0,446**	-0,227	-0,191	-0,496	-0,140	-0,314*	-0,355	0,201
Diversity (No of genera)	0,692**	-0,534**	-0,163	-0,199	-0,266	0,011	-0,388**	-0,964**	0,343
Total microfauna density	0,368**	-0,139	0,220	-0,326*	-0,527	0,161	-0,324*	-0,500	0,550**

3.3.3.1. Testate amoebae

Testate amoebae showed a strong positive association with the efficiency of COD removal, with a correlation coefficient value of 0,775 and 0,735 for AE 1 and AE 2, respectively. Consequently, this keygroup also have a strong negative correlation with the effluent COD. The results also indicate that testate amoebae increase in density with increasing temperature (ρ = 0,526 and 0,361, for AE 1 and AE 2) and when N-ammoniacal concentration is low (ρ = -0,396 for AE 1). Although for AE 2 this last observation is not statistically significant.

These results agree with previous studies once testate amoebae are associated with systems with very low sludge load and with N-removal plants, as they are possible predators of nitrifying bacterial aggregates (Madoni, 1994; Pérez-Uz *et al.*, 2010). This group is also often seasonal being more common in summer when the temperature and growth rate increase (Madoni, 1994; Zhou *et al.*, 2008). Literature indicates that they are associated with plants with high SRT (Madoni, 1994), although in this case the results obtained are contradictory. As explained before the SRT values calculated are inaccurate since is not known the correct value of sludge purged out of the system. This may be the reason for the results obtained.

The correlations found suggest that this group can be used as bioindicators of the performance of the activated sludge process. They are associated with high quality of the final effluent and high efficiency of the plant.

3.3.3.2. Small flagellates

Small flagellates are associated with high effluent COD (ρ = 0,622 and 0,485) and low COD removal (ρ = -0,568 and -0,433), thus poor operation of the plant. The results also indicate that they increase in number when temperature decreases (ρ = -0,482 and -0,464). And according to results from AE 1, small flagellates are indicators of high MLSS concentration (ρ = 0,414).

Previous studies demonstrate that small heterotrophic flagellates presence in a mature activated sludge is associated with bad performance of the biological depuration due to (a) poorly aerated sludge, (b) over loading and/or (c) fermenting substances involved. They become the only protozoan present when the sludge is strongly loaded (F/M > 0,9). In a normally functioning plant these protozoa are strongly subjected to predacious activity of other protozoa and then their presence are limited to few individuals. This means that the increasing number of small flagellates indicates a dysfunction of the plant (Madoni, 1994). Bento *et al.* (2005) identified this group as the main indicator of an effluent with high suspended solids.

In summary, the presence of small flagellates is related with bad performance of the plant and low quality of the effluent. Thus this group can be used as a negative control group.

3.3.3.3. Large flagellates

Large flagellates only occurred in mixed liquor on the last samples analysed thus the results obtain are not conclusive. However, published studies reveal that large flagellates, such as *Peramena* sp., are infrequently observed and hardly in large numbers (Madoni, 1994). They are also related to good performance of N-elimination, very diluted organic matter and good performance of the depuration system (Madoni, 1994; Bento *et al.*, 2005; Pérez-Uz *et al.*, 2010).

The results of this study, although inclusive, tend to corroborate the hypothesis of using this group as good performance indicator since the correlation coefficient found to COD removal is positive and for effluent COD is negative.

3.3.3.4. Free-swimming ciliates

Usually free-swimming ciliates are more abundant in the early stages of a developing plant, when sludge flocs are still rare and consequently sessile ciliates are absent. They appear in a mature sludge if there are problems with the process (Madoni, 1994; Dubber and Gray, 2011). They dominate the microfauna of plants operating at low SRT or at high sludge loading (0,6 < F/M < 0,9) combined with lack of oxygen. These bacterivorous protozoans require high concentrations of dispersed bacteria and survive better than other components of the microfauna to the toxicity of the influent and to the lack of oxygen. This last observation can be attributed to the microaerophilic character of some species (Madoni, 1994; Papadimitriou *et al.*, 2010). This group of protozoa are also sometimes associated with heterotrophic flagellates and these two keygroups occasionally co-dominate the microfauna (Madoni, 1994). Free-swimming ciliates dominate when lower effluent quality is obtained, and are indicators of poor settling proprieties of the activated sludge and bad performance of the treatment (Lee *et al.*, 2004).

For the activated sludge system in study, free swimming ciliates are associated with poor COD removal (ρ = -0,613 and -0,617) and final effluent with high organic matter (ρ = 0,592 and 0,582). Once again the results obtained for the correlation with SRT are inconsistent with what would be expected (ρ = 0,327 and 0,345). This keygroup is also correlated with low temperature (ρ = -0,675 and -0,632), what is explained by the presence of ciliates bellowing to *Tetrahymena* genus (Serrano *et al.*, 2008). In particular *Tetrahymena thermophila* abundance increases when BOD increases in biological reactors and when low water temperatures are experienced in the aeration tanks (Esteban *et al.*, 1991).

Thus, free-swimming ciliates group can be used as a bioindicator of malfunction of the activated sludge process.

3.3.3.5. Sessile ciliates

Peritrich ciliates are normally co-dominant in the activated sludge along with crawling ciliates. A massive increase in their number occurs in occasion of transient conditions, which reduces the plant performance. Sessile ciliates are able to grow through a large range of sludge loadings, nevertheless at F/M values ranging from 0,3 to 0,6 they dominate and for F/M values of 0,6 – 0,9 sessile ciliates and flagellates co-dominate (Madoni, 1994). Sessile ciliates are one of the most representative groups in a stable aeration tank, since they are best adapted to the activated sludge environment through their ability to associate to the flocs (Zhou *et al.*, 2008). Bento *et al.* (2005) identified this group of ciliates as indicator of low organic matter concentration in the effluent and high BOD removal.

The result of the correlation analysis shows that for this system sessile ciliates presence is indicative of high performance of the plant. This group increases in number when the COD removal increases (ρ = 0,600 and 0,612) and consequently the effluent produced is of higher quality (ρ = -0,622 and -0,626). Contrary to results reported in literature, in this system the dominance of sessile ciliates does not suggest transient conditions that reduce the plant performance. Moreover when the higher performance of the plant was achieved, sessile ciliates were the dominant microfaunal group. However it must bear in mind that sessile ciliates were the dominant group, but not the only one observed in these conditions.

For this activated sludge system, this group of ciliates can be used as a bioindicator of high depuration efficiency.

3.3.3.6. Crawling ciliates

Crawling ciliates were never observed in aeration tank 2 and only observed once in the aeration tank 1. So, nothing can be concluded concerning the influence of the physicochemical parameters in the crawling ciliates population for this system.

Dubber and Gray (2011) concluded that *T. cucullulus*, a specie of crawling ciliates, only occurs in high abundances in plants where ammonium and phosphate concentrations are lower than 3 mg/L NH_4-N and 2 mg/L PO_4-P respectively. Thus the absence of this group can be, possibly, related to the phosphorous concentration, Figure 3.6a.

3.3.3.7. *Opercularia* sp.

According to literature the genus Opercularia must be excluded by the sessile keygroup. These ciliates are often observed in low number in the activated sludge, nevertheless they have association with the variables concerned with the quality of the activated sludge. Their numbers increase when the activated sludge is of bad quality. *Opercularia* sp. are associated with high final effluent BOD concentration and high N-ammoniacal concentration. These species are among the most abundant forms at high loadings and are able to survive to severe lack of oxygen (Madoni, 1994; Lee *et al.*, 2004).

Although *Opercularia* sp. are suggested as indicators of a bad performance, the results of this study tend to point in the opposite direction. This can be explained by the fact that *Opercularia* sp. only appeared in the system after re-inoculation, when the higher COD removal values were noticed, and always with low abundance. Before the re-inoculation, peritrich ciliates were almost absent.

3.3.3.8. Carnivorous ciliates

Carnivorous ciliates as the suctorids *Podophrya* and *Tokophrya* are common in WWTP but with low abundances. They are associated with a high effluent quality and low F/M (Lee *et al.*, 2004; Serrano *et al.*, 2008).

The results obtained reveal that these ciliates are related with high COD removal (ρ = 0,489 and 0,523) and high effluent quality (ρ = -0,560 and -0,516). In relation to F/M ratio the results are contradictory to what would be expected (ρ = 0,415 and 0,362). Once again this can be explained by the re-inoculation. Before re-inoculation, when the F/M ratio was very low, carnivorous ciliates was not observed because they are associated with process with well-functioning and before re-inoculation the system was not working properly.

Therefor this group can also be used as an indicator of good performance of the system.

3.3.3.9. Metazoan

Metazoan density was always low, thus the results obtained do not allow a correlation between the physiochemical parameters and the metazoan population. Although they only appeared in the system after the re-inoculation and the results from the aeration tank 2 suggest that they are associated with high COD removal. So, this group can possibly be used as an indicator of proper functioning. Metazoans are associated with high SRT, unless they do not have enough time to reproduce (Bento *et al.*, 2005; Gerardi, 2006). However the results show a negative association with SRT, which can be explained by the inaccuracy of the SRT determination, as explained for testate amoebae.

3.3.3.10. Diversity and density

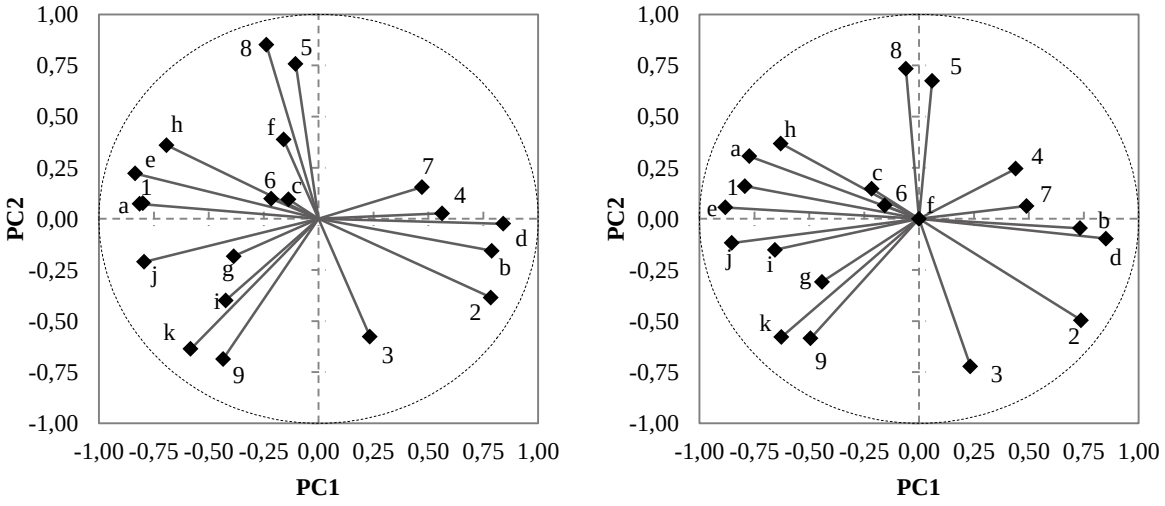
The determination of sludge biotic index (SBI), discussed before, considers both density and diversity of the microfauna. In other words, the density and diversity of the microfauna are highly correlated with the plant performance (Madoni, 2011). The activated sludge system can be categorized in three classes according to microfaunal abundance, specifically protozoa density: (a) inefficient systems, with approximately 10 individuals/mL; (b) systems with low efficiency, densities between 10 – 10^3 individuals/mL; and (c) efficient systems with more than 10^3 individuals/mL (Madoni, 1994; Bento *et al.*, 2005). A normally functioning system has a microfauna highly diversified, namely composed by different groups of organisms. By the other side, a microfauna that is dominated by one group is almost indicative of trophic imbalance, due to the existence of limiting factors impeding the development of other species (Madoni, 2011).

In summary, an activated sludge system with good functioning must have highly diversified and abundant microfauna. The results of this study supports this conclusion, since the correlation coefficients found to both diversity and density shows positive correlation with COD removal efficiency and negative correlation with effluent COD.

3.3.3.11. Correlation circles

The correlation circle enables a graphic visualization of the correlations between microfauna groups and physicochemical parameters discussed. In Figure X can be observed that small flagellates and free-swimming ciliates are related to effluent COD and MLVSS. Testate amoebae, sessile ciliates and carnivorous ciliates are correlated with high COD removal and inversely associated to MLVSS and effluent COD. Also, diversity and density of total microfauna appears to be temperature dependent. The circle of correlations also shows that the first component contrast COD removal efficiency (1) with effluent COD (2), and bioindicators of good performance (a and e) with bioindicators of poor performance (b and d). This means that the first component is related to process performance and thus confirms that the groups identified in Figure 3.20 are also related to process efficiency.

a) b)



Legend:

1	% COD removed	**a**	Testate amoebae
2	Effluent COD	**b**	Small flagellates
3	MLVSS	**c**	Large flagellates
4	N-ammoniacal	**d**	Free-swimming ciliates
5	Phosphorous	**e**	Sessile ciliates
6	F/M	**f**	Crawling ciliates
7	SRT	**g**	*Opercularia* sp.
8	SVI	**h**	Carnivorous ciliates
9	Temperature	**i**	Metazoans
		j	Diversity (Number of genera identified)
		k	Density

Figure 3.21: Correlation circles of measured parameters obtained by PCA (PC1 and PC2).

4. CONCLUSIONS

One of the features of industrial wastewater is the change in effluent characteristics over time, due to unusual discharges for instance. This feature was observed in the wastewater treated in the IWWTP in study, since the results revealed that the effluent produced by the biodiesel production unit has COD values very variable over time. This has impact in the physicochemical treatment performance. Usually this stage of treatment removes over 70% of suspended solids and consequently the majority of the insoluble COD present in wastewater. Although when the influent COD is low, the performance of the treatment decreases. Possibly this is related with the oil concentration present in the influent. Also, pH control is crucial to ensure maximum efficiency of the physicochemical treatment.

The biological treatment is more complex and has more variables that must be controlled. This treatment generally removes over 80% of the influent soluble COD, and the removal efficiency was improved after re-inoculation. The results showed a nutrient imbalance that can be softened with the introduction of a chemical coagulant in DAF 1, which would precipitate a percentage of the phosphorous present in water. Furthermore, the purge or waste sludge removal system is ineffective, causing a fluctuation in MLVSS concentration and in SRT. An improvement of the waste sludge removal system and determination of the desired sludge purge considering a standard SRT value is recommended. The F/M ratio shows instability over time. In some cases this ratio value was so low that caused cell lysis. The results indicate that the activated sludge system was designed taking into account a higher flow of effluent to be treated. Thus, it is suggested to reduce the useful volume of the bioreactors to 80 m^3 and to work with a MLVSS concentration between 5,0 and 7,0 g/L. These new conditions allow maintaining the F/M ratio more constant and around 0,3.

Three distinct phases were identified in the functioning of the activated sludge. The first corresponds to the beginning of the study and is characterized by a malfunction of the system. In this stage the microfauna was dominated by free-swimming ciliates and small flagellates were found very frequently. The second phase refers to a stabilization period after the re-inoculation, where oscillation and succession of microorganisms population were observed until a dynamic equilibrium was reached. The last phase is the equilibrium and is characterized by a proper operation of the system. The microfauna in this phase is diversified and dominated by sessile ciliates. A correlation analysis between physiochemical operation parameters and the relative abundance of microfauna groups

enabled the establishment of positive and negative control groups. The negative control group is composed by free-swimming ciliates and small flagellates and the positive control group comprises sessile ciliates, testate amoebae and carnivorous ciliates. Although inconclusive, the results suggest the inclusion of large flagellates in the positive control group.

The microscopic observation of the activated sludge, using the control groups found, for the evaluation of the efficiency of the system, should be included in the daily control of the IWWTP. This analysis allows a quick and easy classification of the process performance and allows anticipation of future problems.

5. REFERENCES

Abdi, H. and Williams, L.J. (2010) Principal component analysis, *Wiley Interdisciplinary Reviews: Computational Statistics*, **2**(4), 433-459.

Adlan, M.N.; Palaniandy, P. and Aziz, H.A. (2011) Optimization of coagulation and dissolved air flotation (DAF) treatment of semi-aerobic landfill leachate using response surface methodology (RSM), *Desalination*, **277**(1-3), 74-82.

Al-Shamrani, A.A.; James, A. and Xiao, H. (2002a) Destabilisation of oil-water emulsions and separation by dissolved air flotation, *Water research*, **36**(6), 1503-1512.

Al-Shamrani, A.A.; James, A. and Xiao, H. (2002b) Separation of oil from water by dissolved air flotation, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **209**(1), 15-26.

Ali, M.A.J.; Abeer, A.-F.A.; Ali, A.-H.A. and Suhaib, A.-K.A. (2011) The Effect of Suspended Solid Concentration and Oil Droplet Size Distribution on Particle Coalesces and Emulsion Stability, em SPE/DGS Annual Technical Symposium and Exhibition: Society of Petroleum Engineers.

Alley, E.R. (2007) Water quality control handbook, 2 ed., New York; Alexandria, Va.: McGraw-Hill.

American Public Health Association (1999) Standard methods for the examination of water and wastewater, 19 ed., Washington DC.

Amjad, Z. (2010) The science and technology of industrial water treatment, 1 ed., Boca Raton: Taylor & Francis.

Avella, A.C.; Görner, T.; Yvon, J.; Chappe, P.; Guinot-Thomas, P. and de Donato, P. (2011) A combined approach for a better understanding of wastewater treatment plants operation: Statistical analysis of monitoring database and sludge physico-chemical characterization, *Water research*, **45**(3), 981-992.

Bento, A.P.; Sezerino, P.H.; Philippi, L.S.; Reginatto, V. and Lapolli, F.R. (2005) Caracterização da microfauna em estação de tratamento de esgotos do tipo lodos ativados: um instrumento de avaliação e controle do processo, *Engenharia sanitária e ambiental*, **10**(4), 329-338.

Bick, H. (1972) Ciliated protozoa: an illustrated guide to the species used as biological indicators in freshwater biology, 1 ed., Geneva: World Health Organization.

Bitton, G. (2005) Wastewater microbiology, 1 ed., Hoboken, N.J.: Wiley-Liss, John Wiley & Sons.

Black, J.G. (2002) Microbiology: principles and explorations, 1 ed., New York: John Wiley &Sons.

Burgess, J.E.; Mayhew, M.E.; Stephenson, T. and Clark, T.A. (2002) Assessment of Microbial Populations in Activated Sludge Using an Organism Diversity Index, *Water and Environment Journal*, **16**(1), 40-45.

Chatzakis, M.K.; Lyrintzis, A.G.; Mara, D.D. and Angelakis, A.N. (2006) Sedimentation tanks through the ages, em 1st International Symposium on Water and Wastewater Technologies in Ancient Civilizations, Iraklio, Greece.

Cheremisinoff, N.P. (2006) Handbook of water and wastewater treatment technologies, 1 ed., Boston: Butterworth-Heinemann.

Chua, H.; Yu, P.H.F.; Sin, S.N. and Tan, K.N. (2000) Effect of food:microrganism ratio in activated sludge foam control, *Applied Biochemistry and Biotechnology*, **84-86**(1-9), 1127-1135.

Crossley, I.A. and Valade, M.T. (2006) A review of the technological developments of dissolved air flotation, *Journal of Water Supply: Research and Technology*, (7-8), 479-491.

Csuros, M. and Csuros, C. (1999) Microbiological examination of water and wastewater, 1 ed., Boca Raton, FL: Lewis Publishers.

Czaczyk, K. and Myszka, K. (2007) Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation, *Polish Journal of Environmental Studies*, **16**(6), 799-806.

Dafnopatidou, E.K. and Lazaridis, N.K. (2008) Dyes Removal from Simulated and Industrial Textile Effluents by Dissolved-Air and Dispersed-Air Flotation Techniques, *Industrial & Engineering Chemistry Research*, **47**(15), 5594-5601.

de Nardi, I.R.; Fuzi, T.P. and Del Nery, V. (2008) Performance evaluation and operating strategies of dissolved-air flotation system treating poultry slaughterhouse wastewater, *Resources, Conservation and Recycling*, **52**(3), 533-544.

Decreto-Lei n.º 73/2011 (2011) Decreto-Lei n.º 73/2011 de 1 de julho.

Decreto-Lei n.º 236/98 (1998) Decreto-Lei n.º 236/98 de 1 de agosto.

Deng, S.; Yu, G.; Chen, Z.; Wu, D.; Xia, F. and Jiang, N. (2009) Characterization of suspended solids in produced water in Daqing oilfield, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **332**(1), 63-69.

Drzewicki, A. and Kulikowska, D. (2011) Limitation of Sludge Biotic Index application for control of a wastewater treatment plant working with shock organic and ammonium loadings, *European Journal of Protistology*, **47**(4), 287-294.

Dubber, D. and Gray, N.F. (2009) Enumeration of protozoan ciliates in activated sludge: Determination of replicate number using probability, *Water research*, **43**(14), 3443-3452.

Dubber, D. and Gray, N.F. (2011) The influence of fundamental design parameters on ciliates community structure in Irish activated sludge systems, *European Journal of Protistology*, **47**(4), 274-286.

Ebel, L.A. (1994) Preliminary treatment for wastewater facilities: manual of pratice OM-2, 1 ed., Alexandria: Water environment federation.

Eckenfelder, W.W. and Grau, P. (1992) Activated sludge process design and control: theory and practice, 1 ed., Lancaster, Basel: Technomic Publishing Company.

Edzwald, J.K. (2010) Dissolved air flotation and me, *Water research*, **44**(7), 2077-2106.

Edzwald, J.K. and W.W.A.A. (2011) Water quality & treatment: a handbook on drinking water, 1 ed., New York: McGraw-Hill.

Ennos, A.R. (2007) Statistical and Data Handling Skills in Biology, 1 ed.: Pearson/Prentice Hall.

Esteban, G.; Tellez, C. and Miguel Bautista, L. (1991) Effects of habitat quality on ciliated protozoa communities in sewage treatment plants, *Environmental Technology*, **12**(4), 381-386.

Eurostat (2010) Environmental statistics and accounts in Europe, 1 ed., Luxembourg: Publications Office of the European Union.

Fujii, S.; Okada, M. and Furuzono, T. (2007) Hydroxyapatite nanoparticles as stimulus-responsive particulate emulsifiers and building block for porous materials, *Journal of Colloid and Interface Science*, **315**(1), 287-296.

Ganczarczyk, J.J. (1983) Activated sludge process: theory and practice, 1 ed., New York: Marcel Dekker.

Gerardi, M.H. (2006) Wastewater bacteria, 1 ed., Hoboken, N.J.: Wiley-Interscience.

Ginoris, Y.P.; Amaral, A.L.; Nicolau, A.; Coelho, M.A.Z. and Ferreira, E.C. (2007a) Development of an image analysis procedure for identifying protozoa and metazoa typical of activated sludge system, *Water research*, **41**(12), 2581-2589.

Ginoris, Y.P.; Amaral, A.L.; Nicolau, A.; Coelho, M.A.Z. and Ferreira, E.C. (2007b) Raw data pre-processing in the protozoa and metazoa identification by image analysis and multivariate statistical techniques, *Journal of Chemometrics*, **21**(3-4), 156-164.

Gray, N.F. (2004) Biology of wastewater treatment, 2 ed., London: Imperial College Press.

Gray, N.F. (2005) Water technology, 1 ed., Oxford; Amsterdam; Paris: Elsevier.

Han, M.; Kim, T.-i. and Kim, J. (2007) Effects of floc and bubble size on the efficiency of the dissolved air flotation (DAF) process, *Water science and technology*, **56**(10), 109-115.

Hanafy, M. and Nabih, H.I. (2007) Treatment of oily wastewater using dissolved air flotation technique, *Energy sources, part a: recovery, utilization, and environmental effects*, **29**(2), 143-159.

Henze, M.; Harremoës, P.; Jansen, J.l.C. and Arvin, E. (1997) Wastewater treatment: biological and chemical processes, 2 ed., Berlin; New York: Springer.

Jefferson, B.; Burgess, J.E.; Pichon, A.; Harkness, J. and Judd, S.J. (2001) Nutrient addition to enhance biological treatment of greywater, *Water research*, **35**(11), 2702-2710.

Jenkins, D. (1993) Manual on the causes and control of activated sludge bulking and foaming, 2 ed., Boca Raton: Lewis Publishers.

Jern, N.W. (2006) Industrial wastewater treatment, 1 ed., London: Imperial College Press.

Jördening, H.-J. and Winter, J. (2005) Environmental biotechnology: concepts and applications, 1 ed., Weinheim: Wiley-VCH.

Koivunen, J. and Heinonen-Tanski, H. (2008) Dissolved air flotation (DAF) for primary and tertiary treatment of municipal wastewaters, *Environmental Technology*, **29**(1), 101-109.

Lee, S.; Basu, S.; Tyler, C.W. and Wei, I.W. (2004) Ciliate populations as bio-indicators at Deer Island Treatment Plant, *Advances in Environmental Research*, **8**(3–4), 371-378.

Li, A.-j.; Li, X.-y. and Yu, H.-q. (2011) Effect of the food-to-microorganism (F/M) ratio on the formation and size of aerobic sludge granules, *Process Biochemistry*, **46**(12), 2269-2276.

Lin, S.D. and Lee, C.C. (2007) Water and wastewater calculations manual, 2 ed., New York: McGraw-Hill.

Liu, Y. and Fang, H.H.P. (2003) Influences of extracellular polymeric substances (EPS) on flocculation, settling, and dewatering of activated sludge, *Critical reviews in environmental science and technology*, **33**(3), 237-273.

Lofrano, G. and Brown, J. (2010) Wastewater management through the ages: A history of mankind, *Science of the Total Environment*, **408**(22), 5254-5264.

Lopez, C.; Pons, M.N. and Morgenroth, E. (2005) Evaluation of microscopic techniques (epifluorescence microscopy, CLSM, TPE-LSM) as a basis for the quantitative image analysis of activated sludge, *Water research*, **39**(2–3), 456-468.

Madoni, P. (1994) A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis, *Water research*, **28**(1), 67-75.

Madoni, P. (2011) Protozoa in wastewater treatment processes: A minireview, *Italian Journal of Zoology*, **78**(1), 3-11.

Martifer and Prio (2008a) Neutralização/Desgomagem, in Manual de Operações.

Martifer and Prio (2008b) Transesterificação, in Manual de Operações.

Miller, J.N. and Miller, J.C. (2000) Statistics and chemometrics for analytical chemistry, 1 ed.: Prentice Hall.

Moussa, M.S.; Hooijmans, C.M.; Lubberding, H.J.; Gijzen, H.J. and van Loosdrecht, M.C.M. (2005) Modelling nitrification, heterotrophic growth and predation in activated sludge, *Water research*, **39**(20), 5080-5098.

Ni, B.-J.; Rittmann, B.E. and Yu, H.-Q. (2010) Modeling predation processes in activated sludge, *Biotechnology and Bioengineering*, **105**(6), 1021-1030.

Ntougias, S.; Tanasidis, S. and Melidis, P. (2011) Microfaunal indicators, Ciliophora phylogeny and protozoan population shifts in an intermittently aerated and fed bioreactor, *Journal of Hazardous Materials*, **186**(2–3), 1862-1869.

Ouano, E.A.R. (1983) Principles of wastewater treatment. vol: 1, Biological processes, 1 ed., Manila; Philippines: National Science Development Board.

Papadimitriou, C.A.; Papatheodoulou, A.; Takavakoglou, V.; Zdragas, A.; Samaras, P.; Sakellaropoulos, G.P.; Lazaridou, M. and Zalidis, G. (2010) Investigation of protozoa as indicators of wastewater treatment efficiency in constructed wetlands, *Desalination*, **250**(1), 378-382.

Pérez-Uz, B.; Arregui, L.; Calvo, P.; Salvadó, H.; Fernández, N.; Rodríguez, E.; Zornoza, A. and Serrano, S. (2010) Assessment of plausible bioindicators for plant performance in advanced wastewater treatment systems, *Water research*, **44**(17), 5059-5069.

Ratledge, C. and Kristiansen, B. (2006) Basic biotechnology, 3 ed., Cambridge, U.K.; New York: Cambridge University Press.

Rattanapan, C.; Sawain, A.; Suksaroj, T. and Suksaroj, C. (2011) Enhanced efficiency of dissolved air flotation for biodiesel wastewater treatment by acidification and coagulation processes, *Desalination*, **280**(1-3), 370-377.

Redox (2010) Manual FBR Martifer, 1 ed..

Ribeiro, R.R.; Lima, A.P.; Ginoris, Y.P. and Coelho, M.A.Z. (2005) Remoção biológica de fenol e nitrogênio amoniacal de efluentes em reator batelada sequencial, em COBEQIC, S. Paulo, Brasil.

Rojas, C.; Rincón, N.; Díaz, A.; Colina, G.; Behling, E.; Chacín, E. and Fernández, N. (2008) Evaluation of dissolved air flotation unit for oil produced water, *Evaluación de una unidad de flotación con aire disuelto para el tratamiento de aguas aceitosas*, **31**(1), 50-57.

Rubio, J.; Souza, M.L. and Smith, R.W. (2002) Overview of flotation as a wastewater treatment technique, *Minerals Engineering*, **15**(3), 139-155.

Serrano, S.; Arregui, L.; Perez-Uz, B.; Calvo, P. and Guinea, A. (2008) Guidelines for the identification of ciliates in wastewater treatment plants, 1 ed., London: IWA Publishing.

Shon, H.K.; Vigneswaran, S. and Snyder, S.A. (2006) Effluent organic matter (EfOM) in wastewater: Constituents, effects, and treatment, *Critical reviews in environmental science and technology*, **36**(4), 327-374.

SNF Ambientagua (2008) Ficha de especificações técnicas: Ambifloc BIO 865 F.

Spellman, F.R. (2003) Handbook of water & wastewater treatment plant operations, 1 ed., Boca Raton: Lewis.

Tchobanoglous, G.; Burton, F.L.; Stensel, H.D.; Metcalf and Eddy (2003) Wastewater engineering: treatment and reuse, 1 ed., Boston: McGraw-Hill.

Vesilind, P.A. (2003) Wastewater treatment plant design, 1 ed., Alexandria, Va.: Water Environment Federation.

Wiesmann, U.; Choi, I.S. and Dombrowski, E.-M. (2007) Fundamentals of biological wastewater treatment, 1 ed., Weinheim: Wiley-VCH.

Wilén, B.-M.; Onuki, M.; Hermansson, M.; Lumley, D. and Mino, T. (2008) Microbial community structure in activated sludge floc analysed by fluorescence in situ hybridization and its relation to floc stability, *Water research*, **42**(8–9), 2300-2308.

Yiannakopoulou, T.V. (2010) An ecosystem analysis of the activated sludge microbial community, *Journal of Environmental Science and Health, Part A*, **45**(5), 587-602.

Yücesoy, E.; Lüdemann, N.; Lucas, H.; Tan, J. and Denecke, M. (2012) Protein analysis as a measure of active biomass in activated sludge, *Water science and technology*, **65**(8), 1483-1489.

Zhou, K.; Xu, M.; Liu, B. and Cao, H. (2008) Characteristics of microfauna and their relationships with the performance of an activated sludge plant in China, *Journal of Environmental Sciences*, **20**(4), 482-486.

6. ANNEXES

6.1. Physicochemical analysis

Table A.1: Results of the physicochemical analysis of the influent and effluent from DAF 1.

Sample	Date	COD in (mg/L)	COD out (mg/L)	% COD removed	TS in (g/L)	TS out (g/L)	% TS removed	pH in	pH out
1	03-01-2012	13970 ± 10	12130 ± 10	13,17 ± 0,10	2,778 ± 0,023	1,930 ± 0,020	30,32 ± 1,13	4,80 ± 0,01	4,79 ± 0,01
2	04-01-2012	12980 ± 10	10420 ± 10	19,72 ± 0,11	2,513 ± 0,021	1,701 ± 0,020	32,27 ± 1,19	4,90 ± 0,01	4,90 ± 0,01
3	05-01-2012	11000 ± 10	9930 ± 10	9,730 ± 0,13	2,435 ± 0,017	1,690 ± 0,020	30,45 ± 1,10	4,87 ± 0,01	4,82 ± 0,01
4	09-01-2012	11330 ± 10	8780 ± 10	22,51 ± 0,13	2,256 ± 0,016	1,410 ± 0,020	37,33 ± 1,17	4,86 ± 0,01	4,89 ± 0,01
5	10-01-2012	9900 ± 10	8240 ± 10	16,77 ± 0,14	2,016 ± 0,025	1,150 ± 0,020	43,07 ± 1,68	4,81 ± 0,01	4,85 ± 0,01
6	11-01-2012	17930 ± 10	14160 ± 10	21,03 ± 0,08	1,989 ± 0,013	1,160 ± 0,021	41,71 ± 1,27	5,01 ± 0,01	5,08 ± 0,01
7	12-01-2012	18810 ± 10	12100 ± 10	35,67 ± 0,08	2,984 ± 0,026	1,350 ± 0,020	54,70 ± 1,20	5,45 ± 0,01	5,43 ± 0,01
8	13-01-2012	25800 ± 10	9800 ± 10	62,02 ± 0,06	2,312 ± 0,023	1,410 ± 0,020	38,96 ± 1,37	6,64 ± 0,01	6,67 ± 0,01
9	16-01-2012	19580 ± 10	7270 ± 10	62,87 ± 0,08	4,075 ± 0,022	1,630 ± 0,021	60,05 ± 0,81	5,92 ± 0,01	5,79 ± 0,01
10	17-01-2012	15180 ± 10	6490 ± 10	57,25 ± 0,10	3,584 ± 0,027	1,310 ± 0,021	63,41 ± 1,07	6,03 ± 0,01	5,91 ± 0,01
11	18-01-2012	14410 ± 10	5990 ± 10	58,43 ± 0,11	3,791 ± 0,012	1,360 ± 0,021	64,12 ± 0,67	6,24 ± 0,01	6,09 ± 0,01
12	19-01-2012	16940 ± 10	6400 ± 10	62,22 ± 0,09	3,875 ± 0,015	1,640 ± 0,022	57,73 ± 0,72	5,78 ± 0,01	5,71 ± 0,01
13	20-01-2012	20020 ± 10	6990 ± 10	65,08 ± 0,08	3,834 ± 0,022	1,490 ± 0,020	61,10 ± 0,85	5,53 ± 0,01	5,60 ± 0,01
14	21-01-2012	9240 ± 10	5520 ± 10	40,26 ± 0,16	3,832 ± 0,028	1,430 ± 0,020	62,66 ± 1,01	5,53 ± 0,01	5,60 ± 0,01
15	22-01-2012	17160 ± 10	7280 ± 10	57,58 ± 0,09	3,287 ± 0,023	1,430 ± 0,022	56,53 ± 1,05	5,48 ± 0,01	5,42 ± 0,01
16	23-01-2012	12430 ± 10	5910 ± 10	52,45 ± 0,12	2,133 ± 0,023	1,230 ± 0,021	42,25 ± 1,53	5,53 ± 0,01	5,48 ± 0,01
17	24-01-2012	9020 ± 10	5640 ± 10	37,47 ± 0,16	1,846 ± 0,024	1,020 ± 0,019	44,86 ± 1,76	5,50 ± 0,01	5,42 ± 0,01
18	25-01-2012	8800 ± 10	5700 ± 10	35,23 ± 0,17	1,614 ± 0,021	1,080 ± 0,020	32,92 ± 1,85	5,12 ± 0,01	5,23 ± 0,01
19	26-01-2012	13200 ± 10	6300 ± 10	52,27 ± 0,11	2,631 ± 0,017	1,650 ± 0,020	37,26 ± 1,03	5,08 ± 0,01	5,02 ± 0,01
20	27-01-2012	15950 ± 10	8140 ± 10	48,97 ± 0,09	2,059 ± 0,018	1,250 ± 0,020	39,32 ± 1,35	5,01 ± 0,01	5,08 ± 0,01
21	29-01-2012	16490 ± 10	6660 ± 10	59,61 ± 0,09	2,057 ± 0,023	1,250 ± 0,020	39,32 ± 1,55	5,77 ± 0,01	5,83 ± 0,01
22	30-01-2012	16490 ± 10	6660 ± 10	59,61 ± 0,09	8,144 ± 0,025	2,210 ± 0,019	72,85 ± 0,45	5,54 ± 0,01	5,45 ± 0,01
23	31-01-2012	16490 ± 10	7598 ± 10	53,92 ± 0,09	6,612 ± 0,025	1,110 ± 0,020	83,18 ± 0,58	5,72 ± 0,01	5,63 ± 0,01

24	01-02-2012	16490 ± 10	9683 ± 10	41,28 ± 0,09	6,085 ± 0,029	1,310 ± 0,020	78,65 ± 0,69	5,49 ± 0,01	5,46 ± 0,01
25	02-02-2012	16490 ± 10	10500 ± 10	36,33 ± 0,09	5,487 ± 0,009	1,640 ± 0,019	70,13 ± 0,40	5,95 ± 0,01	5,65 ± 0,01
26	08-02-2012	16490 ± 10	7190 ± 10	56,40 ± 0,09	14,61 ± 0,012	3,520 ± 0,020	75,91 ± 0,17	6,06 ± 0,01	6,62 ± 0,01
27	09-02-2012	16490 ± 10	8200 ± 10	50,27 ± 0,09	26,26 ± 0,020	3,190 ± 0,020	87,85 ± 0,13	6,71 ± 0,01	6,54 ± 0,01
28	10-02-2012	16490 ± 10	7610 ± 10	53,85 ± 0,09	20,44 ± 0,031	3,190 ± 0,020	84,39 ± 0,22	6,58 ± 0,01	6,40 ± 0,01
29	11-02-2012	16490 ± 10	7610 ± 10	53,85 ± 0,09	20,44 ± 0,009	3,190 ± 0,021	84,39 ± 0,12	5,27 ± 0,01	5,34 ± 0,01
30	12-02-2012	16490 ± 10	7610 ± 10	53,85 ± 0,09	20,44 ± 0,013	3,190 ± 0,021	84,39 ± 0,13	5,25 ± 0,01	5,21 ± 0,01
31	13-02-2012	16490 ± 10	7108 ± 10	56,89 ± 0,09	15,93 ± 0,023	1,710 ± 0,020	89,33 ± 0,23	5,14 ± 0,01	5,11 ± 0,01
32	20-02-2012	44990 ± 10	3210 ± 10	92,87 ± 0,04	18,93 ± 0,027	1,390 ± 0,021	92,66 ± 0,22	5,30 ± 0,01	5,23 ± 0,01
33	22-02-2012	40040 ± 10	3440 ± 10	91,41 ± 0,04	14,39 ± 0,028	1,260 ± 0,022	91,24 ± 0,30	5,26 ± 0,01	5,20 ± 0,01
34	23-02-2012	65780 ± 10	3530 ± 10	94,63 ± 0,03	22,29 ± 0,022	1,310 ± 0,020	94,12 ± 0,16	5,11 ± 0,01	5,08 ± 0,01
35	24-02-2012	82060 ± 10	3890 ± 10	95,26 ± 0,02	22,29 ± 0,024	1,310 ± 0,020	94,12 ± 0,17	5,15 ± 0,01	5,08 ± 0,01
36	26-02-2012	146300 ± 10	5640 ± 10	96,14 ± 0,01	22,29 ± 0,023	1,310 ± 0,021	94,12 ± 0,17	5,12 ± 0,01	5,07 ± 0,01
37	27-02-2012	50050 ± 10	5710 ± 10	88,59 ± 0,03	15,04 ± 0,020	1,090 ± 0,021	92,75 ± 0,23	5,09 ± 0,01	5,05 ± 0,01
38	28-02-2012	43340 ± 10	6780 ± 10	84,36 ± 0,04	15,44 ± 0,020	1,440 ± 0,020	90,67 ± 0,22	5,01 ± 0,01	4,98 ± 0,01
39	29-02-2012	75900 ± 10	8250 ± 10	89,13 ± 0,02	20,30 ± 0,018	1,580 ± 0,020	92,22 ± 0,16	5,21 ± 0,01	5,09 ± 0,01
40	01-03-2012	58190 ± 10	7850 ± 10	86,51 ± 0,03	16,06 ± 0,015	1,310 ± 0,020	91,91 ± 0,18	5,13 ± 0,01	5,09 ± 0,01
41	02-03-2012	63030 ± 10	8550 ± 10	86,44 ± 0,03	16,06 ± 0,018	1,320 ± 0,019	91,91 ± 0,19	5,19 ± 0,01	5,07 ± 0,01
42	05-03-2012	69410 ± 10	6790 ± 10	90,22 ± 0,02	18,07 ± 0,019	1,030 ± 0,021	94,30 ± 0,19	5,27 ± 0,01	5,14 ± 0,01
43	06-03-2012	77110 ± 10	7350 ± 10	90,47 ± 0,02	21,47 ± 0,032	1,390 ± 0,020	93,53 ± 0,22	5,20 ± 0,01	5,30 ± 0,01
44	07-03-2012	181390 ± 10	15920 ± 10	91,22 ± 0,01	34,71 ± 0,028	1,720 ± 0,020	95,04 ± 0,13	5,14 ± 0,01	5,23 ± 0,01
45	08-03-2012	182080 ± 10	13700 ± 10	92,48 ± 0,01	30,52 ± 0,023	1,540 ± 0,021	94,95 ± 0,12	4,90 ± 0,01	4,93 ± 0,01
46	12-03-2012	89100 ± 10	8340 ± 10	90,64 ± 0,02	24,74 ± 0,024	1,910 ± 0,020	92,28 ± 0,15	5,37 ± 0,01	5,38 ± 0,01
47	13-03-2012	85030 ± 10	12320 ± 10	85,51 ± 0,02	19,67 ± 0,027	2,450 ± 0,020	87,54 ± 0,21	4,71 ± 0,01	4,75 ± 0,01
48	14-03-2012	66330 ± 10	14360 ± 10	78,35 ± 0,02	17,36 ± 0,023	2,160 ± 0,021	87,56 ± 0,21	4,67 ± 0,01	4,65 ± 0,01
49	15-03-2012	59840 ± 10	15260 ± 10	74,50 ± 0,03	16,43 ± 0,023	2,830 ± 0,022	82,78 ± 0,23	4,95 ± 0,01	4,89 ± 0,01
50	19-03-2012	64130 ± 10	13740 ± 10	78,57 ± 0,03	17,34 ± 0,022	2,060 ± 0,022	88,12 ± 0,21	5,11 ± 0,01	4,98 ± 0,01
51	20-03-2012	72380 ± 10	14040 ± 10	80,60 ± 0,02	18,25 ± 0,022	2,310 ± 0,022	87,34 ± 0,20	4,99 ± 0,01	4,95 ± 0,01

52	21-03-2012	74470 ± 10	15780 ± 10	78,81 ± 0,02	19,53 ± 0,021	2,070 ± 0,020	89,40 ± 0,18	4,99 ± 0,01	4,96 ± 0,01
53	26-03-2012	124190 ± 10	14900 ± 10	88,00 ± 0,01	30,56 ± 0,026	2,330 ± 0,020	92,38 ± 0,13	5,22 ± 0,01	5,05 ± 0,01
54	27-03-2012	109340 ± 10	12900 ± 10	88,20 ± 0,02	38,58 ± 0,025	1,210 ± 0,020	96,86 ± 0,10	5,08 ± 0,01	5,07 ± 0,01
55	28-03-2012	127270 ± 10	10460 ± 10	91,78 ± 0,01	33,84 ± 0,025	2,450 ± 0,020	92,76 ± 0,12	5,23 ± 0,01	5,20 ± 0,01
56	29-03-2012	181390 ± 10	5810 ± 10	96,80 ± 0,01	35,24 ± 0,017	2,610 ± 0,020	92,62 ± 0,09	5,95 ± 0,01	5,99 ± 0,01
57	02-04-2012	100320 ± 10	5040 ± 10	94,98 ± 0,02	31,76 ± 0,018	1,080 ± 0,020	96,60 ± 0,10	6,25 ± 0,01	6,12 ± 0,01
58	03-04-2012	86790 ± 10	4560 ± 10	94,75 ± 0,02	27,64 ± 0,018	1,220 ± 0,020	95,59 ± 0,12	6,31 ± 0,01	6,19 ± 0,01
59	04-04-2012	118250 ± 10	4740 ± 10	95,99 ± 0,01	37,13 ± 0,015	1,030 ± 0,020	97,23 ± 0,08	6,22 ± 0,01	6,38 ± 0,01
60	05-04-2012	109450 ± 10	4210 ± 10	96,15 ± 0,02	38,51 ± 0,008	1,040 ± 0,019	97,30 ± 0,06	6,72 ± 0,01	6,61 ± 0,01
61	10-04-2012	70950 ± 10	377 ± 100	94,69 ± 0,02	23,87 ± 0,021	1,320 ± 0,019	94,47 ± 0,14	5,98 ± 0,01	5,96 ± 0,01
62	11-04-2012	72710 ± 10	4480 ± 10	93,84 ± 0,02	21,57 ± 0,022	1,080 ± 0,020	94,99 ± 0,17	5,94 ± 0,01	5,85 ± 0,01
63	12-04-2012	75020 ± 10	5020 ± 10	93,31 ± 0,02	22,82 ± 0,023	1,30 ± 0,020	94,30 ± 0,16	6,14 ± 0,01	6,28 ± 0,01
64	16-04-2012	56100 ± 10	6720 ± 10	88,02 ± 0,03	14,75 ± 0,024	0,930 ± 0,020	93,69 ± 0,26	5,92 ± 0,01	5,95 ± 0,01
65	17-04-2012	48510 ± 10	7120 ± 10	85,32 ± 0,03	10,69 ± 0,024	1,140 ± 0,022	89,34 ± 0,36	5,88 ± 0,01	5,84 ± 0,01
66	18-04-2012	43670 ± 10	7500 ± 10	82,83 ± 0,04	10,53 ± 0,026	1,090 ± 0,020	89,65 ± 0,38	5,72 ± 0,01	5,63 ± 0,01
67	19-04-2012	38720 ± 10	6760 ± 10	82,54 ± 0,04	12,98 ± 0,021	2,90 ± 0,020	77,66 ± 0,26	4,66 ± 0,01	4,66 ± 0,01
68	20-04-2012	47630 ± 10	8710 ± 10	81,71 ± 0,03	12,16 ± 0,022	3,120 ± 0,020	74,51 ± 0,28	4,16 ± 0,01	4,24 ± 0,01
69	24-04-2012	41030 ± 10	14330 ± 10	65,07 ± 0,04	8,540 ± 0,024	3,150 ± 0,020	63,11 ± 0,41	4,88 ± 0,01	4,81 ± 0,01
70	26-04-2012	31460 ± 10	13240 ± 10	57,91 ± 0,05	7,380 ± 0,021	2,790 ± 0,022	62,20 ± 0,45	5,36 ± 0,01	5,34 ± 0,01

Table A.2.1: Results of the physicochemical analysis of the influent, effluent and mixed liquor from the activated sludge system.
a) WWTP without loading

Sample	Date	COD in (mg/L)	COD AE 1 (mg/L)	COD AE 2 (mg/L)	COD out (mg/L)	% COD removed	MLVSS AE 1 (g/L)	MLVSS AE 2 (g/L)
1	19-01-2012	6400 ± 10	2040 ± 10	2450 ± 10	1611 ± 1	74,83 ± 0,20	8,512 ± 0,018	8,304 ± 0,019
2	20-01-2012	6990 ± 10	2380 ± 10	2200 ± 10	1589 ± 1	77,27 ± 0,18	8,000 ± 0,019	7,904 ± 0,019
3	23-01-2012	5910 ± 10	1650 ± 10	1780 ± 10	1296 ± 1	78,07 ± 0,22	8,120 ± 0,020	8,072 ± 0,018
4	24-01-2012	5640 ± 10	1390 ± 10	1570 ± 10	1276 ± 1	77,38 ± 0,22	7,992 ± 0,021	8,064 ± 0,018
5	25-01-2012	5700 ± 10	1470 ± 10	1610 ± 10	1460 ± 1	74,39 ± 0,22	8,392 ± 0,020	8,232 ± 0,020
6	30-01-2012	6660 ± 10	1680 ± 10	1760 ± 10	1420 ± 1	78,68 ± 0,19	7,536 ± 0,018	7,144 ± 0,018
7	02-02-2012	10500 ± 10	1680 ± 10	2420 ± 10	1420 ± 1	86,48 ± 0,13	6,560 ± 0,020	7,224 ± 0,021
8	06-02-2012	17220 ± 10	1680 ± 10	1980 ± 10	1420 ± 1	91,75 ± 0,08	7,920 ± 0,020	8,168 ± 0,020
9	07-02-2012	11250 ± 10	1844,3 ± 10,1	2075,5 ± 12,1	1420 ± 1	87,38 ± 0,12	7,920 ± 0,019	8,168 ± 0,020
10	08-02-2012	7190 ± 10	1393,2 ± 9,2	942,3 ± 9,1	1420 ± 1	80,25 ± 0,18	7,872 ± 0,019	7,888 ± 0,020
11	09-02-2012	8200 ± 10	1393,6 ± 12,4	704,6 ± 10,1	1420 ± 1	82,68 ± 0,16	7,008 ± 0,018	8,080 ± 0,020
12	13-02-2012	7108 ± 10	1393,5 ± 9,1	2500,4 ± 10,1	1420 ± 1	80,02 ± 0,18	7,664 ± 0,020	7,400 ± 0,019
13	14-02-2012	7422 ± 10	2304,0 ± 9,1	2816,2 ± 9,1	1420 ± 1	80,87 ± 0,17	8,408 ± 0,021	8,136 ± 0,018
14	15-02-2012	0 a	2359,0 ± 10,1	2731,5 ± 11,1	1420 ± 1	38,37 ± 0,47	6,432 ± 0,021	5,400 ± 0,018
15	22-02-2012	3440 ± 10	270 ± 10	320 ± 10	147 ± 1	95,73 ± 0,40	6,480 ± 0,022	6,536 ± 0,018
16	23-02-2012	3530 ± 10	320 ± 10	430 ± 10	103 ± 1	97,08 ± 0,40	6,600 ± 0,018	6,680 ± 0,021
17	27-02-2012	5710 ± 10	440 ± 10	510 ± 10	86 ± 1	98,49 ± 0,25	6,904 ± 0,019	6,704 ± 0,018
18	28-02-2012	6780 ± 10	460 ± 10	530 ± 10	77 ± 1	98,86 ± 0,21	7,008 ± 0,019	7,128 ± 0,018
19	29-02-2012	8250 ± 10	340 ± 10	430 ± 10	118 ± 1	98,57 ± 0,17	7,280 ± 0,018	7,096 ± 0,018
20	01-03-2012	7850 ± 10	380 ± 10	520 ± 10	113 ± 1	98,56 ± 0,18	7,368 ± 0,017	7,600 ± 0,022
21	05-03-2012	6790 ± 10	410 ± 10	660 ± 10	146 ± 1	97,85 ± 0,21	7,584 ± 0,023	7,632 ± 0,018
22	06-03-2012	7350 ± 10	350 ± 10	530 ± 10	145 ± 1	98,03 ± 0,19	7,488 ± 0,022	7,848 ± 0,019
23	07-03-2012	15920 ± 10	860 ± 10	1310 ± 10	210 ± 1	98,68 ± 0,09	7,952 ± 0,021	8,056 ± 0,018

24	08-03-2012	13700 ± 10	640 ± 10	980 ± 10	257 ± 1	98,12 ± 0,10	7,584 ± 0,020	7,784 ± 0,018
25	12-03-2012	8340 ± 10	380 ± 10	740 ± 10	259 ± 1	96,89 ± 0,17	8,008 ± 0,020	8,056 ± 0,021
26	13-03-2012	12320 ± 10	420 ± 10	570 ± 10	393 ± 1	96,81 ± 0,11	7,304 ± 0,021	9,024 ± 0,020
27	14-03-2012	14360 ± 10	500 ± 10	560 ± 10	249 ± 1	98,27 ± 0,10	8,504 ± 0,018	8,704 ± 0,018
28	15-03-2012	15260 ± 10	870 ± 10	1270 ± 10	371 ± 1	97,57 ± 0,09	8,600 ± 0,019	8,416 ± 0,018
29	19-03-2012	13740 ± 10	423 ± 1	516 ± 1	338 ± 1	97,54 ± 0,10	7,448 ± 0,019	7,392 ± 0,021
30	20-03-2012	14040 ± 10	395 ± 1	426 ± 1	185 ± 1	98,68 ± 0,10	7,720 ± 0,018	7,872 ± 0,018
31	21-03-2012	15780 ± 10	352 ± 1	405 ± 1	260 ± 1	98,35 ± 0,09	7,160 ± 0,021	7,864 ± 0,022
32	26-03-2012	14900 ± 10	327 ± 1	501 ± 1	178 ± 1	98,81 ± 0,09	6,712 ± 0,022	7,816 ± 0,018
33	27-03-2012	12900 ± 10	322 ± 1	861 ± 1	211 ± 1	98,36 ± 0,11	7,872 ± 0,023	8,024 ± 0,019
34	28-03-2012	10460 ± 10	312 ± 1	572 ± 1	160 ± 1	98,47 ± 0,13	8,080 ± 0,021	8,008 ± 0,018
35	29-03-2012	5810 ± 10	244 ± 1	421 ± 1	261 ± 1	95,51 ± 0,24	8,456 ± 0,023	8,288 ± 0,019
36	02-04-2012	5040 ± 10	244 ± 1	482 ± 1	140 ± 1	97,22 ± 0,28	7,040 ± 0,019	6,800 ± 0,019
37	03-04-2012	4560 ± 10	210 ± 1	410 ± 1	162 ± 1	96,45 ± 0,31	6,960 ± 0,019	7,144 ± 0,019
38	04-04-2012	4740 ± 10	224 ± 1	447 ± 1	182 ± 1	96,16 ± 0,29	6,976 ± 0,019	7,112 ± 0,018
39	05-04-2012	4210 ± 10	225 ± 1	412 ± 1	133 ± 1	96,84 ± 0,33	6,864 ± 0,020	6,848 ± 0,018
40	10-04-2012	3770 ± 10	329 ± 1	434 ± 1	128 ± 1	96,60 ± 0,37	7,008 ± 0,020	6,800 ± 0,021
41	11-04-2012	4480 ± 10	265 ± 1	469 ± 1	132 ± 1	97,05 ± 0,31	6,992 ± 0,021	7,088 ± 0,020
42	12-04-2012	5020 ± 10	262 ± 1	526 ± 1	155 ± 1	96,91 ± 0,28	7,336 ± 0,018	7,160 ± 0,018
43	16-04-2012	6720 ± 10	255 ± 1	975 ± 1	137 ± 1	97,96 ± 0,21	6,920 ± 0,018	6,992 ± 0,020
44	17-04-2012	7120 ± 10	245 ± 1	685 ± 1	168 ± 1	97,64 ± 0,20	7,056 ± 0,018	6,520 ± 0,018
45	18-04-2012	7500 ± 10	267 ± 1	697 ± 1	199 ± 1	97,35 ± 0,19	6,656 ± 0,020	6,880 ± 0,017
46	19-04-2012	6760 ± 10	355 ± 1	459 ± 1	133 ± 1	98,03 ± 0,21	7,752 ± 0,018	7,824 ± 0,019
47	20-04-2012	8710 ± 10	364 ± 1	637 ± 1	169 ± 1	98,06 ± 0,16	7,272 ± 0,018	7,064 ± 0,018
48	24-04-2012	14330 ± 10	779 ± 1	847 ± 1	167 ± 1	98,83 ± 0,10	7,792 ± 0,019	7,808 ± 0,020
49	26-04-2012	13240 ± 10	732 ± 1	1210 ± 1	259 ± 1	98,04 ± 0,11	7,664 ± 0,019	7,536 ± 0,020

Table A.2.2: Results of the physicochemical analysis of the influent, effluent and mixed liquor from the activated sludge system.
b) Without results

Sample	Date	pH AE 1	pH AE 2	N-ammoniacal AE 1 (mg/L)	N-ammoniacal AE 2 (mg/L)	Phosphorus (mg/L)	Temp. (ºC)	SRT (d)	F/M	Organic loading (kg/d)
1	19-01-2012	7,54 ± 0,01	7,48 ± 0,01	2,43 ± 0,01	b	b	18,0 ± 0,5	16 ± 0,13	0,22 ± 0,0008	407,36 ± 0,64
2	20-01-2012	6,94 ± 0,01	6,91 ± 0,01	b	b	b	19,0 ± 0,5	58 ± 1,62	0,22 ± 0,0008	389,48 ± 0,56
3	23-01-2012	7,54 ± 0,01	5,52 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	45,9 ± 0,1	19,0 ± 0,5	31 ± 0,51	0,19 ± 0,0007	344,49 ± 0,58
4	24-01-2012	7,49 ± 0,01	7,45 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	b	19,0 ± 0,5	42 ± 1,04	0,20 ± 0,0008	351,88 ± 0,62
5	25-01-2012	7,37 ± 0,01	7,39 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	b	20,0 ± 0,5	17 ± 0,15	0,17 ± 0,0007	310,59 ± 0,54
6	30-01-2012	7,19 ± 0,01	7,30 ± 0,01	3,64 ± 0,01	3,64 ± 0,01	b	25,0 ± 0,5	46 ± 0,78	0,17 ± 0,0006	268,46 ± 0,40
7	02-02-2012	7,36 ± 0,01	7,41 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	b	28,0 ± 0,5	250 ± 5,02	0,05 ± 0,0002	77,91 ± 0,07
8	06-02-2012	7,39 ± 0,01	7,44 ± 0,01	b	b	b	28,0 ± 0,5	1033 ± 20,57	0,02 ± 0,0001	36,16 ± 0,02
9	07-02-2012	7,45 ± 0,01	7,47 ± 0,01	3,64 ± 0,01	3,64 ± 0,01	b	28,0 ± 0,5	70 ± 1,32	0,20 ± 0,0007	350,21 ± 0,31
10	08-02-2012	7,47 ± 0,01	7,43 ± 0,01	3,64 ± 0,01	3,64 ± 0,01	b	28,0 ± 0,5	20 ± 0,11	0,10 ± 0,0004	181,19 ± 0,25
11	09-02-2012	7,41 ± 0,01	7,44 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	b	28,0 ± 0,5	13 ± 0,09	0,24 ± 0,0009	404,75 ± 0,49
12	13-02-2012	6,78 ± 0,01	6,76 ± 0,01	4,86 ± 0,01	4,86 ± 0,01	b	21,0 ± 0,5	23 ± 0,24	0,20 ± 0,0008	323,56 ± 0,46
13	14-02-2012	7,02 ± 0,01	7,04 ± 0,01	4,44 ± 0,01	5,61 ± 0,01	b	22,0 ± 0,5	1047 ± 14,79	0,01 ± 0,0000	10,54 ± 0,01
14	15-02-2012	7,26 ± 0,01	7,12 ± 0,01	5,24 ± 0,01	5,54 ± 0,01	191,1 ± 0,1	24,5 ± 0,5	b	b	b
15	22-02-2012	7,40 ± 0,01	7,35 ± 0,01	1,87 ± 0,01	1,95 ± 0,01	98,3 ± 0,1	27,6 ± 0,5	19 ± 0,30	0,16 ± 0,0009	232,85 ± 0,68
16	23-02-2012	7,40 ± 0,01	7,42 ± 0,01	1,82 ± 0,01	1,91 ± 0,01	b	25,0 ± 0,5	23 ± 0,34	0,15 ± 0,0008	222,67 ± 0,63
17	27-02-2012	7,52 ± 0,01	7,46 ± 0,01	1,08 ± 0,01	1,91 ± 0,01	77,1 ± 0,1	25,0 ± 0,5	25 ± 0,37	0,22 ± 0,0009	334,04 ± 0,59
18	28-02-2012	7,44 ± 0,01	7,41 ± 0,01	1,17 ± 0,01	1,94 ± 0,01	b	25,0 ± 0,5	25 ± 0,43	0,29 ± 0,0012	455,89 ± 0,67
19	29-02-2012	7,37 ± 0,01	7,35 ± 0,01	0,87 ± 0,01	1,99 ± 0,01	b	28,0 ± 0,5	11 ± 0,07	0,32 ± 0,0012	508,70 ± 0,62
20	01-03-2012	7,33 ± 0,01	7,25 ± 0,01	0,95 ± 0,01	1,88 ± 0,01	b	30,5 ± 0,5	10 ± 0,06	0,26 ± 0,0010	424,76 ± 0,54
21	05-03-2012	7,46 ± 0,01	7,47 ± 0,01	1,21 ± 0,01	1,35 ± 0,01	68,9 ± 0,1	30,5 ± 0,5	14 ± 0,15	0,26 ± 0,0011	442,71 ± 0,65
22	06-03-2012	7,59 ± 0,01	7,41 ± 0,01	1,02 ± 0,01	1,78 ± 0,01	b	29,8 ± 0,5	11 ± 0,06	0,16 ± 0,0007	277,02 ± 0,38
23	07-03-2012	7,42 ± 0,01	7,39 ± 0,01	1,26 ± 0,01	2,06 ± 0,01	b	28,0 ± 0,5	14 ± 0,10	0,43 ± 0,0015	760,50 ± 0,48

24	08-03-2012	7,42 ± 0,01	7,39 ± 0,01	1,23 ± 0,01	1,69 ± 0,01	b	36,0 ± 0,5	15 ± 0,11	0,37 ± 0,0013	632,53 ± 0,46
25	12-03-2012	7,45 ± 0,01	7,51 ± 0,01	0,87 ± 0,01	0,36 ± 0,01	69,9 ± 0,1	36,2 ± 0,5	12 ± 0,12	0,41 ± 0,0016	725,00 ± 0,87
26	13-03-2012	7,42 ± 0,01	7,37 ± 0,01	0,33 ± 0,01	0,33 ± 0,01	b	34,0 ± 0,5	9 ± 0,03	0,17 ± 0,0006	308,49 ± 0,25
27	14-03-2012	7,35 ± 0,01	7,38 ± 0,01	0,14 ± 0,01	0,14 ± 0,01	b	25,5 ± 0,5	12 ± 0,04	0,17 ± 0,0005	327,98 ± 0,23
28	15-03-2012	7,45 ± 0,01	7,46 ± 0,01	0,43 ± 0,01	0,46 ± 0,01	b	28,0 ± 0,5	12 ± 0,04	0,12 ± 0,0004	221,73 ± 0,15
29	19-03-2012	7,50 ± 0,01	7,56 ± 0,01	41 ± 1	39 ± 1	78,5 ± 0,1	24,6 ± 0,5	269 ± 3,03	0,04 ± 0,0001	58,26 ± 0,04
30	20-03-2012	7,57 ± 0,01	7,58 ± 0,01	38 ± 1	39 ± 1	b	28,0 ± 0,5	33 ± 0,17	0,13 ± 0,0004	225,76 ± 0,16
31	21-03-2012	7,53 ± 0,01	7,54 ± 0,01	29 ± 1	29 ± 1	b	28,0 ± 0,5	b	0,21 ± 0,0009	343,69 ± 0,22
32	26-03-2012	7,49 ± 0,01	7,54 ± 0,01	1,21 ± 0,01	1,31 ± 0,01	67,1 ± 0,1	28,0 ± 0,5	15 ± 0,11	0,37 ± 0,0015	587,95 ± 0,39
33	27-03-2012	7,61 ± 0,01	7,61 ± 0,01	1,51 ± 0,01	2,48 ± 0,01	b	28,0 ± 0,5	19 ± 0,11	0,20 ± 0,0008	348,04 ± 0,27
34	28-03-2012	7,61 ± 0,01	7,55 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	b	28,0 ± 0,5	7 ± 0,03	0,22 ± 0,0008	394,34 ± 0,38
35	29-03-2012	7,60 ± 0,01	7,51 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	b	28,0 ± 0,5	8 ± 0,03	0,09 ± 0,0004	167,97 ± 0,29
36	02-04-2012	7,56 ± 0,01	7,43 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	58,5 ± 0,1	28,0 ± 0,5	9 ± 0,04	0,12 ± 0,0005	181,29 ± 0,36
37	03-04-2012	7,45 ± 0,01	7,48 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	b	28,0 ± 0,5	6 ± 0,02	0,12 ± 0,0005	181,35 ± 0,40
38	04-04-2012	7,45 ± 0,01	7,41 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	b	28,0 ± 0,5	4 ± 0,02	0,15 ± 0,0007	238,56 ± 0,50
39	05-04-2012	7,46 ± 0,01	7,35 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	b	28,0 ± 0,5	6 ± 0,02	0,13 ± 0,0006	193,53 ± 0,46
40	10-04-2012	7,06 ± 0,01	7,16 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	120,8 ± 0,1	28,0 ± 0,5	6 ± 0,03	0,10 ± 0,0005	149,78 ± 0,40
41	11-04-2012	7,23 ± 0,01	7,16 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	b	25,0 ± 0,5	6 ± 0,03	0,16 ± 0,0007	247,74 ± 0,55
42	12-04-2012	7,25 ± 0,01	7,16 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	b	25,0 ± 0,5	7 ± 0,04	0,21 ± 0,0008	332,32 ± 0,66
43	16-04-2012	7,27 ± 0,01	7,22 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	75,7 ± 0,1	25,0 ± 0,5	57 ± 1,18	0,17 ± 0,0007	256,84 ± 0,38
44	17-04-2012	7,30 ± 0,01	7,21 ± 0,01	2,43 ± 0,01	2,43 ± 0,01	b	25,0 ± 0,5	3 ± 0,01	0,19 ± 0,0008	284,30 ± 0,40
45	18-04-2012	7,15 ± 0,01	7,18 ± 0,01	1,21 ± 0,01	1,21 ± 0,01	b	30,0 ± 0,5	18 ± 0,09	0,10 ± 0,0004	143,10 ± 0,19
46	19-04-2012	7,23 ± 0,01	7,12 ± 0,01	1,77 ± 0,01	1,72 ± 0,01	b	30,0 ± 0,5	13 ± 0,08	0,18 ± 0,0006	301,56 ± 0,45
47	20-04-2012	7,12 ± 0,01	7,00 ± 0,01	1,34 ± 0,01	2,45 ± 0,01	b	25,0 ± 0,5	116 ± 1,70	0,07 ± 0,0003	117,93 ± 0,14
48	24-04-2012	7,20 ± 0,01	7,18 ± 0,01	4,27 ± 0,01	1,99 ± 0,01	b	30,0 ± 0,5	12 ± 0,04	0,10 ± 0,0004	171,96 ± 0,12
49	26-04-2012	7,04 ± 0,01	6,95 ± 0,01	2,66 ± 0,01	3,50 ± 0,01	b	30,0 ± 0,5	15 ± 0,06	0,09 ± 0,0003	153,05 ± 0,12

6.2. Microfauna analysis

Table A.3: Results of the microfauna analysis for aeration tank 1, small flagellates abundance evaluated through a subjective scoring and the others in individuals/mL.

Sample	Date	Testate amoebae	Small flagellates	Large flagellates	Free swimming ciliates	Sessile ciliates	Crawling	*Opercularia* sp.	Carnivorous ciliates	Metazoa
1	19-01-2012	0	++++	0	1100	0	0	0	0	5
2	20-01-2012	5	++++	0	6050	0	0	0	0	5
3	23-01-2012	0	+++	0	1105	0	0	0	0	0
4	24-01-2012	0	+++	0	1092	0	0	0	0	0
5	25-01-2012	0	+++	0	1191	5	0	0	0	0
6	30-01-2012	0	++++	0	6102	0	0	0	0	0
7	02-02-2012	0	++	0	605	0	0	0	0	0
8	06-02-2012	0	++	0	604	0	0	0	0	0
9	07-02-2012	0	++	0	554	0	0	0	0	0
10	08-02-2012	0	++	0	548	5	0	0	0	0
11	09-02-2012	0	+	0	605	0	0	0	0	0
12	13-02-2012	0	+	0	5	0	0	0	0	0
13	14-02-2012	0	+	0	8	0	0	0	0	0
14	15-02-2012	0	-	0	5	0	0	0	0	0
15	22-02-2012	0	-	0	60	543	0	5	370	0
16	23-02-2012	58	-	0	5	498	0	5	57	0
17	27-02-2012	606	-	0	0	54	5	5	190	0
18	28-02-2012	304	-	0	23	54	0	5	350	0
19	29-02-2012	107	-	0	5	69	0	5	570	5
20	01-03-2012	3	-	0	7	605	0	5	245	0
21	05-03-2012	550	-	0	0	2098	0	0	43	5
22	06-03-2012	240	-	0	0	4680	0	280	320	40

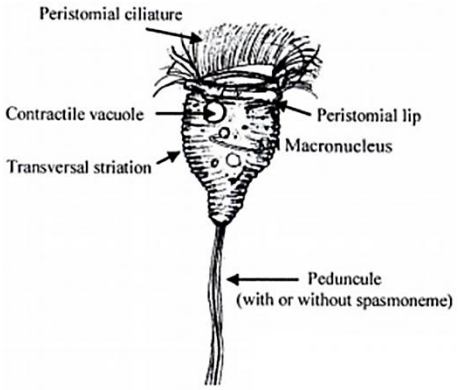
23	07-03-2012	600	-	0	0	13040	0	160	280	40
24	08-03-2012	240	-	0	0	10680	0	240	200	40
25	12-03-2012	240	+	0	80	11280	0	40	160	40
26	13-03-2012	350	-	0	50	10600	0	300	100	50
27	14-03-2012	200	-	0	0	9300	0	600	100	50
28	15-03-2012	200	-	0	100	10300	0	600	100	0
29	19-03-2012	100	-	0	100	9650	0	450	0	50
30	20-03-2012	300	-	0	50	16100	0	1100	0	0
31	21-03-2012	50	-	0	150	8750	0	650	50	0
32	26-03-2012	100	-	0	4000	3600	0	4000	0	0
33	27-03-2012	150	-	0	900	7050	0	2850	0	100
34	28-03-2012	100	-	0	1050	7750	0	1700	1	50
35	29-03-2012	100	-	0	550	9100	0	400	1	50
36	02-04-2012	100	-	0	950	5150	0	400	1	0
37	03-04-2012	150	-	0	1450	7750	0	0	0	100
38	04-04-2012	50	-	0	1400	10200	0	0	1	50
39	05-04-2012	50	-	0	1050	7450	0	1	0	50
40	10-04-2012	50	-	50	50	6900	0	150	0	0
41	11-04-2012	50	-	0	0	6100	0	100	0	50
42	12-04-2012	100	-	1	100	4900	0	100	50	0
43	16-04-2012	100	-	1	100	1900	0	150	1	0
44	17-04-2012	50	++	0	200	1400	0	100	0	0
45	18-04-2012	50	++	0	400	1450	0	150	1	50
46	19-04-2012	50	++	0	750	1800	0	100	1	0
47	20-04-2012	50	++	50	650	1850	0	100	0	0
48	24-04-2012	100	++	0	450	1300	0	100	0	50
49	26-04-2012	100	++	0	50	2050	0	0	0	100

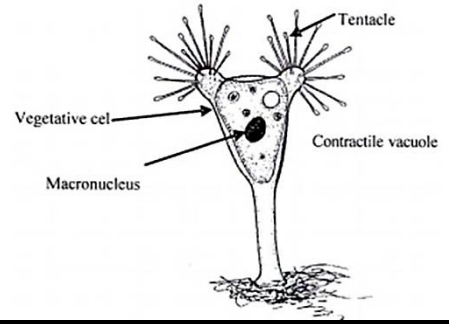
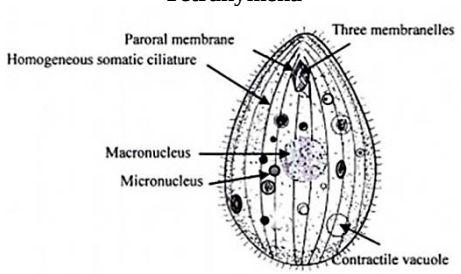
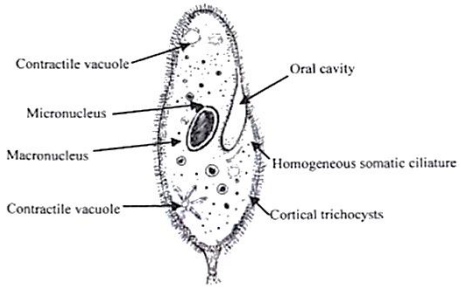
Table A.4: Results of the microfauna analysis for aeration tank 2, small flagellates abundance evaluated through a subjective scoring and the others in individuals/mL.

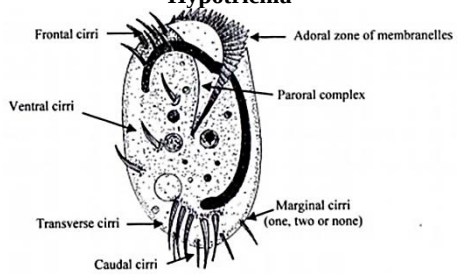
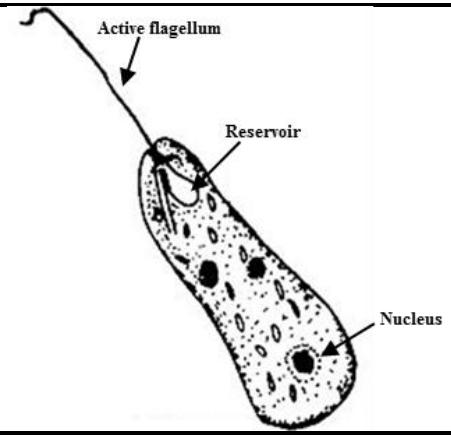
Sample	Date	Testate amoebae	Small flagellates	Large flagellates	Free swimming ciliates	Sessile ciliates	Crawling	*Opercularia* sp.	Carnivorous ciliates	Metazoa
1	19-01-2012	0	++	0	1108	0	0	0	0	0
2	20-01-2012	0	++++	0	1003	0	0	0	0	0
3	23-01-2012	0	+++	0	6082	0	0	0	0	0
4	24-01-2012	0	+++	0	6021	0	0	0	0	0
5	25-01-2012	0	+++	0	1067	0	0	0	0	0
6	30-01-2012	0	++++	0	6201	0	0	0	0	0
7	02-02-2012	0	++	0	610	0	0	0	0	0
8	06-02-2012	0	+	0	398	0	0	0	0	0
9	07-02-2012	0	+	0	902	5	0	0	0	0
10	08-02-2012	0	++	0	551	0	0	0	0	0
11	09-02-2012	0	+	0	609	5	0	0	0	0
12	13-02-2012	0	+	0	7	0	0	0	0	0
13	14-02-2012	0	+	0	4	0	0	0	0	0
14	15-02-2012	0	-	0	6	0	0	0	0	0
15	22-02-2012	0	-	0	5	560	0	5	478	0
16	23-02-2012	56	-	0	0	501	0	5	108	5
17	27-02-2012	597	-	0	5	55	0	8	55	5
18	28-02-2012	506	++	0	5	59	0	0	205	0
19	29-02-2012	205	+	0	5	58	0	0	604	0
20	01-03-2012	23	+	0	5	634	0	6	134	0
21	05-03-2012	550	-	0	5	1790	0	5	36	5
22	06-03-2012	280	-	0	40	6240	0	160	160	80
23	07-03-2012	280	-	0	0	10840	0	320	160	80
24	08-03-2012	360	-	0	40	10040	0	320	200	80

25	12-03-2012	200	+	0	0	11250	0	100	200	50
26	13-03-2012	150	-	0	0	9750	0	650	150	0
27	14-03-2012	100	-	0	50	10500	0	350	200	50
28	15-03-2012	100	-	0	100	9600	0	800	100	0
29	19-03-2012	150	-	0	50	10300	0	450	50	50
30	20-03-2012	150	-	0	100	12450	0	900	50	50
31	21-03-2012	50	-	0	100	10350	0	500	50	50
32	26-03-2012	100	-	0	2800	3600	0	4700	0	50
33	27-03-2012	50	-	0	800	5350	0	2800	0	100
34	28-03-2012	100	-	0	500	7850	0	2650	1	150
35	29-03-2012	50	-	0	500	8250	0	450	1	50
36	02-04-2012	50	-	0	550	5600	0	500	0	100
37	03-04-2012	150	-	0	900	8100	0	300	0	100
38	04-04-2012	50	-	0	950	8450	0	0	0	50
39	05-04-2012	50	-	0	950	8350	0	1	0	50
40	10-04-2012	50	-	0	50	5150	0	150	0	0
41	11-04-2012	50	-	50	50	5850	0	100	1	50
42	12-04-2012	50	-	1	50	5550	0	50	50	1
43	16-04-2012	50	-	0	150	2350	0	100	1	0
44	17-04-2012	50	++	1	100	1550	0	100	1	1
45	18-04-2012	50	++	1	300	1700	0	100	1	100
46	19-04-2012	50	++	50	500	1650	0	0	0	50
47	20-04-2012	50	++	0	500	1450	0	0	0	50
48	24-04-2012	0	++	1	250	1200	0	100	0	50
49	26-04-2012	100	++	0	50	2650	0	150	0	100

Table A.5: Identification key of the microorganism found in the activated sludge samples.

Groups	Subclass	Genera	Identification key	Reference
	(overall)		➢ Firmly attached to the sludge flocs by a stalk which may be either rigid or contractile ➢ Cilia in the anterior region of the body near oral cavity	Jenkins (1993) Bento *et al.* (2005) Madoni (2010)
Sessile ciliates	**Peritrichia**  	*Vorticella*	➢ Sessile bell-like ciliates ➢ Single zooids with a contractile peduncle (with internal spasmoneme) ➢ One long macronucleus extending more or less along the longitudinal axis of the cell ➢ Contractile vacuole located near the buccal cavity ➢ Buccal ciliation that winds counterclockwise to the buccal cavity	Bick (1972) Serrano *et al.* (2008)
		Carchesium	➢ Colonial species with branched stalk with self-contained discontinuous spasmonemes ➢ Myonemes in stalk not continuous therefore each stalked member of the colony contracts independently ➢ One long band-like macronucleus that extends along the longitudinal axis of the cell	Bick (1972) Serrano *et al.* (2008)
		Zoothamnium	➢ Colonial species with branched stalk with continuous spasmonemes ➢ All the zooids of the colony contract simultaneously ➢ Myomenes of all stalks of the colony are continuous	Serrano *et al.* (2008)
		Epistylis	➢ Colonial species ➢ Stalk without myonemes, thus not contractile ➢ Contracted individuals with characteristic folds at posterior end ➢ With a large peristomial lip ➢ Rigid stalks	Bick (1972) Serrano *et al.* (2008)
		Opercularia	➢ Colonial species ➢ Stalk without myonemes, thus not contractile ➢ Without peristomial lip ➢ With opercula ➢ Body elongated	Bick (1972) Serrano *et al.* (2008)

Free swimming ciliates	**Suctoria** [1]  	*Podophrya*	➢ Spherical ciliate ➢ Tentacles homogenously distributed ➢ Spherical macronucleus ➢ One contractile vacuole	Bick (1972) Serrano *et al.* (2008)
		Tokophrya	➢ Triangular shaped cells ➢ Two clusters of tentacles	Serrano *et al.* (2008)
	(overall)		➢ Round to oval shape ➢ Cilia evenly distributed through the cell ➢ Actively motile by rows of short, hair-like cilia ➢ Swim in the liquor phase	Jenkins (1993) Bento *et al.* (2005) Madoni (2010)
	Tetrahymena  	*Tetrahymena*	➢ Pyriform swimming cells ➢ Posterior contractile vacuole	Serrano *et al.* (2008)
		Dexiostoma / Colpidium	➢ Ovoid cells with a torsion at the anterior body end ➢ Uniform ciliation except for a group of longer cilia at the posterior pole ➢ One spherical macronucleus and one micronucleus ➢ One contractile vacuole	Bick (1972) Serrano *et al.* (2008)
	Peniculida  	*Paramecium*	➢ Fast swimming cells ➢ Oval or elongated foot-shaped cells ➢ Equatorial torsion where the oral cavity is clearly distinguished ➢ Caudal cilia ➢ One ellipsoid macronucleus and one compact micronucleus ➢ Two contractile vacuoles	Bick (1972) Serrano *et al.* (2008)

	(overall)		➢ Move on the surface of the sludge flocs ➢ Flattened body ➢ Cilia grouped on the body part that contact the sludge flocs	Jenkins (1993) Bento *et al.* (2005) Madoni (2010)
Crawling ciliates	**Hypotrichia**  	*Euplotes*	➢ Ciliates with a well-developed anterior oral opening ➢ Well-developed adoral zone of membranes (numerous membranelles consecutively arranged from the anterior part of the oral cavity to the left side) ➢ Long fronto-ventral and transverse cilia that moves as a unit (cirri) ➢ Macronucleus C-shaped	Serrano *et al.* (2008)
		Apidisca	➢ Small adoral zone of membranes ➢ Long frontal and transverse cirri ➢ Small, ovoid ➢ Macronucleus horseshoe-shaped	Bick (1972) Serrano *et al.* (2008)
Testate Amoeba		*Arcella*	➢ Motile by pseudopodia ➢ External shell	Jenkins (1993) Bento *et al.* (2005)
Large Flagellates	 	*Peranema*	➢ Long, thick flagellum ➢ The cell is usually 20-30 µm long	Eikelboom (2000)
Small Flagellates		*Bodo* [(2)] *Polytoma* [(2)] *Tetramitus* [(2)]	➢ Small (5-20 µm) ➢ Oval or elongated forms ➢ Actively motile by one or more long flagellate	Jenkins (1993) Bento *et al.* (2005)

Metazoan	➢ Multicellular organisms of various phyla	Bento *et al.* (2005)

(1) Carnivorous ciliates, that must be excluded by the "sessile ciliates" keygroups
(2) Small flagellates commonly found in activated sludge

6.3. Statistical analysis

Table A.6: Two-sample Student's *t*-test for the comparison between 9 hours and 2 hours of oven evaporation for total solids determination.

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Results	9 hours	30	7,5220	4,65308	0,84953
	2 hours	30	7,6637	4,66718	0,85211

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Results	Equal variances assumed	0,005	0,946	-0,118	58	0,907	-,14167	1,20324	-2,55022	2,26688
	Equal variances not assumed			-0,118	57,999	0,907	-,14167	1,20324	-2,55022	2,26688

Table A.7: One-sample Student's t-test for the comparison between the experimental value found for the relation between TS and VSS and the hypothesized value of 80%.

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Relation (%)	13	81,1854	2,58144	0,71596

One-Sample Test

	Test Value = 80					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Relation (%)	1,656	12	0,124	1,18538	-0,3746	2,7453

Table A.8: Two-sample Student's *t*-test for the comparison between the COD removal efficiency before and after the re-inoculation.

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
% COD removed	before re-inoculation	14	80,7800	4,90482	1,31087
	after re-inoculation	35	97,6677	0,91078	0,15395

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
% COD removed	Equal variances assumed	28,047	0,000	-19,828	47	0,000	-16,88771	0,85172	-18,60115	-15,17428
	Equal variances not assumed			-12,795	13,360	0,000	-16,88771	1,31988	-19,73134	-14,04409

Table A.9: Two-sample Student's *t*-test for the comparison between the influent COD concentration before and after the re-inoculation.

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Influent COD	before re-inoculation	14	8115,1429	3093,40019	826,74598
	after re-inoculation	35	8955,1429	4180,15858	706,57576

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Influent COD	Equal variances assumed	6,543	0,014	-0,679	47	0,500	-840,00000	1236,42010	-3327,35641	1647,35641
	Equal variances not assumed			-0,772	32,331	0,445	-840,00000	1087,54688	-3054,37048	1374,37048

Table A.10: Two-sample Student's *t*-test for the comparison between the effluent COD concentration before and after the re-inoculation.

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Effluent COD	before re-inoculation	14	1429,4286	88,36525	23,61661
	after re-inoculation	35	186,6000	75,83116	12,81781

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Effluent COD	Equal variances assumed	0,109	0,743	49,439	47	0,000	1242,82857	25,13884	1192,25575	1293,40140
	Equal variances not assumed			46,252	21,087	0,000	1242,82857	26,87081	1186,96175	1298,69539

Table A.11: Pearson correlation analysis for the evaluation of the relation between F/M and organic loading.

Correlations

		F/M	Organic loading
F/M	Pearson Correlation	1	0,991**
	Sig. (2-tailed)		0,000
	N	47	48
Organic loading	Pearson Correlation	0,9991**	1
	Sig. (2-tailed)	0,000	
	N	48	49

**. Correlation is significant at the 0.01 level (2-tailed).

Table A.12: Pearson correlation analysis for the evaluation of the relation between COD removal efficiency and TS removal efficiency.

Correlations

		% COD removed	% TS removed
% COD removed	Pearson Correlation	1	,851**
	Sig. (2-tailed)		,000
	N	70	70
% TS removed	Pearson Correlation	,851**	1
	Sig. (2-tailed)	,000	
	N	70	70

**. Correlation is significant at the 0.01 level (2-tailed).

Table A.13: Spearmans'rho correlation analysis for the evaluation of the relation between functional groups and physiochemical parameters for aeration tank 1.

Correlations

		% COD removed	Effluent COD	MLSS	N-NH4+	Total Phosphorous	pH	F/M	SRT	SVI	Temp (ºC)
Testate amoebae	Corr. Coef.	0,775**	-0,678**	-0,202	-0,396**	-0,294	0,022	0,194	-0,207	0,000	0,526**
	Sig. (2-tailed)	0,000	0,000	0,163	0,007	0,381	0,883	0,191	0,163	1,000	0,002
	N	49	49	49	45	11	49	47	47	7	33
Small flagellates	Corr. Coef.	-0,568**	0,622**	0,414**	0,310*	-0,472	-0,389**	-0,150	0,413**		-0,482**
	Sig. (2-tailed)	0,000	0,000	0,003	0,039	0,143	0,006	0,314	0,004		0,005
	N	49	49	49	45	11	49	47	47	7	33
Large flagellates	Corr. Coef.	0,055	-0,258	-0,190	0,144	0,324	-0,335*	-0,147	0,011	-0,612	-0,054
	Sig. (2-tailed)	0,705	0,074	0,191	0,346	0,332	0,019	0,325	0,941	0,144	0,763
	N	49	49	49	45	11	49	47	47	7	33
Free-swimming ciliates	Corr. Coef.	-0,613**	0,592**	0,082	0,381**	-0,179	-0,185	-0,193	0,327*	0,342	-0,675**
	Sig. (2-tailed)	0,000	0,000	0,575	0,010	0,598	0,204	0,195	0,025	0,452	0,000
	N	49	49	49	45	11	49	47	47	7	33
Sessile ciliates	Corr. Coef.	0,600**	-0,622**	-0,164	-0,203	0,074	0,122	-0,121	-0,503**	-0,039	0,447**
	Sig. (2-tailed)	0,000	0,000	0,260	0,182	0,829	0,402	0,416	0,000	0,933	0,009
	N	49	49	49	45	11	49	47	47	7	33
Crawling ciliates	Corr. Coef.	0,174	-0,235	-0,174	-0,175	0,100	0,153	0,125	0,098	0,204	0,152
	Sig. (2-tailed)	0,233	0,103	0,233	0,251	0,770	0,293	0,402	0,513	0,661	0,397
	N	49	49	49	45	11	49	47	47	7	33
Opercularia sp.	Corr. Coef.	0,701**	-0,475**	-0,111	-0,127	-0,092	0,210	-0,010	-0,192	-0,607	0,208
	Sig. (2-tailed)	0,000	0,001	0,447	0,404	0,788	0,148	0,948	0,197	0,148	0,246
	N	49	49	49	45	11	49	47	47	7	33
Carnivorous ciliates	Corr. Coef.	0,489**	-0,560**	-0,132	-0,597**	0,019	0,144	0,415**	-0,184	0,414	0,371*
	Sig. (2-tailed)	0,000	0,000	0,368	0,000	0,956	0,322	0,004	0,215	0,355	0,033
	N	49	49	49	45	11	49	47	47	7	33
Metazoa	Corr. Coef.	0,252	-0,139	0,106	-0,221	-0,017	0,114	-0,075	-0,370*	-0,445	0,392*
	Sig. (2-tailed)	0,081	0,342	0,469	0,145	0,960	0,437	0,615	0,011	0,317	0,024
	N	49	49	49	45	11	49	47	47	7	33
Diversity (No of genera)	Corr. Coef.	0,604**	-0,550**	-0,037	-0,357*	-0,108	0,025	0,038	-0,403**	-0,869*	0,322
	Sig. (2-tailed)	0,000	0,000	0,801	0,016	0,751	0,863	0,800	0,005	0,011	0,068
	N	49	49	49	45	11	49	47	47	7	33
Total microfauna density	Corr. Coef.	0,384**	-0,163	0,143	-0,228	-0,318	0,352*	0,151	-0,368*	-0,500	0,570**
	Sig. (2-tailed)	0,007	0,264	0,326	0,132	0,340	0,013	0,310	0,011	0,253	0,001
	N	49	49	49	45	11	49	47	47	7	33

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table A.14: Spearmans'rho correlation analysis for the evaluation of the relation between functional groups and physiochemical parameters for aeration tank 2.

Correlations

		% COD removed	Effluent COD	MLSS	N-NH4+	Total Phosphorous	pH	F/M	SRT	SVI	Temp (ºC)
Testate amoebae	Corr. Coef.	0,735**	-0,711**	-0,326*	-0,265	-0,193	0,083	0,253	-0,198	-0,126	0,361*
	Sig. (2-tailed)	0,000	0,000	0,022	0,078	0,570	0,570	0,087	0,182	0,788	0,039
	N	49	49	49	45	11	49	47	47	7	33
Small flagellates	Corr. Coef.	-0,433**	0,485**	0,218	0,229	-0,472	-0,422**	-0,062	0,394**	0,134	-0,464**
	Sig. (2-tailed)	0,002	0,000	0,133	0,130	0,143	0,003	0,678	0,006	0,775	0,007
	N	49	49	49	45	11	49	47	47	7	33
Large flagellates	Corr. Coef.	0,164	-0,265	-0,234	-0,006		-0,369**	-0,093	-0,304*		-0,114
	Sig. (2-tailed)	0,261	0,066	0,106	0,970		0,009	0,533	0,038		0,528
	N	49	49	49	45	11	49	47	47	7	33
Free-swimming ciliates	Corr. Coef.	-0,617**	0,582**	0,123	0,266	-0,055	-0,231	-0,220	0,345*	0,143	-0,632**
	Sig. (2-tailed)	0,000	0,000	0,399	0,078	0,873	0,110	0,136	0,018	0,760	0,000
	N	49	49	49	45	11	49	47	47	7	33
Sessile ciliates	Corr. Coef.	0,612**	-0,626**	-0,231	-0,266	0,074	0,125	-0,095	-0,478**	0,039	0,447**
	Sig. (2-tailed)	0,000	0,000	0,110	0,077	0,829	0,392	0,526	0,001	0,933	0,009
	N	49	49	49	45	11	49	47	47	7	33
Crawling ciliates	Corr. Coef.										
	Sig. (2-tailed)										
	N	49	49	49	45	11	49	47	47	7	33
Opercularia sp.	Corr. Coef.	0,589**	-0,331*	-0,030	-0,214	-0,146	0,333*	-0,088	-0,289*	-0,559	0,463**
	Sig. (2-tailed)	0,000	0,020	0,836	0,159	0,669	0,019	0,556	0,049	0,192	0,007
	N	49	49	49	45	11	49	47	47	7	33
Carnivorous ciliates	Corr. Coef.	0,523**	-0,516**	-0,143	-0,264	0,210	0,168	0,362*	-0,115	0,786*	0,349*
	Sig. (2-tailed)	0,000	0,000	0,327	0,079	0,536	0,249	0,012	0,441	0,036	0,046
	N	49	49	49	45	11	49	47	47	7	33
Metazoa	Corr. Coef.	0,552**	-0,446**	-0,227	-0,191	-0,496	0,128	-0,140	-0,314*	-0,355	0,201
	Sig. (2-tailed)	0,000	0,001	0,117	0,209	0,121	0,381	0,349	0,032	0,435	0,261
	N	49	49	49	45	11	49	47	47	7	33
Diversity (No of genera)	Corr. Coef.	0,692**	-0,534**	-0,163	-0,199	-0,266	0,110	0,011	-0,388**	-0,964**	0,343
	Sig. (2-tailed)	0,000	0,000	0,263	0,189	0,429	0,452	0,943	0,007	0,000	0,051
	N	49	49	49	45	11	49	47	47	7	33
Total microfauna density	Corr. Coef.	0,368**	-0,139	0,220	-0,326*	-0,527	0,411**	0,161	-0,324*	-0,500	0,550**
	Sig. (2-tailed)	0,009	0,341	0,128	0,029	0,096	0,003	0,280	0,026	0,253	0,001
	N	49	49	49	45	11	49	47	47	7	33

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).