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Advanced biological wastewater treatment – MBBR process for dairy wastewater

Master Thesis in the scientific area of Chemical Engineering, supervised by Professor Dr. Rosa Quinta-Ferreira and Professor Dr. Luís M. Castro, submitted to the Department of Chemical Engineering , Faculty of Science and Technology, University of Coimbra

September, 2015

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Universidade de Coimbra

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Coimbra
2015

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UNIVERSIDADE DE COIMBRA

“Learn from yesterday, live for today and hope for tomorrow.

The important thing is not stop questioning”

-Albert Einstein

To my Grandmother Carma,
to not only made me the person who I am now and all the wise, but also to teach me face all the adversity in life, and fight for what I believe. Thank you and we will meet again one day.

ACKNOWLEDGEMENTS

During this journey there were times that giving up seemed to be the best option, but there was people that was there for me and always had the right words, for that I couldn't be more grateful.

First I would like to acknowledge Professor Dr. Rosa Quinta-Ferreira and Professor Dr. Luís M. Castro, for the opportunity to investigate and create this research. Also to Professor Dr. Rui C. Cardoso Martins for all the help, advice and fun.

My family that is my backbone and the best in all world, Mom and Dad thank you for giving me this opportunity and for always believe in my and in my crazy ideas. Ju and Sá thanks for being the best sister and brother that anyone could have. My aunts Lu, Té for being like sisters. My others aunts Mimi, Olga and Aurora, and my uncles Ti Tiago, Quim, João, Luís e Herminio, for just being there, and also my Grandmothers and Grandfathers, thank you all.

My little Princes and Princesses, Kiko, Mary, Pipa, Gui, Nono and Dudu, I love you from the button of my heart you are the ones that make all the bad days seams less painful and more happier.

Catarina the older cousin/sister, you know that I'm always here for you as you are for me, thank you and your beautiful family.

Rita, a fofita, thank you for the best years of my live, you always had the right word and action for every occasion.

Marco, the top of flop, thanks for showing me that a sort time is enough to leave a mark, and for the time that we spend together it was amazing, next time will be in Italy.

Vicky, my rebel punk, I have to acknowledge you for being that special friend that just you know how to be.

Tufinhas, you are irreplaceable, thank you for sharing this experience with me.

Last but not the least I'm grateful for all that persons, you know who you are, that appeared in my life and somehow make a positive change in my life. For everything my biggest gratitude to you, all have a special place in my heart.

Abstract

Over the last decades, potable water has become a more scarce resource, so there is a constant need to develop new ways to treat the residual waters, in order to send it back to the natural resources without change the ecosystem equilibrium.

The Moving Bed Biofilm Reactor (MBBR) system is considered an Advanced Wastewater Treatment (AWT), which combine the best of Conventional Activated Sludge (CAS) and biofilter processes, making use of suspended biomass and attached biomass, as biofilter. This process requires less space than CAS to process the same amount of wastewater, and can be adapted to the existing structures.

The dairy wastewater is obtained during the milk transformation and cleaning operations, and for each litter of processed 4 up to 15 litters of water are used. This residue is characterized by its high content of organic matter and carbonic compounds which contribute to its biodegradability, allowing the use of biological processes.

This work used a simulated wastewater, made with 1 parte of low fat milk and 200 parts of municipal water, showing a Chemical Oxygen Demand, (COD), of 640 mg O_2/L and a Biochemical Oxygen Demand, (BOD), of 320 mg O_2/L, resulting on a biodegradability (COD/BOD_5) of 0.5, which allow the application of a biological treatment for this wastewater. Also, the synthetic effluent had 28 mg /L, of Total Kjeldahl Nitrogen, (TKN), and 12 mg P/L, of Total Phosphorus.

The MBBR used in this project was a Beaker glass with an operable volume of 900 mL, originals Kaldness® carrier K1 were used as carriers and the air was provided with an air pump, for the sludge survival and the carriers movement. Two Filling Ratios (FR), 20% and 40%, were tested, as well as the impact on the system behaviour of the initial Organic Load Ratio (OLR). Moreover, batch and Continuous operations were carried out.

As expected the operations with a FR of 40% allow more efficient results in COD reduction, from 1100 mg O_2/L to 60 - 80 mg O_2/L, and TKN removal from 60 mg /L to 6.8 mg /L.

Resumo

Ao longo das últimas décadas, a água potável tornou-se um recurso cada vez mais escasso, havendo por isso, uma necessidade constante de desenvolver novas formas de tratar as águas residuais, a fim de o colocar de novo na natureza, sem alteração do equilíbrio do ecossistema.

O MBBR é considerado um Tratamento Avançado de Águas Residuais (AWT), que combinam o melhor das Lamas Ativadas convencionais (CAS) e os processos de filtros biológicos, que atua combinando a biomassa em suspensão e aderida aos suportes. Este processo requer menos espaço que a CAS, para processar a mesma quantidade de efluente, podendo ser adaptado a estruturas existentes.

O efluente de laticínios é produzido durante a operação de transformação de leite e limpeza, sendo que por cada litro de leite processado faz-se uso de 4 a 15 litros de água. Este efluente é caracterizado pelo seu elevado teor de matéria orgânica e compostos carbónicos que contribuem para a sua biodegradabilidade, permitindo a utilização de processos biológicos no seu tratamento.

Neste trabalho foi utilizado um efluente simulado, feito com uma parte de leite, de baixo teor de gordura, e 200 partes de água municipal, apesentando uma Carência Química de Oxigênio (CQO) de 640 mg O2 / L e uma Carência Bioquímica de Oxigênio (CBO) de 320 mg O2 / L, o que resulta numa biodegradabilidade de 0,5, permitindo a aplicação de um tratamento biológico. Tinha também um valor de 28 mg / L, para o azoto de Kjeldahl total (TKN) e 12 mg P / L, para o Fósforo Total.

O sistema MBBR utilizado neste projeto foi um goblet com um volume de operação de 900 mL, os suportes utilizados foram os originais *Kaldness® carrier* K1, o ar foi fornecido por uma bomba de ar, que também proporciona o movimento dos suportes. Foram testadas duas frações de enchimento, (FR), 20% e 40%, várias cargas orgânicas iniciais, (OLR), e a operação continua e descontinua.

Conforme esperado as operações com um FR de 40% revelaram resultados mais eficazes quer na redução do CQO, desde 1.100 mg O2 / L até 60-80 mg O2 / L, e de TKN de 60 mg / L a 6,8 mg / L.

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I. Introduction

This chapter has as objective to make a reflection on the global environmental problems, such as the contamination either by industry or by domestic effluents and water abusive consumption. So the main environmental issues and some ways to prevent them are described in this section. Moreover, focus is given to the different kinds of biological treatment, especially MBBR. Finally it is referred how the thesis is organized and the main objectives behind it.

1.1 Water issues

The developed countries industrialization is a considerable cause of pollution in air, soil and water.

Water is a natural resource that in its pure form is nowadays more and more scarce, which gives this resource a high economic value. The presence of water is crucial for the survival of the ecosystems and life as we well know, and the uncontrolled consumption of this asset is a huge concern for general population, as well as the unequal distribution of this resource. These facts show the importance of establishing strict rules for a more conscientious consume and distribution.

Besides the inappropriate use of water, the pollution of watercourses is another important issue, because a lot of substances produced in industries are toxic for environment and for all species of life. In this context, it is essential searching for new and better ways to treat the wastewaters before discharging them throughout the natural water courses.

1.2 Wastewater treatment

The processes used, to treat industrial wastewater, can be divided as biological or physic-chemical. A typical plant is composed by three steps: primary, secondary and tertiary. The primary treatment has as objective the removal of settleable organic and inorganic solids and the removal of float material, using sedimentation and flotation. The secondary processes take place afterwards, and have the aim of removing the residual suspended and dissolved organic solids usually through biological systems. In the tertiary step advanced wastewater treatments are applied to remove specific compounds that secondary treatment could not remove.

Furthermore, advanced oxidation processes targeting biodegradability enhancement can also be used previously to the biological treatment.

1.2.1 Biological Processes

The Biological treatments are the ones mostly used in industry, due their lower economic costs of operation and investment, and can be divided into aerobic and anaerobic processes if they require oxygen or not. In the Anaerobic process the organic impurities are assimilated in the absence of air, resulting on methane and carbon dioxide gases and biomass, according with the equation 1.

$$(CH_2)_n \xrightarrow{bacteria} nCO_3COOH \xrightarrow{bacteria} CH_4 + CO_2 \qquad (1)$$

The aerobic treatment occurs at the presence of oxygen, and the microorganisms, that can be bacteria, fungi, protozoa, rotifers, assimilate organic matter and convert them into carbon dioxide, water and biomass. This process can be explained by the equation 2.

$$(CH_2)_n + O_2 \xrightarrow{bacteria} CO_2 + H_2O + NH_3 + Energy + New\ cells + New\ products \qquad (2)$$

The technologies applied in this process involve aeration tanks that can be designed as plug-flow, completely mixed, percolation filters or sequencing batch reactor.

The most common and old technology is the Conventional Activated Sludge (CAS), but there are also stabilization lagoons and biological filters.

One of the disadvantages of the traditional aerobic process is that it leads to a high production of biomass. This will require a bigger plant, for sludge management. A possible approach to overcome this drawback is to grow the biomass in a carrier, producing a biofilm.

Over the last few years, biofilm systems aroused the interest of scientific community. This technology when compared with activated sludge requires less space, the concentration of biomass to be separated is ten times lower, and at a given point the biomass becomes more specialized, once there is no need to sludge recirculation (Odegaard, 1999).

There are many kinds of biofilms systems in use, like trickling filters, rotating biological contactors (RBC), fixed media submerged biofilter, fluidized bed reactors etc. Yet they all have disadvantages, the not volume-effective of trickling filter or the mechanical failures of the RBC's, besides that in the fixed media biofilter is difficult to obtain an even load in the carriers and the granular media biofilter shows hydraulic instability. All these reasons promote the demands for the development of an efficient moving bed biofilm process (Odegaard, 1999).

1.3 The MBBR History

The Moving Bed Biofilm Reactor (MBBR) came up for the first time during the late 1980 and the early 1990, in Norway (Qiqi *et al*., 2012), when the biofilms systems were not sufficient to process successfully the nitrogen removal from an effluent (Rusten *et al*., 1995). Until then, most of the full-scale nitrogen removal plants were based on conventional activated sludge (CAS) systems, and the application of biofilms was limited.

There are different biofilm reactors as referenced above (Rusten *et al*., 1995). However these processes are complicated to operate, because it is difficult to obtain an even distribution of the load on the whole carrier surface, and the granular media biofilters have to be operated discontinuously, due the need of the backwashing and hydraulic instability of the reactor (Odegaard, 1999). So, all these reasons contributed to studies focusing the MBBR development.

1.3.1 The MBBR Technology

The MBBR is considered an Advanced Wastewater Treatment (AWT), and the main idea behind this reactor was to combine the best from both CAS and biofilter processes (Qiqi, *et al*., 2012).

The MBBR process uses suspended biomass, similar to CAS, and attached biomass, as biofilter. To promote the attachment of the biomass, small pieces of High Density Polyethylene (HDPE), known as carriers, are added into the tank, where the biofilm will be formed and will further grow (Qiqi *et al*., 2012). This will allow the elimination of sludge recycling. This reactor can be used for aerobic, anoxic or anaerobic processes (Odegaard, 1999) (Qiqi *et al*, 2012).

Figure 1 is an exemplification of the agitation and the carrier movement in both processes. In the aerobic process, the stirring is promoted by the air supplying. Contrarily, the anoxic and anaerobic reactors use a mixer to keep the carrier on moving. The carriers are kept inside the reactor by an outlet sieve (Odegaard, 1999).

The main advantage of this process compared to the CAS is that the MBBR needs less space to process the same amount of the wastewater, once there is no need of a sedimentation tank, or periodical washes. This mechanism can be adapted in the existent structures with small changes. However the MBBR has operational costs higher than CAS, because the aeration needed require energetic costs.

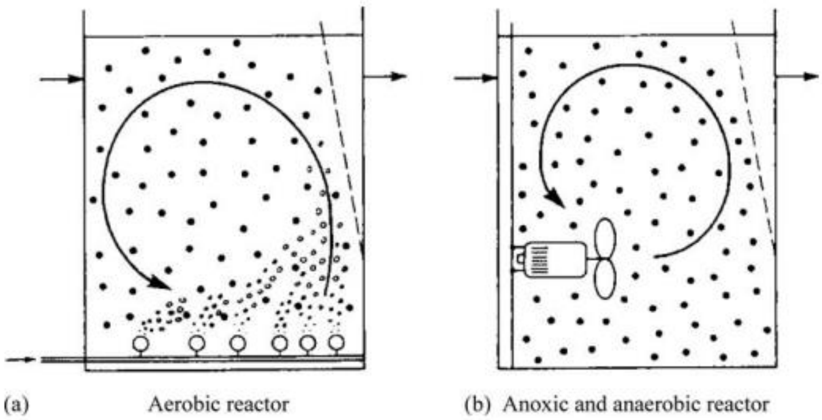

Figure 1 - Principal of the MBBR (Rusten *et al*., 2006).

1.3.2 The carrier elements

The biofilm carriers, developed by AnoxKaldness®, are made of polyethylene with a density close to 1 g/cm^3 and various diameters and height, and the volume occupied by carriers in empty reactor, filling fraction (FF) must be up to 70%, so that the carriers can move easily.

There are many kinds of carriers in the market such as the originals Kaldness® carrier K1 in a cylinder shape, that is the most used, but there are also the K2, ANOX, BiofilmChip, etc. Table 1 resumes some of the characteristic for the different kind of the carriers used and Figure 2 shows the most studied carriers.

Table 1 - Characteristic of different carriers. (Adopted from (Rusten *et al*., 2006) and ((Anon., 2015a))).

	Kind of Carriers				
	K1	K3	Natrix F3	BiofilmChip P	BiofilmChip M
Nominal diameter (mm)	9.1	25	64	48	45
Nominal length (mm)	7.2	12	50	2.2	3.0
Bulk density (kg/m^3)	150	100	-	-	-
Specific biofilm surface area (m^2/m^3)	500	500	200	1 200	900
Specific biofilm surface area at 60% fill (m^2/m^3)	300	300	132	720	540

Figure 2 - The biofilm carriers (A) K1, (B) K3, (C) Natrix F3, (D) BiofilmChip M and (E) BiofilmChip P (Adopted from ((Anon., 2015a) (Anon., 2015b)).

1.3.3 <u>The biofilm formation</u>

The biofilms are complex systems of microbial cells and colonies inserted in a polysaccharide gel, whose structure and composition change with age and environmental conditions.

The way the biofilm is forming inside the bio carrier is not an exact science. In the beginning the process is slow due to the turbulence caused by aeration, and when the aeration is too high, the carriers movement promote the clash between them, making difficult the adherence of microorganisms. Usually the start-up is made with an inoculum of sludge from a CAS (Dezotti *et al*., 2011).

The biofilm formation process begins with the adherence of the microorganisms at carrier surface, then the cells start to grow, and release extracellular materials, exopolymers, then the cells present in the liquid medium initiate to adhere to the biofilm, this process is show in the Figure 3.

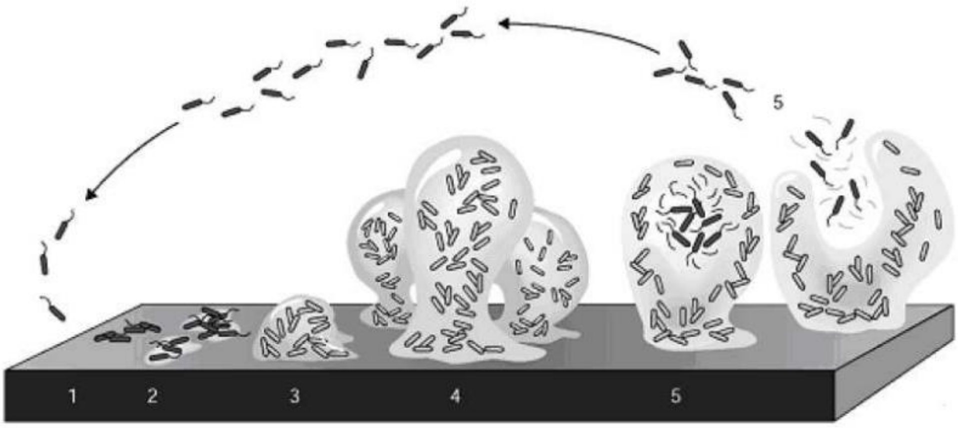

Figure 3 - Biofilm formation. 1 - Initial adherence of cells to carrier surface; 2 and 3- Exopolymers production; 4 - Biofilm development; 5 - Dispersion of biofilm cells (Adapted from (Sauer, 2003)).

The biofilm characteristics change in accordance with the MBBR application. For the organic matter removal the biofilm is thicker than the applied to nitrogen removal (Dezotti *et al*., 2011).

1.3.4 Operational conditions

The efficiency of the MBBR is conditioned by operational conditions as Filling Ratio and Dissolved Oxygen.

Filling Ratio

The amount of carriers inside the MBBR is referred as the Filling Ratio, which is the ratio between the volume occupied by the supports and the total volume of the reactor – $V_S/ V_{R.}$. This value can be changed and adapted to the desired conditions, but is referred that the optimal range is between 20 and 70% (Chen *et al*., 2007).

***Dissolved* Oxygen**

This parameter is one of the limiting variables in this process; usually the value 2 mg O_2/L is the minimal concentration to use in biological reactors for the removal of the organic matter, although in biofilm systems this value can be higher.

In the aerobic processes the carriers movement is proportionate by the aeration system; so in this case the air flow is conditioned by the movement, because it has to be in a way that the clash between the carriers is not to strong, to avoid the biofilm release.

This issue leads to the development of new designs for the reactor and the aeration system.

1.4 Dairy industry

Milk has a huge role in daily life of human beings. The dairy industry is responsible to process and transform milk into yogurts, cheese, ice-cream, butter and others sub-products. During this transformation the residues produced, combined with large water consumption make the dairy industry the most polluting of the food industries (Chaiudhari & Dhoble, 2010). The transformation process could use from 4 up to 15 L of water per litter of milk processed (Patil *et al*., 2014; Figueiredo *et al*., 2001).

The effluent from dairy industries is characterized by is highly biodegradable nature and presence of soluble organics, suspended solids and trace organics (Tikariha & Sahu, 2014).

In Portugal, 153.3 thousand tons of milk were processed in the year of 2014 corresponding to a huge quantity of residues (solid, liquid and gases) generated as well as high levels of water and energy consumed.

The wastewater from the dairy industry is produced during the cleaning process of the milk equipment and pipelines. This process is usually made in four cycles.

First rinsing is where about 92% of the suspend solids are removed. ***Detergent wash*** is used to remove attached organic material. ***Acid rinsing*** is to remove the inorganic deposits from the piping and neutralize the alkaline detergent residue. ***Sanitize rinsing*** it is to ensure that the milk lines are free of any microorganisms (Janni *et al*., 2007).

Although the cleaning process, the dairy effluent is also caused by spills and leaks due to the improper use of the equipment or to the lack of maintenance, as well as losses during the filling operation and whey resulting from the cheese manufacturing process (Figueiredo *et al*., 2001)

The table 2 shows the average values of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), suspend solids, phosphorus (P), nitrogen (ammonia, nitrate, and total Kjeldahl), pH and fats/oils/grease (FOG).

Table 2 - Average values of parameters for dairy effluent. (Figueiredo *et al*., 2001) (Janni *et al*., 2007).

Parameter	Average value
COD (mg O_2/L)	1 000 – 12 000
BOD_5 (mg O_2/L)	500 – 2 600
TSS (mg/L)	200 – 1 000
Fats, Oils & Grease (mg/L)	90 – 500
Total Nitrogen (mg N/L)	30 – 100
Total Phosphorus (mg P/L)	20 – 100
pH	6.2 – 8.0

The discharge of effluent in the aquatic environment have limit values. In Portugal it is regulated by the Decree Law number 236/98 of 1 of August. Wherein the COD cannot be superior to 150 mg O_2/L, BOD_5 must be inferior to 40 mg O_2/L, the total nitrogen has a limit of 15 mg N/L and the Total Kjeldahl Nitrogen (TKN) has a limit of 10 mg NH_4^+/L.

1.5 Objectives of this study and structure of the thesis

This study has as major purpose the installation of a MBBR unit, determine the optimal operation conditions and make a comparison between a batch and a continuous operation of the MBBR process.

This report is organized in five chapters. In this first one a brief introductory note about the global environmental problems associated to the waste management is referred. The biological process more used nowadays, with a special focus on MBBR history and technology and also the wastewater is intended to be treated. It also presents the objectives and the report's organization.

In Chapter II – Bibliographic Review, a bibliographic search about the Moving Bed Biofilm Reactor and the dairy wastewater treatment is described, which was organized and commented.

Chapter III – Experimental Procedures describes all the laboratory work done during the thesis period, including the analytical techniques used along the research and the methodologies followed to evaluate the MBBR performance.

The Results and Discussion are presented in Chapter IV. Finally the Chapter V – Conclusions and Future Works, includes a resume of the overall important conclusions that emerged during this work, and the future work proposals as well, to continue the study of the MBBR process.

II. Bibliographic review

In this section of the thesis a bibliographic review is made about the Moving Bed Biofilm Reactor and the theme of the dairy wastewater treatment was approached. All the collected information was organized in tables and commented.

2.1 The Moving Bed Biofilm Reactor (MBBR)

The MBBR process was applied in different kinds of wastewater and operational conditions. In the Table 3 is presented the studies already made, comparing the carrier and effluent, as well as the operational conditions and the principal conclusions of each author.

Table 3 - Bibliographic review on MBBR process.

Support kind	Effluent kind	Experimental Conditions	Principal Conclusions	Reference
K1	Dairy wastewater	• A pilot-plant with two aerobic MBBR; • Hydraulic retention time between 3.5 and 11.2 hours; • Air flow rate 18-30m^3/h. • Temperature 15 °C; • Pressure 1 atm; • Specific biofilm surface area 276 m^2/m^3.	• The specific biofilm surface area can be regulated up to a maximum of 400 m^2/m^3; • The pilot-plant shows 85% COD removal, at volumetric organic load of 500 g COD/m^3h and 60% COD removal at a volumetric organic load of 900 g COD/m^3h.	Rusten *et al*, 1992.
K3	Paper mill wastewater	• The MBBR has a useful volume of 0.85 L; • Was filled with 200 carriers; • Temperature 19.1 ±2.1° C; • Dissolved oxygen 6.0 – 7.6 mg/L.	• BOD_5 removal efficiency above 98.7% under an organic load rate of 0.13 kg BOD_5/m^3.d.	Jarpa *et al*, 2012.

K3	Pulp and paper mill effluent	• Total volume of liquid of 5 L; • The carriers occupyed 11% of the reactor's liquid volume; • Dissolved oxygen 6.0 – 7 mg/L.	• COD removal from 66% to 96% and BOD removal from 57% to 97/% increasing with the hydraulic retention time; • The maximum efficiency was obtained at 10 h.	Vaidhegi, 2013.
AQWISE K1 BIOCONS	Urban wastewater	• Three reactors with an operating volume of 3 L each; • Equipped with a porous plate and a stirring system; • Operational time of three weeks, in three phases of 7 days with different filling ratios and influent flows of 0.6, 0.3 and 0.2 L/h, corresponding to hydraulic retention time of 5, 10 and 15 h.	• Using filling ratios of 50% shows a more mature biofilm and better colonized carrier surface.	Calderón *et al*, 2012.
BiofilmChip-P	Pulp and paper mill wastewater	• Pilot-plant with an usable volume of 20 m^3; • Airflow rate of 3.0 m^3/min; • Dissolved oxygen 2 to 3 mg/L; • Average effluent flow of 6.2 m^3/h; • Carriers volume equal to 10% of the usable volume; • Temperature 44 °C and pH ranging between 6.5 and 8.5.	• Achieving a high adhered biomass formation, equal to 14.6 VSS m^{-2}; • The temperature was not a limiting factor.	Oliveira *et al*, 2014 .

Tube chip shaped	Pesticide wastewater	• MBBR with a working volume of 5 L; • Filing ratio of 50 %; • Organic loading rate about 3 kg COD/ (m^3 day).	• This bio-carrier could tolerate inlet COD loading higher than 37.5 g COD/(m^2 carrier day); • The COD removal efficiency was more than 85% for a bio-carrier volume more than 20%.	Chen *et al*, 2007.
K1	Synthetic wastewater with sugar beet molasses	• Two MBBR of 22 L volume; • Filing ratio of 70%; • Temperature 23±2 °C; • Dissolved oxygen above 4.5 mg O_{2*}.	• Reducing a HTR from 24 to 8 h, shows a gradual decrease of the COD removal efficiency; • MBBRs are very stable against the hydraulic and toxic shocks.	Borghei, and Hosseini 2002.
K1	Coal gasification wastewater	• Effective volume of the MBBR was 4 L; • Filing ratio about 50 %; • Dissolved oxygen around 5 mg O_2; • Temperature 33±1 °C.	• The MBBR exhibited good performance on shock loading ; • Removal efficiencies of SCN^- and NH_4^- decreased with the reduction of HRT ; • The effected of attached growth biomass on pollutant removal was much better than suspended growth biomass in the MBBR.	Li *et al*, 2011.

Rusten *et al*. (1992) used a MBBR to treat a dairy wastewater, in a pilot-plant in Oslo. The wastewater was received in the pilot-plant every day of the week from 5 to 10 pm. Influent COD concentrations varied from 1 400 to 4 700 mg/L and pH value from 5 to 10. The pilot-plant was composed by two biorreactores of 0.53 m^3 volume, and in order to keep the moving bed biofilm media inside, a wire mesh screen with opening was used to covered the outlets. The biofilm media are short hallow cylinders with a cross in the middle, forming four separated channels. The pieces had a density of 0.97 g/cm^3 and 9.7 ± 0.3 mm of external diameter and 8.1 ± 0.4 mm long. The two biofilms reactors had a total hydraulic retention time that varied from 3.5 to 11.2 h, and a normal air flow rate of 18-30m^3/h for each. Those operational conditions allow a removal efficiency of total CQO above 85% for organic loads up to 500 g total CQO/m^{3}h, corresponding a biofilm surface area of 1.8 g total CQO/m^{3}h. This removal efficiency was obtained at a HRT of 7 h that is really short for a biological treatment of food industry.

MBBR was also applied to treat paper mill wastewater as Jarpa *et al*. (2012) studied using a reactor of a useful volume of 0.85 L, filled with 200 carriers with a density of 0.98 g/cm^3, a specific surface area of 850 m^2/m^3 and 85% of porosity. The paper mill wastewater treated had a pH variation between 6.33 – 7.67, a COD of 839.00 mg O_2/L and a BOD_5 of 441.03 mg O_2/L resulting on an organic matter removal efficiency of 98.7%, when operated with an Organic Load Rate of 0.13 kg BOD_5/m^3.d. Likewise Vaidhegi (2013) studied the same effluent in a laboratory scale using a batch reactor with 5 L volume operating with different filling ratios of biocarries to reactor volume (40, 50, 60 and 70) as well as various hydraulic retention times (2 h, 4h, 6h and 8h). These experiments showed the maximum removal percentage when using 50 percent of the biocarries filling in 8 h, also pointing out that the filling ratio is an important factor, because the organic removal depends on the biomass attached to the biocarriers. Oliveira *et al*.(2014) studied the same application for MBBR, in a pilot plant using a reactor with a usable volume of 20 m3. This pilot plant was working with an average HRT of 3.3 hours and 10% of carriers volume.

This kind of equipment was also applied to an urban wastewater, where the study had as principal focus the bacterial diversity and the effect of different conditions. Using a lab-scale plant Calderón, *et al*. (2012) that consisted in three reactor operating in parallel and fed from a common tank. Each reactor had different kinds of biocarriers, but the same filling percentage. This study reveal that for a 50% filling ratio was observable a more mature and better colonized biofilm on the carrier surface

The MBBR can be used as a secondary treatment, as Chen *et al*. (2007), studied the use of Fenton-Coagulation as pre-treatment of a pesticide wastewater. The Fenton-Coagulation process was used to reduce the COD and improve the biodegradability, then proceed to the MBBR. In this work the MBBR had a working volume of 5L. The parameters changed during the test were the organic loading rate, the carrier volume and the hydraulic retention time, whose variations allowed to conclude that the biocarriers volume must be between 20% and 85%.

The removal of phenolic compounds is also an application of the MBBR system. Using a synthetic wastewater, Borghei & Hosseini (2002) used two reactors at laboratory scale operating in simultaneous with different hydraulic retention times. This study showed that the MBBR had a good response to the hydraulic toxic shocks, reflected in a good COD removal.

Similarly, Li *et al*. (2011) studied the MBBR efficiency to remove phenol, thiocyanate and ammonium in coal gasification wastewater, using a 4L reactor with a filling ratio of 50%. One more time the system revealed a good performance to shock loading, but the reducing of the HRT affected the thiocyanate and ammonium degradation, showing that 48 h was the optimum HRT for the removal of pollutant compounds.

2.2 The Dairy Wastewater Treatment

The dairy wastewater is usually characterized by a high biodegradation level, carrying high organic matter, fat, oil and grease as well as suspended solids. Most of the treatment plants apply biological treatment. Table 4 resumes a few kind of treatments used in this kind of wastewater.

Table 4 - Bibliographic review on dairy wastewater treatment processes.

Treatment used	Principal Conclusions	Reference
Biological treatment with an aeration tank.	• Dairy wastewater is highly biodegradable, which makes the aerobic biological treatment more feasible;	Patil *et al*. (2014)
Anaerobic biological treatment.	• The biogas production decreases with the HRT and the influent concentrations; • The BOD and COD value were quite high which indicates the wastewater polluted nature.	Tikarilha & Sahu (2014)
Injection of pure oxygen	• The oxygen injection system is more environmental friendly and simpler; • The operational costs are lower than the traditional anaerobic system.	Matín-Rilo *et al*. (2014)

Electrocoagulation using mild steel electrodes	• Due to the high content of oil and grease of the dairy wastewater, electrocoagulation process is feasible for treating it;	Sengil & Özacar (2006)
MBBR	• The upgrading of the biological-chemical treatment plant aimed to handle higher loads and achieve better removal efficiencies in organic matter and phosphorous; • The final result demonstrated a more efficient treatment plant, able to handle a variable organic load.	Rusten, *et al*., 1996

Patil, *et al*. (2014) describe a case study of dairy wastewater, where the main objectives were to identify the sources of the wastewater and find a way to minimize them and minimize the exit of the treatment facility.

The effluent treatment plant was composed by a balancing tank, which had no aerators, then had the screens and then had the screens. Next was the aeration tank, and afterwards there was a circular settling tank. The wastewater characterization show a highly biodegradable waste, the pH was slightly acidic, and the treatment led to a safe waste disposal. The most important conclusion is that the wastewater is extremely biodegradable and the aerobic treatment is the most viable process.

Tikarilha & Sahu (2014) studied the biogas production during the dairy wastewater treatment. During this process there was a physic-chemical control showing a temperature range from 26.2 to 35.4 ° C, a pH between 6.1 and 7.7, while the TDS values were in the range 180.2 – 445.4 ppm, the dissolved oxygen varied from 0.38 to 1.42 mg/L, and a BOD of 9033 mg/L and a COD of 4958 mg/L were observed. During this study the COD and BOD values showed small variations however this decreased with the HRT. During the anaerobic process the increase of the production of volatile acids affects the COD removal efficiency.

In 2014 Matín-Rilo *et al*. (2014) studied the injection of pure oxygen to promote the oxidation of COD in the wastewater, this technique aims to avoid the main problems related to the Up Flow Anaerobic Sludge Blanket (UASB) reactors, such as the accumulation of the fats oils and grass in the surface, preventing the biogas release and causing biomasses flotation. The loss of solids is an evident problem added to the large space needed by this type of equipment. Using the injection system appears to be the solution to the problems above, since the dissolution of pure oxygen promotes a homogenization of the biological raft avoiding the development of odours. And using pure oxygen instead of air, the saturation concentration in water is almost five times higher than using air, producing sludge with much higher efficiency. with the implementation of this system the COD removal increased from 35% to 75% corresponding to a final COD value below 500 mg/L. Even though this system has a higher investment cost, the results are more appealing.

The electrocoagulation process to remove the COD and oil-grease in dairy wastewater was also studied by Sengil & Özacar (2006). This process had great results in removing oil/grease and suspended solids (SS) in a variety of industrial wastewater. Sengil & Özacar (2006), used an iron sacrificial anode which was affected by the initial pH, current density, amount of NaCl and the concentrations of the COD and oil-grease, showing a removal efficiency of 98% and 99% of COD and oil-grease respectively.

Rusten *et al*. (1996) followed the upgrading of a conventional aerobic biological treatment into a MBBR. In the old treatment plant the average organic load was about 250kg COD/d achieving an average COD removal efficiency of 88% and 95% in total P. During the upgrading the existing aeration basin was converted to an aerated equalization tank and the two trickling were replaced with two MBBRs. After these changes the treatment plant had a capacity to process an average organic load of 347 kg COD/d, and obtained a removal efficiency of 98% in booth COD and P.

III. Experimental Procedures

In this chapter the laboratory work done during the thesis is described, including the analytical techniques used during the research and the methodologies followed to evaluate the MBBR performance.

3.1 Analytic techniques

In this section the analytical techniques used for the determination of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Dissolved Oxygen (DO), Total Suspend Solids (TSS), Volatile Suspend Solids (VSS), nitrogen and phosphorous are described.

3.1.1 Chemical Oxygen Demand (COD)

This technic allows the indirect measure of the amount of oxygen needed to degrade the organic matter present in the sample, COD that was determined by the 52220 D method established in the *Standard Methods (Closed Reflux)*. This method uses a digestion solution of Potassium Dichromate, and for its preparation 12g of Potassium Dichromate (K_2SO_4) are firstly put on an oven for 2h at 105 °C, and after cooling in a desiccator, 11.77g are weighted and 800 mL of distillated water are added in a volumetric flask of 1L. 100 mL of concentrate Sulfuric Acid (H_2SO_4) are also added and finished with distilled water. For the COD determination an acid solution is also need, made by measuring 9.6 g of Silver Sulfate (Ag_2SO_4), dissolving it in 1L of concentrate Sulfuric Acid (H_2SO_4) and maintained under rest for two days.

Figure 4 - Vials of calibration curve after reaction.

To apply this technique in each Vials tube 1.5 mL of the digestion solution, 3.5 mL of acid solution and 2.5 mL of sample were added. The Vials were placed in the termoractor *(ECO 25)* during 2h at 150 °C. Finished this time the samples were placed in the dark, for 1h till they are cooled. After the absorbance was read at 605nm, in a photometer *(Photolab S6 WTW).*

Once it is the absorbance that is read, is needed to make a calibration curve, that was made with different concentrations of Potassium Hydrogen Phthalate (KHP), which correspond to different values of COD in mg O_2/L. In this work the operational range to determinate COD was 100 – 1 000 mg O_2/L, so the calibration curve had the points 100 mg O_2/L, 250 mg O_2/L, 500 mg O_2/L, 750 mg O_2/L and 1 000 mg O_2/L, that after the reaction had the characteristics colours shown in Figure 4.

3.1.2 Biochemical Oxygen Demand (BOD_5)

The Biochemical Oxygen Demand (BOD) corresponds to the amount of the biodegradable pollutants of a wastewater, is express in mg O_2/L. Usually the BOD is measured after 5 days of consumption, and it's called BOD_5.

For the BOD_5 determination it is needed to prepare a dilution water that contains a solution of Calcium Chloride ($CaCL_2$) and Iron Chloride Hexahydrate ($FeCl_3.6H_2O$), and a buffer solution, with Potassium Dihydrogen Phosphate (KH_2PO_4), Dipotassium Phosphate (K_2HPO_4), Ammonium Chloride (NH_4Cl) and Sodium Monohydrogen Phosphate Heptahydrate ($Na_2HPO_4.7H_2O$). After the preparation the water was aerated for 24h before the test. The inoculum was the same sludge used for the MBBR process. For control a standard-solution of Glucose and Glutamic acid was used.

300 µL of inoculum, dilution water and a certain volume of sample according with the COD of the sample, as showed in Table 5, were introduced in a 300 mL Erlenmeyer.

Table 5 - ml of sample added in order of the COD range. (Adapted from (Metcalf 2003))

mL	COD range
0.02	30 000 – 105 000
0.05	12 000 – 42 000
0.10	6 000 – 21 000
0.20	3 000 – 10 500
0.50	1 200 – 4 200
1.0	600 – 2 100
2.0	300 – 1 050
5.0	120 – 420
10.0	60 – 210
20.0	30 – 105
50.0	12 – 42
100.0	6 – 21
300.0	0 - 7

After the Erlenmeyer full with dilution water the initial Dissolved Oxygen $[O_2]_i$ is measured with a *WTW inoLab 740*. Next the Erlenmeyer was closed and put in a thermostatic bath at 20 °C for five days. Finished that time the Dissolved Oxygen $[O_2]_f$ was measured again. The BOD_5 was determined by the Equation (3).

$$CBO_5 = \frac{([O_2]_i - [O_2]_f) - ([O_2]_{bi} - [O_2]_{bf})}{\frac{V_{Sample}}{V_{total}}} \qquad (3)$$

Where $[O_2]_{bi}$ and $[O_2]_{bf}$ are the values of DO of the blank in the beginning and the end of the five days, V_{Sample} is the volume of the sample and V_{total} is the volume of the Erlenmeyer.

3.1.3 Solids Determination

The solids determination requires the utilization of a vacuum filtration system that involves a few steps, and all the samples were made in duplicated. First it is need to weigh some watch glasses, then wash the filter paper on the vacuum filtration system, with distilled water and take it to the oven during 30 min at 105 °C. After that time is cooled in a desiccator, and weighed again (mass 1).

*3.1.3.1**<u>Total Suspend Solids (TSS)</u>***

After the steps above it was needed to measure 30 mL of sample and filter it, remove the filter, put again in the watch glass and take it to the oven during 1h at 105 °C. Then cooled in a desiccator, and weighed again (mass 2). The TSS could be determined with the Equation (4).

$$mg\ TSS/_L = \frac{(mass\ 2 - mass\ 1) * 1000}{V, mL} \qquad (4)$$

*3.1.3.2**<u>Volatile Suspend Solids (VSS)</u>***

To evaluate the VSS a ceramic dish is weighed, and then put it in the filter paper with the solids, and weighted again. Next it goes to a muffle furnace and is ignited at 550 °C for 1h. Then cooled in a desiccator, and weighed again (mass 3). The Equation 5 give us the VSS.

$VSS = TSS - FSS$ (5) where $mg\ FSS/_L = \frac{(massa3 - massa2) * 1000}{V, mL}$

3.1.4 pH measure

The pH is read automatically (*Crison micropH 2002*), Figure 5.

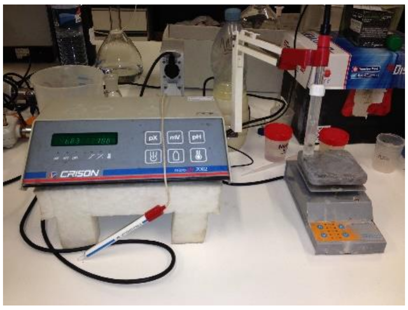

Figure 5 - pH measure.

3.1.5 Nitrogen

To the determination of nitrogen in the samples the Kjeldahl 's method was used, which comprises a few steps that are described next:

Frist 50 mL of sample water is added in each digestion tube, next the reagents for digestion, 7 g potassium sulphate anhydrous K_2SO_4, 350 mg mercuric oxide red HgO and 10 mL of sulphuric acid concentrated H_2SO_4 are introduced.

The second process is to the digestion, VELP SCIENTIFICA DKL Fully Automatic Digestion Units, by heating at 200 °C during 60 minutes, to promote the water evaporation, and after at 370 °C for 120 minutes. Then the digestion tubes era let cool down until 50-60 °C.

Third step involves the distillation, VELP UDK 129. The distillation program takes 3 minutes and the program requires 50 mL of Sodium Hydroxide (25–35 % w/w), NaOH, and Mil-Q water steam at 100 %. Before putting the digestion tubes in position, 25 mL of Boric acid (40g/L), H_3BO_3, is added in each tube. The distillate is collected in an Erlenmeyer flask.

The fourth and the last step is the titration of the distillate, where tree drops of indicator and 0.01 M H_2SO_4 are added where 1 mL of H_2SO_4 correspond to 0.028 mg of N-NH_4. The indicator used in the titration is the Tashiro's indicator which is made by dissolving 0.6 g of methyl red in 50 mL of 95% ethyl alcohol and then added to a methylene blue solution (2 g/L). The green colour correspond to an alkaline range and change from grey to pink (pH 4.9) in acid medium, up to red with excess of acid.

In every determination is needed to make a blank, using distillate water as sample and a Nitrogen standard solution. This solution is obtained by dissolving 153 mg of Ammonium Chloride in 100 mL of ammonia-free water, next adding 25 mL of the previous solution with 10 mL of 1 N Sulfuric Acid, H_2SO_4 and introducing free water ammonia to a total volume of 1 L. This final solution must have 0.01 mg of N-NH_3 per 1 mL. The sample needs to have an organic Nitrogen range of 2 – 150 mg.

3.1.6 Phosphorous

In order to evaluate the content of phosphorous in the wastewater, the Standard Method, # 365.3 was followed, which determines specific forms of phosphorus in water. In this method the ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphorus-molybdate complex. That is reduces to an intensely blue-colored complex by the ascorbic acid. The colour is proportional to the phosphorous concentration.

The determination procedure involving the digestion phase, was carried out by using the digestion unit VELP SCIENTIFICA DKL. In each digestion tube 50 ml of sample, 1 mL of 11 N sulfuric acid and 0.4 g ammonium persulfate was added and put in the digester at 100 °C during 30 minutes. After cooling 5 mL of sodium bisulfite were mixed and put in the digestion unit for 30 minutes at 95 °C. After this time the mixture was let to cool down. Afterwards 4 mL of the ammonium molybdate-antimony potassium tartrate were introduced and mixed and 2 mL of the ascorbic acid solution were added and mixed again. After waiting 5 minutes the absorbance was measured at 650 nm using a spectrophotometer. The standard curve to determine the phosphorous concentration was obtained with a Stock phosphorous solution, where 1.0 mL = 0.1 mg P.

3.2 <u>Experimental procedures</u>

During the laboratory work, in order to install a MBBR unit and treat the effluent, it was needed to follow a few steps, including the effluent choice, the inoculum adherence to the carriers and treatment tests in a continuous MBBR operation.

3.2.1 The effluent in study

Once the MBBR was first applied to a dairy industry in a previous work, this was also the effluent in study in the present research. The mixture was a simulated dairy wastewater composed by 1 part of low fat milk and 200 part of water.

3.2.2 Sludge used in the reactor

The sludge used in this experimentation was from the conventional activated sludge (CAS) in Ribeira de Frades Wastewater Treatment Station.

3.2.3 Carriers used in the reactor

The kind of carriers used in the MBBR was the “Kaldnes Evolution Aqua K1”as shown in Figure 6. These carriers have a diameter of 12 mm and 7mm of height, a filter area of 836 $m^2/m^{3,}$ a protected area of 494 m^2/m^3 and a density of 0.84 kg/dm^3.

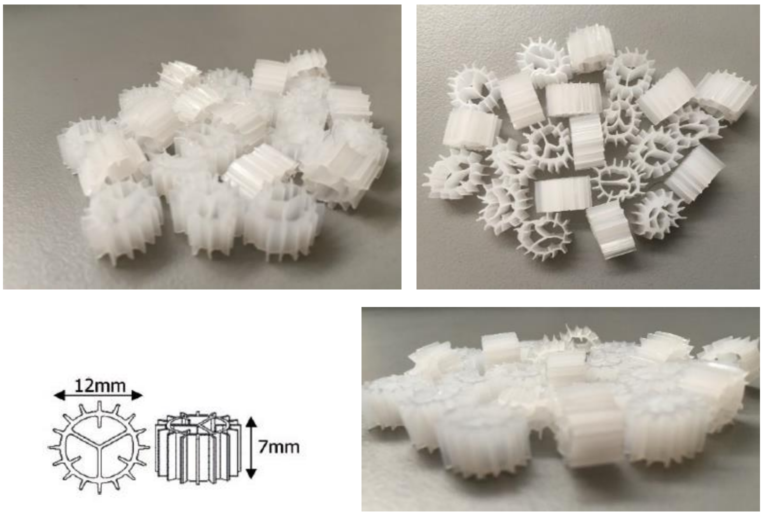

Figure 6 - Kaldness Evolution Aqua K1 carriers.

3.2.4 Inoculum preparation

In order to promote the attachment of the biomass to the bio-carrier i an inoculum reactor is demanded. For this, a Beaker glass of 1L volume is used with the inoculum media with 10% of sludge, a filling ratio of the carriers of 20% and effluent with a hydraulic retention time of 24h. This allows the biomass growth in a medium with constant values of nutrients and conditions (Zilli, (2013)).

The air was supplied with a pump which allowed to control the air flow that was kept in the minimum in the beginning, and the oxygen dissolved was around 6 mg O_2/L; in this way there is only the sludge moving free in the reactor while the carrier has a minimum movement. This allowed the biomass start to form biofilm inside the carriers.

The inoculum operation took about one month, and during this time the pH and COD were measured every day in order to check if there was any change in the system.

3.2.5 Tests with MBBR

After the carriers being inoculated, the MBBR is now operated. So in order to evaluate its performance, some teste were carried out changing the hydrodynamic retention time, the organic load rate and the filling ratio. Table 6 summarizes the testes made in the MBBR system.

Table 6 - Tests realized in the MBBR.

Test type	**Test code**
Effect of the organic load	TEST 1
Effect of the biocarriers fraction	TEST 2
Continuous operation	TEST 3

3.2.5.1TEST 1

In this test the effect of the initial organic load was evaluated, maintaining the same hydrodynamic retention time HRT of 8 h and filling ratio FR about 20% only change the organic load. The test was made with 4 different organic loads.

In the first day the effluent was added with a dilution of 1:200, and in the next three days was used a dilution of 2:200, 3:200 and 4:200, respectively.

During the test samples were collected every hour, and pH and COD were analysed. Figure 7 represents the lab-scale MBBR used during this operation.

Figure 7 - Lab scale MBBR.

3.2.5.2 TEST 2

In order to evaluate which is the optimal biocarriers fraction, a test similar to the TEST 1 was performed using now the FR equal to 40% and 60% for the same conditions referred above. The same operational parameters were assessed.

3.2.5.3TEST 3

As major purpose of this work, the continuous operation of the MBBR, this test was made to evaluate its performance. In figure 8 the continuous system of the MBBR is represented.

Figure 8 - MBBR in a continuous operation.

IV. Results and Discussion

Along this section the results obtained during the thesis elaboration, in all the operations described in Chapter 3 will be analysed.

4.1 Synthetic wastewater characteristics

As described in the previous chapter the effluent used during this study was a synthetic effluent with the characteristic presented in Table 7.

Table 7 - Characteristics of the synthetic wastewater.

Parameter	Value
pH	6.70
COD (mg O_2/L)	640
BOD_5 (mg O_2/L)	320
BOD_5 / COD	0.50
TSS (mg/L)	58
TKN (mg /L)	28
Total Phosphorus (mg P/L)	12

Being a synthetic wastewater, even though the characteristic slightly changed a little during the work, the values of the parameters were almost the same. This effluent show an organic load somehow low when comparing with the real wastewater (Table 2), as well as the Kjeldahl nitrogen and the total Phosphorous. A possible reason for this was, that low fat milk was used and, therefore, the fat oils and grease content was lower than usual, causing a decreasing of the other values.

The ratio BOD_5 / COD is an indicator of the wastewater biodegradability and a value of 0.5 represents an effluent with a good biodegradability, allowing the use of biological treatment.

Once the wastewater was treated in a biological way, there was a concerning about the nutrients ratio, COD:N:P = 100:5:1. The wastewater in study has values that generally correspond to this ratio and although N:P is around 2 is not a limitation within an aerobic biological treatment.

4.2 Sludge characteristics

To obtain a characterization of the sludge used in this study, the Chemical oxygen demand, pH and the solids were evaluated. The results are presented in Table 8.

Table 8 - Characteristics of the sludge.

Parameter	Value
pH	5.14
COD sludge (mg O2/L)	2180
COD filtrated (mg O2/L)	244
TSS (mg/L)	1065
VSS (mg/L)	945

4.3 Inoculum preparation

The time needed to promote the biofilm formation in the carriers, was called the inoculation period. During this time the air supplied was maintained constant and it was found that if it was kept in the minimum, 2 mg O_2/L, the sludge moved free in the reactor while the carrier had a minimum movement. This dynamic allows to biomass adhere and start to form biofilm inside the carriers. The inoculation test has occurred in two distinct periods.

4.3.1 First inoculation test

The first test took place in two Beaker glasses, one of 1L and other one of 2L, with different carriers, as seen in Figure 9. The Reactor A had operational volume of 900 mL, and the "Kaldness Evolution Aqua K1"were used. In the reactor B the Natrix F3 carriers were used, and had a working volume of 1800 mL was available.

Figure 9 - Reactors A and B used in the test.

During this test of 35 days the reactors were fed once a week in the first two weeks (day 7 and 14) and after two weeks (day 28), whereas the organic load and solids content were evaluated in these days and also in between.

Figure 10 represent the COD values that were measured in that period of 35 days.

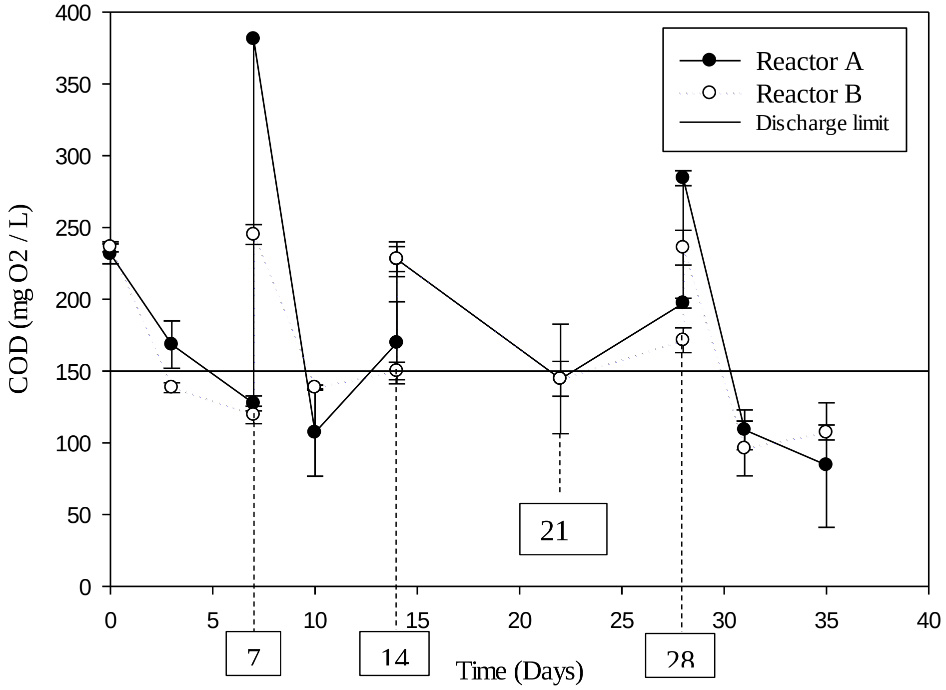

Figure 10 - COD variation in time, in the first inoculation test.

In this Figure it is possible to identify when the wastewater was added, because in these days two points are visible, with the lower value corresponding to the sample after degradation and the higher one obtained when the fresh wastewater is added.

Both reactors had a similar behaviour, relative to COD values; the last samples have a smaller amount of COD compared to the others, because of the acclimation of the sludge to the wastewater.

The evaluation of suspended solids was made for some samples being possible to observe in some cases that total and volatile solids amounts were about the same, whereas in other cases some decrease could be detected probably due to sludge removal jointly with the withdrawn samples, Figure 11.

In the reactor A, the total suspended solids had initially less variation; this happens because in the beginning the feed had the same period of time (one week), and after there is a 15 days interval between the next feed, so that the biomass didn't had nutrients and started to die; once dead they become food to the ones alive, so the dead ones become dissolved solids giving organic compounds to the medium. That explained the increase of the COD value observed in Figure 10 from day 21 until day 27 without adding wastewater. Regarding reactor B, results are somehow different due to the different carriers that were used.

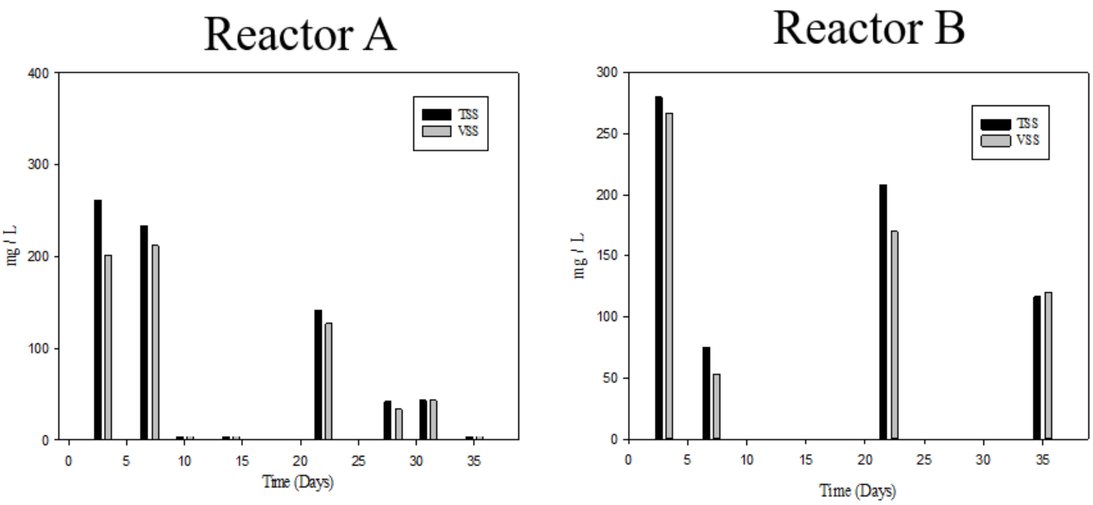

Figure 11 - Biomass behavior during experimental time for reactor A and B.

At the end of this test the biomass attachment and biofilm formation was not visible, so it was decided to feed the systems daily, once the biomass need to have a medium with constant conditions of pH, temperature and nutrients to growth.

4.3.2 Second inoculation test

In order to maintain the conditions of the medium constant, both reactors were every morning, except during the weekend, and the COD was evaluated in the instant that the wastewater was added to the reactor and approximately 24 hours after, as shown in Figure 12.

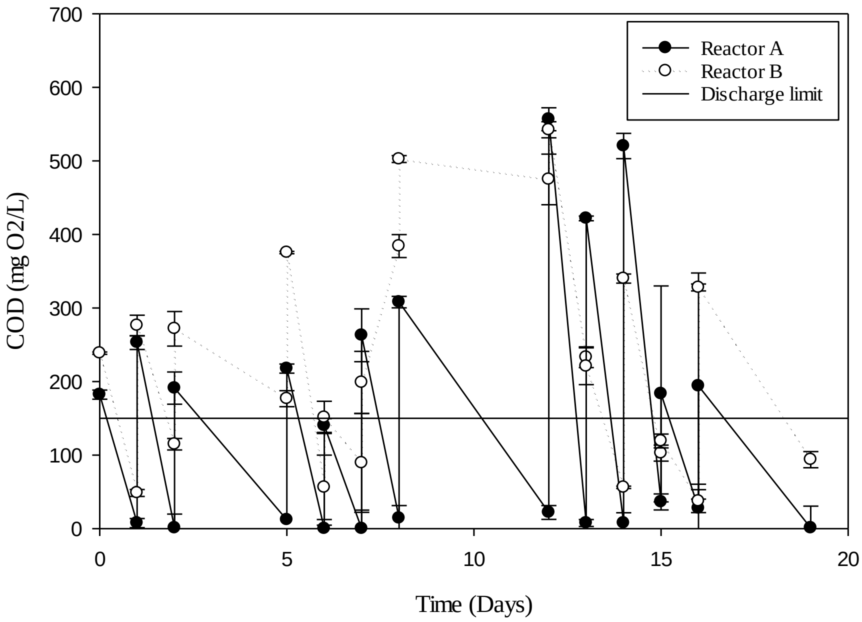

Figure 12 - COD variation in time, in the first inoculation test.

Throughout this test the initial organic load was almost the same during the first 8 days of experience, but after this time this value changed, maybe due to the contamination of the milk package in the fridge, where it was conserved.

The value of the residual COD in reactor A is almost 0 in every measure in consecutive days, resulting on a removal efficiency of 100%. In reactor B the COD removal capacity shows a different behaviour that was probably due to the different carriers that were used, pointing out in this case lower degradation efficiencies. In particular in day 7 an unexpected COD increase was observed maybe caused by biomass death whose corresponding organic matter might be dissolved in the liquid medium.

In this time the sludge colour was changed in reactor B. Figure 9 shows that in the beginning the sludge was brown and afterwards became white and at the end of the 19 days were white and smelly, Figure 13.

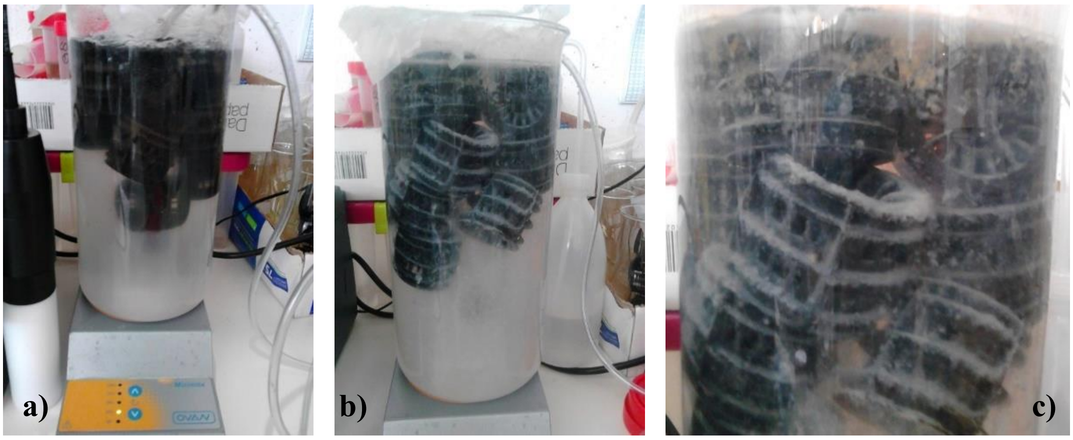

Figure 13 - Sludge changes in reactor B.

In reactor A it was visible a different sludge behaviour, since firstly the sludge was suspended in the reaction medium, then it started to adhere to the carriers and forming biofilm, as show in Figure 14.

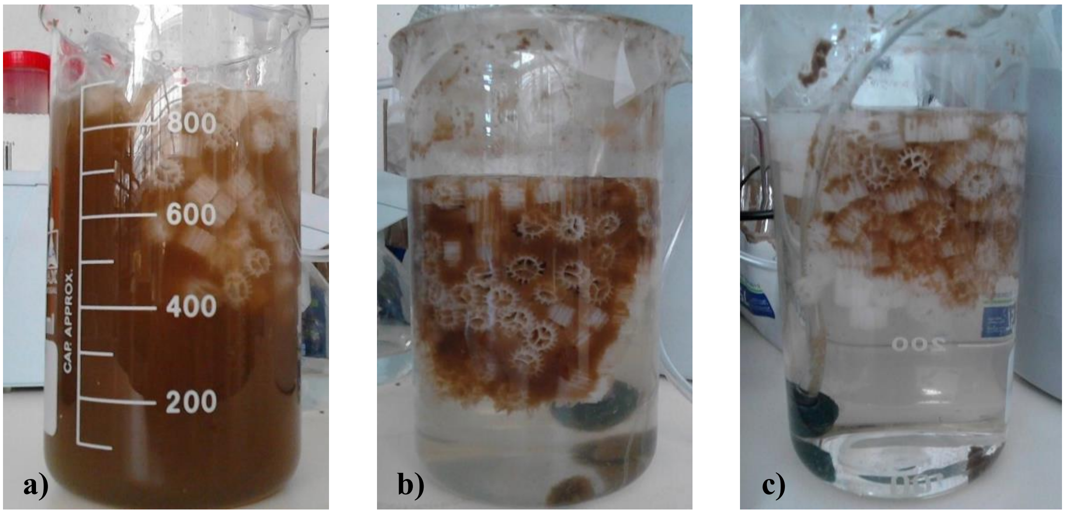

Figure 14 - Inoculum in reactor A. a) beginning, b) attachment of the biomass and c) biofilm formation.

Once only the biomass adherence to the carriers was noticed in reactor A, and this one had a higher COD efficiency removal, and the only difference between them was the carriers kind, the operation with reactor B was stopped wile reactor A was continued to be fed with fresh wastewater, until the biofilm formation, the only initial difference between them was the carrier kind,. During this time the COD and the pH were measured daily; the COD removal was between 75 and 100%, and the pH had values between 6.5 and 7.5 .

Figure 15 represents the COD values in the following 14 days, where only reactor A was fed diurnal. In the 38th day the test was stopped once the biofilm formation was observed.

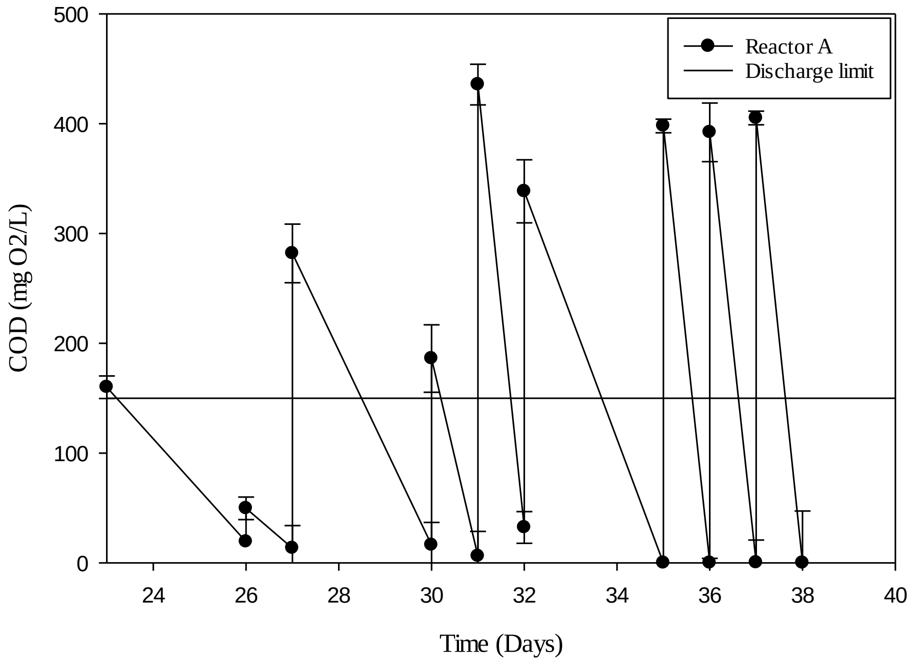

Figure 15 - COD variation in reactor A, during the following days.

Once more carriers with biofilm were needed to increase the process efficiency, to proceed with future tests, another Beaker glass with a different shape was tested (reactor C) as seen in Figure 16, without giving the result expected, biofilm formation.

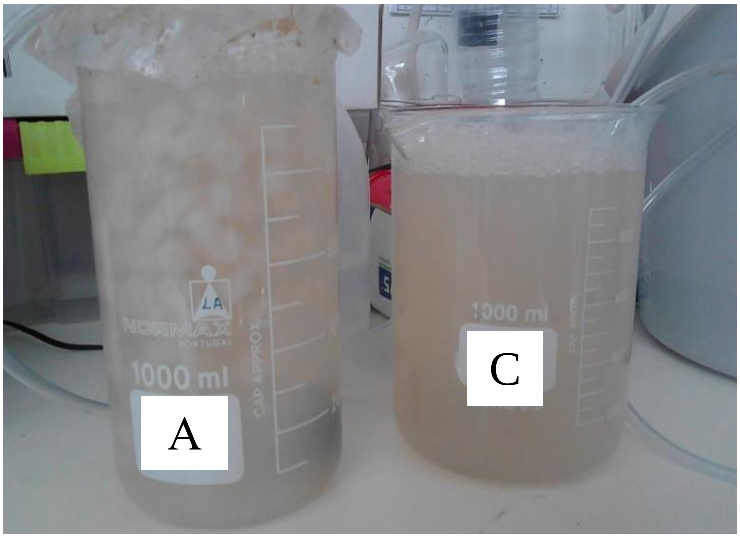

Figure 16 - Becker glass used in inoculation.

However, this change of reactor was useful to verify that shape is a parameter which may contribute to promote the attachment of the sludge and biofilm formation. With less diameter and higher height, reactor A, the biomass and sludge dispersion is avoided, promoting the sludge to stay near the carrier and start to forming biofilm, Figure 16. When the reactor C was used that didn’t happen, so the Beaker glass A revealed to have the optimal shape to promote the attachment.

Figure 17 - Steps to the biofilm formation.

In Figure 17 the steps to the biofilm formation cam be observed in three position of the same three supports; in the initial white carrier there is no sludge, while in the middle one some biomass is attached, and in the third one the biofilm is well visible, due to the brownish colour of the carrier.

4.4 <u>Study of the MBBR performance</u>

In order to evaluate the performance of the MBBR, some tests were performed, with different operational conditions, as Filling Ratio, Hydraulic Retention Time, and operation mode, batch or continuous.

4.4.1 Test 1

This test had as major purpose to study the impact of different initial organic loads. During this test the reactor A with a FR of 20%, was fed with different initial organic loads corresponding to a different concentration of the wastewater. In the first day the lower concentration, 1:200, corresponding to a COD of about 600 mg O_2/L, and in the following three days the concentration were 2:200, 3:200 and 4:200, corresponding to COD values of 800, 1100 and 1200 mg O_2/L respectively.

The reactor was filled with wastewater in the beginning of the test and the samples were collected from the reactional medium every hour until the experiment was completed, after a total reaction time of 8 hours.

Therefore, the initial sample correspond to the wastewater before being added to the reactor and the last one to the sample collected 8 hours after the reaction start.

Figure 18 shows a graphic representation of the normalized COD (COD/COD_0) evolution during the 8 hours of each experiment in four sequential days (day 1 to day 4).

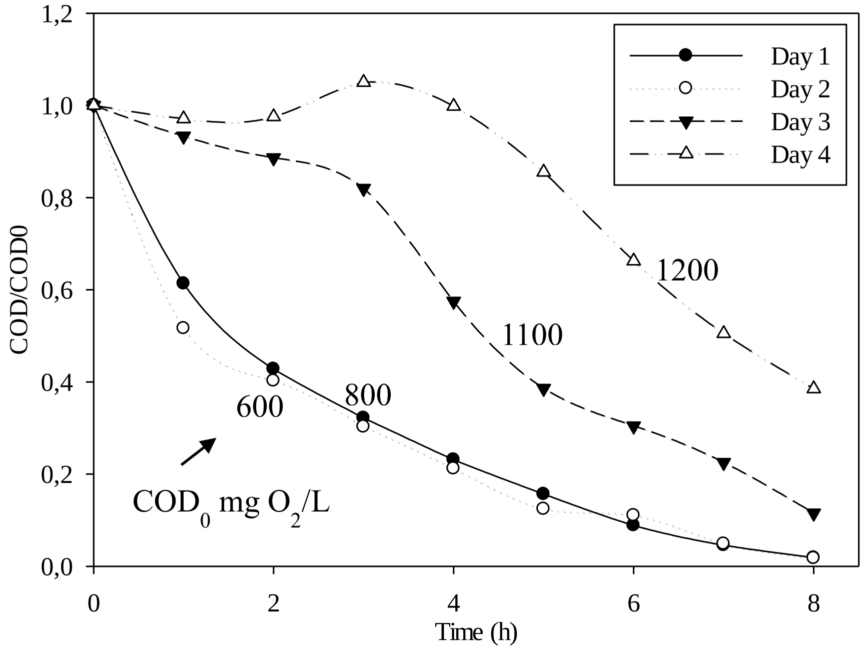

Figure 18 - COD evolution during time, for each of the four days of experience for test 1 with different initial organic loads COD_0.

During these four days the MBBR was operated in batch mode with different initial organic load COD_0, corresponding, however, all the cases to unit normalized COD/COD0 values In the day 1 the reactor was charged with 600 mg O_2/L and in the day 2 with 800 mg O_2/L, resulting on the same removal efficiency, about 98%. Indeed, booth expressed a similar behaviour, though starting with different OLR, what is a result of the sludge acclimation to the wastewater.

On the 3rd day the organic load was about 1100 mg O_2/L, and the MBBR system show a removal efficiency of 88%, which being lower than in the previous days led to a remaining COD of 135 mg O_2/L so that the effluent could be still able to discharge without other treatment. A slower degradation rate was, nevertheless, initially observed due to the higher organic amount.

In the last day only after 4 hours reaction the COD started to be removed, and at the end of the 8 hours the COD was 466 mg O_2/L. This is an indicator that the HRT used was not enough to treat a wastewater with an initial OLR of 1200 mg O_2/L.

Therefore, under these conditions of HRT and FR, is was possible to conclude that the maximum initial organic load, that the system can handle, is the one used in the day 3 corresponding to a COD value of 1100 mg O_2/L.

4.4.2 Test 2

Test 2 aimed to study the effect of the Filling Ratio in a batch operation. During this test the conditions were the same as referred above, i.e., during four days the initial organic load was changed and samples were collected during 8 hours, but this time the FR was 40% instead of 20%.

Figure 19 shows the behaviour of the system in reducing the initial organic load over the experience time.

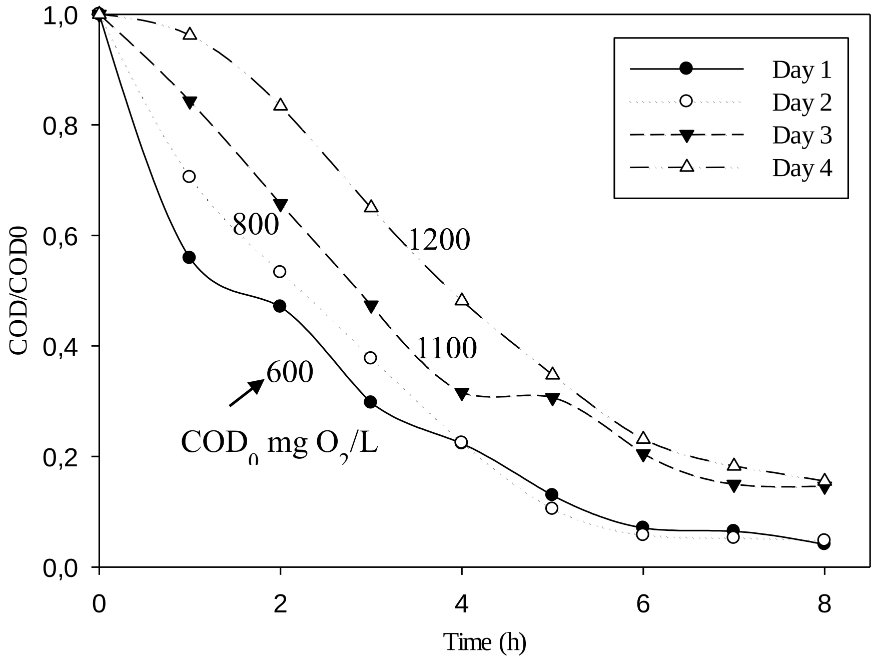

Figure 19 - COD evolution during experimental time, for the four days of experience for test 2.

As expected in day 1 and 2 the curves have a similar behaviour, such as had happened in test 1, whereas in day 3 and 4 higher initial rates were detected. A tendency for stable COD values after 6 hours of HRT, allows to conclude that, as expected, the higher filing ratio contributes to the efficacy of the system.

Once the objective of this test was to study the FR influence in the system performance, it makes sense to compare both test for each day separately, Figures 20 and 21.

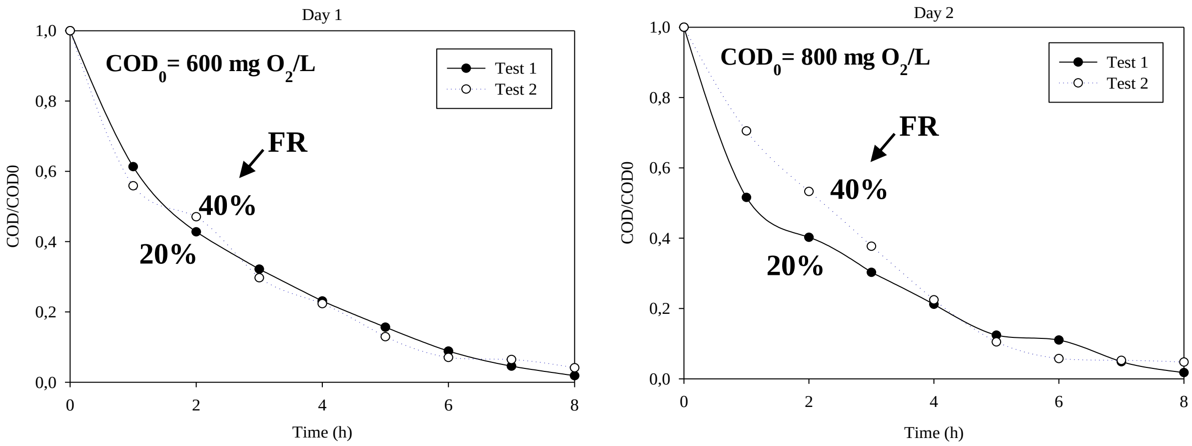

Figure 20 Comparison of COD/COD$_0$ for days 1 and 2 in both tests.

In day 1, both tests start with an initial OLR about 600 mg O_2/L, and an efficiency of 98% was obtain. For test 2, the system reveals a stable COD value after 6 hours, which did not happen in test 1, showing the need to extend the reaction time. For day 2 the initial OLR was around 800 mg O_2/L, and had an efficiency of 98% too, so in these two tests it is possible to conclude that with an initial OLR of 800 mg O_2/L the same results were obtained when using 600 mg O_2/L, but with a FR of 40% the HRT can be reduced from 8 to 6 hours in both tests.

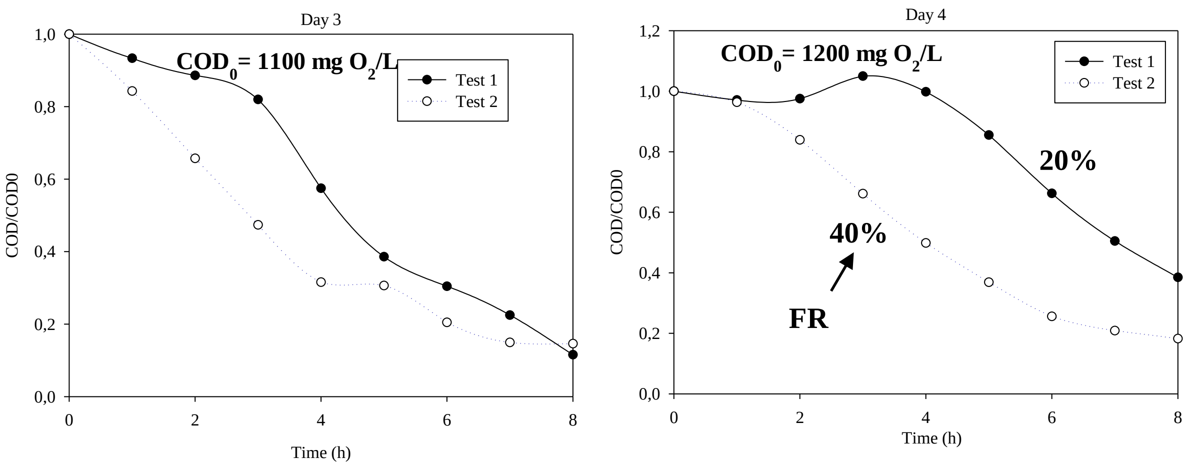

Figure 21 Comparison of COD/COD$_0$ for days 3 and 4 in both tests.

For days 3 and 4 system 2 reaches an almost stable value of COD, after 6 hours, even when the initial OLR is around 1100 and 1200 mg O_2/L respectively, which did not occur in test 1. All these evidences show that using a FR of 40% is more efficient than using 20%, because of the increase of the carrier specific area and consequently the area for the biofilm to degrade the organic matter.

During this test the difficulty for the carriers movement was visible, so that the test for a FR of 60% was not carried out, although some studies referred a FR of 70%. .

The MBBR was also applied as a nitrifying system (Rusten, *et al*., (1995)), and in order to evaluate this characteristic, the nitrogen presence in the samples was also assessed, by using the Kjeldahl's method, to measure the Total Kjeldahl's Nitrogen (TKN). Figure 22 is represents the values of the TKN in the wastewater, before and after treatment in each day for Test 2.

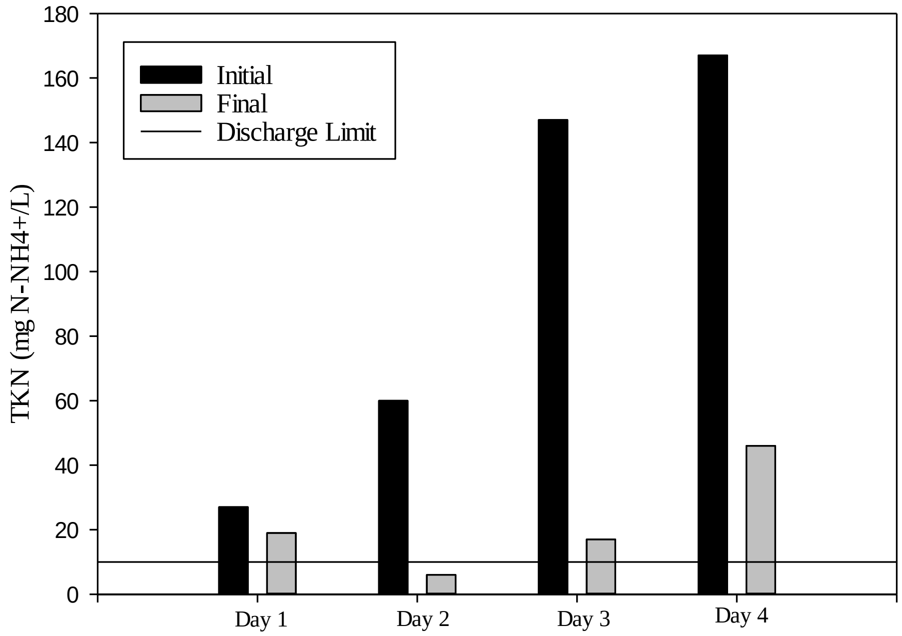

Figure 22 - Ammonia evolution in the four days of the Test 2.

The amount of the nitrogen present in the initial wastewater is proportional to the concentration used. After the treatment only in day 2 a value of nitrogen that allows discharge in water courses is obtained, (Decree Law number 236/98 of 1 of August).

4.4.3 Test 3

The continuous operation of the MBBR is the main idea of this project, so that some experiments, were run to check the influence of the HRT ant the FR. In this operation the reactor A was continuously fed with fresh wastewater by using a pump, and the treated water was also continuously removed.

4.4.3.1 The influence of the Hydraulic Retention Time HRT

For the process start-up the same conditions of the TEST 1 in day 2 were used (FR=20%), with a flow of 1.73 mL/min that corresponding a HRT of 8H, which proved in Test 1 to be a possible to reach a final effluent with optimal characteristics for discharge.

During the start-up the COD values were measured to check the system stability; the samples were collected at 24, 28, 32, 48, 72 and 76 hours after the start-up, and the results are showed in Figure 23.

The initial organic load was 610 mg O_2/L, and it is visible that after 24h the system is stable and the COD is lower than the discharge limit. The variations in the COD values my be due to the fact that the room where the experiment took place didn´t have controlled temperature, what may alter the properties of the feed and inside the reactor, and since there is no also control in sludge production, biomass death may contribute to increase COD.

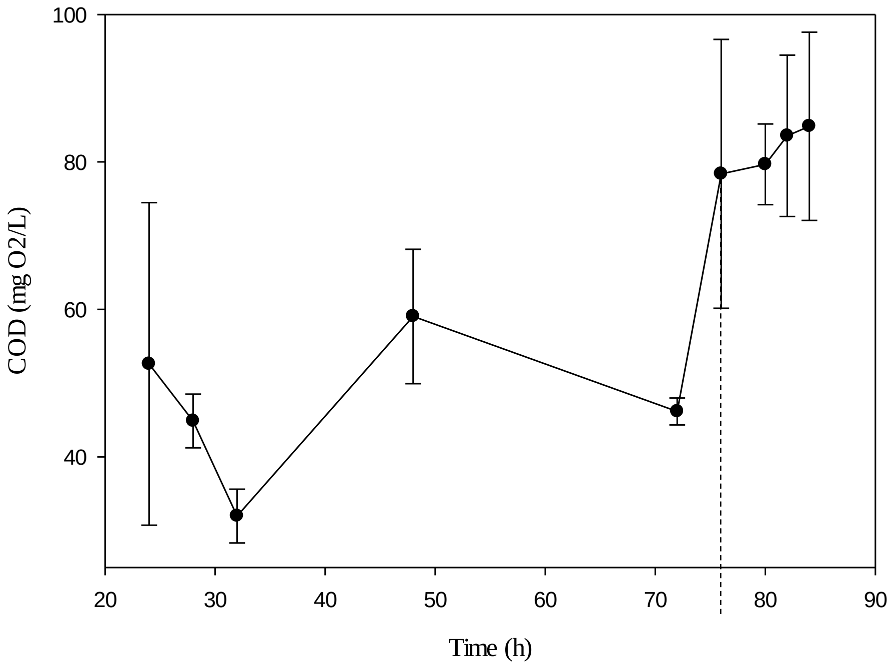

Figure 23 - COD variations in the continuous system during 84 hours.

In order to check the stability of the system, the flow was changed from 1.73 mL/min to 2.28 mL/min, changing the HRT from 8.6 hours to 6.5. This change happened at 76 hours, as seen in Figure 23, where an increase of the COD value was detected, although it still remained under the discharge limit; besides small variation within the following 6 hours were observed.

The next step taken, were to replace all the remaining wastewater in the MBBR, with fresh one, with an initial OLR of 900 mg O_2/L, and control the system by measuring the COD every hour since time zero till 9 hours after. This data are organized in Figure 24.

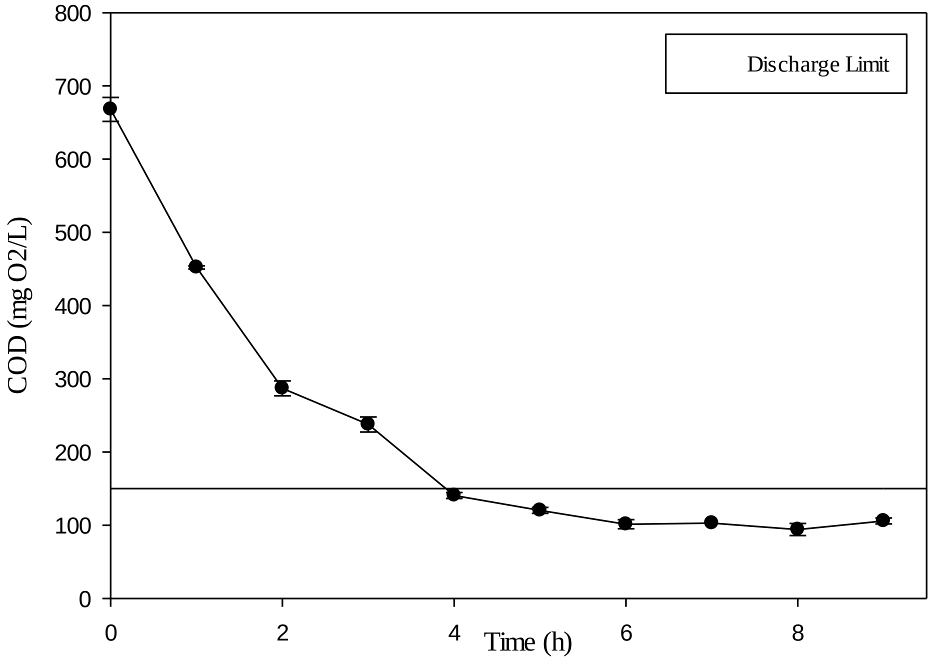

Figure 24 - COD evaluation of the MBBR with an HRT of 6 hours during 9 hours.

As occurred in Tests 1 and 2 for this initial organic load, after 4 hours of reaction the system shows a COD value under the discharge limit, and reached a constant value after 6 hours, as expected.

Once after 4 hours of reaction the COD attained the 150 mgO2/L threshold the inlet flow was increased from 2.28 mL/min to 3.85 mL/min, corresponding now to 4 h of HRT maintaining FR equal to 20%.

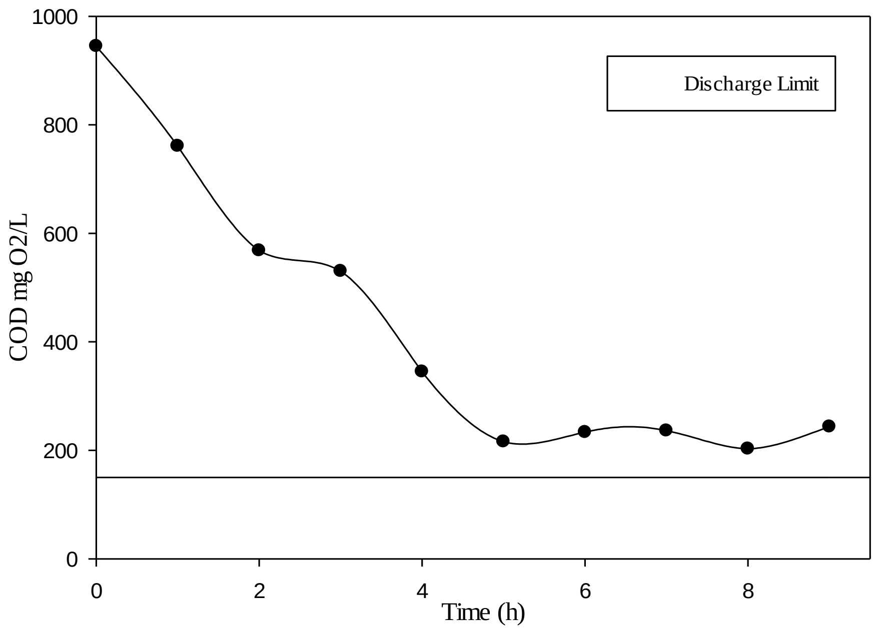

Figure 25 - COD evaluation of the MBBR with an HRT of 4 hours during 9 hours.

Figure 25 shows the COD behaviour of the MBBR operated in continuous being visible that after 4 hours the system reach a stationary point: however, this HRT under these conditions is not enough to obtain a COD value that allows its discharge, once is higher than the discharge limit of 150 mg O_2/L

4.4.3.2The influence of the Filling Ratio, FR

As demonstrated in the Test 2 the filling ratio has an influence in the MBBR efficacy, so a similar test was performed in the continuous operation. The initial organic load was maintained about 1100 mg O_2/L and the samples were collected during 9 hours.

In Figure 26 the data regarding the tests with the MBBR at different FR, 20 and 40% are presented.

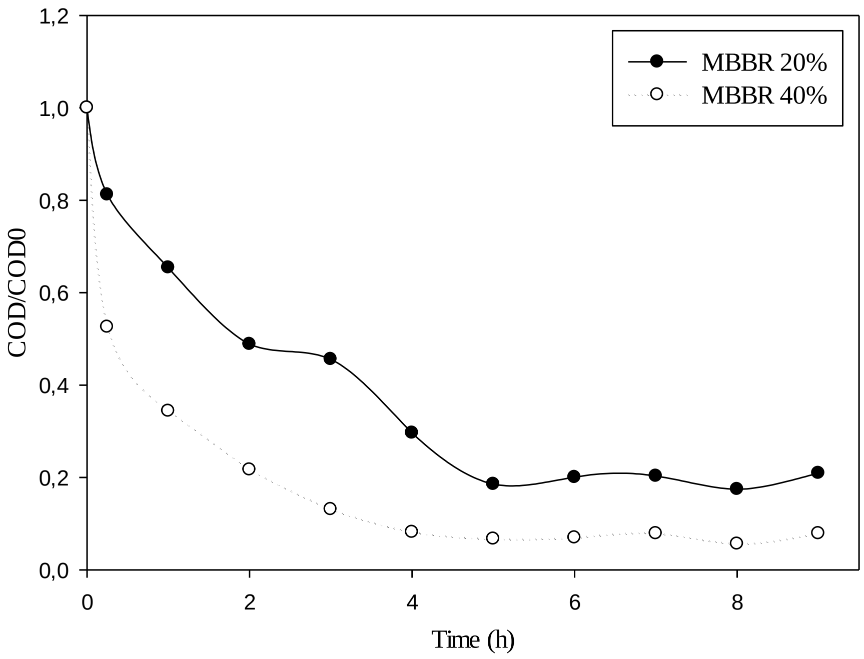

Figure 26 - COD/COD0 for the MBBR operated in continuous, with a Filling Ratio of 20 and 40%

Using a FR of 40% shows to be more efficient than using 20%, both achieving constant values of COD after 4 h, but when a higher amount of biocarries was used, the system had a more linear response, reaching COD values of 60-80 mg O_2/L after 4 h instead of 200 mg O_2/L detected for the case with FR equal to 20%. However when using a FR of 40% an intensification of the aeration system was required in order to promote the carrier movement, which will lead to higher energy costs.

Regarding the nitrogen removal the system an efficiency of 90% on the operation with FR 20% and 88% when using 40% of FR. In the Table 9 the values for the wastewater before and after treatment are presented, as well as the Discharge Limit, according to the Decree Law number 236/98 of 1 of August.

Table 9 - Data for Total Kjeldahl's Nitrogen

Sample	TKN (mg N/L)
Wastewater before treatment	60
Wastewater after treatment with MBBR at 20%	5.8
Wastewater after treatment with MBBR at 40%	6.8
Discharge Limit	10

After all the tests and analyses carried out along this work, it is possible to conclude that a Filling Ratio of 40% reproduce better results, even if that requires more costs for energy supplying, in order to promote the carriers movement.

The MBBR with a FR of 40% can be operated at an HRT of 4 hours, presenting a final COD value of 60-80 mg O_2/L and a TKN of 6.8 mg /L both values under the legal thresholds thus allowing direct discharge of the wastewater.

V. Conclusions and Future Work

The main objective of this work was the development and the performance evaluation of a lab-scale moving bed biofilm reactor, MBBR, for the treatment of a dairy wastewater.

First of all it was needed to promote the biofilm formation inside the carriers, usually called the inoculation stage. This is the most time consuming step because there are a lot of variables that require to be optimized, such as air flow, the reactor feed period, the filling ratio and the reactor shape. After a lot of attempts, it was possible to come to the conclusion that the air flow must be sufficient to promote the movement of the sludge, but not the carriers. A daily feed with fresh wastewater create a medium with constant conditions of pH, temperature and enough nutrients to the sludge growth. The optimal configuration of the reactor is the one that is preferentially narrow and tall. Such conditions promoted a sludge attachment and biofilm formation in 38 days.

Relatively to the MBBR performance, the continuous operation with a filling ratio, FR, of 40% revealed a more efficient process when compared with a FR of 20%, even if this FR increase the energy cost associated to the process. In this system the initial organic load rate, OLR, was around 1100 mg O2/L, and when operating with a HRT of 4 h COD values of 60 - 80 mg O2/L were attained. The nitrogen removal reached 88% of efficiency. Those values allow the wastewater discharge without other treatment, proving the efficiency of the MBBR.

The MBBR performance with a FR of 60% was not evaluated due the restricted movement of the carriers, already observed when tested with 40% of FR.

After the work that was developed, it can be concluded that the MBBR system application in order to treat the dairy wastewater shows a great potential, because the concentration of pollutants in the treated wastewater allows the direct discharge into the aquatic environment. That way, this process may be a great alternative to the conventional activated sludge, once it allows the fulfilment of the Law Decreed 236/98 of 1 August, and there is no need to install a final sedimentation unit.

As future works, it will be interesting to analyze of the MBBR behavior in a pilot plant and the costs associated, and compare the conventional activated sludge system with this advanced biological process. Also the application to other wastewaters and testing different kinds of carriers will be highly relevant.

Bibliography

Anon., 2015a. *AnoxKaldnes.* [Online]Available at: www.anoxkaldnes.com[Acedido em Fevereiro 2015].

Anon., 2015b. *Veolia Water Technologies.* [Online]Available at:ww.veoliawaterst.com [Acedido em Fevereiro 2015].

Borghei, S. & Hosseini, S., 2002. The treatment of phenolic wastewater using a moving bed biofilm rector. *Process Biochemistry,* pp. 1177-1181.

Calderón, K., J. Martín-Pascoal, J. M. Poyatos, B. Rodelas, A. González-Martínez, e J. González-López. “Comparative analysis of the bacterial diversity in a lab-scale miving ber biofilm reactor (MBBR) applied to treat urban wastewater under different operational conditions.” *Bioresource Technology,* 2012: 119-126.

Chaiudhari, D. H. & Dhoble, R. M., 2010. Performance evaluation of the effluent treatment plant of a dayri industry. *Current World Environmental,* Volume 5, p. 373.378.

Chen, S., Sun, D. & Chung, J., 2007. Treatment of pesticide wastwater by moving-bed biofiml reactor combined with Fenton-coagulation pretreatment. *Journal of Hazardous Material,* pp. 577-584.

Dezotti, M., Sant'Anna Jr., G. L. & Bassin, J. P., 2011. Reator de Leito Móvel com Biofilm; Moving Bed Biofilm Reactor - MBBR. Em: *Processos Biológicos Avançados.* s.l.:Interciência, pp. 43-87.

Dvorák, L., T. Ledere, V. Jirku, J. Masak, e L. Novák. “Removal of aniline, cyanides and diphenylguanidine from industrial wastewater using a full-scale moving bed biofilm reactor.” *Process Biochemestry*, 2014: 102-109.

Figueiredo, J. M., V. Fernandes, V. Limpo, L. Gonçalves, F. Pedrosa, e C. Diniz. *Guia Técnico - Indústria de Laticínios.* Lisboa: INETI, 2001.

Janni, K. A., Schmidt, D. R. & Christipherson, S. H., 2007. *Milk House Wastewater Characteristics,* Minnesota: University of Minnesota.

Jarpa, M., G. Pozo, R. Baeza, M. Martínez, e G. Vidal. “Polyhydroxyalkanoate biosynthesis from paper mill wastewater treated by a moving bed biofilm rector.” *Journal of Environmental Science and Health, Part A*, 2012: 2052-2059.

Li, H.-q., Han, H.-j. & Wang, W., 2011. Removal of phenols, thiocyanate and ammonium from coal gasification wastewater using moving bed biofilm reactor. *Bioresource Technology,* pp. 4667-4673.

Matín-Rilo, S., Coimbra, R. N., Martín-Villacorta, J. & Otero, M., 2014. Treatment of dairyindustry wastewater by oxygen injection performance and outlay parameters from the full scale implementation. *Journal of Cleaner Productions,* pp. 1-9.

Metcalf, L. and Eddy, H. *) Wastewater Engineering: Treatment and Reuse.* McGraw-Hill., 2003.

Odegaard, H., 1999. The moving bed biofilm reactor. *Water Environmental Engeneering and Reuse of Water,* pp. 250-305.

Odegaard, H., Gisvold, B. & Strickland, J., 2000. The influence of carrier size and shape in the moving bed biofiml process. *Water Science Tecnhonogy* , pp. 383-391.

Oliveira, D. V. M., Rabelo, M. D. & Nariyoshi, Y. N., 2014. Evaluation ofa MBBR (moving bed biofilm reactor) pilot plant for treatment of pulp and paper mill wastewater. *International Journal of Environmental Monitoring and Analysis,* pp. 220-225.

Patil, S. A., Ahire, V. V. & Hussain, M. H., 2014. Dairy wastewater - A case study. *Internation Journal of Research in Engineering and Technology* , Volume 3, pp. 30-34.

Porwal, H., Mane, A. & Velhal, S., 2015. Biodegradation of dairy effluente by using microbial isolates fromactivated sludge. *Water Resouces and Industry,* Volume 9, pp. 1-15.

Qiqi, Y., Qiang, H. & Ibrahim, H. T., 2012. Review on moving bed biofilm processes. *Pakistan Journal of Nutrition,* pp. 804-811.

Rusten, B., Eikebrokk, B., Ulgenes, Y. & Lygren, E., 2006. Design and operations of the Kaldnes moving bed biofilm reactors. *Aquacultural Engineering,* pp. 322-331.

Rusten, B., Hem, L. J. & Odegaard, H., 1995. Nitrification of municipal wastewater in moving ber biofilm reactors. *Water Environment Research,* Volume 67, pp. 75-86.

Rusten, B., Odegeer, H. & Lundar, A., 1992. Treatment of Dairy Wastewater in a Novel Moving Bed Biofilm Reactor. *Wat. Sci. Tech,* pp. 703-711.

Rusten, B., Siljudalen, J. & Strand, H., 1996. Upgrading of a biological-chemical treatment plant for cheese factory wastewater. *Water Science Technology* , Volume 34, pp. 41-49.

Sauer, K., 2003. The Genomics and Proteomics of Biofilm Formation. *Genome Biology,* pp. 219.1 - 219.5.

Sengil, I. A. & Özacar, M., 2006. Treatment of dairy wastewater by electrocoalation using mild steel electrodes. *Journal of Hazardous Materials,* p. 1197.1205.

Tikarilha, A. & Sahu, O., 2014. Study of Characteristics and Treatment of Dairy Industry Waste Water. *Journal of Applied & Environmental Microbiology,* Volume 2, pp. 16-22.

Vaidhegi, K., 2013. Treatment of Bagasse Based Pulp and Paper Industry Effluent Using Moving Bed Biofilm Reactor. *International Journal of ChemTech Research*, pp. 1313-1319.

Zilli, R. P., 2013. *Influência Do Tempo De Retenção Hidráulica E Da Velocidade Superficial Do Ar No Desempenho De Um Reator De Leito Móvel Com Biofilme (MBBR)*, Florianópolis.