UNIVERSITAT DE BARCELONA

A contribution to resource recovery from wastewater

Anaerobic processes for organic matter and nitrogen treatment

Núria Basset Olivé

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Programa de doctorat d'*Enginyeria i Tecnologies Avançades*

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El Dr. **JOAN MATA ÁLVAREZ**, catedràtic del Departament d'Enginyeria Química de la Universitat de Barcelona i el Dr. **JOAN DOSTA PARRAS**, professor agregat interí del mateix departament,

CERTIFIQUEN QUE:

El treball d'investigació titulat "**A CONTRIBUTION TO RESOURCE RECOVERY FROM WASTEWATER. ANAEROBIC PROCESSES FOR ORGANIC MATTER AND NITROGEN REMOVAL**" constitueix la memòria que presenta l'Enginyera Química **Núria Basset Olivé** per a aspirar al grau de Doctor per la Universitat de Barcelona. Aquesta tesi doctoral ha estat realitzada dins del programa de Doctorat "*Enginyeria i Tecnologies Avançades"*, en el Departament d'Enginyeria Química de la Universitat de Barcelona.

I perquè així consti als efectes oportuns, signen el present certificat a Barcelona, Juliol de 2015.

Dr. Joan Mata Álvarez i Dr. Joan Dosta Parras

Directors de la tesi doctoral

I have not failed. I've just found 10,000 ways that won't work.

Thomas A. Edison

Agraïments

Són moltes les persones que haig de mencionar en aquest capítol, de ben segur el que serà el més llegit de tots. Des de fa 4 anys, i si comptem bé des de fa més de 6 anys (entre el TFC i el màster), que vaig aterrar al departament on m'he creuat amb molta gent de qui més qui menys n'he après coses interessants.

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Abstract

Organic matter and nutrients present in urban and industrial wastewater should be removed or valorised to reduce its impact on the environment. Conventional wastewater treatments are focused on the removal of these pollution sources at the minimum cost. The idea of resource recovery from wastewater is changing the concept of the conventional wastewater treatment plants that tend to incorporate little by little processes as anaerobic digestion, MBR, biofilm, granulation, etc. However, their application to obtain reusable by-products from wastes should be cost effective.

Anaerobic digestion processes are well-known to achieve high organic matter removal efficiencies without oxygen requirement, low biomass production and energy generation from biogas. Conventional digesters usually treat organic rich streams (e.g. sewage sludge, manure, etc.) operating at high hydraulic and solid retention times (HRT and SRT). However, for low strength wastewater treatment, biomass immobilisation technologies should be applied in order to uncouple the HRT from the SRT; thus avoiding biomass wash-out. For this reason, anaerobic membrane bioreactor (AnMBR) was considered as a possibly feasible system for wastewater treatment.

Winery wastewater is an effluent with a highly biodegradable organic load, worth considering for biogas production, and low nutrient content. A sidestream AnMBR was started up and operated under organic load oscillations in order to determine its feasibility for winery wastewater treatment. The stable operation was assured by keeping a ratio between intermediate alkalinity and total alkalinity (IA/TA) below 0.3 achieving a 96.7±2.7% COD removal efficiency. The maximum organic loading rate (OLR) achieved was 3.4 kgCOD m^{-3} d^{-1}. The biogas production varied according with the OLR that was on average 0.50±0.17 m^3_{biogas} $m^{-3}_{digester}$ d^{-1} with an 87.1±3.0% of methane. The external membrane module reached a flux of 20.2 ± 8.5 LMH operating at a mixed liquor suspended solids (MLSS) concentration of 4.78±1.9 g L^{-1} and a crossflow velocity of 0.64 m s^{-1}. The crossflow velocity helped to remove the cake layer attached on the membrane that was the main contribution (>80%) to flux decline.

The energy demand of the AnMBR was calculated considering the net energy production of a combined heat and power (CHP) unit, and the expenses of pumping, stirring, influent heating and heat losses. The energy production was calculated regarding the production of biogas obtained and the HRT at which the digester operated at lab-scale. It was concluded that only when influent COD was over 3.25 gCOD L^{-1} the energy balance was positive. Since winery wastewater suffers seasonal variations, to achieve a positive energy balance at mesophilic temperature is not always possible. During vintage, there is enough COD to cover energy expenses. However, the rest of the year COD concentration can be around 500 - 1000 mg L^{-1} so that the requirements would be higher than the energy recovered from biogas. However, considering the upscaling of the AnMBR of the present study the submerged membrane configuration would be a more feasible option due to its lower operational costs, especially in winter season. Therefore, taking into account that a submerged membrane configuration requires around 0.3 kWh m^{-3}, the energy balance becomes positive when

influent COD is over 460 mg L^{-1} which corresponds to a P_B=0.06 m^3_{biogas} $m^{-3}_{digester}$ d^{-1}. Hence, submerged AnMBR for winery wastewater treatment would be a suitable option at full-scale application.

Simulating the conditions of winter season, winery wastewater was treated in the AnMBR at low temperatures of 25°C and 15°C. Since the organic load of winery wastewater in winter is much lower than in summer (vintage season), the average OLR applied was 0.32 and 0.29 kgCOD $m^{-3}_{digester}$ d^{-1} and the average COD removal reached was 80% and 71% at 25°C and 15°C., respectively. As expected, the efficiency of the system was lower than in mesophilic conditions and higher amount of VFA were accumulated in the digester that promoted the enrichment of the biomass in Methanosarcina as the main methanogen observed. Due to the operation at low temperatures, methane got dissolved in the permeate and low biogas production was obtained. Following Henry's law, it was determined that the methane lost dissolved in the liquid phase corresponded to a 6.7% and 10.2% at 25°C and 15°C., respectively. Moreover, higher degree of fouling was observed despite the amount of suspended solids was lower. Frequent cleanings were necessary, although they were carried out without chemicals since the main resistance was due to the cake layer on the surface, thus a high crossflow velocity was enough to recover the initial flux.

The upflow anaerobic sludge blanket (UASB), which is a more typical configuration for anaerobic wastewater treatment, was also studied in order to compare the advantages and drawbacks of both systems. An OLR up to 5.5±1.2 kgCOD m^{-3} d^{-1} was applied reaching an 84±9% of total COD removal. A significant amount of SS was observed in the UASB effluent that led to a high effluent COD over the discharge limits. For this reason, the UASB was coupled with a membrane unit (UASB-MBR) reaching higher COD removal efficiency of 92±4% with an effluent COD of 0.11±0.06 mg L^{-1} and free of suspended solids. However, compared with the membrane performance in the AnMBR at low temperatures, the membrane filtration was not significantly improved. The flux and the flux decline determined were only slightly better, although the solids in contact with the membrane unit were considerably lower. Despite the UASB could treat a higher OLR, the granules suffered disaggregation with the sharp oscillations of OLR, typical from winery wastewater.

The effluent of anaerobic digestion often requires a post-treatment to remove nutrients, especially nitrogen. Compared with conventional biological nitrogen removal, nitritation/denitritation (N/DN) via nitrite represent a 25% less aeration and 40% less external carbon source. In order to reach higher flexibility and reducing space requirements, N/DN can be carried out in a sequencing batch reactor (SBR). Under feast and famine conditions applied in the SBR, storage compounds can be served as internal carbon sources for post-anoxic denitrification. A novel scheme was developed for the treatment of municipal wastewater; consisting in an UASB reactor followed by a short cut sequencing batch reactor (scSBR) in the main water line. Nitritation/denitritation was integrated with the selection of polyhydroxyalkanoate (PHA) storing biomass. An aerobic-feast and anoxic-famine regime was adopted, thus denitritation was driven by internally stored PHA. Biowaste fermented liquid was applied as carbon source in the feast regime. The SBR was operated at a nitrogen loading rate of 0.075 kgN m^{-3} d^{-1}. The average nitrogen removal was 83%. PHA stored reached its maximum at the

time VFA were depleted, and it progressively decreased under aerobic conditions when nitritation took place. After achieving an acceptable ammonia removal of 93%, there was enough available PHA for the subsequent denitritation, reaching a maximum nitrite removal of 98%. The PHA accumulation capacity was evaluated in fed batch tests. The maximum PHA content was 10.6% (gPHA $gTSS^{-1}$) after 10 h of accumulation when biowaste fermented liquid (C/N/P= 100/4.5/0.42) was applied. Nitrogen removal limits could be successfully met while PHA-storing biomass was selected. Although higher PHA yields can be achieved under complete aerobic conditions, this novel scheme presents an added value due to the integration of the PHA production in the nitritation/denitritation process.

Another cost-effective treatment of anaerobic digestion effluents is the Anammox process, combined with a previous step of partial nitritation (PN). When compared with conventional biological nitrogen removal process, the PN - Anammox process avoids the requirement of organic carbon source to denitrify, produces about 85% less of sludge and allows saving around 60% of the oxygen supply, thus reducing energy requirements. A two-step PN/Anammox process was carried out at lab-scale conditions to treat reject water of a municipal WWTP. PN was achieved in a granular SBR obtaining an effluent with a NH_4^+-N/NO_2^--N molar ratio around 1.0. The microbial characterization of this reactor revealed a predominance of Betaproteobacteria, with a member of Nitrosomonas as the main autotrophic ammonium oxidizing bacteria. Nitrite oxidizing bacteria were under the detection limit of 16S rRNA gene pyrosequencing, indicating their effective inhibition. The effluent of the PN reactor was fed to an Anammox SBR where stable operation was achieved with an observed NH_4^+-N:NO_2^--N:NO_3^--N stoichiometry of 1:1.25:0.14. The slight deviation to the theoretical stoichiometry could be attributed to the presence of heterotrophic biomass in the Anammox reactor (mainly members of Chlorobi and Chloroflexi). Planctomycetes accounted for 7.9% of the global community, being members of Brocadia the main anaerobic ammonium oxidizer detected.

1. Introduction

Nowadays, the preservation of hydraulic resources and water reclamation are becoming a necessary task among industries and also society. The major motivation falls on water scarcity, which is a present fact favoured by the increase of population and human activities. However, not only water saving is an important issue, but reducing the costs and taking benefit from the depuration by-products can turn a wastewater treatment plant (WWTP) into a profitable business.

In 2009, the project *"De Energiefabrike" (Energy Factory)* was born in Holland to promote the design of new plants that incorporate advanced technologies (i.e. granulation, biofilms or membranes) trying to minimise the operational costs and reach a neutral or even a positive energy balance (López-Palau and de Kreuk, 2010). The objective of this concept is to improve the treatment efficiency in order to obtain products from wastewater that have an added value. For instance, processes already applied as anaerobic digestion can be placed in the main water line converting the organic matter into biogas and reducing significantly the overall sludge production. Ammonium would be removed latter by Anammox or via nitrite nitritation/denitritation reducing considerably the aeration and external carbon sources requirements. Particularly, the use of an anaerobic membrane bioreactor coupled with an Anammox unit in the main water line, would lead to an increase in biogas production of 13% and a reduction of oxygen demand around 85% (Lema, 2012).

More recently, the storage compounds that are accumulated in the biomass, as fat for humans, are of interest due to their possibilities to replace petroleum based plastics. Although the application of bioplastic production in a WWTP is still in an early stage, since 1990's several industries are producing it by pure cultures. The cost of the accumulation and especially the extraction of the bioplastic is very high. For this reason, pure cultures, which have the capacity to accumulate up to 90% in weight of storage compounds, are widely used in industries. Despite the process to accumulate and extract bioplastic is complex and expensive, the current price ranges between 3€ kg^{-1} and 5€ kg^{-1}. It is estimated that the benefit obtained from the organic matter in terms of chemical oxygen demand (COD) would be 1.75€ kg^{-1}COD; while biogas production can reach 0.07 € kg^{-1}COD (Fatone et al., 2014).

These attempts to recover resources from wastewater would only be implemented if the operational costs are lower than the conventional processes. Therefore, the scientific research is focused on reducing the costs of these technologies to become a feasible option.

1.1. LEGISLATION

Due to the raising concern about water pollution, stringent regulations appear to protect the environment from the adverse effects of wastewater. National and European legislations have been introduced to protect environment from human damaging activities. They regulate the maximum allowed concentrations of organic matter, nitrogen and phosphorus in wastewater discharged to the rivers and other water sources. In the European Union, the Council Directive 91/271/EEC concerning urban wastewater treatment was adopted to protect the water environment from the adverse effects of discharges of urban and certain industrial wastewater discharges. There is a general need for secondary treatment to prevent the environment from being adversely affected by the disposal of insufficiently treated wastewater. Member states shall ensure that urban wastewater entering collecting systems should be subjected to secondary treatment or equivalent treatment before discharge.

On 1998, the European Commission issued Directive 98/15/EC amending Directive 91/271/EEC to clarify the requirements of the Directive in relation to discharges from WWTP to sensitive areas which are subject to eutrophication Table 1.1.

Table 1.1. Requirements for discharges from urban WWTP to sensitive areas which are subject to eutrophication (Directive 91/271/EEC amended by Directive 98/15/EC)

Parameter	Concentration	Minimum reduction
BOD_5 ($mgO_2\ L^{-1}$)	25	70-90%
COD ($mgO_2\ L^{-1}$)	125	75%
Suspended solids ($mgSS\ L^{-1}$)	35	90%
Total nitrogen ($mgN\ L^{-1}$)	15 (10,000-100,000 p.e.) 10 (>100,000 p.e.)	70-80%
Total phosphosrus ($mgP\ L^{-1}$)	2 (10,000-100,000 p.e.) 1 (>100,000 p.e.)	80%

1.2. INCREASING TREATMENT CAPACITY AND WATER REUSE BY MBR TECHNOLOGY

The legal requirements about water discharge are getting stricter, thus new technologies are considered to accomplish the legislation and satisfy the water demand. In recent years, membrane bioreactors (MBR) have been developed and put into practise in wastewater treatment field improving the results in terms of water quality. MBR technology consists of a combination of a biological treatment followed by a solid-liquid separation by means of a membrane. The bioreactor and the membrane should be considered as an only one unit operation because their interactions affect positively the final result (Drews, 2010). In Table 1.2, the main advantages and disadvantages of the MBR technology are summarized.

Table 1.2. Advantages and disadvantages of the MBR technology (Wang et al., 2009b)

Advantages	Disadvantages
- High quality effluent able to be reused - Compact facility and modular design - High biomass concentration and short hydraulic retention time - Complete solid-liquid separation, independent on settling velocity. - Less sludge production - Flexible and simple operation	- High energy consumption (aeration to reduce membrane fouling) - Periodic maintenance cleanings

The MBR technology in urban wastewater treatment, which is the earliest application, has some advantages; for instance the high effluent quality practically free of suspended solids and disinfected, because the pore size is generally below 0.1 μm (Santos et al., 2011). The task of the membrane, in comparison with conventional activated sludge (CAS) treatment, is to replace the secondary clarifier reducing considerably the space requirement. Moreover, the presence of a membrane makes the hydraulic retention time (HRT) completely independent of the sludge retention time (SRT), which leads to a better control of the biological processes, a decrease of sludge production and a minimisation of the reactor volume. The longer the SRT, the higher the biomass concentration in the bioreactor, which in WWTPs ranges normally between 8 and 18 g L^{-1} of mixed liquor suspended solids (MLSS) (Brepols et al., 2008; Judd, 2011).

The capital cost required for MBR implementation in a WWTP is relatively high, although it is decreasing appreciably due to the standardisation of the modules, which can be applied to an existing facility (Buer and Cumin, 2010). Anyway, the major contribution to costs is associated to the energy consumption and the maintenance of the membrane, especially to reduce fouling. This is the reason why the energy optimisation (aeration, filtration and relaxation cycles, cleaning, etc.) is really of utmost importance in order to save in terms of operational costs and modules replacement.

Another factor involved in the optimisation of a MBR is the membrane material, which is normally polymeric based. However, it has been observed that hydrophobic materials are more prone to fouling than hydrophilic materials (Meng et al., 2009). Furthermore, the anti-fouling capacity of membranes can be improved by providing polar groups that enhance filtration, therefore membrane surface can be treated with NH_3 and CO_2, TiO_2, copolymers, etc. (Asatekin et al., 2006; Bae and Tak, 2005a; Yu et al., 2008).

The high operational costs are compensated by the excellent effluent quality obtained, but sometimes such a high quality is not necessary to meet the legal requirements. Hence, in order to reduce costs, there is the possibility of replacing the microfiltration (MF) and ultrafiltration (UF) membranes by filters of larger pore size and thus cheaper (Iversen et al., 2007; Seo et al., 2007; Ye et al., 2006). However, the main limitation of the application of these filters is the pore blockage by the sludge flocs. The roughness of the surface area and the greater pore size favour the deposition of solids on the filter surface, which are difficult to remove by aeration. Another alternative consists of covering the surface of the filter with activated carbon, which mitigates fouling and improves effluent quality (Ye et al., 2006).

Only when effluent reuse is considered, operational and maintenance costs of a MBR-WWTP are lower than a CAS-WWTP, because CAS process will always require tertiary treatments to achieve an effluent able to be reused as advanced oxidation processes (Brepols et al., 2010; Fenu et al., 2010a). In other words, to obtain the same effluent quality CAS treatment implies a higher cost than MBR treatment.

1.1.1. Possible MBR configurations

There exist two possible configurations of MBR: submerged/immersed or sidestream. In the late twentieth and early twenty-first century, sidestream MBR was the most used. However, regarding both the number of applications and the capacity, immersed MBR seems to be more successful for the time being. There exist different types of membranes, but only three of them are commercialised for urban wastewater treatment: flat sheet (FS) and hollow fibre (HF) for immersed MBR, and multitubular for sidestream MBR (Judd, 2011).

Immersed MBR. The main feature of immersed MBR configuration (Figure 1.1a) is that it involves aeration, clarification and filtration processes in the same reaction vessel. A MBR plant can be made from the beginning as well as *a posteriori* modifying a CAS already built. During its operation, air is supplied by means of diffusers placed in the base of the reactor. Coarse bubbles help to reduce membrane fouling, since high shear forces improve the removal of solids accumulated on the membrane surface (Kraume and Drews, 2010). However, their oxygen transfer is worse than supplying smaller bubbles, therefore the bubble size should be optimised or simply different sizes of diffusers can be installed. Anyway, periodic physical and chemical cleanings are required to recover permeability.

Immersed MBR configuration is the most used, but to make its control and optimisation easier, membrane can be placed in an external tank outside the biological reactor (Figure 1.1b). As both processes are physically separated, the system becomes much more flexible because each step can be independently optimised.

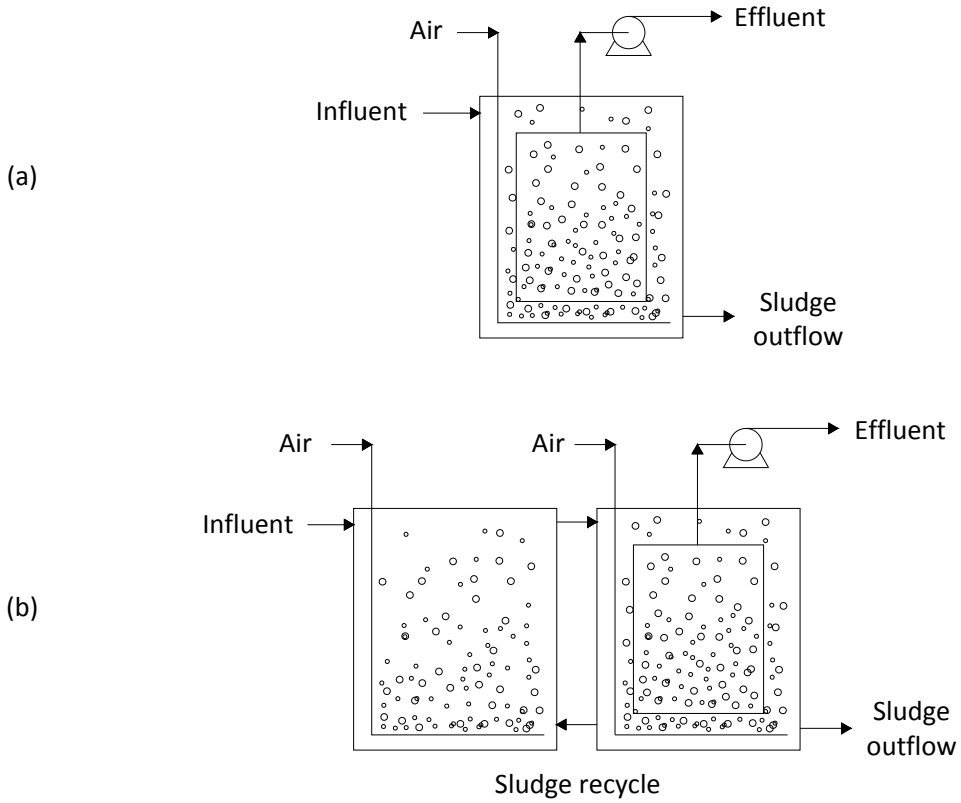

Figure 1.1. Submerged MBR (a) and submerged MBR with an external tank (b)

Sidestream MBR. This configuration consists of a membrane unit located aside from the biological reactor (Figure 1.2), thus cleaning and operation flexibility are its main advantages. Nevertheless, a higher space requirement and an additional pumping unit are required. In this case the filtration is driven by pressure while vacuum is the driving force in immersed systems.

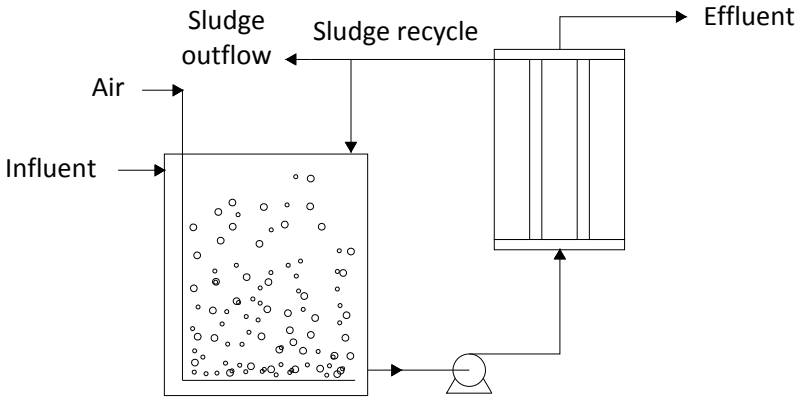

Figure 1.2. Sidestream MBR

1.1.2. MBR application for aerobic processes

Compared with CAS, MBR has greater organic matter and nutrient removal potential since biomass concentration can be higher. In CAS process MLSS concentration is limited at about 3.5 g L^{-1} due to its settling properties, among other factors. The higher biomass concentration, the worse settling performance, and thus the content of suspended solids in the clarified effluent may easily exceed the legal limit. This is the reason why organic matter and nutrient removal is limited by the maximum biomass concentration that CAS can accept. The main advantage of MBR lies in that achieving high MLSS concentration is possible due to the biomass immobilisation by means of a membrane, which allows a high quality effluent regardless of the settling properties. The increase of biomass concentration leads to a higher yield and consequently a reduction of the reactor volume. Nevertheless, a large quantity of biomass in the mixed liquor leads to an increase of viscosity, which implies higher energy consumption for stirring and aerating.

As MBR can operate with longer SRT, it allows the presence of slow growing microorganisms, like nitrifying bacteria and others which take part in the biodegradation of organic macromolecules that are difficult to remove (micropollutants). Nutrient removal in MBR takes place combining anaerobic, anoxic and aerobic zones (Di Trapani et al., 2011;

Fenu et al., 2010a; Kim et al., 2007; Lyko et al., 2008; Monclús et al., 2010). This system is similar to CAS, but there is a membrane instead of a secondary clarifier. The membrane should be placed in the aerobic zone or in an external aerobic tank, because aeration helps to remove the solids attached on its surface.

On the other hand, phosphorus is removed by polyphosphate accumulating organisms (PAO) whose growth is stimulated when they are subjected to feast and famine periods. In anaerobic conditions, PAO assimilate the organic matter in the mixed liquor as polyhydroxybutyrate (PHB) whereas phosphorus is released. In aerobic or anoxic conditions, where organic matter content is low, PAO use the PHB previously accumulated to produce energy and during this process phosphorus is taken up. The point in this process is that PAO are able to take more phosphorus than it is released. In a CAS process, the anaerobic conditions of the clarifier enable PAO to release phosphorus; therefore a concentration below 1 mg L^{-1} is difficult to achieve. On the contrary, in the MBR phosphorus concentration is as low as possible since the permeate is obtained from the aerobic zone. However, the study of Monclús et al. (2010) revealed that an insufficient recirculation between the aerobic and anaerobic zones can seriously affect PAO performance, because they require intermittent aerobic/anaerobic cycles.

Concerning the biokinetic parameters, Di Trapani et al. (2011) evaluated the biomass from a University of Cape Town (UCT)-MBR pilot plant by means of respirometric techniques, concluding that the operational conditions (SRT in particular) strongly influence the kinetic behaviour. In comparison with a CAS system, a decrease in the heterotrophic kinetic parameters and a higher biomass activity was observed with lower SRT values. Regarding nitrifying bacteria, the parameters were within the range proposed for nitrification in CAS system, although a high SRT is essential to reach a complete nitrification.

In order to achieve an efficient organic matter and nutrient removal not only biomass concentration is important, but other operational parameters (HRT, SRT, flux, organic loading rate, etc.) and design parameters (reactor configuration, membrane type and material, etc.). The optimal values of these parameters cannot be generalised, because each MBR plant has different configuration and its optimisation will provide own results. In Table 1.3 some published examples of MBR at municipal scale are shown.

Table 1.3. Published examples of urban scale MBR facilities

Author	Flux ($Lm^{-2}h^{-1}$)	Flow rate (m^3d^{-1})	Membrane		Cycle (s)	SRT (d)	HRT (h)	SS ($g\ L^{-1}$)	% Removal			
			Type	Area (m^2)					COD	BOD	TN	TP
(Brannock et al., 2010)	11.8	1,100	FS	-	-	16.6	-	11.3	92	99	99*	-
	29	1,100	HF	-	-	9.9	-	5.0	94	98	100*	-
(Fenu et al., 2010b)	22-34	230	HF	10,160	300/ 25	14-21	3.5-5	9-12	82	93	48	78
(Kim et al., 2007)	14.5-20.8	210	HF	11.7	420/ 180	30	6	8	95.2	98.2	72.7	71.4
(Lousada-Ferreira et al., 2010)	33.6	-	HF	10,560	-	21	-	11	-	-	-	-
	24.3	-	FS	4,110	-	20	-	12	-	-	-	-
(Lyko et al., 2008)	12.1-23.4	16,000 - 45,000	HF	84,480	400/ 50	25	-	10-12	97.6	99	82.8	95.4
(Zanetti et al., 2010)	21	-	FS	3,840	-	-	-	-	97	-	-	-

*% NH_4^+-N removal
FS: flat sheet
HF: hollow fibre
COD: chemical oxygen demand
BOD: biological oxygen demand

1.1.3. MBR application for anaerobic processes

Anaerobic digestion is a biochemical process which, in the absence of oxygen, biodegradable organic matter is decomposed into biogas. The conversion of the organic matter into biogas is a process which involves several reactions, both in series and in parallel, and different groups of microorganisms (bacteria and archaea). The anaerobic digestion process may be subdivided into the following four phases (Figure 1.3):

1. *Hydrolysis:* Complex and undissolved organic matter is decomposed into simple soluble organic molecules which can pass through the cell walls and membranes of the fermentative bacteria.
2. *Fermentation or Acidogenesis:* The dissolved compounds present in cells of fermentative bacteria are converted into simple compounds (volatile fatty acids, alcohols, lactic acid, CO_2, H_2, NH_3 and H_2S).

3. *Acetogenesis:* The fermentation products are converted into acetate, hydrogen and carbon dioxide by what are known as acetogenic bacteria.
4. *Methanogenesis:* Acetate and hydrogen/carbon dioxide are converted into methane and CO_2 by methanogenic bacteria.

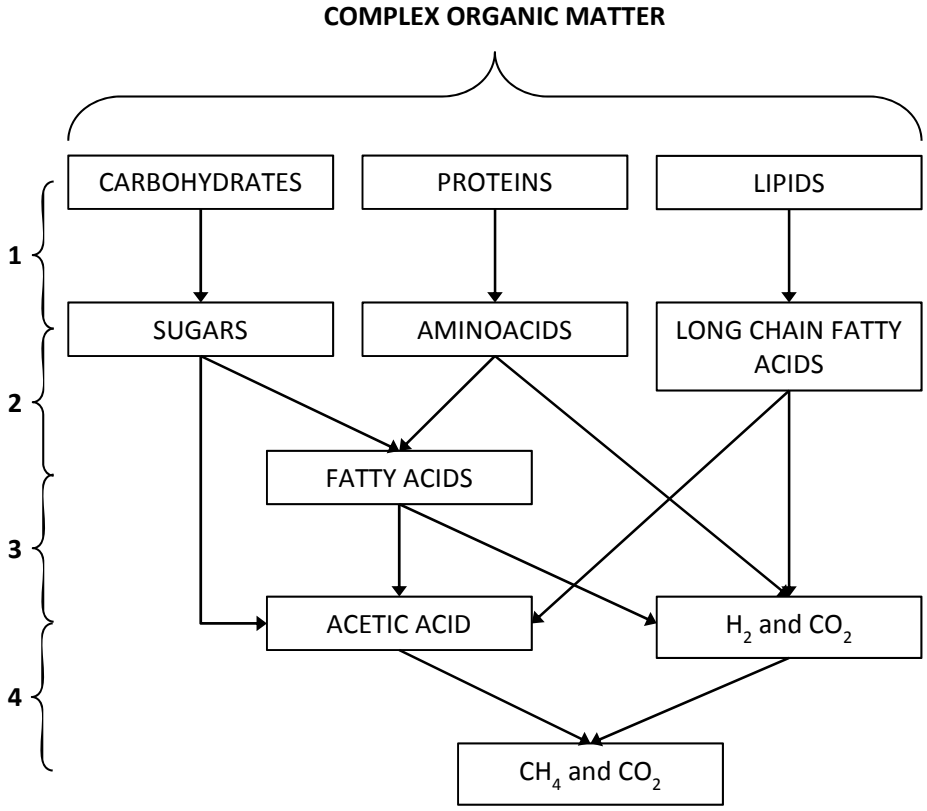

Figure 1.3. Anaerobic digestion pathway

Analysing the reasons why anaerobic digestion is rapidly growing, the following advantages of anaerobic treatment over conventional aerobic treatment can be given:

- **Reduction of excess sludge production up to 90%.** Since the anaerobic processes result in lower biomass production by a factor of about 6 to 8 times, sludge processing and disposal costs are reduced greatly.
- **Less energy required.** Anaerobic processes may be net energy producers instead of energy users, as is the case for aerobic processes. This is a very significant benefit, especially when it exists an insistent need for cost reduction.

- **Higher Volumetric Loads.** Anaerobic processes generally can achieve higher volumetric organic loads than aerobic processes, so smaller reactor volumes and less space may be required for treatment.
- **Lower nutrient demand.** Many industrial wastewaters present a lack of sufficient nutrients to support aerobic growth. The cost for nutrient addition is much less for anaerobic processes because less biomass is produced.

However, potential disadvantages also can be found:

- **Longer start-up periods.** It usually takes months for anaerobic versus days for most of aerobic processes.
- **Need for alkalinity addition.** The most significant negative factor that can affect the feasibility of anaerobic versus aerobic treatment is the possible need of extra alkalinity. Alkalinity concentrations of 2,000 to 3,000 mg$CaCO_3$ L^{-1} may be needed in anaerobic processes to maintain an acceptable pH.
- **Need for further treatment.** Anaerobic processes can also be followed by polishing aerobic processes to remove nutrients. Two-step reactors of anaerobic-aerobic processes have been shown feasible for treating municipal wastewater in warmer climates resulting in lower energy requirements and less sludge production.
- **Increased potential of odours and corrosive gases production**.
- **Heating requirements.** Mesophilic conditions (37ºC) are the most desirable to achieve high biogas production rates. However, when wastewater to treat has low organic content, the operation at high temperature would not be feasible.
- **Less stability after 'toxic shock'.**

In general, for municipal wastewaters with lower concentrations of biodegradable COD, lower temperatures, higher effluent quality needs, and nutrient removal requirements, aerobic processes are preferred currently. For industrial wastewaters with much higher biodegradable COD concentrations and elevated temperatures, anaerobic processes may result more economical.

The interest in the anaerobic MBRs (AnMBR) is increasing, due to the advantages of the combination of an anaerobic digester with membrane filtration. The AnMBR removes efficiently biodegradable organic matter without oxygen requirement, low biomass production, energy generation from biogas and offers many advantages over other anaerobic processes which must operate at high HRT to allow the growth of methanogenic bacteria.

The AnMBR can be defined as an anaerobic bioreactor coupled with a membrane filtration unit. The possible AnMBR configurations are analogous to those of aerobic MBR (Judd, 2011). However, whereas the aerobic MBR facilities have clearly opted for submerged membrane configuration, the anaerobic MBR still has no clear trend. An increase of the submerged membrane applications can be found in recent literature (Dereli et al., 2012; Ferrer et al., 2015; Smith et al., 2012), as a result of the positive experience in aerobic MBR. Nevertheless, as a tool to reduce membrane fouling, the produced biogas can be used instead of aeration (Akram and Stuckey, 2008). However, if not enough biogas is obtained, it should be compressed to be recirculated, therefore the energy cost of the AnMBR might not be lower than an aerobic MBR.

With AnMBR it is possible to treat high organic loads (10-15 kgCOD m^{-3} d^{-1}), due to biomass retention, which implies a reduction of the digester volume and space requirement. Moreover, in this case, a previous solid separation is not necessary and thus the global process becomes simpler, the sludge production is reduced and the biogas generation even improves.

AnMBR generally works under mesophilic (30-45°C) or thermophilic (55-65°C) temperatures (Liao et al., 2006a). Under thermophilic conditions, the reaction rate and the biogas production are higher. However, the thermophilic processes are much sensitive to changes in the influent conditions (Ahn and Forster, 2002). In addition, several studies indicate that membrane fouling is more important in thermophilic AnMBR due to smaller flock size, more production of soluble microbial products and more dense and compact fouling cake (Jeison and van Lier, 2008; Lin et al., 2009).

The literature about AnMBR at psychrophilic conditions (<25°C) is increasing in the last five years. The operation at low temperatures would be appropriate for low organic loaded wastewaters (i.e. urban wastewater) as it does not consume energy on heating. Anaerobic digestion treatment for urban wastewater at psychrophilic temperatures was performed with upflow anaerobic sludge blanket (UASB) reactors and similar (Elmitwalli et al., 2001; Foresti, 2002; Mahmoud et al., 2004). In this sense, AnMBR appears as a promising technology due to the total biomass retention.

Giménez et al. (2011) worked on the implementation of a pilot-scale AnMBR (submerged UF hollow fibre membranes) for urban wastewater treatment at a temperature of 35°C, with the aim of reducing it at 20°C. High and stable biogas production was achieved (>70% of CH_4 v/v) as well as low volatile fatty acid concentration in the effluent (<10 mg L^{-1}). Furthermore, Ho and Sung (2010) studied the start-up of two lab-scale AnMBRs (tubular MF membranes) for synthetic wastewater treatment reaching more than the 85% of COD removal.

Some drawbacks of the AnMBR technology have also been reported as the lack of nutrient removal and the long start-up period. Another aspect to consider is the presence of sulphates that can be reduced to H_2S by means of sulphate-reducing bacteria, causing the inhibition of methanogenic bacteria, bad odours, corrosion, etc.

Moreover, it should be noted that the effluent requires a post-treatment due to the dissolved methane that can be recovered, oxidised aerobically or used as carbon source in the denitrification process reducing the potential greenhouse gas emissions (Foresti, 2002). This fact is very important at low temperatures because gas solubility increases. For instance, methane solubility at 15°C is 1.5 times higher than at 35°C, which can be a very important fraction of total methane production (Smith et al., 2012). There exist several processes to recover dissolved methane:

- **Methane stripping with air** (McCarty et al., 2011): the energy demand associated is less than 0.05 kWh m^{-3}. However, the resulting mixture from the stripping has potential explosion hazards, and also the efficiency of removing dissolved methane from AnMBR effluent with this practice is not well established yet.
- **Degassing membrane** (Bandara et al., 2011): this type of membranes are permeable to gases but not to liquids. Higher efficiencies at lower temperature are reached as a result of a higher methane solubility at lower temperatures. However, the energy requirements for degassing can be higher than the energy recovered.
- **Down-flow Hanging Sponge (DHS) reactor** (Hatamoto et al., 2010): dissolved methane is consumed biologically up to 95%. However, energy recovering is not possible since dissolved methane is oxidized by microorganisms.

1.1.3.1. Application of AnMBR for industrial wastewater treatment

The AnMBR is an alternative to other types of reactors for intensive anaerobic digestion, such as the Upflow Anaerobic Sludge Bed (UASB), the Expanded Granular Sludge Bed (EGSB) and the Internal Circulation (IC), based on the biofilm technology and granules, commonly applied for the industrial water treatment with high organic load (Van Lier, 2008). While these technologies can cope with higher loads, up to 40 kg COD m^{-3} d^{-1} (Liao et al., 2006a), one of the advantages of the AnMBR is the total retention of biomass, regardless of its aggregation or sedimentation properties. Hence, when the wastewater characteristics negatively affect biofilm or granular formation (e.g. high solid content, high temperature, toxicity, high salinity, drastic changes in organic loads or in HRT), the retention of slow growing biomass able to acclimate to extreme conditions is crucial (Dereli et al., 2012). Due to the aim of saving and reuse water, industrial wastewater will tend to increase the concentration of pollutants (Van Lier, 2008); therefore the SRT should be long enough for

biomass acclimation and slowly biodegradable organic matter removal. Hence, membrane filtration is favoured because no bacteria are discriminated. In contrast, other systems of biomass immobilisation promote the selection depending on sedimentation or aggregation capacity. The following Table 1.4 summarizes the main advantages and disadvantages of the AnMBR according to the characteristics of industrial wastewater.

Table 1.4. Advantages and disadvantages of the AnMBR for industrial wastewater treatment in extreme conditions (Dereli et al., 2012)

Conditions	Advantages	Disadvantages
High suspended solid content	– Effluent free of SS – Degradation of slowly biodegradable SS due to the high SRT.	– Inert SS accumulation.
Thermophilic temperature	– Total retention of biomass although aggregation properties get worse. – Lower mixed liquor viscosity	– Increase of membrane fouling due to a higher decay constant. – Lower fluxes than mesophilic due to a higher fouling.
Toxicity	– Higher biomass adaptation, due to total retention. – Enhanced growth of specialized bacteria improving degradation.	– Suspended biomass is more sensitive to toxics than granular or biofilm biomass.
High salinity	– High biomass adaptation, due to total retention.	– High biomass decay due to the osmotic pressure stress.
Drastic changes in organic load and HRT	– No risk of biomass wash-out. – Possible changes in HRT are adjusted according to the installed membrane area.	– Suspended biomass is more susceptible to acidification. – Unexpected changes in load promote the extracellular material liberation, increasing the fouling.

Since 2000, AnMBR technology has been applied at industrial scale, mainly in Japan, for the treatment of organic waste and industrial wastewater with high organic content from distilleries, septic tanks, food and paper industries, etc. (Grant et al., 2008; Kanai et al., 2010). Among these AnMBR applications, it should be noted the major AnMBR plant worldwide treating wastewater from food industry, which is equipped with submerged Kubota membranes achieving a COD removal of 99.4% (475 m^3 d^{-1}; 39 g COD L^{-1}; ratio biochemical oxygen demand (BOD/COD = 0.46) at a temperature of 33°C (Christian et al., 2011). In Table 1.5 examples of full-scale AnMBR for industrial wastewater treatment are summarized.

Table 1.5. AnMBR examples for industrial wastewater treatment

Wastewater	Configuration	Removal (%COD)	TSS ($g\ L^{-1}$)	OLR ($kgCODm^{-3}d^{-1}$)	Flux (LMH)	HRT (d)	SRT (d)	T (°C)	Biogas production	Authors
Ethanol thin stillage	iMBR (flat sheet; 0.08 µm; 18 m^2)	98	24	4.5-7	4.3±1.1	16	200	37	0.31 $m^3CH_4\ kg^{-1}COD$	(Dereli et al., 2012)
Snacks factory wastewater	iMBR (hollow fibre; 0.4 µm; 2 m^2)	97	7.9-10.4	5.1	6.5-8	-	-	35	-	(Diez et al., 2012)
Thermomechanical pulping pressate	iMBR (flat sheet; 70 kDa; 0.03 m^2)	76-83	10.9±0.5	2.59±0.53	5.7-6.9	-	350	37	0.21 $m^3biogas\ kg^{-1}COD$	(Gao et al., 2012)
Palm oil mill	sMBR (tubular; 0.1 µm; 0.024 m^2)	96-99	11.8-20.8	1-11	-	6.8	12.1	-	0.25-0.57 $m^3CH_4\ kg^{-1}COD\ d^{-1}$	(Abdurahman et al., 2011)
Food processing	iMBR (flat sheet; 0.4 µm)	99.4	23	1.2	2.5-4.2	29		33	-	(Christian et al., 2011)
Thermomechanical pulping whitewater	iMBR (flat sheet; 70 kDa; 0.03 m^2)	90	6.7-9.1	2.6-4.8	4.8-9.1	-	280	37	0.25-0.30 $m^3CH_4\ kg^{-1}COD$	(Lin et al., 2011)
Brewery with surplus yeast	sMBR (tubular; 0.2 µm)	>97	25	1-12	6-20	-	-	30	-	(Torres et al., 2011)
Stillage from tequila production	iMBR (flat sheet)	95	-	4.8	-	12.4	70	37	-	(Grant et al., 2010)
Potato processing	iMBR (flat sheet; 0.4 µm)	99	40	2-12	0.83-5	3.5-14	80	35	0.34 $m^3CH_4\ kg^{-1}COD$	(Singh et al., 2010)
Acidified cheese whey	sMBR (0.2 µm; 0.4 m^2)	98.5	6.4-10	5-20	137-140	4	30-79	37	0.3 $m^3CH_4\ kg^{-1}COD$	(Saddoud et al., 2007)
Food processing	sMBR (flat sheet; 70 kDa; 0.32 m^2)	81-94	6-8	0.88-4.52	13.1-18.9	2.5	50	37	0.136 $m^3biogas\ kg^{-1}COD$	(He et al., 2005)

iMBR: immersed or submerged membrane bioreactor
sMBR: sidestream membrane bioreactor

1.1.3.2. Application of AnMBR for urban wastewater treatment

The AnMBR technology is beginning to be applied for urban wastewater treatment. However, it is not understood as an intensive technology concept but as a necessarily low temperature treatment, which produces a high quality effluent (Smith *et al.*, 2012). The main limitation of the mesophilic anaerobic digestion of urban wastewater is the biogas production, which does not cover the heating requirements. Therefore, since urban wastewater is low loaded influent, anaerobic digestion would only feasible at ambient temperature, thus it would be more appropriate for warm climates (Liao et al., 2006a). In addition, the organic matter degradation in anaerobic conditions is much more complex than in aerobic conditions. The growth of anaerobic bacteria is slower, thus longer start-up periods are required. For these reasons, historically the aerobic treatments have been developed faster and were preferably applied. The AnMBR technology aims to reduce the limitations of the conventional anaerobic digestion by the total biomass retention. The filtration unit prevents the biomass washout allowing their growth and acclimatization to hardly biodegradable compounds.

During the last decade, the popularity of aerobic MBR have significantly increased due to its higher treatment capacity and effluent quality compared to conventional aerobic treatments. But MBR establishment was specially favoured by the reduction of membrane costs. Although the capital costs have been adjusted to the market needs, the operational costs of the MBR technology are still high because of the aeration required to reduce fouling on the membrane surface. For this reason, the interest in AnMBR technology is increasing, but it is still under research at lab- or pilot-scale. According to Martin et al. (2011), when COD concentration is higher than 4-5 g L^{-1}, there is enough biogas production to cover the heating costs of mesophilic digestion. Since urban wastewater usually has a COD concentration about ten times lower, AnMBR would only be energetically feasible operating at ambient temperature.

The operation of AnMBR at low temperature negatively affects the kinetics of organic matter degradation, especially the hydrolysis of particulate organic matter (Lettinga et al., 2001). Due to total retention of particulate matter in the AnMBR, an acceptable efficiency of particulate COD removal have been observed even in psychrophilic temperature (about 25°C), although it is reduced when temperature decreases in winter (Bandara et al., 2012). Hence, the SRT must be long enough to achieve high particulate organic matter removal. In addition, as urban wastewater has low organic load, short HRT is preferable in order to minimize the bioreactor size. Therefore, AnMBR operation should try to minimize the HRT and maximize the SRT. Nevertheless, these parameters are limited. A high SRT involves an

increase of the biomass concentration and the production of extracellular polymeric substances (EPS) and soluble microbial products (SMP) (Huang et al., 2011), which have a direct effect on the membrane fouling. On the other hand, a short HRT also promotes production of EPS and SMP, particularly in the case of the AnMBR with external membrane module due to the high crossflow velocities that stress the biomass (Salazar-Peláez et al., 2011). Hence, a short HRT promotes the accumulation of soluble COD in permeate, reducing its quality (Baek et al., 2010). For these reasons, the HRT should be minimised, but it is important to define a minimum limit taking into account the process efficiency and the degree of membrane fouling.

The origin of the inoculum may have a significant impact during the start-up of the AnMBR at low temperature (Smith et al., 2012). Psychrophilic microorganisms found in natural habitats are the most promising and feasible option (Xing et al., 2010), due to the large number of psychrophilic methanogenic and acetogenic microorganisms that have been isolated (O'Flaherty et al., 2006). However, according to Smith et al. (2012) studies, which compared mesophilic and psychrophilic inoculum, it was observed that after 275 days of operation at 15°C the microbial communities were similar, concluding that the communities were mainly formed by psychrotolerant mesophilic microorganisms. Even though the AnMBR works properly with a psychrophilic inoculum, its efficiency at higher temperatures should be considered since the temperature of urban wastewater may vary up to 20°C throughout the year. For this reason, a combination of both communities (psychrophilic and mesophilic) would be worth considering in order to deal with the seasonal variations.

Some examples of AnMBR for urban wastewater treatment are listed in Table 1.6.

Table 1.6. AnMBR examples for urban wastewater treatment

Configuration	COD_{inf} (mg L^{-1})	COD_{eff} (mg L^{-1})	VSS (g L^{-1})	Organic Load	Flux (LMH)	HRT (h)	SRT (d)	T (°C)	Biogas production	Authors
iMBR 5L (flat sheet; 0.45 µm; 0.118 m^2)	426.8	60.8	6	0.18 kgCOD kg^{-1}VSS d^{-1}	-	10	30	25-30	0.19 L CH_4 d^{-1}	(Huang et al., 2013)
iMBR 5L (flat sheet; 0.45 µm; 0.118 m^2)	426.8	60.8	9.3	0.12 kgCOD kg^{-1}VSS d^{-1}	-	10	60	25-30	0.45 L CH_4 d^{-1}	
iMBR 5L (flat sheet; 0.45 µm; 0.118 m^2)	426.8	61.9	9.9	0.13 kgCOD kg^{-1}VSS d^{-1}	-	10	90	25-30	0.50 L CH_4 d^{-1}	
iMBR 1.3 m^3 (hollow fibre; 0.05 µm; 30 m^2)	459	<100	5-25	-	13.3	24.5	70	20	100 L d^{-1}	(Robles et al., 2013)
iMBR 1.3 m^3 (hollow fibre; 0.05 µm; 30 m^2)	459	<100	5-25	-	12	5.5	70	25	100 L d^{-1}	
iMBR 1.3 m^3 (hollow fibre; 0.05 µm; 30 m^2)	459	<100	5-25	-	10-13.3	5.5 - 16.5	70	33	100 L d^{-1}	
UASB 4.3 L + external membrane (tubular; 100 kDa; 0.0085 m^2)	452	96-98% removal	-	1.2 kgCOD m^{-3} d^{-1}	4.2-5.2	8	-	21-24	-	(Cerón-Vivas et al., 2012)
UASB 12 L + external membrane (tubular; 100 kDa)	350	64 - 41	-	-	<40	4-12	-	-	-	(Salazar-Peláez et al., 2011)
sMBR 12.9L (tubular; 0.64 µm; 0.98 m^2)	162.3 - 603.2	77.5	-	2.36 kgCOD m^{-3} d^{-1}	5	2.6	-	15-20	-	(An et al., 2009)
sMBR 180L (hollow fibre; 0.2 µm; 4.0 m^2)	540	65	14-80	1.08-4.32 kgCOD m^{-3} d^{-1}	3.75 - 11.25	4.5 - 12	-	25	-	(Lew et al., 2009)
sMBR 50 L (100 kDa; 1.0 m^2)	685	87	10	0.23-2.32 kgCOD m^{-3} d^{-1}	9-13	60-15	-	37	28 L d^{-1} (70% CH_4)	(Saddoud et al., 2007)
EGSB 5 L + immersed membrane (hollow fibre; 0.1 µm; 0.1 m^2)	383-849	76-96	15.8	1.6-4.5 kgCOD m^{-3} d^{-1}		3.5 - 5.7	145	11-25	0.28-0.58 L L^{-1} d^{-1} (63-72% CH_4)	(Chu et al., 2005)

iMBR: immersed or submerged membrane bioreactor

sMBR: sidestream membrane bioreactor

1.1.4. Fouling: general aspects and causes

During the operation of a membrane, an unwanted solid layer is formed on its surface (fouling) due to the high suspended solid concentration in the bulk, and these solids can also accumulate in the pores (clogging). The word fouling is generally used to describe all the mechanisms that cause the flux and permeability to decline. The particles that originate fouling can be microorganisms, colloids, solutes and cell wastes. There are some factors like aeration, relaxation and backwash cycles, periodically chemical cleanings, etc. worth considering that help fouling reduction. Finally, normally after several years of operation, the remaining option is the membrane replacement.

Fouling phenomenon is directly related with the TMP increase. Three steps of fouling behaviour can be observed as TMP variation at an increasing flux (Figure 1.4). At the very first moments, TMP rapidly increases until it stabilises at a nearly constant value due to a cake formation on the membrane surface. After a long period of soft TMP variation, it sharply and irreversibly increases due to a severe fouling, called TMP jump, which determines the critical flux. The main goal of fouling control strategies is to avoid the TMP jump working below the critical flux. However, the sub-critical flux operation at long term leads to fouling problems as well, although the fouling can be removed chemically (Meng et al., 2009).

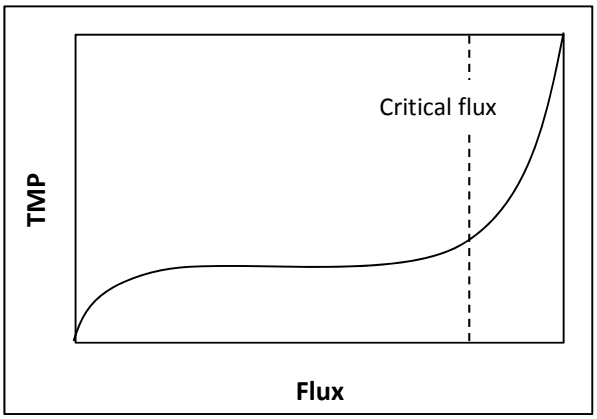

Figure 1.4. TMP evolution versus flux.

There are different types of fouling that can be classified depending on its removal (reversible and irreversible) and its origin (biologic, organic and inorganic). Concerning urban wastewater treatment, fouling observed is mostly of biological origin due to the presence of microorganisms that leads to an accumulation of EPS. Hence, EPS are

considered the main cause of fouling in MBR systems. Laspidou and Rittmann (2002) clarified the concept of EPS: bound EPS act as glue for the microbial aggregates, whereas soluble EPS, also called SMP, are released in the supernatant. Bound EPS consist of proteins, polysaccharides, nucleic acids, lipids, humic acids, etc. situated on the cell surface. On the other hand, soluble EPS arise during the substrate metabolism and the biomass decay.

Several studies about EPS as a cause of fouling suggest that there exist a close relationship between bound EPS concentration and sludge characteristics (Drews, 2010). Moreover, SMP concentration affects the sludge, thus it becomes more prone to fouling (Geng and Hall, 2007). Patsios and Karabelas (2011) state that the SRT is the main factor affecting the concentration of dissolved organic matter (DOM), which mainly consists of SMP, and bound EPS. A large SRT results in a reduction of DOM, even though bound EPS slightly increase. It should be considered that a low DOM and bound EPS concentration inhibit the self-accelerating phenomena, which causes a TMP increase, so that a longer sustainable filtration period can be performed. Hence, bound EPS and SMP are factors that should be taken into account in order to reduce fouling.

EPS and SMP analysis are complex, tedious and expensive, therefore indirect methods as total organic carbon (TOC) (Wang and Li, 2008) and dissolved organic carbon (DOC) (Lyko et al., 2008) are employed to determine them. For instance, Wang and Li (2008) state that the SMP correspond to the TOC compounds those are able to pass through the membrane. Hence, the difference between TOC concentrations in permeate and in supernatant of the bioreactor corresponds to the concentration of biopolymer clusters that are agglomerated SMP with such a size that are retained in the membrane.

1.1.4.1. Factors affecting fouling

While MBR capital costs have decreased in recent years, energy demand to reduce fouling is the main contribution to the operational costs (Verrecht et al., 2010). Membrane fouling notably affects MBR performance as follows (Kraume and Drews, 2010):

- Productivity and efficiency decrease due to interruptions during filtration in order to remove the solid cake by relaxation or backwash. This period lasts 15-60 seconds each 3-12 minutes of filtration. In addition, each 2-7 days maintenance cleanings are carried out, and once or twice a year an exhaustive chemical cleaning is needed.
- An ineffective and late chemical cleaning results in a reduction of the membrane lifespan which supposes a huge increase of replacement costs. The frequency of chemical cleaning should be adjusted to seasonal variations and equipment in order to be efficient and prolong membrane lifespan.

- Membrane aeration supposes a 60-70% of the energy costs, which are the major contribution to the operational costs.

There are three main factors that influence MBR fouling: biomass characteristics, operation conditions and membrane characteristics (Judd, 2011). The factors related with membrane operation that have an impact on fouling include filtration mode, air scouring and type and frequency of cleaning (Delrue et al., 2011). It should be noted that cleanings play an important role, thus its misuse can modify membrane surface becoming more susceptible to fouling and shortening its lifespan. Furthermore, biological parameters as MLSS concentration, SRT, dissolved oxygen (DO) and ratio Feed/Microorganisms (F/M) have not a direct effect on membrane fouling, even though they determine sludge characteristics which means that they have an indirect effect (Meng et al., 2009). Hence, optimising these parameters, biomass characteristics are modified as well in such a way that fouling is reduced, although it should be considered aeration and periodic cleanings optimisation as well. Since the nature of the activated sludge is very complex, it is not surprising that fouling appears to be also a very complicated phenomenon.

The MLSS concentration is a key factor of the MBR operation. As mentioned before, one of the advantages of an MBR is the possibility to operate with a high MLSS concentration. Nevertheless, some authors state that fouling increases with the increase of MLSS (Meng et al., 2010), and it directly affects the viscosity of the mixed liquor (Delrue et al., 2011). Hence, if the viscosity is considerable and the cross flow is not enough, the formation of a cake of solids is favoured. In aerobic systems, the MLSS concentration and the SRT are generally limited at 16 g L^{-1} and 28 days, respectively (Judd, 2011), in order to avoid a too high MLSS concentration and viscosity, which would involve an increase of energy demand due to aeration, pumping and stirring.

1.1.4.2. Fouling reduction strategies

Membrane fouling is the most important limitation of MBR technology because of the energy implications. Therefore, new control and optimisation strategies are now emerging. Table 1.7 summarises some of the strategies studied, among those are included changes in the membrane surface, use of flux enhancers, use of enzymes to prevent communication between microorganisms and synthetic particles addition.

Table 1.7. Fouling reduction strategies (Kraume and Drews, 2010)

Strategy	Characteristics
Antifouling membranes	Dynamic membranes with a protective layer of fouling-causing substances
	Membrane surface modification by introducing polar groups (NH_3 and CO_2)
	TiO_2-embedded membranes (more hydrophilic)
Advanced control	Feedback control based in a simple polynomial model which is calibrated after each filtration cycle and generates new decision variables for the next
	Feedback control of backwash reaching a 25% reduction of the backflush period and avoiding permeate losses
	Mechanistic model which describes filtration and fouling mechanisms
Modules and aeration optimisation	Cross-flow velocity and shear stress increase with the maximum bubble size and the minimum space between modules of flat sheet membranes
	Lower bend loss and higher circulation velocities by means of introducing smoother draft tube edge
Configuration of MBR	MLSS reduction near the surface of the membrane by raising it creating two sections in the bioreactor
Addition of flux enhancers	Filterability improvement by means of flux enhancers as cationic polymers, activated carbon and starch
Quorum sensing interruption	Quorum sensing interruption (bacterial communication) that initiates biofilm formation by signal molecules
Pre-treatment as screening or settling	Membrane lifespan increase by retaining large particles; settling results better due to the retention of small particles (100µm) that damage the membrane
Fine particles addition	Formation of a more porous and less compressive cake layer by adding sub-micron synthetic particles of latex and melamine

1.1.5. Energy consumption

Initially, the MBR process for the treatment of wastewater was based on pressurized MF or UF tubular membranes. However, this technology was limited to the treatment of wastewater with high pollutant load (Buer and Cumin, 2010), for example from the pharmaceutical industry and landfill leachate, due to the high energy consumption (3-6 kWh m^{-3}) compared to conventional treatments for urban (0.4-0.8 kWh m^{-3}) and industrial wastewater (up to several kWh m^{-3}).

Energy consumption decreased up to 20% compared with tubular technology (about 1 kWh m^{-3}) with the development of immersed membrane systems, becoming more

competitive in the market. As described in the introduction, MBR technology is growing exponentially thanks to its rapid decentralized commercialisation in WWTPs, as well as in small and medium industries, which has become a reliable solution even for large volumes of wastewater. MBRs currently require less power and accept a flow rate 5-10 times greater, which implies a lower cost per unit of treated water than 20 years ago.

Nevertheless, the competitiveness of the MBR technology is threatened by the low cost of operation of CAS treatment. For this reason, it is necessary to consider the progress on technology and its applications at full scale. Table 1.8 shows examples of the energy cost of some WWTP equipped with MBR. It should be noted that the energy requirement of an MBR can be reduced significantly with a hybrid MBR-CAS process (Verrecht et al., 2010). MBR plants consume more energy than hybrid MBR-CAS because they are oversized, designed to treat maximum influent flow. In contrast, hybrid MBR is designed to treat a constant flow, so that CAS is only used to treat flow overloads.

The scientific literature is mainly focused on optimising the reduction of fouling in order to minimise energy consumption, because more than the 50% of the energy is used in the filtration process (Fenu et al., 2010b). As research on MBR technology progresses, the energy required during operation decreases, although the cost of CAS treatment is still lower.

Table 1.8. Energy consumption of WWTPs with MBR and CAS processes

Process	Consumption ($kWh \cdot m^{-3}$)	Capacity or Average flow	Authors
Tubular sMBR	1-4	-	(Cornel and Krause, 2006)
Flat sheet iMBR	0.8-1.2	10,600 p.e.	(Mulder, 2009)
Hollow fibre iMBR	0.64	28,000 p.e.	(Fenu et al., 2010b)
CAS	0.19	18,000 p.e.	
Hollow fibre iMBR	0.5-1.8	20,851 m^3d^{-1}	(Verrecht et al., 2010)
Hybrid iMBR-CAS	0.4	20,851 m^3d^{-1}	
Hollow fibre iMBR	0.3	-	(Martin et al., 2011)
Tubular sMBR	3.7	-	

1.3. REDUCING OPERATIONAL COSTS FOR BIOLOGICAL NITROGEN REMOVAL

Traditionally, biological nitrogen removal through nitrification/denitrification (N/DN) processes has a significant energy demand mainly due to oxygen and carbon source requirements. During nitrification step ammonium present in wastewater is converted into nitrite and nitrate autotrophically, under aerobic conditions. Ammonium oxidation is carried out by ammonium oxidising bacteria (AOB) (e.g. *Nitrosomonas, Nitrosococcus* and *Nitrosospira*) following the stoichiometry described in Equation 1.1 (Fernández, 2010):

$$NH_4^+ + 1.5\ O_2 + 2\ HCO_3^- \rightarrow NO_2^- + 3\ H_2O + 2\ CO_2 \quad (1.1)$$

When the biomass growth is taken into account, the stoichiometry is estimated to be (Equation 1.2):

$$NH_4^+ + 1.382\ O_2 + 1.982\ HCO_3^- \rightarrow 0.018\ C_5H_7O_2N + 0.982\ NO_2^- + 2.927\ H_2O + 1.891\ CO_2 \quad (1.2)$$

Nitrite oxidation is performed by nitrite oxidising bacteria (NOB) (e.g. *Nitrobacter, Nitrospira, Nitrospina* and *Nitrococcus*) following Equation 1.3:

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (1.3)$$

When the biomass growth is taken into account, the stoichiometry is estimated to be (Equation 1.4):

$$NO_2^- + 0.003\ NH_4^+ + 0.488\ O_2 + 0.010\ H_2CO_3 + 0.003\ HCO_3^- \rightarrow 0.003\ C_5H_7O_2N + NO_3^- + 0.008\ H_2O \quad (1.4)$$

Subsequently, during denitrification step nitrate is reduced to nitrogen gas under anoxic conditions and available carbon. Conventional WWTPs usually operate with a pre-denitrification step in order to use the influent organic matter for nitrate reduction and afterwards nitrification takes place under aerobic conditions and low organic matter content. The reduction of nitrate to nitrogen gas is carried by heterotrophic bacteria as *Pseudomonas, Alcaligenes, Paracoccus* or *Thiobacillus.* The stoichiometry considering acid acetic as carbon source is described in Equation 1.5:

$$8\ NO_3^- + 5\ CH_3COOH \rightarrow 4\ N_2 + 10\ CO_2 + 6\ H_2O + 8\ OH^- \quad (1.5)$$

Considering this stoichiometry, total consumption of oxygen in order to convert completely ammonium into nitrate is about 4.2-4.5 gO_2 $(gNH_4^+\text{-}N)^{-1}$. Furthermore, nitrification causes an alkalinity consumption of 7.1 $gCaCO_3$ $(gNH_4^+\text{-}N)^{-1}$. When biodegradable organic matter is not present in the wastewater to be treated or when its concentration is not enough in order to complete denitrification, the addition of an external carbon source is necessary. Some typical electron donors are small chain alcohols (methanol, ethanol), acetate and glucose. Usually, methanol is the cheapest available carbon source, thus it is the most used compound (Park and Yoo, 2009). The need of organic matter per unit of mass of nitrogen is about 3.7 $gCOD\ (gN)^{-1}$ when methanol is employed.

The conventional N/DN scheme can be improved in order to save costs, especially in terms of oxygen and carbon demand. Because ammonium is oxidised from state -3 to +6 and then reduced to state 0, several strategies can be applied in order to achieve a more direct way to nitrogen gas. In Figure 1.5, the oxidation state of the different nitrogen forms that appear during N/DN is shown.

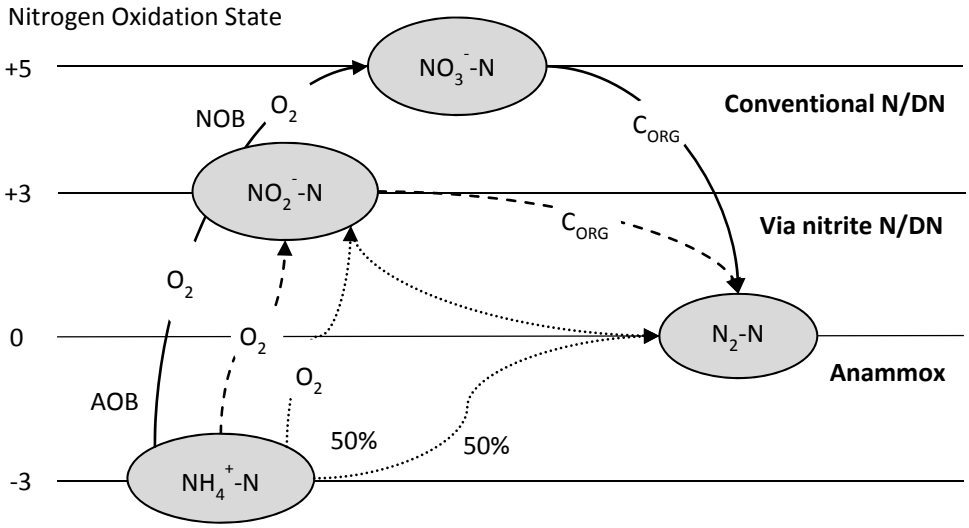

Figure 1.5. Nitrogen oxidation states during N/DN processes

Via nitrite N/DN consists in oxidising ammonium (-3) to nitrite (+3) and then reduced it to nitrogen (0). Another possibility is the Anammox process consisting in a first partial nitritation where the 50% of ammonium is oxidised to nitrite. Afterwards, Anammox bacteria are able to produce nitrogen from ammonium and nitrite without external carbon supply. Both processes give an alternative way to remove nitrogen from wastewater reducing the operational costs.

1.1.6. Via nitrite nitrification/denitrification

Nitrite route is an interesting option for nitrogen removal in a WWTP because it supposes a significant reduction in aeration and carbon source costs as well as a smaller reactor size due to a shorter reaction pathway (Wang et al., 2009a). Considering the stoichiometry described in Equation 1.1, total consumption of oxygen in order to convert completely ammonium into nitrite would be 3.4 gO_2 $(gNH_4^+\text{-}N)^{-1}$ and methanol requirement for denitritation would be 2.3 gCOD $(gN)^{-1}$. Compared with the conventional N/DN, a 25% of aeration and 40% of external carbon source are saved and production of sludge decreases a 30% (Gu et al., 2012).

In order to achieve a shortened nitrification pathway (i.e. nitritation), nitrite production should be promoted. The three main factors have been found that affect nitritation rate are (Wang et al., 2009a):

- The relative specific growth rate of AOB and NOB.
- The level of free ammonia.
- The level of dissolved oxygen.

The enrichment of AOB and limitation of NOB is the key point for stable nitritation. Most of the scientific publications deal with the factors that inhibit NOB activity instead of optimising AOB growth rate. Several parameters such as dissolved oxygen, temperature, sludge retention time, substrate concentration and aeration pattern have been found to inhibit or washout NOB (Aslan et al., 2009; Ganigué et al., 2012; Peng and Zhu, 2006; Yuan et al., 2008).

1.1.6.1. Specific growth rate

The specific growth rates of AOB and NOB are closely affected by the temperature. As observed in Figure 1.6, at low temperatures NOB growth rate is higher than AOB growth rate, thus ammonium would be completely oxidised to nitrate. However, at temperatures over 20ºC, the growth rate of AOB becomes higher than the one of NOB. Therefore, at a high temperature and a low SRT, NOB can be washout from the system. This control strategy can be feasibly applied when the operation temperature is relatively high, for instance, for the treatment of the supernatant of sewage sludge anaerobic digestion (Hellinga et al., 1998). Nevertheless, in the main water line of a urban WWTP that commonly operates at 10-20ºC, nitrite would be hardly accumulated and easily oxidised to nitrate because NOB would grow faster than AOB (Wett and Rauch, 2003).

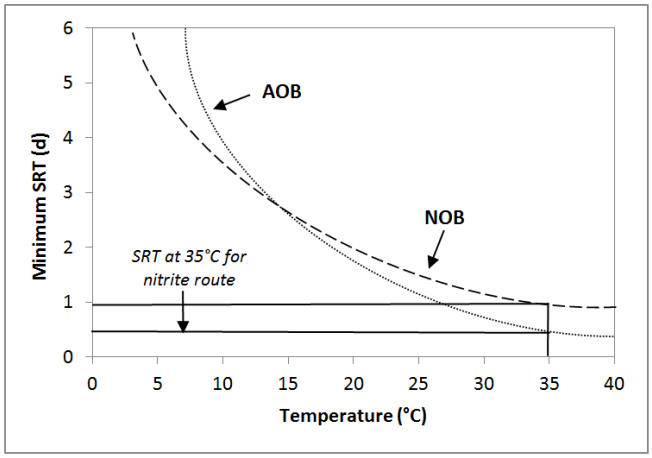

Figure 1.6. Minimum SRT for the growth of AOB and NOB (Hellinga et al., 1998)

Via nitrite N/DN strategy has been implemented in different configurations of reactors. One of the most common is the SHARON (Single reactor system for High-activity Ammonia Removal over Nitrite) process (Hellinga et al., 1998). This process is carried out in a continuous stirred tank reactor (CSTR) with suspended biomass. It is operated at temperatures between 30°C and 40°C and low SRT of 1-1.5 days (in this type of continuous reactor, the hydraulic retention time is equal to the sludge retention time). In these conditions, AOB are selectively retained while the slower growing NOB are washed out. Both nitritation and denitritation may take place in the same stirred reactor using intermittent aeration. This principle for the selective enrichment of the AOB is not only restricted to CSTR configuration; via nitrite N/DN was also successfully applied in sequencing batch reactors (SBR) (Frison et al., 2013; Fux and Siegrist, 2004; Sílvia López-Palau et al., 2011).

1.1.6.2. Level of dissolved oxygen

Dissolved oxygen (DO) can be considered as a co-substrate in the nitrification reaction. Therefore, its concentration affects the kinetics of the reaction. The affinity of NOB to oxygen is much lower than AOB due to their different oxygen half-saturation coefficients: 1.1 and 0.3 mgO_2 L^{-1} for NOB and AOB, respectively (Wiesmann, 1994). Therefore, at low DO conditions NOB have less affinity for oxygen than AOB, thus nitrite will accumulate in the system (Wang et al., 2009a). When DO is below 1 mg L^{-1}, the growth rate of AOB is 2.6 times faster than that of NOB (Tokutomi, 2004). It has been observed that at a DO between 0.7 mg L^{-1} and 1.4 mg L^{-1} the activity of NOB was avoided (Ruiz et al., 2003).

This strategy has the advantage that DO concentration and aeration duration are economically feasible control parameters, since low DO concentration and appropriated aeration duration can save aeration consumption. However, compared with other control parameters (e.g. pH or temperature), DO control is more difficult to implement (Rongsayamanont et al., 2010) and may lead to negative effects such as bulking (Guo et al., 2010) and N_2O emissions (Colliver and Stephenson, 2000).

1.1.6.3. Level of free ammonia

Another strategy to achieve NOB inhibition consists in keeping a significant free ammonia (FA) and/or free nitrous acid (FNA) concentration in the mixed liquor of FA > 1 mgNH_3-N L^{-1} and FNA > 0.02 mgHNO_2-N L^{-1}. AOB are also favoured against NOB at alkaline pH (8–8.8). Anthonisen et al. (1976) observed that the inhibition of NOB occurred at a concentration of FNA between 0.2 and 2.8 mg L^{-1} and/or 0.1-1 mg FA L^{-1}, while AOB became inhibited at 150 mg FA L^{-1}. Figure 1.7 shows the different zones of inhibition of AOB and NOB depending on the pH value and the concentrations of FA and FNA. Two zones can be distinguished, in the white zone nitrite accumulation is expected due to the inhibition of NOB.

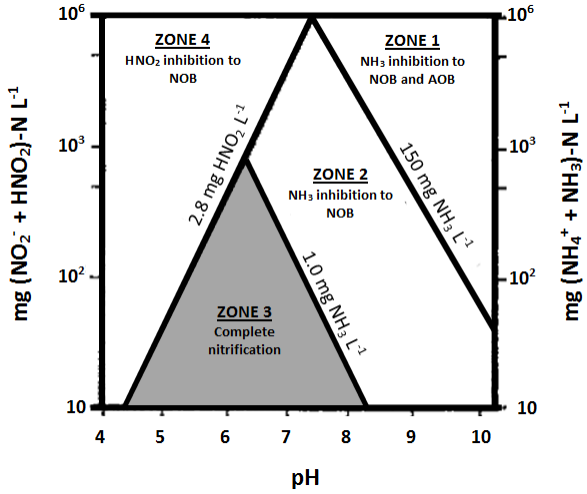

Figure 1.7. Effect of FA and FNA on the activity of AOB and NOB (Anthonisen et al., 1976)

Frison et al. (2012) accomplished stable growth of AOB and inhibition of NOB after 20 days of SBR operation for the treatment of supernatant of anaerobic digestion of organic waste. The stable AOB growth was obtained at a wide temperature range (13-28°C), due to the significant FA concentration in the reactor (1-3 mgNH_3-N L^{-1}).

1.1.6.4. Via nitrite N/DN applications

A large amount of references are available reporting via nitrite N/DN processes using the different control strategies mentioned above. Recent studies deal with real effluents with a high N/COD ratio, such as sludge reject water, piggery wastewater, landfill leachate and digested organic waste, but also examples for domestic wastewater can be found. In Table 1.9 the main characteristics of several studies that achieved via nitrite N/DN are summarised. It can be observed that the control strategy applied depends on the type of wastewater. For low loaded wastewater (e.g. domestic wastewater), via nitrite N/DN is achieved by controlling aeration keeping very low DO values (DO < 2 mg L^{-1}). In contrast, for high loaded wastewaters, such as landfill leachate or supernatant of anaerobic digestion, the preferred control parameters are the FA and FNA, because high free ammonia concentrations are easy to achieve.

In addition, it should be noted that the SBR is the type of reactor most used even at pilot-scale facilities. Because SBR reactors have the flexibility to operate at multiple aerobic and anoxic periods with low space requirement, they have become a very interesting option for the via nitrite nitrogen removal (Ge et al., 2015).

Table 1.9. Via nitrite N/DN published examples

Control strategy	Operational parameters	NO_2-N/ NO_x-N	Authors
Domestic wastewater			
Aeration real-time control	Reactor type: SBR V=14L; T=32°C; SRT=14d; HRT=8-12h	98%	(Peng et al., 2004)
Intermittent aeration	Reactor type: SBR V=2m^3; T=12-24°C; SRT=16d; pH=7.2-7.6; DO<0.2mgL^{-1}	>95%	(Kornaros et al., 2008)
DO	Reactor type: A/O V=300L; T=21°C; SRT=15d; HRT=10h	>95%	(Ma et al., 2009)
Aeration real-time control	Reactor type: SBR V=10L; T=12-25°C; SRT=30d; HRT=8-12h	>90%	(Guo et al., 2010)
Landfill leachate			
FA	Reactor type: A/O V=15L; T=17-30°C; HRT=1.5-4.1h	90-99	(Peng et al., 2008)
FA, FNA	Reactor type: SBR V=250L; T=25-35°C; HRT=4.5-12d; pH=8.0; DO=2.0mgL^{-1}	-	(Gabarró et al., 2012)
Supernatant of anaerobic digestion			
FA, FNA	Reactor type: SBR V=3L; T=30°C; HRT=0.42-0.83d; pH=7.8; DO=4-6mgL^{-1}	>95%	(López-Palau et al., 2011)
FA, FNA	Reactor type: SBR V=2.8m^3; T=20°C; SRT=15d; HRT=0.75d; DO=1.48mgL^{-1}	>98%	(Frison et al., 2013)

1.1.7. Partial nitritation - Anammox

The anaerobic ammonium oxidation (Anammox) had been widely reported in the environmental engineering field (Wang et al., 2009a), which can be used for cost-effective and space-saving nitrogen removal from high-strength wastewater. The autotrophic ammonium oxidation occurs with nitrite as the electron acceptor under strict anoxic conditions. The Anammox process must be preceded by a partial nitritation unit, where the half of the ammonium is oxidised to nitrite. In order to achieve the partial nitritation, not only NOB should be inhibited, the key parameter that limits the ammonium oxidation is the ratio alkalinity/NH_4^+-N. As described in Equation 1.1, 2 moles of alkalinity (HCO_3^-) are required to completely nitrify 1 mole of ammonium. Therefore, a ratio alkalinity/NH_4^+-N around 1 would result in the oxidation of the 50% of total nitrogen.

Subsequently, the Anammox process converts ammonium together with nitrite directly to nitrogen gas under anoxic conditions in the absence of any organic carbon source. The stoichiometry of the process was established by Strous et al. (1998) (Equation 1.6):

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (1.6)$$

It was determined that 1.32 moles of nitrite are required to transform 1 mole of ammonium into nitrogen gas. However, it should be noted that 0.26 moles of nitrate are produced during the reaction. Therefore, when high nitrogen loads are treated, a polishing post-treatment would be required to remove the remaining nitrate.

Several microorganisms have been detected that are able to perform Anammox process belonging to *Planctomycetes* branch. The most important ones are *Candidatus Brocadia Anammoxidans, Candidatus Kuenenia stuttgartiensis* and *Candidatus Scalindua* species (Fernández, 2010).

Anammox process is considered as a promising technology to treat high ammonium loaded wastewaters with a low C/N ratio (Van Loosdrecht and Jetten, 1998), such as supernatant of sewage sludge anaerobic digestion, piggery manure, livestock wastewater, landfill leachate and some industrial waters (Fux and Siegrist, 2004; Qiao et al., 2010; Van Dongen et al., 2001; Yamamoto et al., 2011). Nevertheless, Anammox bacteria are especially sensitive to the numerous factors that can inhibit its activity. These factors are reviewed by Jin et al. (2012) and summarised in Table 1.10.

Table 1.10. Anammox inhibition factors (Jin et al., 2012)

Factor	Description
Ammonium	Anammox process is not inhibited by NH_4^+-N or by the by-product NO_3^--N since a concentration of 1 gN L^{-1}. Dapena-Mora et al. (2007) determined that the IC_{50} was 770 mgNH_4^+-N L^{-1}, but the true inhibitor was free ammonia. Stable operation was maintained when free ammonia was less than 20-25 mg L^{-1} (Fernández et al., 2012).
Nitrite	NO_2^--N has a negative effect on Anammox activity that has been reported widely. Although the adverse effect is clear, conflicting reports exist on the level at which it occurs. The threshold values of nitrite inhibition are not uniform in literature varying from 5 to 280 mg L^{-1} depending on the different operational conditions and species. Strous et al. (1999) found that the Anammox bacteria were completely inhibited in the presence of more than 100 mgNO_2^--N L^{-1}. Later, Fux and Siegrist (2004) observed the inactivation of Anammox organisms when 40 mgNO_2^--N L^{-1} were maintained for some days. However, Dapena-Mora et al. (2007) determined that the 50% of the Anammox bacteria were inhibited with a nitrite concentration of 350 mgNO_2^--N L^{-1}. Nitrite toxicity is generally related to the presence of nitrous acid (HNO_2). Fernández et al. (2012) stated that concentrations higher than 0.5 mgHNO_2-N L^{-1} should be avoided to maintain stable operation of Anammox systems, which agree with Jung et al. (2007), who reported a significant long-term inhibition in the presence of 0.8-1.2 mgHNO_2-N L^{-1}. The inhibition by substrate causes a decrease in activity that can be partially restored by adding trace amounts of the Anammox intermediates hydroxylamine and hydrazine, even after long-term exposure to high concentrations of nitrite (Strous et al., 1999). Lotti et al. (2012) demonstrated that nitrite inhibition levels are rather high (IC_{50}=400 mgN L^{-1}) and biomass can recover relatively fast from high nitrite concentrations.
Organic matter	The biodegradable organic matter present in an Anammox system leads to the growth of heterotrophic denitrifying biomass. Autotrophic bacteria is not able to compete for nitrite with heterotrophic biomass, thus conventional denitrification will prevail. The presence of a limited amount of organic matter can be advantageous in order to denitrify the remaining nitrite and the nitrate produced during Anammox reaction. It has been reported that alcohols can inhibit Anammox activity. 3-4 mmol L^{-1} methanol can almost completely inhibit the activity of Anammox bacteria (Jensen et al., 2007). Convincing evidence has shown that Anammox activity is inhibited also by antibiotics (Fernández et al., 2009). However, there are few studies on antibiotic inhibition of Anammox; those that have been published are limited to only three kinds of antibiotics: chloramphenicol, β-lactams and tetracycline.
Oxygen	Since Anammox bacteria are strictly anaerobic, they are inhibited by dissolved oxygen. Egli et al. (2001) stated that oxygen inhibits Anammox reversibly at low oxygen levels (air saturation 0.25 - 2%), but probably irreversibly at high levels.

Factor	Description
Temperature	The optimum temperature range for Anammox is 30-40°C. At a temperature higher than 45°C, an irreversible decline in Anammox activity occurs. Operating at a low temperature (below 15°C) the Anammox activity would be also inhibited (Dosta et al., 2008). However, at ambient temperatures (23±2°C) Anammox process is feasible achieving a high nitrogen load of 20.5 kgN m^{-3} d^{-1} (Yang et al., 2011).
Inorganic carbon	Anammox bacteria are chemoautotrophic and CO_2 acts as their main carbon source. Therefore, the influent bicarbonate concentration is an important factor that affects the Anammox enrichment and there is evidence that the Anammox activity deteriorates with a decrease in the influent inorganic carbon (Jin et al., 2012). Liao et al. (2008) demonstrated that the optimum bicarbonate:ammonium ratio ranges between 2.3:1 and 4.7:1; lower ratios resulted in a low activity due to the lack of carbon source, while higher ratios also led to a decrease of Anammox activity, probably due to the formation of high amount of free ammonia.
Salinity	High salinity would result in high osmotic pressure that could severely inhibit bacteria. Dapena-Mora et al. (2007) stated that NaCl did not affect Anammox activity when the concentration was below 8.78 g L^{-1}. When KCl concentration was higher than 7.45 g L^{-1} or Na_2SO_4 concentrations was greater than 7.10 g L^{-1}, Anammox bacteria was inhibited. With long-term operation at high salts concentrations, the function of the Anammox system is reduced, however the adaptation of Anammox bacteria is successfully improved by acclimatization. Jin et al. (2012) reported that the salinity inhibition level of Anammox was reduced from 67.5% to 43.1% by acclimatization.
Phosphate and sulphide	The different Anammox species show different tolerance for phosphate. Van De Graaf et al. (1995) reported that 155 mgP L^{-1} caused a loss of activity of *Brocadia Anammoxidans*. However, Egli et al. (2001) showed no inhibition when a culture of *Kuenenia stuttgartiensis* was supplied with up to 620 mgP L^{-1}, the same concentration that caused the 50% inhibition to Dapena-Mora et al. (2007). Dapena-Mora et al. (2007) showed an Anammox inhibition of 50% at low sulphide concentrations of 9.6 mgS L^{-1}, while Van De Graaf et al. (1995) showed a resistance of Anammox to at least 64 mgS L^{-1}.
pH	The desired pH interval for Anammox is 6.7-8.3, with an optimum of 8.0 (Strous et al., 1999). Egli et al. (2001) observed the maximum activity in the range between 7.5 and 8.0. These authors did not observe activity when pH was lower than 6.5, but Anammox activity was not completely inhibited when pH overcame 8.5.
Stirring speed	Stirring speeds up to 180 rpm have no negative effect on the performance of the Anammox process, whereas Anammox activity decreased to 40% when a rotating speed of 250 rpm was tested (Arrojo et al., 2006).
Visible light	The direct exposure to visible light caused a decrease in activity of 30 to 50% (Van De Graaf et al., 1996).

Partial nitritation – Anammox processes can be performed in two different units, such as SHARON-Anammox, or even in a one single reactor. This second option allows important savings in equipment but has quite sensitive operational characteristics in dissolved oxygen, nitrogen load, biofilm thickness and temperature.

1.1.7.1. Two stage configuration

The two stage configuration (Figure 1.8) consists in two different units. The first reactor is operated under aerobic conditions in order to convert approximately half of the ammonium into nitrite. The second reactor is the Anammox anoxic reactor where autotrophic denitrification is achieved.

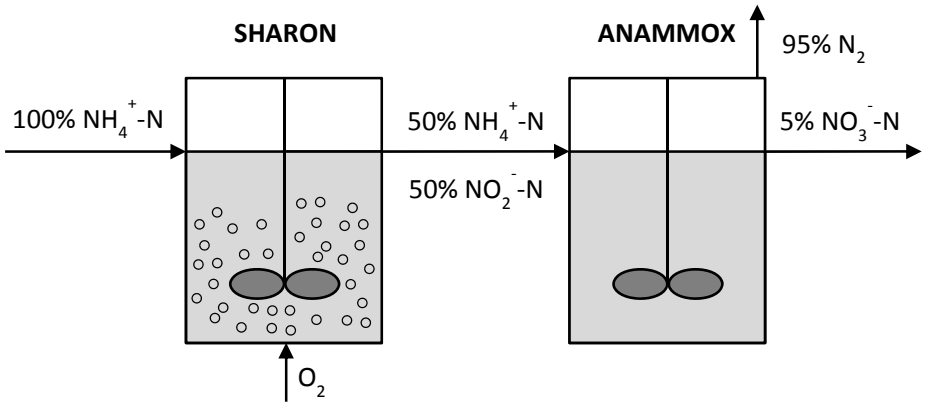

Figure 1.8. Two stage partial nitritation – Anammox process

The most common system to reach partial nitritation is the SHARON reactor (described in section 1.3.1.1). This reactor is operated under aerobic conditions and at temperatures over 30°C, controlling the hydraulic retention time (HRT) to wash out NOB but keeping AOB. Usually the employed HRT is 1 d (Fernández, 2010). By controlling dissolved oxygen and pH in the system, it is possible to keep the optimum ammonium/nitrite ratio in the effluent. Partial nitritation can also be carried out in a sequencing batch reactor (SBR) operated at relatively high temperature and controlled SRT. Galí et al. (2007) performed a comparative study to produce the correct influent for Anammox process from anaerobic digestion reject water. They demonstrated that both systems (SBR and SHARON) were able to achieve the same specific conversion rate (40 mgNH_4^+-N g^{-1}VSS h^{-1}) but the SBR achieved a higher value of absolute nitrogen removal (1.1 gN L^{-1} d^{-1} versus 0.35 gN L^{-1} d^{-1}), due to the different HRT used. The SHARON process showed however, a better stability. One more recent option is the use of aerobic granules in order to perform the conversion of half of the ammonium into nitrite (Fernández et al., 2008; López-Palau et al., 2011). When effluents from anaerobic

digesters are treated, the optimum ammonium/nitrite ratio can be achieved without any special control system, because these effluents have an ammonium/bicarbonate molar ratio around 1. Therefore, when the 50% of ammonium is oxidised nitritation stops due to the lack of alkalinity that causes a drop of pH.

Afterwards, the Anammox step can be carried out in different reactors like UASB (Upflow Anaerobic Sludge Blanket), similar to the ones used in the anaerobic digestion processes; gas-lift; continuous stirred tanks; etc. At lab-scale, the SBR is widely used due to its flexibility of operation and control. Dapena-Mora et al. (2007) showed that the SBR is a suitable system to grow and enrich Anammox biomass in the form of granular sludge. Recently, SBRs are also being employed as full scale Anammox reactors, but in this case with one stage configuration.

1.1.7.2. One stage configuration

Partial nitritation and Anammox can occur in the same unit where the different species co-exist in a biofilm or aggregates/granules. The AOB are active in the outer layers, producing a suitable amount of nitrite for the Anammox organisms that are located in the inner layers Figure 1.9. The transport of ammonium and the produced nitrite occur by diffusion (Van Hulle et al., 2010).

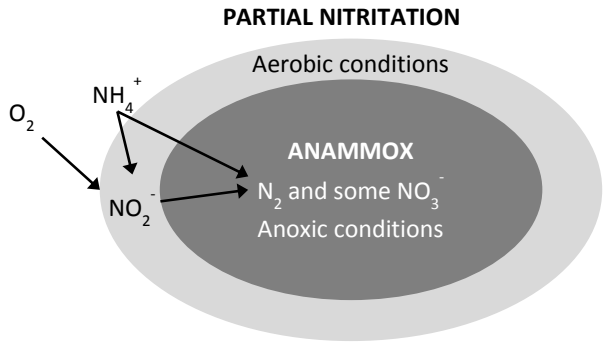

Figure 1.9. Biomass aggregates in a one stage partial nitritation – Anammox system

This technology has received different names: CANON (Completely Autotrophic Nitrogen removal Over Nitrite) (Third et al., 2001); OLAND (Oxygen-Limited Autotrophic Nitrification-Denitrification) (Windey et al., 2005) and DEMON (DEaMmONification) (Hippen et al., 1997). The main difference among them is that CANON process employs suspended biomass growing in a mixed reaction medium, while OLAND and DEMON are biofilm based processes, thus biomass is growing on biodiscs or on moving plastic carriers.

1.1.7.3. Partial nitritation/Anammox applications

Anammox process was discovered in Delft in the early nineties, and within 15 years after its discovery, the first full scale Anammox plant was implemented at the wastewater treatment plant of Dokhaven (The Netherlands), treating the wastewater of the city of Rotterdam (1 million p.e.). After the very long start-up of the first full scale Anammox reactor, which took more than three years, the time to start the new reactors was progressively reduced. This improvement had several causes, but the most important is the fact that unlike in the case of the first reactors, enriched Anammox biomass was afterwards available to inoculate new plants. Because of this, the most recent Anammox systems were started up in few months.

The Anammox process was initially implemented only in wastewater treatment plants in the Netherlands, but it has been quickly adopted globally, as it can be applied to any stream with relatively high ammonium concentrations, such as reject water from anaerobic digestion, effluents from landfills and composting, or wastewaters from some industries, such as food, fertilizers, petrochemical or manure processing.

In 2015 there exist more than 20 referenced full-scale Anammox plants implemented by Paques and 100 full-scale plants worldwide. Regarding the partial nitritation/Anammox installations; which have been successfully developed for high-strength ammonium wastewaters with low C:N ratios and elevated temperatures; more than 50% are sequencing batch reactors (80% configured as DEMON technology), 88% are being operated as single-stage systems, and 75% are treating reject water (Lackner et al., 2014). In Table 1.11, some full-scale DEMON SBR examples treating reject water are summarised.

Table 1.11. Full-scale SBR Anammox applications (Lackner et al., 2014)

Place	Apeldoorn	Balingen	Heidelberg	Zürich
Volume (m^3)	2400	705	2 x 570	2 x 1400
TSS ($g\ L^{-1}$)	3.5-4	1.2	1.0-2.5	3.5-4.5
HRT (h)	58	94	114	45
Nitrogen load ($kgN\ m^{-3}\ d^{-1}$)	0.54	0.04-0.11	0.20	0.4
Energy demand ($kWh\ kg^{-1}N$)	1.10	0.92	1.67	1.11

1.1.8. Economical aspects of biological nitrogen removal processes

The different N/DN processes described in this section were compared in terms of operational costs based on 1 $kgNH_4^+$-N removed. The cost estimation was carried out following the guidelines of Fernández (2010), updating the costs to the present market. Two scenarios were supposed: when there is enough carbon in wastewater to denitrify, and when an external carbon source is required. It was assumed that the wastewater to be treated has enough alkalinity, thus carbonate or an alkaline reagent is not necessary. Investment cost to build the plant (very influenced by the land cost) and salaries are not considered.

The estimated costs are presented in Table 1.12, which included the electricity demand due to oxygen supply, the methanol required as external carbon source and the sludge management. The oxygen required was considered to be 4.3 kgO_2 kgN^{-1} for conventional N/DN; 3.4 kgO_2 kgN^{-1} for via nitrite N/DN; and 2.0 kgO_2 kgN^{-1} for partial nitritation. The electricity demand of oxygen transferred in clean water ranged between 3 to 6 kgO_2 kWh^{-1}, if aeration panels (MESSNER) are employed. An average value was taken for the calculations (i.e. 4.5 kgO_2 kWh^{-1}). However, the transfer efficiency decreases to about 80% when dealing with mixed liquor containing suspended solids (Metcalf and Eddy, 2003). The current cost of the electricity is around 0.15 € kWh^{-1}. The cost of methanol is about 400 € t^{-1} in the international market. Regarding the sludge production, biomass yield is about 1 kgVSS kgN^{-1} for conventional N/DN; 0.40 kgVSS kgN^{-1} for via nitrite N/DN; and 0.15 kgVSS kgN^{-1} for partial nitritation/Anammox (Fux and Siegrist, 2004). The percentage of solids of the dehydrated sludge was assumed about 22% and the sludge management cost is about 30 € t^{-1} when it is used for agricultural or composting purposes.

Table 1.12. Cost estimation of the different N/DN processes based on 1 $kgNH_4^+$-N removed (updated from Fernández (2010))

Technology	O_2	Electricity		Methanol		Sludge		Total cost
	kg	kWh	€	kg MeOH	€	kg	€	€
Conventional N/DN	4.3	1.19	0.18	-	-	1.00	0.14	0.32
Conventional N/DN external C source	4.3	1.19	0.18	2.5	1.00	1.00	0.14	1.32
Via nitrite N/DN	3.4	0.94	0.14	-	-	0.60	0.08	0.22
Via nitrite N/DN external C source	3.4	0.94	0.14	1.5	0.60	0.60	0.08	0.82
Partial N /Anammox	2.0	0.56	0.08	-	-	0.15	0.02	0.10

Clearly partial nitritation/Anammox had a significant lower cost than other N/DN processes, basically because is an autotrophic system with low requirements. In the heterotrophic N/DN processes, the main cost was attributed to the external carbon requirement (>70%). Therefore, in the scenario where wastewater had enough carbon to denitrify, the cost even for the conventional N/DN became more reasonable, around 0.32 € kgN^{-1}. Considering the conventional treatment as the basis, via nitrite N/DN and partial nitritation/Anammox processes supposed a cost reduction of 29% (0.22 € kgN^{-1}) and 67% (0.10 € kgN^{-1}), respectively. Comparing the results shown in Table 1.12 with the cost estimation of Fernández (2010), it is highlighted that the total costs have decreased. Despite electricity has almost doubled the price from 0.09 € kWh^{-1} to 0.15 € kWh^{-1} in 5 years, the aeration systems are nowadays much more efficient. Hence, the overall costs became more competitive.

1.4. BIOPLASTIC PRODUCTION IN A WASTEWATER TREATMENT PLANT

Wastewater treatment plants (WWTP) are considered as end-of-pipe processes. According to this definition, a WWTP is the last stage before a stream is disposed. However, there is an increasing interest in changing the point of view towards a bio-based society, in which a WWTP would become a biorefinery where wastes are converted into resources (De Vegt et al., 2012).

Apart from reducing the energy demand of a WWTP by applying treatments as anaerobic digestion or Anammox, the possibility of producing bioplastics from wastewater is also of great interest. Environmentally degradable polymers (biopolymers) are one of the possible solutions to replace some petroleum-based polymers. Based on the definition given by the European Bioplastics Association, biopolymers are based on renewable resources complying with ASTMD-6866 and can be degraded to comply with international standards such as EN13432, ASTM D6400, and ISO17088. In Europe, the criteria for biodegradability are set out within the standard EN13432, 2000, which is binding when applied to compostable packing under the EU directive on Packaging and Packaging Waste (94/62/EC) (Chanprateep, 2010). Members of the European Union have already established policies against petroleum based consumer packaging. For example, Belgium has established an eco-tax of 3€ kg^{-1} on packaging such as shopping bags, whereas compostable shopping bags that conform to the European Standard (EN) 13432 for compostable packaging material are exempted. The Netherlands has established a carbon based packaging tax based on CO_2 emissions from the production of packaging material and the carbon content of the packaging (Chanprateep, 2010).

Numerous bacteria have been found able to synthesize storage compounds under nutrient dynamic conditions in the natural environment (Jiang et al., 2012). One of those storage compounds is polyhydroxyalkanoate (PHA), which is a type of polyester with thermal properties similar to petroleum based plastics (Figure 1.10). Unlike conventional plastics, PHA is fully biodegradable and derived from renewable resources (Sudesh et al., 2000). However, until now the main obstacle for the replacement of synthetic plastics by biopolymers is the high production cost.

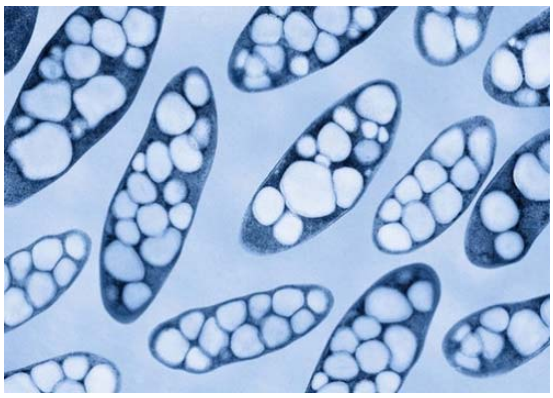

Figure 1.10. PHA accumulated in *E. coli* cytoplasm (Quan and Tian, 2009)

Poly(3-hydroxybutyrate) [P(3HB)] is the most common PHA and was first described by Lemoigne, a French scientist in year 1925; since then, various bacterial strains (e.g. archaebacteria and photosynthetic bacteria) have been identified to accumulate P(3HB) both aerobically and anaerobically (Chee et al., 2010).

About 150 different monomers of PHA have been found (Steinbüchel, 2001). A large variety of PHA monomers have been listed by Witholt and Kessler (1999) with straight, branched, saturated, unsaturated and also aromatic structures. PHA can be classified according to the monomer size. There are two major groups of PHA; short-chain-length (SCL) PHA with five or less carbon atoms in a monomer, and medium-chain-length (MCL) PHA with six to fourteen carbon atoms in a monomer. *Alcaligenes eutrophus* is a well studied bacterium capable of producing SCL-PHA and it has been identified to produce PHA polymers consisting of 3HB (hydroxybutyrate), 3HV (hydroxyvalerate) and 4HB monomers (Kunioka et al., 1989). *P. oleovorans* and *Pseudomonas putida* are known to synthesize MCL-PHA consisting of 3HO (hydroxyoctanoate) and 3HD (hydroxydecanoate) monomers as major components (Lee et al., 2000).

Despite the numerous advantages of using biodegradable plastics, the commercialization of PHA has great limitations. The high production cost of PHA is the major drawback for the replacement of petrochemical plastics. Considering the price of most commodity plastics derived from petroleum, such as polyethylene and polypropylene, are below 1€ kg^{-1} (Chee et al., 2010). Therefore, PHA cannot currently compete with the huge production of petrochemical plastics.

PHAs are industrially produced by pure cultures applying as main substrates glucose and propionic acid (Reis et al., 2003). The major costs of the PHA production are due to the cost of the substrate and the extraction of the polymer from the cells. Industries are currently working towards decreasing the cost of these biopolymers by increasing production capacity and improving process technology, especially the downstream extraction. In Table 1.13 the commercialised PHA are summarised.

Table 1.13. Large volume manufacturers of PHA (Chanprateep, 2010)

Polymer	Trade name	Manufacturer	Capacity (tons)	Price (€ kg^{-1})
PHB	Biogreen®	Mitsubishi Gas Chemical Company Inc. (Japan)	10000	2.5-3.0
PHB	Mirel™	Telles (US)	50000	1.50
PHB	Biocycle®	PHB Industrial Company (Brazil)	50	-
PHBV, PHB	Biomer®	Biomer Inc. (Germany)	50	3.0-5.0
PHBV, PHBV	Enmat®	Tianan Biologic, Ningbo (China)	10000	3.26
PHBH	Nodax™	P&G (US)	20000-50000	2.50
PHBH	Nodax™	Lianyi Biotech (China)	2000	3.70
PHBH	Kaneka PHBH	Kaneka Corporation (Japan)	1000	-
P(3HB-co-4HB)	Green Bio	Tianjin Gree Bio-Science	10000	-
PHA	Meredian	Meredian (US)	272000	-

PHA (polyhydroxyalkanoate); PHB (polyhydroxybutyrate); PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)); PHBH (poly(3-hydroxybutyrate-co-3-hydroxyhexanoate))

In order to reduce production costs, two different strategies have been developed. The first strategy consist in the use of pure cultures of microorganisms fed with waste streams as carbon source (Koller et al., 2010). Because the feedstock is estimated to be the 30-50% of the total production cost (Jiang et al., 2012), the overall production costs are expected to be reduced by using waste streams instead of pure substrates. Several cheap wastes can be used from agriculture and food industry (e.g. whey, molasses). The organic fraction of municipal solid waste (OFMSW) and primary sludge (PS) are also of interest, because a high content in VFA can be achieved after its fermentation.

The second strategy consists in using mixed cultures, as activated sludge, instead of pure cultures avoiding the costs of isolating a specific bacteria and sterile conditions. The use of mixed cultures and waste substrates can reduce up to a 50% the PHA production cost (Reis et al., 2003). However, the efficiency of PHA accumulation is lower when using mixed cultures. PHA accumulated can reach a 60% of sludge dry weight, whereas by using pure cultures PHA accumulated is above 80% of dry weight.

PHA production from biomass implies a first step of growth and enrichment in PHA storing bacteria. Dynamic conditions, alternating carbon or electron donor availability and unavailability, should be applied in order to favour the development of biomass able to produce storage compounds. For instance, aerobic and anaerobic conditions or feast and famine regime are the most common operation strategies for the PHA storing biomass selection. The metabolic pathway for acetate conversion into bioplastic under transient conditions is shown in Figure 1.11. Under these dynamic conditions, during excess of external carbon substrate, the uptake is driven to simultaneous growth of biomass and polymer storage, and after substrate consumption, stored polymer can be used as a carbon source. In these cases storage polymers are formed under conditions that are not limiting for growth. The storage phenomena is usually dominant (70%) over growth, but under conditions in which substrate is present for a long time, growth becomes more important (Dionisi et al., 2002). The ability to store internal reserves gives to these microorganisms a competitive advantage over those without this capacity under transient substrate supply.

The most known groups of bacteria that are able to store PHA are the polyphosphate accumulating organisms (PAO) and the glycogen accumulating organisms (GAO). The metabolic pathway to convert organic matter into PHA is similar in both cases. The difference lies on that PAO require mainly polyphosphate to obtain energy (ATP) while GAO require glycogen. Under anaerobic/aerobic conditions with predominance of PAO and acetate as carbon source, it is produced a polymer mainly composed of HB (69-100%) and HV (0-31%). In contrast, when GAO are predominant, HV appeared in higher proportion (25-30%) because glycogen contributes to its formation (Reis et al., 2003). If propionate is used as carbon source instead of acetate, an inversion of the proportion HB/HV is observed (2-28% of HB and 45-72% HV) because propionate is the main precursor of PHV. A mixture of propionate and acetate leads to the production of a copolymer composed by HB and HV with improved characteristics in relation to the homopolymer of HB. The final composition of the polymer can be defined by adjusting the relative concentrations of acetic and propionic acid (Takabatake et al., 2000).

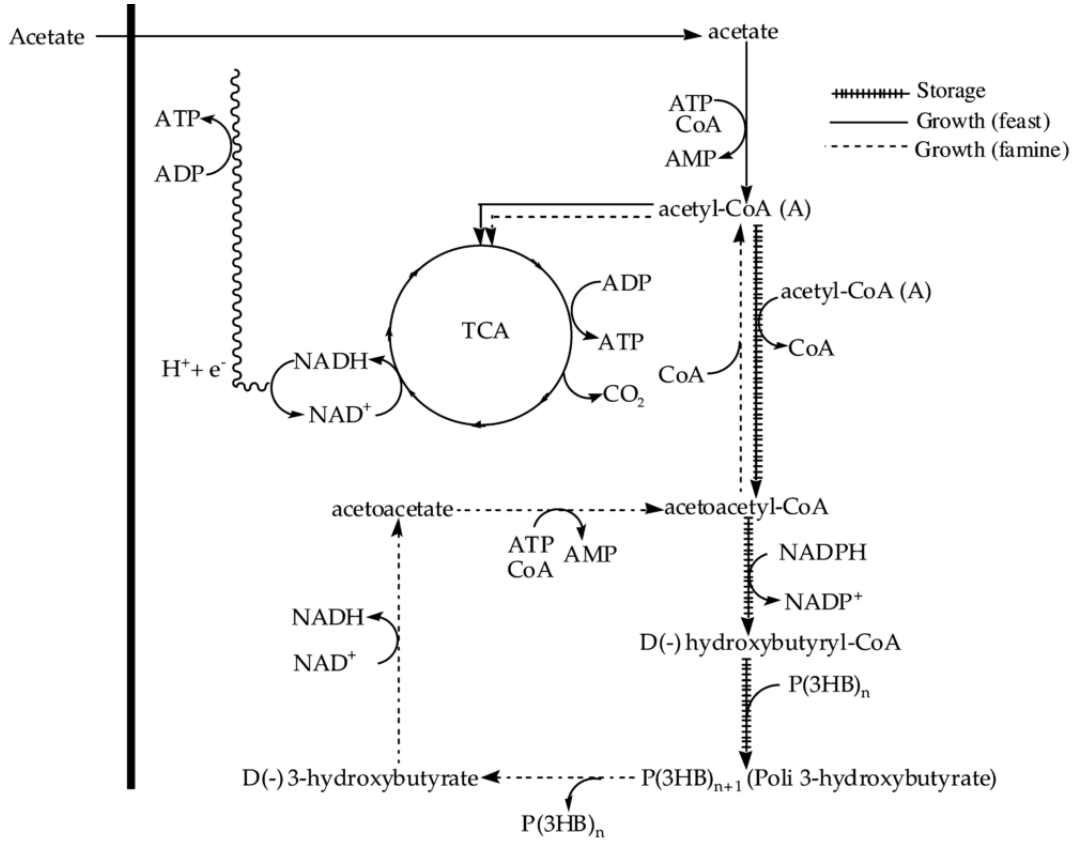

Figure 1.11. Metabolic pathway for acetate consumption under feast/famine aerobic conditions (Reis et al., 2003)

Subsequently, once the biomass is enriched in PHA storing microorganisms, an accumulation step is applied. In this step carbon is supplied continuously in order to accumulate PHA as much as possible under aerobic conditions. The key point in this step is the minimisation of the nutrient content and non-VFA organic matter of the carbon source. If nutrients are present growth would be favoured, and the non-VFA organic matter also contributes to decrease the PHA production yield (Morgan-Sagastume et al., 2010a). The success in the accumulation step is closely related to the operating conditions in the selection step. When the selection step is operated under optimal conditions, the accumulation step would result in a high storage yield.

The most common method for the extraction of PHA from biomass is solvent extraction by using chloroform. Chloroform extraction is a very simple and effective method to separate PHA granules from the biomass. By using this method, highly purified PHA can be obtained

without degradation of PHA molecules (Lanham et al., 2013). Other halogenated hydrocarbon solvents such as dichloromethane, dichlorethane and chloropropane can be also used to extract and purify PHA from the cell biomass (Chee et al., 2010). However, these methods are not suitable for the mass production of PHA as the solvent is potentially hazardous to health and environment.

A part from solvent extraction, another simple and effective method that has been employed to recover PHA is cell lysis by using sodium hypochlorite. In this method, the cell biomass is initially treated with sodium hypochlorite solution before the PHA granules are isolated from the cell debris by centrifugation. The use of sodium hypochlorite to extract PHA from biomass results in severe degradation of PHA and yields PHA (60%-80%) with a lower molecular weight (Samorì et al., 2015).

1.1.9. The role of the carbon source for PHA storage

Research studies have demonstrated the technical feasibility of producing PHA in mixed cultures using single and simple VFA mixtures (Dionisi et al., 2004; Pijuan et al., 2009) and more complex substrates, including several fermented waste streams: food waste (Rhu et al., 2003), olive oil (Dionisi et al., 2005) and palm oil (Din et al., 2006) mill effluents, sugar cane molasses (Albuquerque et al., 2007), paper mill effluent (Bengtsson et al., 2008)), and municipal sludge (Mengmeng et al., 2009). Volatile fatty acids are the main products of organic waste fermentation and the most suitable substrate for PHA storage (Mengmeng et al., 2009). The feasibility of producing PHAs from complex waste streams would depend on the effects of non-VFA organic matter and the high levels of organics and nutrients that may negatively affect the overall PHA yield, especially in the accumulation step.

The presence of N and P in the carbon source in the enrichment is advantageous because it favours biomass growth and the limited carbon promotes only the growth of PHA accumulating microorganisms. However, during the accumulation step without carbon limitations, nutrient may hinder the PHA storage. Several studies have been performed to evaluate the effect of N and P on the storage yield. In general, the highest PHA accumulation yield was obtained when nutrient content was low (Bengtsson et al., 2008; Dionisi et al., 2005, 2004). Biomass growth during PHA accumulation is considered to be a negative influence due to anticipated production of non-PHA storing biomass resulting in dilution of final PHA content (Jiang et al., 2012).

In contrast, Valentino et al. (2015) determined that the growth during PHA accumulation step can be advantageous achieving higher productivities than under nutrient starvation. Productivity increased due to active PHA storing biomass growth without risk of non-storing

biomass development. PHA production with respect to the initial active biomass was significantly higher even in cases of excess nutrient additions when compared to the cases of nutrient starvation. The best results were found to be with combined N and P limitation and N/COD between 2 mg g^{-1} and 15 mg g^{-1} and P/COD between 0.5 mg g^{-1} and 3 mg g^{-1}. The 24-h PHA productivities were enhanced from a base value of 1.35 g-PHA per gram initial active biomass with respect to nutrient starvation feedstock. The method of feed-on-demand accumulation batch, that regulates the rate of organic matter supply depending on the biomass demand, were considered to be important towards achieving selective growth of PHA-storing bacteria rather than non-storing populations.

1.1.10. Integration of nutrient removal and PHA storing biomass selection

A significant amount of scientific literature has focused on the aerobic storage of PHA and its subsequent degradation in activated sludge (Chee et al., 2010; Dionisi et al., 2006; Morgan-Sagastume et al., 2010b; Salehizadeh and Van Loosdrecht, 2004). However, less information is available about the role of the stored PHA in nutrient removal systems. In order to achieve simultaneous N and P removal, anaerobic/anoxic/aerobic conditions are applied, which at the same time imply a feast and famine regime. This configuration can induce the bacteria to store external substrates as internal storage compounds in the feast period. As ammonium oxidation is a relatively slow process, nitrogen removal processes require a slowly degradable carbon substrate that should be available for the subsequent denitrification step, instead of being rapidly oxidised to CO_2 within the early stages of aeration. Stored PHA are degraded much slower than soluble substrate and can be used as the electron donor for denitrification when no external substrate is available (Third et al., 2003).

The storage compounds can be served as internal carbon sources for post-anoxic denitrification. Although the endogenous denitrification efficiency is low, the limited internal carbon sources can be used to satisfy the need of denitrification via nitrite (Chen et al., 2013), which require much less organic carbon than the conventional denitrification. Post-anoxic denitrification via nitrite driven by storage compounds was successfully achieved in a SBR operated as aerobic/anoxic/extended-idle regime by Chen et al. (2013). The results indicated that this treatment process could achieve desirable and stable nitrogen and phosphorus removal efficiencies of 95% and 99%, respectively.

In Figure 1.12, a possible sequence for nitritation/denitritation driven by stored PHA is shown. During the aerobic phase, the carbon source containing VFA is spiked which is consumed and transformed into storage compounds. At the same time, ammonium is oxidised in to nitrite. Subsequently, aeration is stopped leading to the anoxic phase where

nitrite is reduced endogenously driven by the stored PHA. The ratio feast/famine applied has great importance in order to favour the storage instead of growth. Albuquerque et al. (2010) determined that feast/famine ratios between 0.2 and 0.6, the storage and growth yield have a linear and inversely proportional tendency. Hence, at the lowest ratio of 0.2, the storage yield was around 0.8 Cmol PHA $Cmol^{-1}$ VFA while the growth yield was 0.1 Cmol X $Cmol^{-1}$ VFA. By increasing the feast and famine ratio, the PHA yield decreases to 0.4 Cmol PHA $Cmol^{-1}$ VFA.

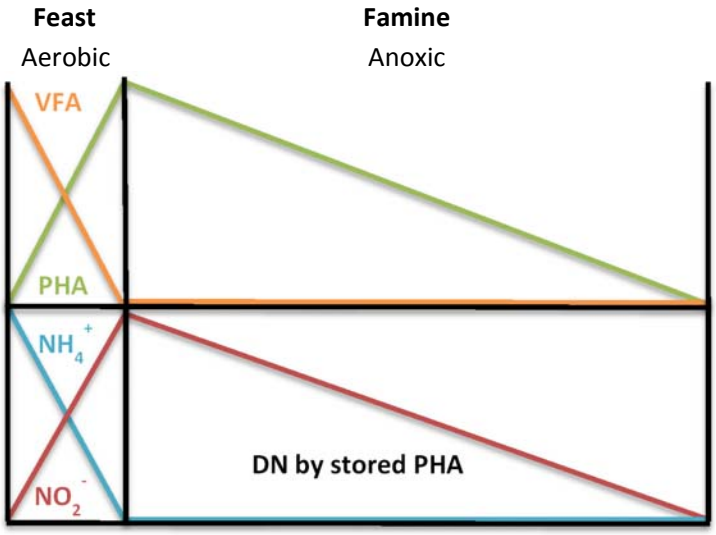

Figure 1.12. N/DN via nitrite driven by stored PHA

Phosphorus can also be removed in this kind of sequences by applying an idle phase (anaerobic) at the beginning of the cycle (Chen et al., 2013). Therefore, P can be released during the first anaerobic phase while VFA are converted in storage compounds. During aerobic phase ammonium is oxidised into nitrite while P is uptaken and, afterwards, nitrite is removed by the stored compounds under anoxic conditions. However, it should be noted that nitrite must be completely removed to assure strictly anaerobic conditions at the beginning of the cycle; otherwise, the spike of carbon source would promote the conventional denitritation of remaining nitrite.

2. Objectives and thesis structure

In this Chapter, the motivation and the objectives of this thesis are presented and they are related with the thesis structure.

2.1. MOTIVATION AND OBJECTIVES

As stated in Chapter 1, organic matter and nutrients present in urban and industrial wastewater should be removed or valorised to reduce its impact on the environment. Conventional wastewater treatments are focused on the removal of these pollution sources at the minimum cost. The idea of resource recovery from wastewater is changing the concept of the conventional wastewater treatment plants that tend to incorporate little by little processes as anaerobic digestion, MBR, biofilm, granulation, etc. However, their application to obtain reusable by-products from wastes should be cost effective.

Anaerobic digestion processes are well-known to achieve high organic matter removal efficiencies without oxygen requirement, low biomass production and energy generation from biogas (Metcalf and Eddy, 2003). Conventional digesters usually treat organic rich streams (e.g. sewage sludge, manure, etc.) operating at high hydraulic and solid retention times (HRT and SRT). However, for low strength wastewater treatment, biomass immobilisation technologies should be applied in order to uncouple the HRT from the SRT; thus avoiding biomass wash-out. For this reason, anaerobic membrane bioreactor (AnMBR) was considered as a possibly feasible system for wastewater treatment. The upflow anaerobic sludge blanket (UASB), which is a more typical configuration for anaerobic wastewater treatment, was also studied in order to compare the advantages and drawbacks of both systems.

The effluent of anaerobic digestion often requires a post-treatment to remove nutrients, especially nitrogen. Compared with conventional biological nitrogen removal, nitritation/denitritation (N/DN) via nitrite represent a 25% less aeration and 40% less external carbon source (Gu et al., 2012). In order to reach higher flexibility and reducing space requirements, N/DN can be carried out in a sequencing batch reactor (SBR) (Frison et al., 2013). Under feast and famine conditions applied in the SBR, storage compounds can be served as internal carbon sources for post-anoxic denitrification (Chen et al., 2013). Therefore, the via nitrite removal can be integrated with the selection of bioplastic storing biomass (i.e. polyhydroxyalkanotes (PHA)). PHAs are biodegradable and biobased polymers, well known for their application in bioplastics and are produced biologically. PHAs have

thermoplastic and elastomeric properties, while the use of renewable resources for their production has gained attention in recent years (Dias et al., 2006; Gumel et al., 2012).

Another cost-effective treatment of anaerobic digestion effluents is the Anammox process, combined with a previous step of partial nitritation (PN). When compared with conventional biological nitrogen removal process, the PN - Anammox process avoids the requirement of organic carbon source to denitrify, produces about 85% less of sludge and allows saving around 60% of the oxygen supply, thus reducing energy requirements (Fux and Siegrist, 2004). Anammox biomass can be inhibited by nitrite concentration, temperature, pH, visible light exposure, dissolved oxygen (DO), organic matter, among many others (Jin et al., 2012). Therefore, careful control of the Anammox process is required since it becomes easily unstable.

These considerations were the motivation of the present work, which deals with the application of anaerobic systems to obtain biogas from the organic matter and the subsequent biological nitrogen removal step in order to contribute to the knowledge of more sustainable treatment process. Moreover, the flexibility of the anaerobic processes to treat high and low strength wastewaters at different temperatures is an important aspect that may affect its application.

In order to reach this general objective, the following specific goals were proposed:

- To start-up and operate a mesophilic AnMBR using a synthetic substrate simulating winery wastewater, which is high organic loaded stream with low nutrient content.

- To test the flexibility of the AnMBR under influent organic load fluctuations using synthetic and real winery wastewater.

- To operate the AnMBR at low temperature (15°C and 25°C) using a synthetic substrate comparing its efficiency and the biomass activity with the mesophilic conditions.

- To estimate the energy requirements and production of the AnMBR and evaluate the possibilities of full-scale application.

- To start-up and operate a granular UASB at psychrophilic conditions for winery wastewater treatment.

- To couple the UASB with an external membrane unit to improve the membrane performance compared with the AnMBR with suspended biomass.

- To operate a granular UASB at room temperature for low strength wastewater treatment, using a synthetic substrate simulating urban wastewater.

- To operate a short-cut (N/DN via nitrite) SBR treating the UASB effluent under feast and famine conditions achieving denitritation driven by the storage compounds.

- To test different carbon sources in order to increase the PHA accumulation capacity and evaluating the limitations of using fermented wastes for this purpose.

- To start-up a two-step PN – Anammox SBR treating sewage sludge anaerobic digestion effluent with high nitrogen content.

- To optimise the Anammox SBR operation based on activity tests.

- To determine the microbial population in the PN – Anammox SBR and specifically identify the species that play role in the Anammox step.

2.2. THESIS STRUCTURE

Chapter 1: Introduction

This chapter provides a general introduction regarding the main concepts included in this thesis. It is described an overview of the possibilities for energy savings in a wastewater treatment plant, taking benefit from the conversion of the organic matter into biogas and the removal of nitrogen through more sustainable processes.

Chapter 2: Objectives and thesis structure

This chapter summarises the motivation and objectives of the thesis and its structure.

Chapter 3: Materials and methods

In this chapter, the experimental bioreactors and the analytical methods carried out are described in detail.

Chapter 4: Start-up and operation of an AnMBR for winery wastewater treatment at mesophilic temperature

In this chapter an anaerobic membrane bioreactor (AnMBR) is started-up and operated at mesophilic temperature for winery wastewater treatment. The results obtained are discussed in order to assess the impact of the organic load variability on the removal efficiency and the flexibility of the digester.

Chapter 5: Energetic aspects of the AnMBR technology compared with aerobic granulation

The energy balances used for the calculations of energy production and requirement of the AnMBR are presented in this chapter. Moreover, this technology is compared with an intensive aerobic treatment based on granulation. The advantages and drawbacks of both anaerobic and aerobic processes to deal with winery wastewater are discussed.

Chapter 6: Operation of an AnMBR for winery wastewater treatment at low temperature

In this chapter, the AnMBR treating winery wastewater is operated at low temperatures (15°C and 25°C) simulating winter season and also at lower organic load. The performance of the AnMBR is discussed, as well as the evolution of the microbial population considering that it comes from a mesophilic inoculum.

Chapter 7: Winery wastewater treatment by means of an UASB and an UASB-MBR

In this chapter, an upflow anaerobic sludge blanket (UASB) at psychrophilic temperature is started-up and operated to treat winery wastewater. Afterwards, the UASB is coupled with the membrane unit discussing the necessity of the filtration unit and comparing its performance with the AnMBR.

Chapter 8: Integrating the selection of PHA storing biomass and nitrogen removal via nitrite treating UASB effluent

This chapter presents a possible scheme applied in the main water line consisting in an UASB treating urban wastewater followed by a nitritation/denitritation process. It is focused on the integration of the nitritation/denitritation step with the production of bioplastics from the organic matter, thus denitritation is driven by the store compounds.

Chapter 9: Start-up and operation of a two-step partial nitritation – Anammox SBR for reject water treatment

In this chapter, partial nitritation – Anammox process, which is one of the biological nitrogen removal processes with lowest energy demand, is applied for the treatment of reject water. The microbial population, especially in the Anammox step, is evaluated in collaboration with Microbiology Department.

Chapter 10: General conclusions and recommendations

In this chapter, the general conclusions drawn from this work are listed. In addition, recommendations for further research are proposed.

3. Material and methods

3.1. EXPERIMENTAL SET-UP

Different biological reactors were used in this study: an anaerobic membrane bioreactor (AnMBR) for winery wastewater treatment (Chapter 4, 5 and 6); an upflow anaerobic sludge blanket (UASB) for winery wastewater treatment (Chapter 7); an UASB coupled with a sequencing batch reactor (SBR) for urban wastewater treatment (Chapter 8); and a two step SBR for partial nitritation – Anammox for reject water treatment (Chapter 9). The operational conditions of each reactor are described in the corresponding chapter, due to the variety of strategies applied.

3.1.1. Anaerobic membrane bioreactor for winery wastewater treatment

The AnMBR was set-up as a conventional stirred anaerobic digester of 5 L coupled with an external membrane unit (Orelis, Rayflow Module) of 100 cm^2 of membrane area (Figure 3.1). The digester was a jacketed vessel mechanically stirred at 100 rpm and heated at the desired temperature by recirculating water from a heated water bath (HUBER 118A-E). Influent wastewater was fed from a 10 L tank with a cooling system to avoid early degradation. Digester feeding was performed by pressure equilibrium keeping the digester in contact with a 500 mL cylinder at a constant volume of wastewater. Thus, the working volume inside the digester was kept at 3.5 L. Since the membrane unit was placed outside the digester, biogas was easily quantified with an on-line measuring device (Ritter MGC-1) connected to the headspace of the digester.

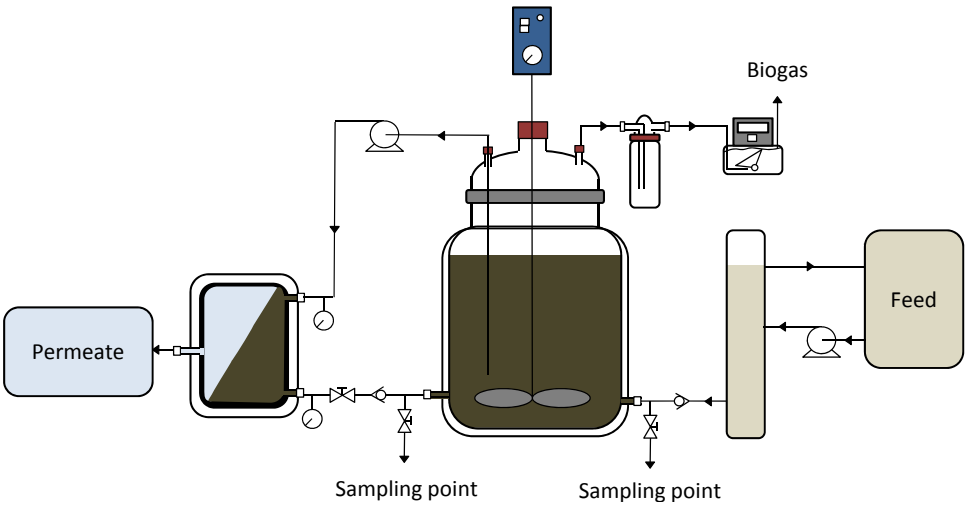

Figure 3.1. Anaerobic membrane bioreactor

3.1.2. Upflow anaerobic sludge blanket for winery wastewater treatment

Winery wastewater was also treated by an UASB reactor. It was set up as a tubular reactor of 1.5 L made of glass fed continuously by a peristaltic pump. The height to diameter ratio was H/D=3.5, which favoured the washout of the biomass with poor settling properties and granular biomass was kept inside the reactor. The UASB was inoculated with granular anaerobic biomass filling about the 50% of the volume. In order to improve biomass fluidisation an internal recirculation was required achieving an upflow velocity of 0.74 m h^{-1}. The biogas was collected in the upper part as shown in Figure 3.2. The gas collector was connected to an on-line measuring device (Ritter MGC-1).

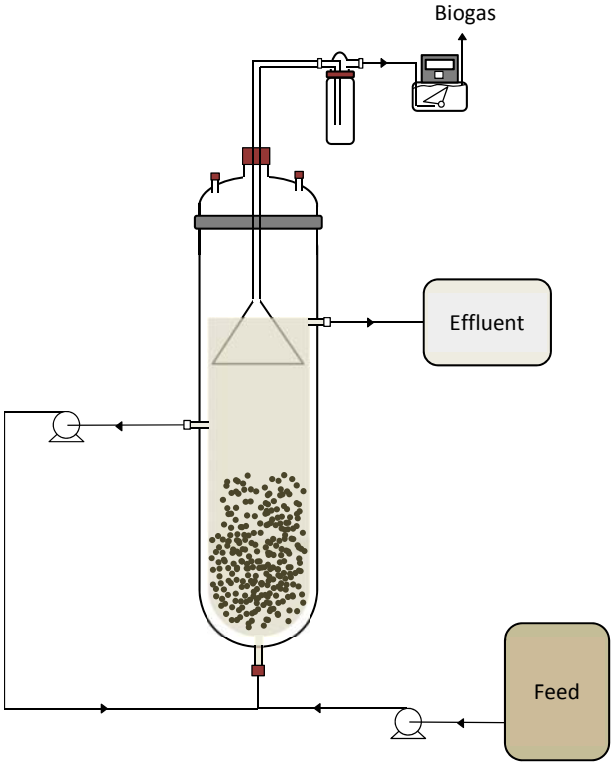

Figure 3.2. Upflow anaerobic sludge blanket

Afterwards, the UASB was coupled with the membrane unit described previously. An intermediate tank was required to collect the UASB effluent and then pumped it through the membrane module. The retentate was directly returned to the UASB. Hence, the internal recirculation was not necessary because the retentate flow rate was enough to assure an upflow velocity around 1 m h^{-1}.

3.1.3. Upflow anaerobic sludge blanket coupled with a sequencing batch reactor for urban wastewater treatment

A pilot-scale UASB reactor was applied for urban wastewater treatment followed by a SBR to remove nutrients. The UASB had a volume of 16 L with the same configuration as the lab-scale UASB shown in Figure 3.2. The UASB was followed by a short cut sequencing batch reactor (scSBR) where via nitrite nitritation/denitritation took place Figure 3.3. The SBR had a working volume of 20 L. On-line submerged probes of dissolved oxygen (DO), pH and oxidation-reduction potential (ORP) were used to monitor the process. The SBR operated at room temperature. The carbon source was added during the last minute of feeding by another peristaltic pump. The probe signals were processed and stored by a programmable logic controller (PLC, Compact Field Point CF-2200, National Instruments). The aeration was provided by blowers located at the bottom of the SBR that were switched on and off based upon the desired DO set point.

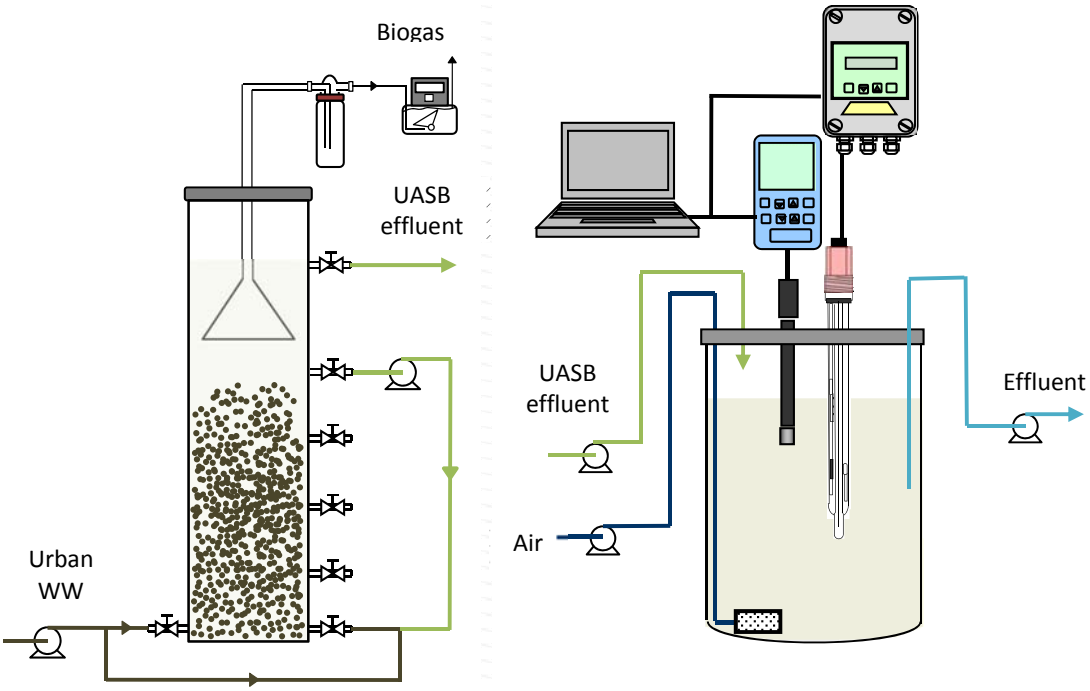

Figure 3.3. UASB and scSBR for urban wastewater treatment

The sequence of the phases in the SBR cycle consisted of fill, aerobic reaction, anoxic reaction, settle, and decant. The aerobic and anoxic reaction length varied, depending on the nitritation-denitritation rates observed, from 130-160 min and from 40-50 min, respectively. During the start-up, the SBR operated without any biomass wasting for 10 days (volatile suspended solids – VSS in the mixed liquor increased up to 2 $g \cdot L^{-1}$); while in the subsequent normal operation activated sludge was wasted to achieve a sludge retention time (SRT) of 25 days (VSS~2.5 $g \cdot L^{-1}$). Biomass was wasted achieving an SRT of 25 days, always keeping a VSS around 2.5 $g \cdot L^{-1}$.

3.1.4. Two step partial nitritation – Anammox sequencing batch reactor

The study of the partial nitritation of the supernatant from anaerobic digestion of sewage sludge was carried out in a lab-scale granular sequencing batch reactor shown in Figure 3.4. Operating temperature was controlled by a heating system (RM6 Lauda). A Programmable Logic Controller (PLC, LOGO SIEMENS 230RC) was usually used to control the length of the different stages of the operational cycles. This reactor was equipped with a pH electrode and ORP electrodes (Crison pH 28) and a dissolved oxygen probe (Oxi 340i, WTW). The 4-20 mA signals of the probes were collected and logged on a PC equipped with the Advantech AdamView software package. Fill and discharge stages were performed by peristaltic pumps. The air flow inside the reactor was supplied by several air blowers (Rena Air 300) connected to a sparging system.

The Anammox process was carried out in a lab-scale SBR of 5 L (Figure 3.4), since it has been reported that good mixing and high nitrogen elimination rates can be easily achieved in sequencing batch reactors with suspended or granulated biomass (Strous et al., 1998). The operating temperature was maintained at 33°C using a thermostatic bath (Haake DC 30). Mixing was provided by means of a mechanical stirrer (IKA Werke RW 16 basic) working at about 120 rpm. Fill and draw stages were performed by two peristaltic pumps (Ismatec Reglo and P-Selecta Percom N-M, respectively). The reactor was continuously flushed with nitrogen to maintain anaerobic conditions and to give some excess pressure to the reactor in order to avoid the entrance of oxygen. Neoprene tubing and connections were used in order to minimize the diffusion of oxygen. Moreover, reactor was covered in order to avoid light incidence. Operational cycles were controlled with a PLC Siemens 230-RC.

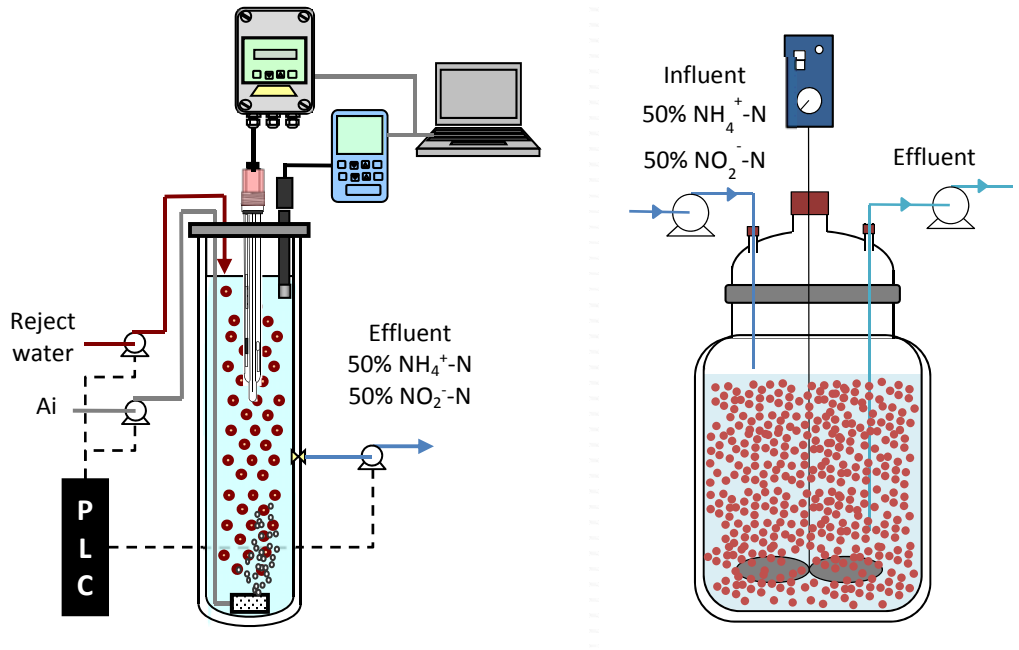

Figure 3.4. Two step PN-Anammox for reject water treatment

3.2. ANALYTICAL METHODS

The analytical methods used in this work were performed according to the *Standard Methods for the examination of Water and Wastewater* (APHA, 2005).

3.2.1. Suspended solids content

Total suspended solids (TSS) and volatile suspended solids (VSS) were determined following the reference methods 2540D and 2540E, respectively. A known volume of sample (V) was filtered through a 1.2 µm Millipore standard filter, previously weighted (W_1). Then, the filter with the TSS was placed at 105°C during 4h, afterwards in a desiccator for 10 minutes and it was weighted (W_2). TSS concentration was calculated according to Equation 3.1. Finally, the filter with TSS was introduced at 550°C for 15 minutes, after that in a desiccator for 10 minutes and was weighted (W_3). The VSS were calculated as shown in Equation 3.2.

$$TSS\ (g/L) = \frac{W_2(g) - W_1(g)}{V\ (L)} \tag{3.1}$$

$$VSS\ (g/L) = \frac{W_2(g) - W_3(g)}{V\ (L)} \tag{3.2}$$

3.2.2. Chemical oxygen demand (COD)

The COD indicates the quantity of matter present in a wastewater sample that is susceptible to be oxidised. This parameter is expressed as $mgO_2\ L^{-1}$, so that the COD is defined as the quantity of oxygen used in biological and non-biological oxidation of materials in water. The reference method 5220C was the standard method utilized to perform the COD measurement. It consisted on the complete oxidation of the matter in a liquid sample with a strong oxidising agent under acidic conditions, by means of potassium dichromate and sulphuric acid (with silver and mercuric sulphate). Equation 3.3 shows the reaction of potassium dichromate with organic compounds. Silver sulphate was used to catalyse the reaction and mercuric sulphate to avoid the interference of chloride (Equation 3.4).

$$C_nH_aO_bN_c + \left(\frac{2n}{3} + \frac{a}{6} - \frac{b}{3} - \frac{c}{2}\right) Cr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \left(\frac{a + 8d - 3c}{2}\right) H_2O + cNH_4^+ + 2dCr^{3+} \quad (3.3)$$

$$6Cl^- + Cr_2O_7^{2-} + 14H^+ \rightarrow 3Cl_2 + 2Cr^{3+} + 7H_2O \quad (3.4)$$

Following the guidelines of the reference method, 2.5 mL of the wastewater sample were mixed with 1.5 mL of sodium dichromate 0.04 mol/L (with 80 g/L of mercuric sulphate) and 3.5 mL of sulphuric acid (with 10 g/L of silver sulphate). Together with the samples, 5 standards of potassium biphtalate with 0, 50, 250, 500 and 1000 mg COD/L were analysed to determine the calibration curve. The samples were maintained at 150ºC during 2h in a digester (**Figure 3.5**a) to ensure the complete reaction. After the digestion, the samples were removed from the digester to cool down and to allow the solids formed to settle at room temperature. Finally, the absorbance at λ = 620 nm of the COD samples was measured by means of a spectrophotometer Shimadzu UV-1203 (**Figure 3.5**b).

(a) (b)

Figure 3.5. COD digester (a) and spectrophotometer Shimadzu UV-1203 (b)

The results of absorbance from the standards were used to obtain a correlation between concentration of COD and absorbance, therefore the COD of the samples was calculated as shown in Equation 3.5.

$$COD\ (mg\ O_2/L) = a \times ABS + b \quad (3.5)$$

3.2.3. Total organic carbon and total nitrogen

Total organic carbon (TOC) is a non-specific indicator of the quality of a wastewater. It is related to the organic matter content, so that the carbon that is quantified is associated with organic compounds. TOC, expressed as mg of Carbon/L, was measured with an analyser Shimadzu TOC-V_{CSN} (**Figure 3.6**). TOC was analysed as non-purgeable organic carbon (NPOC). This method consisted in firstly the acidification of the sample, previously centrifuged and filtered, to convert inorganic carbon to dissolved CO_2 that is removed passing a gas flow through the sample. Afterwards, organic compounds were transformed into CO_2 by means of a catalytic pyrolysis. This CO_2 was detected and quantified by an infrared radiation in a quimioluminescent detector.

Figure 3.6. Total organic carbon analyser

Moreover, TOC analyser can measure total nitrogen (TN) as well. The procedure consisted in an oxidation of the total nitrogen (nitrates, nitrites, ammonia and organic nitrogen) by a catalytic pyrolysis which converted nitrogen compounds into nitrogen monoxide (NO). The NO was detected and quantified by an infrared radiation in a quimioluminescent detector.

3.2.4. Total ammonium nitrogen

Total ammonium nitrogen concentration (N-NH_4^+) was analysed with a specific ammonia electrode (pH/mV Crison MicropH 2002) (

Figure **3.7**) following the standard method 4500-NH_3D. The method is based on the conversion of dissolved ammonium NH_3(aq) and NH_4^+-N(aq) to NH_3(g) by raising the pH

above 11 with a strong base (NaOH) and a subsequent $NH_3(g)$ diffusion through the electrode membrane. Immediately, the electrode was submerged into the sample, and provided a potential (ΔV) in mV. This potential was related to the NH_4^+-N concentration by means of a semilogarithmic expression (Equation 3.6), obtained from 4 standards of 5, 25, 50 and 100 mg NH_4^+-N L^{-1}.

$$Ln(NH_4^+ - N) = a \times \Delta V + b \quad (3.6)$$

Figure 3.7. Ammonia electrode (pH/mV Crison MicropH 2002)

3.2.5. Nitrates, nitrites and ammonium

When nitrogen was in an ion form as nitrate, nitrite or ammonium, it could be measured by ionic chromatography. Ionic chromatograph Metrohm Advanced Compact IC (**Figure 3.8**) can measure the concentration of both anionic and cationic species in a liquid sample. The sample, previously centrifuged and filtered, was automatically injected into the anionic column in which anions were separated giving a response in terms of peaks. Each peak corresponded to a different anionic specie, and its area was related to its concentration in the sample. When nitrates and nitrites were measured and chloride concentration was very high, the peaks overlapped so chlorides were removed adding silver sulphate.

Figure 3.8. Ionic chromatograph (Metrohm Advanced Compact IC)

In the case of the ammonium quantification, a cationic column was used to separate cationic species. The main drawback of this method to determine ammonium concentration was the presence of sodium. Sodium strongly interfered in the ammonium peak and unfortunately it was not possible to remove it using a precipitating agent. When the samples analysed contained huge concentration of sodium due to the addition alkalinity ($NaHCO_3$), ammonium was mostly determined using the specific electrode, detailed before.

3.2.6. Alkalinity

Alkalinity is a measure of the buffering capacity of a water or wastewater to neutralise acids. The buffering capacity of a wastewater is mainly related to the bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) content. However, the presence of other buffering substances such as hydroxide (OH^-), borates, silicates, phosphates, ammonium, sulphides and organic ligands can also provide alkalinity to the wastewater.

The alkalinity was measured using an automatic titration device (CRISON pH Burette 24) equipped with a pH meter (CRISON Basic 20) (**Figure 3.9**). The method consists in a titration into a 25 mL of sample with standard acid (HCl 0.1) to desired end point.

Figure 3.9. Automatic titration device (CRISON pH Burette 24)

The alkalinity, expressed as mg $CaCO_3$ L^{-1}, is calculated with the Equation 3.7.

$$Alkalinity\ (mg\ CaCO_3/L) = \frac{mL_{HCl} \times 0.1N \times 50.000}{mL_{sample}} \quad (3.7)$$

3.2.7. Gas chromatography

Individual VFAs (acetic, propionic, butyric, valeric, hexanoic and heptanoic acids) were analysed by a Shimadzu GC-2010+ gas chromatograph (Figure 3.10) equipped with a capillary column Nukol (0.53 mm ID; 15 m length) and a flame ionization detector (FID). Specifically, the chromatograph oven temperature program was as follows: increase from 85°C to 110°C at 10°C min^{-1}; increase to 145°C at 15°C min^{-1}; increase to 190°C at 20°C min^{-1}, and hold 0.10 min. Injector and detector temperature was set at 280°C and 300°C, respectively. Carrier gas was helium at a rate of 36.9 mL min^{-1} and 17.6 kPa.

Biogas composition as percentage of methane and carbon dioxide was determined by a Shimadzu GC-2010+ gas chromatograph equipped with a capillary column Carboxen 1010 Plot (0.53 mm ID; 30 m length) and a thermal conductivity detector (TCD). The analysis program was as follows: hold 6 min at 40°C; increase to 230°C at a rate of 25°C min^{-1} and hold 2 min at this temperature. Injector and detector temperature was set at 200°C and 230°C, respectively. Helium was the carrier gas at 47 mL min^{-1} and 20.4 kPa.

Figure 3.10. Shimadzu GC 2010+

3.2.8. Fluorescence in situ hybridization (FISH)

The different populations of microorganisms present in the sludge samples of the reactors were studied by means of fluorescent in situ hybridization (FISH). It is a powerful molecular tool for rapid, reliable and cultivation-independent monitoring of phylogenetically defined bacterial populations in environmental samples. With this technique, specific regions in 23S or 16S rRNA are detected with fluorescently labelled probes. If the corresponding domain, phylum, genus or species is present, the probe hybridizes to the targeted sequence and can later be detected microscopically. The procedure described by Amann (1995) includes the fixation and permeabilization of the sample, hybridization of the targeted sequence to the probe, washing steps to remove unbound probe and finally, the detection of labelled cells by microscopy (Figure 3.11).

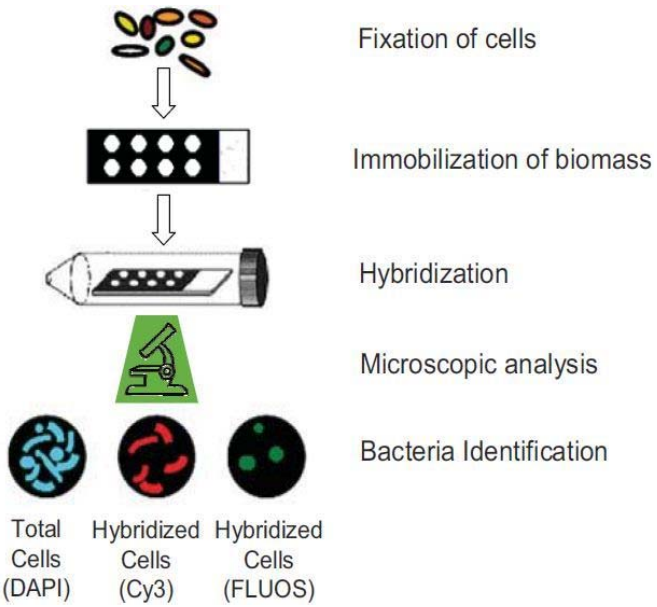

Figure 3.11. Different steps of the typical FISH protocol (López-Palau, 2012)

During hybridization the cells are exposed to high temperatures, detergents and osmotic gradients. Thus fixation of the cells is essential in order to maintain the morphological integrity of the cells. Fixation of cells with glutaraldehide results in considerable autofluorescence of the specimen. Autofluorescence was minimized by fixation in freshly prepared (less than 24 h) 4% paraformaldehyde solution in PBS. After fixation, the cells were immobilized on a microscopic slide and used for hybridization with 16S rRNA probes. In

order to avoid non-specific binding of the rRNA probes, the hybridization was done at stringent conditions (46°C, 0-65% formamide) and specimens were washed with buffer (48°C). The targeted organisms were detected by the characteristic fluorescence.

The fluorochromes used to detect the hybridized rRNA were Fluo (5(6)-carboxyfluorescein-N-hydroxysuccinimide ester) and Cy3 (indocarbocyanine). To visualize all cells in a sample the stain 4,6-diamidino-2-phenylindole (DAPI) was used. Fluorescent signals were recorded with a TCS-SP2 confocal laser scanning microscope (Leica, Germany) equipped with a DPSS 561nm laser for the detection of Cy3 and one Ar ion laser for detection of Fluo. The probes applied are listed and detailed in each chapter.

3.2.9. Scanning Electron Microscope (SEM)

The membrane surface (new and after months of operation) was observed in more detail using scanning electron microscopy (SEM). This technique uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition or and crystalline structure.

The membrane samples were dehydrated with graded ethanol series followed by critical point drying with liquid CO_2. Finally samples were sputter-coated with gold before examining with an ESEM Quanta 200 FEI, XTE 325/D8395 operated at 20 kV.

3.2.10. Biomethane potential (BMP) test

The BMP tests were carried out following the guidelines of Angelidaki et al. (2009). Several physicochemical parameters were determined in order to characterise the initial and final conditions of the BMP test, which were COD, TS, VS, TSS, VSS, ammonium, VFA and pH. BMP tests were performed in 100 mL serum bottles closed with a PTFE/Butyl septum fixed around the rim of the bottle by an aluminium crimp cap (Figure 3.12). The bottles, containing 50 mL of inoculum, were filled up to 80 mL with substrate keeping a ratio $COD_{substrate}/COD_{inoculum}$ of 0.5, therefore substrates were diluted properly. A blank test, adding only biomass and deionized water, was necessarily prepared to evaluate biomass endogenous activity. Before closing the bottles, nitrogen was flushed for one minute to avoid presence of oxygen in the headspace. The digesters were placed at 35°C and manually mixed twice a day. The biogas production during the test was measured by means of a vacumeter (Ebro – VAM 320) and adjusted to normal conditions (0°C and 1 atm) once subtracted the vapour pressure. Finally, the methane content was analysed by gas chromatography after each biogas production measurement.

Figure 3.12. BMP bottle and vacumeter

3.2.11. Biomass activity test to determine sAUR and sNUR

In Chapter 8, in situ and ex situ biomass activity tests were carried out to evaluate the rate of nitrogen removal and VFA uptake. The specific ammonium and nitrogen uptake rates (i.e. sAUR and sNUR) were monitored in the mixed liquor during the operation of the SBR. In the in situ tests the time profile of ammonium (NH_4-N), nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were determined in the reactor during its operation under aerobic and anoxic conditions. In the ex situ batch experiment, the procedure was the following: 500 mL of activated sludge was collected at the end of the cycle operation. It was washed with a solution of 0.9% NaCl to remove any remaining nitrogen and it was placed in 1 L Erlenmeyer flask, under mild agitation and continuous aeration (DO>4 $mg \cdot L^{-1}$). Subsequently, the biomass was spiked with ammonium nitrogen and external carbon source (OFMSW FL or PS & OFMSW FL) at a fixed concentration. After some minutes, depending on the experiment, the aeration was stopped and the top part of the flask was covered with aluminium foil to avoid direct contact with air. The profiles of VFA, ammonium, nitrite and nitrate were recorded during the whole ex situ tests. Respirometric tests were also performed in order to determine the effect of the carbon source on the sAUR as well as its contribution to the oxygen requirements. In these cases, the oxygen uptake rate (OUR) was recorded with a respirometric device (MARTINA, SPESS Italy). All batch activity tests were conducted at room temperature and the pH was maintained in the range of 7.8±0.2. The reported activities have been normalised to the reference temperature of 20^{o}C using the Arrhenius temperature correction equation and to the volatile suspended solids (VSS) of the mixture.

3.2.12. Polyhydroxyalkanoates extraction and quantification

PHA extraction was performed following the method of Lanham et al. (2013) The samples collected from the reactor were centrifuged and immediately frozen in order to stop the

biological activity. Before the PHA extraction, all the collected samples were lyophilized. Around 10 mg of sample was placed in a closed digestion vial together with 1 ml of chloroform.

The mixture was digested at 70ºC for 3 h, thus the PHA was dissolved in the chloroform. Once the samples reached room temperature, the remaining solids were filtered and the organic phase was collected in 3 ml of methanol. PHA species dissolved in chloroform tend to precipitate in contact with methanol (Figure 3.13). Finally, the solid phase obtained was filtered through Whatman 0.45 µm membranes and weighted to calculate the percentage of PHA (%, gPHA·gSS^{-1}) in the biomass.

Figure 3.13. PHA extracted from biomass

3.2.13. Calculation of aeration requirements

The SBR is a batch process; thus it is necessary to consider aeration system being able to cover the variable air requirements in the beginning (maximum) and the end (minimum) of the cycle. The calculation of the average oxygen demand can be performed according to Equation 3.8 (Metcalf and Eddy, 2003):

$$OTR = 0.5 \times L_{BODoxidised(average)} + k_d \times MLVSS \times V + 3.43 \times L_{Nnitrified\ (average)} \quad (3.8)$$

Where: OTR (kgO_2/h) is the oxygen transfer rate estimated for the SBR operating under process conditions at an average DO concentration and temperature, $L_{BODoxidised}$ (kg/h) is the average BOD oxidised aerobically, $L_{Nnitrified}$ is the average NH_4-N oxidised aerobically; is the K_d: decay coefficient 0.06 d^{-1}.

In the current work the average OTR (kgO_2/h) (actual oxygen requirement) was measured using the respirometric device for oxidation and nitritation. The theoretical oxygen demand (standard oxygen requiriments) in standard conditions (via nitrite) was calculated using Equation 3.9:

$$SOTR = \frac{OTR}{F \times a \times \theta^{(T-20)} \times \frac{\beta \times C_s' - DO}{C_{s20℃,1\ atm}}} \quad (3.9)$$

Where: SOTR (kgO_2/h) is the oxygen transfer rate under standard conditions (20°C, 1 atm, C=0 $mg \cdot L^{-1}$), F: fouling factor; for surface aerators is 1, T is the operating temperature of wastewater (°C), θ is a correction factor (1.024 for fine-pore diffusers; dimensionless), DO = dissolved oxygen in wastewater, α factor is the relative oxygen transfer factor in process versus clean water and α is assumed equal to 0.8 (Equation 3.10).

$$\alpha = \frac{k_{La(wastewater)}}{k_{La(tapwater)}} \quad (3.10)$$

ß is the relative DO saturation to clean water; it is calculated by Equation 3.11; β can be assumed 0.9 for wastewater.

$$\beta = \frac{C'_{s(wastewater)}}{C_{s(tapwater)}} \quad (3.11)$$

C_s is the steady state DO saturation concentration obtained for nonlinear regression analysis of clean water test results, $C_{s(20°C,\ 1atm)}$ is the saturated dissolved oxygen (DO) concentration in clean water standard temperature 20°C and 1 atm for diffusion aeration, C'_s is the average DO concentration at saturation in wastewater at temperature T and pressure P for the particular equipment at the submergence.

The specific oxygen transfer efficiency (SOTE) of diffusers (%OTE/m depth) was selected 30% for 4.5m. SOTE can be calculated by assuming 7%/m. The average flow rate of air circulation at the maximum process temperature is calculated from Equation 3.12:

$$Airdemand = \frac{SOTR}{SOTE \times oxygen\ in\ air} \quad (3.12)$$

3.2.14. Anammox activity test

The maximum specific Anammox activity (SAA) was evaluated following the procedure described by Dapena-Mora et al. (2007) (Figure 3.14). This method, based on the overpressure generated in closed vials due to nitrogen production, similar to the BMP test, was carried out as follows:

1. The biomass samples were washed three times with phosphate buffer ($H_2PO_4^-$, HPO_4^{2-}; pH 7.8).
2. 80 mL of mixed liquor were inserted in 120 mL Pyrex vials.

3. The bottles were carefully closed with caps provided with a central rubber zone, where needles can be inserted in order to add the substrates and measure the pressure.
4. The headspace was flushed with nitrogen gas to remove the oxygen. This was done inserting one needle connected to the inert gas line into the rubber zone of the tap for 5 minutes, while another needle was used to allow gas outlet.
5. The bottles were placed in the incubator shaker (150 rpm, 30ºC).
6. After 30 minutes of acclimation, the substrates were injected into the vials. The initial concentrations were 70 mg NH_4^+-N L^{-1} and 70 mg NO_2^--N L^{-1}.
7. In order to release the initial overpressure into the bottles and to achieve an initial pressure close to the atmospheric one, a needle was inserted during some seconds.

The bottles were placed in the incubator shaker at the same conditions than during acclimation and overpressure was measured every 15 minutes until consecutive measurements did not show a significant pressure increase.

The pressure increase is related to nitrogen production according to Equation 3.13, where n is the mole of nitrogen produced per unit of time (mole N d^{-1}), V_G is the volume of the gas phase (L), R is the ideal gas constant (atm L (mol K)$^{-1}$), T is the temperature (K) and α is the slope of the pressure increase inside the vial versus time (atm d^{-1}).

$$n = \alpha \cdot \frac{V_G}{R \cdot T} \tag{3.13}$$

Then, the maximum SAA expressed in gN (gVSS d)$^{-1}$ can be assessed according to Equation 3.14, where M_{N2} is the molecular weight of N_2 (gN mol^{-1}), X is the biomass concentration inside the vial (gVSS L^{-1}) and V_L is the volume of the liquid phase (L).

$$SAA = n \cdot \frac{M_{N_2}}{V_L \cdot X} \tag{3.14}$$

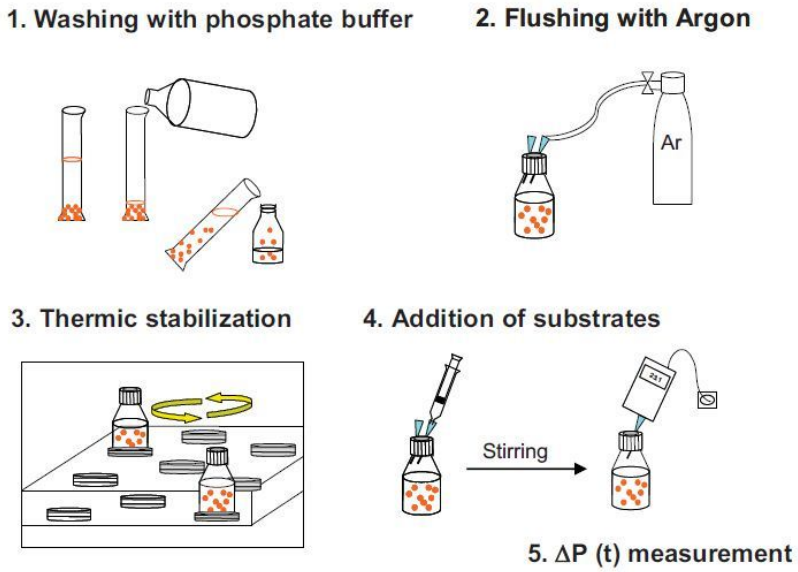

Figure 3.14. Experimental procedure to determine SAA (A. Dapena-Mora et al., 2007)

3.2.15. PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis)

This methodology was performed in collaboration with the Microbiology Department of the University of Barcelona, in particular with Dr. Joaquim Vila and Dr. Magda Grifoll.

Total DNA from triplicate samples (0.25 ml) of the PN and Anammox reactors was extracted using the Power Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA). The V1-V3 regions of the 16S rRNA gene were amplified on an Eppendorf Mastercycler, using the Illustra™ puReTaq Ready-To-Go PCR beads premix (GE Healthcare, Little Chalfont, UK), containing 1 µl of DNA extract and 0.25 pmol of primers GC40-63f and 518r in a final volume of 25 µl, as described previously (Vila et al., 2010). Amplification products were directly used for DGGE analysis on 6% polyacrylamide gels with denaturing gradients ranging from 40% to 70% (100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was performed at a constant voltage of 100 V for 16 h in 1x TAE buffer at 60°C on a DGGE-2001 (CBS Scientific, Del Mar, CA, USA) machine. Gels were stained for 30 min with 1x SYBR Gold nucleic acid gel stain (Molecular Probes Europe BV, Leiden, The Netherlands) and photographed under UV light using a Chemidoc XRS (Bio-Rad Laboratories) camera with Quantity One version 4.5.1 image capture software (Bio-Rad Laboratories). Band intensities were quantified and submitted to correspondence analysis (CA) using SPSS v.20 software to visualize differences between the DGGE profiles.

Bands of interest were extracted from the gel and reamplified with the previous primer set (nonclamped) prior to sequencing on an ABI 3730XL DNA Analyzer (Applied Biosystems) at Macrogene Europe. The retrieved sequences were aligned and manually adjusted using the BioEdit Software, and analyzed for the presence of chimeras with the Decipher chimera detection tool (http://decipher.cee.wisc.edu/FindChimeras.html). Taxonomic affiliation was achieved using the BLAST alignment tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the classifier tool of the Ribosomal Database Project II (RDP II) at http://rdp.cme.msu.edu.

3.2.16. Barcoded 16S rDNA pyrosequencing

This methodology was performed in collaboration with the Microbiology Department of the University of Barcelona, in particular with Dr. Joaquim Vila and Dr. Magda Grifoll.

The eubacterial community structures of the PN and Anammox reactors were analysed by bacterial tag-encoded amplicon pyrosequencing using the same DNA extracts. The V1-V3 regions of 16S rRNA genes were amplified using primers 27f and 519r. Primer 27f was modified to incorporate an 8 bp barcode sequence unique to each sample on the 5' end of the primer sequence. Sample preparation and sequencing on a Roche GS-FLX 454 pyrosequencer (Roche, Branford, CT, USA) was performed at the Molecular Research DNA Lab (Shallowater, TX, USA). Prior to analysis, data was processed to remove barcodes and primers, short sequences (<200 bp) were deleted, and those with ambiguous base calls or homopolymer runs exceeding 6bp removed. Sequences were then denoised and chimeras removed. OTUs were defined by clustering sequences at 3% divergence, and taxonomically classified using BLASTn against a curated GreenGenes database.

Sequences of all detected OTUs related to *Planctomycetes* were selected and their taxonomic affiliation confirmed with the classifier tool of the RDP II. These and a set of reference sequences available at ncbi (www.ncbi.gov) were aligned with the ClustalW tool and manually adjusted using BioEdit. Phylogenetic trees were constructed using the maximum likelihood and neighbour joining methods using MEGA6.0 software with 1000 bootstrap resamplings. Use of different tree construction algorithms resulted in similar dendogram topology.

4. Start-up and operation of an AnMBR for winery wastewater treatment

Abstract

Winery wastewater is an effluent with a high organic load worth considering for biogas production. A sidestream anaerobic membrane bioreactor (AnMBR) was started up and operated under organic load oscillations in order to determine its feasibility for winery wastewater treatment. The stable operation was assured by keeping a ratio between intermediate alkalinity and total alkalinity (IA/TA) below 0.4 achieving a 96.7±2.7% COD removal efficiency. The maximum organic loading rate (OLR) achieved was 3.4 kgCOD m^{-3} d^{-1}*. The biogas production varied according with the OLR that was on average 0.50 ± 0.17* m^3_{biogas} $m^{-3}_{digester}$ d^{-1} *with an 87.1 ± 3.0% of methane.*

The external membrane module reached a flux of 20.2 ± 8.5 LMH operating at a mixed liquor suspended solids (MLSS) concentration of 4.78±1.9 g L^{-1} *and a crossflow velocity of 0.64 m* s^{-1}*. The crossflow velocity helped to remove the cake layer attached on the membrane that was the main contribution (>80%) to flux decline.*

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- Operación de un Bioreactor de Membranas Anaeróbico (BRM-An) para el tratamiento de agua residual vitivinícola. *XI Reunión de la Mesa Española de Tratamiento de Aguas (META)*, Alicante, Spain, 18th – 20th June 2014.

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4.1. INTRODUCTION

Winery wastewater represents an important issue in the major wine producer countries in the world, which are France, Italy and Spain. The interest in winery wastewater treatment lies on that it cannot be discharged to the environment harmlessly due to its particular characteristics: high biodegradable organic load, low nutrient content and acidic pH. During wine production, large volumes of wastewater are generated mainly from washing operations that can be up to 4 L L^{-1} of wine produced (Andreottola et al., 2009) . Wineries usually treat their wastewater by means of aerobic processes (ponds, activated sludge, etc.) which are efficient enough to meet the legal discharge requirements, although bad odours and groundwater pollution might occur (Bories et al., 2007). Since winery wastewater is a high organic loaded effluent, energy generation from anaerobic digestion is worth considering, which aims to obtain at least enough energy to cover process requirements, otherwise the low cost of conventional aerobic treatments would be more feasible.

Anaerobic digestion of winery wastewater has shown good reliability as nutrient demand is much lower than aerobic processes. Due to lower biomass growth rate, the ratio chemical oxygen demand to nitrogen and phosphorus (COD/N/P) could be in the order of 800/5/1 (Moletta, 2005). Nevertheless, winery wastewater characteristics are seasonal, so that during vintage and wine making organic load and flow rate are much higher than the rest of the year. This is a clear drawback for the application of conventional anaerobic digestion due to its lack of flexibility that forces to operate at long hydraulic retention times (HRT) to avoid biomass washout. Hence, biomass immobilisation technologies (as membrane bioreactors, biofilters, fluidized beds, granulation, etc.) should be taken into account in order to enhance process flexibility.

In recent years, membrane bioreactor (MBR) technology has experienced a huge growth due to its numerous advantages compared to conventional treatments, and it is considered a successful technology for urban and industrial wastewater treatment. The main reason for such a success is its capacity to obtain a high effluent quality, free of suspended solids, accomplishing legal requirements with a reduced footprint (Judd, 2011). Several studies about MBR technology at full-scale for winery wastewater treatment have already been performed showing an improvement of efficiency versus conventional activated sludge processes (Artiga et al., 2007; Bolzonella et al., 2010; Ioannou et al., 2014; Valderrama et al., 2012). Nevertheless, the major limitation of MBR technology is membrane fouling, which is the most important factor to bear in mind due to its close relationship with energy requirements (Judd, 2011). For this reason, it is necessary to consider the progress on technology and its application at full-scale that improves energy savings.

Despite aerobic MBRs represent the vast majority of the total MBRs installed at full-scale (Kraume and Drews, 2010), the interest in the anaerobic MBR (AnMBR) is increasing because of the advantages of an anaerobic digester combined with a membrane filtration. Conventional anaerobic processes are well-known to achieve high organic matter removal efficiencies without oxygen requirement, low biomass production and energy generation from biogas (Metcalf and Eddy, 2003). However, AnMBR technology enables a wider range of anaerobic digestion possibilities. It has been introduced for industrial application since 1990s for the treatment of organic waste and industrial wastewater with high organic content from distilleries, septic tanks, food and paper industries, etc. (Skouteris et al., 2012). For instance, AnMBR is used in food processing industries treating high organic loads (10-15 kgCOD $m^{-3}d^{-1}$). The biomass retention allows the uncoupling of the HRT from the sludge retention time (SRT) (Liao et al., 2006a).

A positive energy balance is essential for the AnMBR establishment. Few examples of full-scale AnMBR detailing energy requirements can be found. For example, Kim et al. (2011) concluded that with only a 30% of the methane production, energy demand was covered when treating low strength synthetic wastewater. The configuration of this AnMBR consisted in a two stage anaerobic fluidised bed reactor with granular activated carbon, in which a submerged hollow fibre membrane was placed. The energy requirement of both reactors was 0.058 kWh m^{-3}, being 0.028 kWh m^{-3} the demand of the second stage where the membrane was placed. In the study of Kanai et al. (2010), it was determined that the electricity and heating costs of a submerged AnMBR for food waste treatment were approximately 4 GJ d^{-1} while the energy recovered from biogas was up to 12 GJ d^{-1}. Therefore, the expenses were more than covered. Although AnMBR seems a feasible option, it is still under research and there is little literature about its application to winery wastewater treatment (Ioannou et al., 2014).

The aim of the present work is to determine the efficiency and feasibility of the application of an AnMBR for the treatment of winery wastewater. Biomethane potential of different winery wastewaters was determined by means of anaerobic biodegradability tests. An AnMBR was started-up and operated with synthetic wastewater in order to control organic loading rate applied, which was progressively increased to allow the biomass acclimation. Afterwards, real winery wastewater collected during vintage was fed to the AnMBR evaluating the flexibility of the system. A final energy balance was calculated determining a suitable configuration for full-scale application.

4.2. RESULTS AND DISCUSSION

4.2.1. Experimental set-up and substrate

The AnMBR was set-up as a conventional stirred anaerobic digester of 5 L coupled with an external membrane unit (Orelis, Rayflow Module) of 100 cm^2 of membrane area, described in section 3.1.1. The digester was a jacketed vessel mechanically stirred at 100 rpm and heated at 35°C by recirculating water from a heated water bath (HUBER 118A-E). Influent wastewater was fed from a 10 L tank with a cooling system to avoid early degradation. However, significant oscillations in COD concentration were observed, therefore wastewater was prepared every 2-3 days. Digester feeding was performed by pressure equilibrium keeping the digester in contact with a 500 mL cylinder at a constant volume of wastewater. Thus, the working volume inside the digester was kept at 3.5 L. Since the membrane unit was placed outside the digester, biogas was easily quantified with an on-line measuring device (Ritter MGC-1) connected to the headspace of the digester.

The control of digester conditions, especially during the start-up period, is crucial for an appropriate biomass acclimation. Synthetic wastewater was used to feed the system avoiding the typical winery wastewater variability in terms of COD content. Synthetic wastewater was prepared with diluted white wine (Artiga et al., 2005) and NH_4Cl and K_2HPO_3 that cope the lack of nutrients in accordance to the ratio COD/N/P of 800/5/1. In addition, alkalinity was added (500 - 1000 $mgCaCO_3\ L^{-1}$) to keep the pH at neutral values.

Afterwards, real winery wastewater collected during vintage was fed to the AnMBR. As shown in Table 4.1, the ratio COD/N/P was far from the desirable one. Therefore, the same reagents as in the synthetic mixture were added to the raw wastewater. In addition, sulphate concentration should be taken into account. If the ratio COD/SO_4^{2-} is low, the affinity for COD of sulphate-reducing bacteria would be higher than the methanogenic archaea decreasing the biogas production (Appels et al., 2008). In this case, sulphate content in winery wastewater was very low, thus sulphate reducers contribution was expected to be unnoticeable.

Table 4.1. Winery wastewater characterisation collected during vintage

Parameter	Average value
pH	4.67 ± 0.42
tCOD (mg L^{-1})	6752 ± 663
sCOD (mg L^{-1})	4040 ± 692
ST (mg L^{-1})	2913 ± 26
SV (mg L^{-1})	1533 ± 20
SST (mg L^{-1})	913 ± 21
SSV (mg L^{-1})	763 ± 31
NH_4^+-N (mg L^{-1})	4.31 ± 0.69
PO_4^{3-}-P (mg L^{-1})	5.55 ± 0.46
SO_4^{2-} (mg L^{-1})	4.28 ± 0.40
COD/N	1568
COD/P	1217
COD/SO_4^{2-}	1583

The ratio COD/N/P was far from the desirable one, being COD/N=1568 and COD/P=1217. Therefore, the same reagents as in the synthetic mixture were added to the raw wastewater. In addition, sulphate concentration should be taken into account. If the ratio COD/SO_4^{2-} is below 3, the affinity for COD of sulphate-reducing bacteria would be higher than the methanogenic archaea decreasing the biogas production (Appels et al., 2008). In this case, COD/SO_4^{2-} was 1583, thus sulphate reducers contribution was expected to be unnoticeable.

4.2.2. Membrane characterisation: critical flux and resistances in series

The critical flux was determined in order to characterise the membrane module and the optimal operational flux. The critical flux was measured following the guidelines of Le Clech et al. (2003). They suggested a controlled increase of flux in steps while monitoring the pressure until a severe rise is observed. The critical flux corresponds to the flux below which the pressure does not increase with time, thus dP/dt is close to zero. Steps of 15 minutes from 5 LMH (L m^{-2} h^{-1}) to 45 LMH were carried out monitoring the pressure increase in each step.

The critical flux was determined by representing the slope (dP/dt) in each step of flux versus the flux (Figure 4.1). It was clearly observed that over 23 LMH, dP/dt rapidly increased from 0.5 mbar min^{-1} to 2.85 mbar min^{-1}. Hence, the membrane module should operate below this value, otherwise flux would decline rapidly.

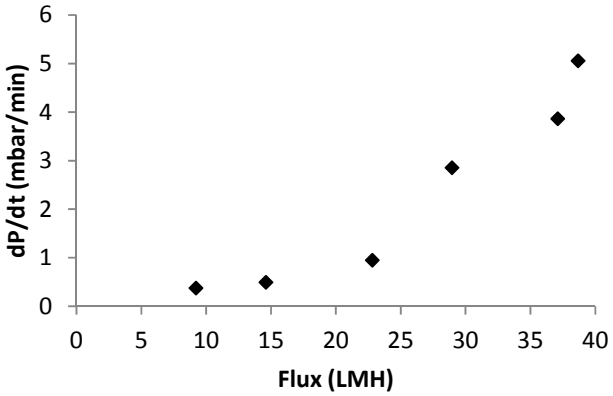


Figure 4.1. Critical flux determination

The filtration resistances in series based on Darcy's law (Equation 4.1) were measured following the procedure of Bae and Tak (2005). They proposed that the total filtration resistance (R_T) can be divided in three resistances in series (Equation 4.2) corresponding to the cake layer (R_c), the pore fouling (R_f) and the membrane itself (R_m).

$$R = \Delta P/(\mu \times J) \quad (4.1)$$

Where R is the resistance (m^{-1}), ΔP is the TMP pressure (mbar), μ is the viscosity (Pa s) and J is the flux ($m^3\ m^{-2}\ s^{-1}$).

$$R_T = R_c + R_f + R_m \quad (4.2)$$

R_m was determined by filtering distilled water during 15 minutes until the flux was stable at a given pressure. R_T was determined by filtering sludge, thus it would include all contributions: the membrane itself, the cake layer on the membrane surface and the pore blocking. R_f was determined by filtering distilled water afterwards. The distilled water helps to remove the cake layer leaving the membrane surface free of attached solids, although it is not able to remove the fouling inside the pores. Finally, R_c was calculated from R_T subtracting R_m and R_f. The test was performed using anaerobic sludge with 4 gSS L^{-1}.

It was determined that the total resistance was R_T = 4.36·10^{12} m^{-1}. The 88% of this total resistance was attributed to R_c; the 11% corresponded to R_m and finally only a 1% was due to R_f. Considering that R_c was the main resistance and the module design (flat sheet, crossflow filtration), the filtration would improve by a higher crossflow velocity to remove the cake layer attached on the membrane surface. By the contrary, R_f (caused by fouling i.e. pore blocking) that can only be removed by chemical cleaning had a very low effect on the total resistance observing a R_f = 3.33·10^{10} m^{-1}. Since the microfiltration membrane pore was quite big (0.2 µm), R_f was not expected to have a huge contribution as it would have in a smaller pore size membrane (i.e. osmosis). However, after long term operation (around 2 years) it increased up to R_f = 3.89·10^{11} m^{-1}, which represented a 8% of the total resistance. This increasing pore blocking would lead at long term operation to perform chemical cleanings more often in order to reduce its contribution.

4.2.3. AnMBR for winery wastewater treatment

The AnMBR was operated during three experimental periods. In **Table 4.2** the main parameters and results of each period are summarised. In all the periods, COD removal efficiencies over 95% were achieved. Instabilities only occurred when a sharp increase in the influent COD promoted the accumulation of VFA and thus a decrease of pH. These instabilities were solved by the addition of enough alkalinity to buffer the amount VFA accumulated. The addition of an external reagent to provide alkalinity should be adjusted because it represents an extra cost. Therefore, at the beginning of the AnMBR operation, 500 mg $CaCO_3$ L^{-1} were added to the synthetic feed. However, during the first 60 days pH decreased to 6 with the presence of around 200 mg L^{-1} of VFA. Hence, alkalinity was increased up to 800 mg$CaCO_3$ L^{-1} in the synthetic feed, which were enough to keep a neutral pH even when 600 mg L^{-1} of VFA were occasionally accumulated. The alkalinity of the AnMBR in the three experimental periods is shown in Table 4.2. Because the amount of VFA in the influent increased from one period to another, alkalinity was added accordingly. The pH was kept around 7 with enough buffer capacity to cope any VFA accumulation due to an increase in the organic loading rate (OLR).

Biogas production was closely related to the OLR, thus the oscillations in OLR led to a variable P_B, especially in Period I. The specific methane production (SMP) was around 0.30 m^3CH_4 kg^{-1}COD and methane concentration in biogas was over 85% in all the experimental periods. According to Henry's law, at mesophilic temperature the desorption rate of methane is higher than the one of carbon dioxide. Therefore, low HRTs promoted the retention of CO_2 in the permeate achieving a high methane content in biogas.

The operational conditions were adjusted from one period to another in order to reach higher OLR assuring stable operation. In this system, the OLR not only depended on the influent COD, but also on the flux through the membrane. Therefore, higher OLRs can be achieved by increasing the inlet COD but also increasing the flux. However, during operation it was observed that OLR decreased even when influent COD was high due to the cake layer formed on the membrane surface led to a decrease of flux.

During Period I, the AnMBR was fed with synthetic winery wastewater increasing progressively its OLR. For 230 days, biomass was not purged in order to favour the growth and the accumulation of the most suited microorganisms for the winery wastewater treatment. The membrane was operated at an average flux of 10.5±2.5 LMH. Since the flux applied was far from the critical one (23 LMH), the flux declined at a low rate of 0.07±0.04 LMH d^{-1}. Subsequently, in Period II an oscillating influent COD was applied in order to determine the flexibility of the system and the needs of alkalinity. At a similar influent COD concentrations, the OLR achieved was higher in Period II than in Period I. A higher flux of 16.0±3.9 LMH was obtained because the crossflow velocity increased from 0.10 to 0.64 m s^{-1}. Despite the average flux increased, the rate of flux decline was similar at 0.10±0.04 LMH d^{-1}. Finally, in Period III real winery wastewater was fed to the AnMBR. Higher OLR and flux were applied up to 1.75±0.63 kgCOD $m^{-3}_{digester}$ d^{-1} and 20.2±8.5 LMH, respectively. This increase in flux was achieved by applying higher TMP of 0.3 bar. However, the flux decline rate significantly increased to 0.54±0.33 LMH d^{-1}, thus cleanings were carried out more frequently.

Table 4.2. Operational parameters and results of each experimental period of the AnMBR for winery wastewater treatment

Operational parameters			
	Period I	**Period II**	**Period III**
Type of wastewater	**Synthetic**	**Synthetic**	**Winery wastewater**
pH	7.2±0.3	7.1±0.2	7.6±0.5
Alkalinity ($mgCaCO_3\ L^{-1}$)	742±519	887±199	1092±88
MLSS ($g\ L^{-1}$)	2.08 - 8.48	6.30±1.33	4.78±1.94
HRT (d)	3.5±1.1	2.2±0.5	2.26±0.27
SRT (d)	790	560	560
COD influent ($g\ L^{-1}$)	0.91 → 9.05	2.92±1.05	5.09±1.27
COD effluent ($g\ L^{-1}$)	0.095±0.15	0.14±0.23	0.28±0.35
VFA influent ($mg\ L^{-1}$)	278±72	668±162	928±661
VFA effluent ($mg\ L^{-1}$)	110±65	114±120	269±409
%COD removal	95.7±3.4	95.7±3.7	96.7±2.7
OLR ($kgCOD\ m^{-3}_{digester}\ d^{-1}$)	0.26 → 2.58	1.32±0.51	1.75±0.63
sOLR ($kgCOD\ kg^{-1}MLSS\ d^{-1}$)	0.15 → 0.70	0.22±0.09	0.30±0.18
Membrane performance			
Flux (LMH)	10.5±2.5	16.0±3.9	20.2±8.5
Flux decline ($LMH\ d^{-1}$)	0.07±0.04	0.10±0.04	0.54±0.33
TMP (bar)	0.2	0.2	0.3
Crossflow velocity ($m\ s^{-1}$)	0.10	0.64	0.64
Biogas production			
P_B ($m^3_{biogas}\ m^{-3}_{digester}\ d^{-1}$)	0.02 → 1.19	0.42±0.26	0.50±0.17
$\%CH_4$ in biogas	86.3±2.4	84.8±1.0	87.1±3.0
SMP ($m^3CH_4\ kg^{-1}COD$)	0.30±0.02	0.28±0.16	0.33±0.15

4.2.4. Period I - Start-up and operation of an AnMBR treating synthetic winery wastewater

The first period of the AnMBR operation was performed using synthetic winery wastewater with a COD concentration progressively increased from 900 to 9,000 mg L^{-1} and a HRT of 3.5±1.1 days. The COD concentration profile of both influent and permeate as well as the ratio between intermediate alkalinity and total alkalinity (IA/TA) are shown in Figure 4.2. It was observed that permeate COD remained below 125 mg L^{-1} during the major part of the

experimentation period. However, some peaks from 130 to 900 mg L^{-1} were obtained in days 50, 130, 185 and 215. These undesired peaks in the permeate COD were related to a sharp increase in the influent COD content that led to VFA accumulation. From the day 50 to 60, a destabilisation period was observed. It was expected that the system could have recovered stable conditions if inlet COD concentration had been reduced. However, it was necessary to add alkalinity in the AnMBR to increase the pH up to 7. During stable operation, VFA content was very low < 5 mg L^{-1} and the ratio IA/TA was around 0.2. When a pH decrease and thus an accumulation of VFA were detected, IA/TA increased over 0.3. Therefore, when the ratio IA/TA was between 0.2 and 0.4, the system was arriving at its limit of buffer capacity. Hence, in order to decrease the ratio IA/TA below 0.4, 800 mg$CaCO_3$ L^{-1} were added to the feed (initially synthetic winery wastewater was prepared with 500 mg$CaCO_3$ L^{-1}).

A maximum OLR of 2.58 kgCOD $m^{-3}_{digester}$ d^{-1} was successfully treated, which is not a high value compared with other AnMBR that can treat up to 25 kgCOD $m^{-3}_{digester}$ d^{-1} (Skouteris et al., 2012). Nevertheless, regarding the specific organic loading rate (sOLR [kgCOD kg^{-1}MLSS d^{-1}]), shown in Figure 4.2**¡Error! No se encuentra el origen de la referencia.**, it is noted that during the first 60 days the average sOLR was 0.37±0.13 kgCOD kg^{-1}MLSS d^{-1}, with a maximum value of 0.70 kgCOD kg^{-1}MLSS d^{-1}. These values are high compared to those reported in the review of Dereli et al. (Dereli et al., 2012), which range from 0.04 to 0.57 kgCOD kg^{-1}MLSS d^{-1}. However, also few studies that achieve higher sOLR up to 2 kgCOD kg^{-1}MLSS d^{-1} can be found (Liu et al., 2013). The sOLR was directly related to the MLSS concentration, which increased from 2.08 g L^{-1} to 8.48 g L^{-1} due to the long SRT of 790 days that led to biomass accumulation. From day 60 on, sOLR decreased to an average value of 0.23±0.08 kgCOD kg^{-1} MLSS d^{-1}. Despite influent COD was progressively increased, the accumulation of biomass helped to avoid instabilities in the digestion process caused by an excessive sOLR.

The sOLR was calculated directly from the daily flux obtained through the membrane and the influent COD, thus it depended on the membrane performance. Solids tended to accumulate on the membrane surface promoting the decrease of flux and therefore sOLR, which were in addition affected by the increase of MLSS. The crossflow velocity kept in the membrane unit during Period I was 0.10 m s^{-1}. The average permeate flux was 10.5±2.5 LMH, although it progressively decreased due to membrane fouling. Membrane unit was cleaned periodically when the flux was below 7 LMH following the procedure given by the manufacturer. Chemical cleanings were performed in order to remove the cake layer and also the fouling accumulated in the membrane. In Figure 4.2 it can be observed that the increase in MLSS promoted a decrease of flux which could be partially recovered after a

chemical cleaning (marked in dash lines). However, flux tended to decrease rapidly after a cleaning especially when MLSS were around 8 g L^{-1} (from day 140 on).

Biogas production (P_B) ranged from 0.02 to 1.19 Nm^3_{biogas} $m^{-3}_{digester}$ d^{-1} with an average methane concentration of 86.3±2.4%. Since the influent COD suffered significant oscillations, P_B was also very variable. Moreover, when a huge accumulation of VFA occurred in the digester (days 50 to 60), P_B decreased until 0.02 Nm^3 $m^{-3}_{digester}$ d^{-1}; because methanogenic bacteria were inhibited at a pH below 6.5 (Appels et al., 2008).

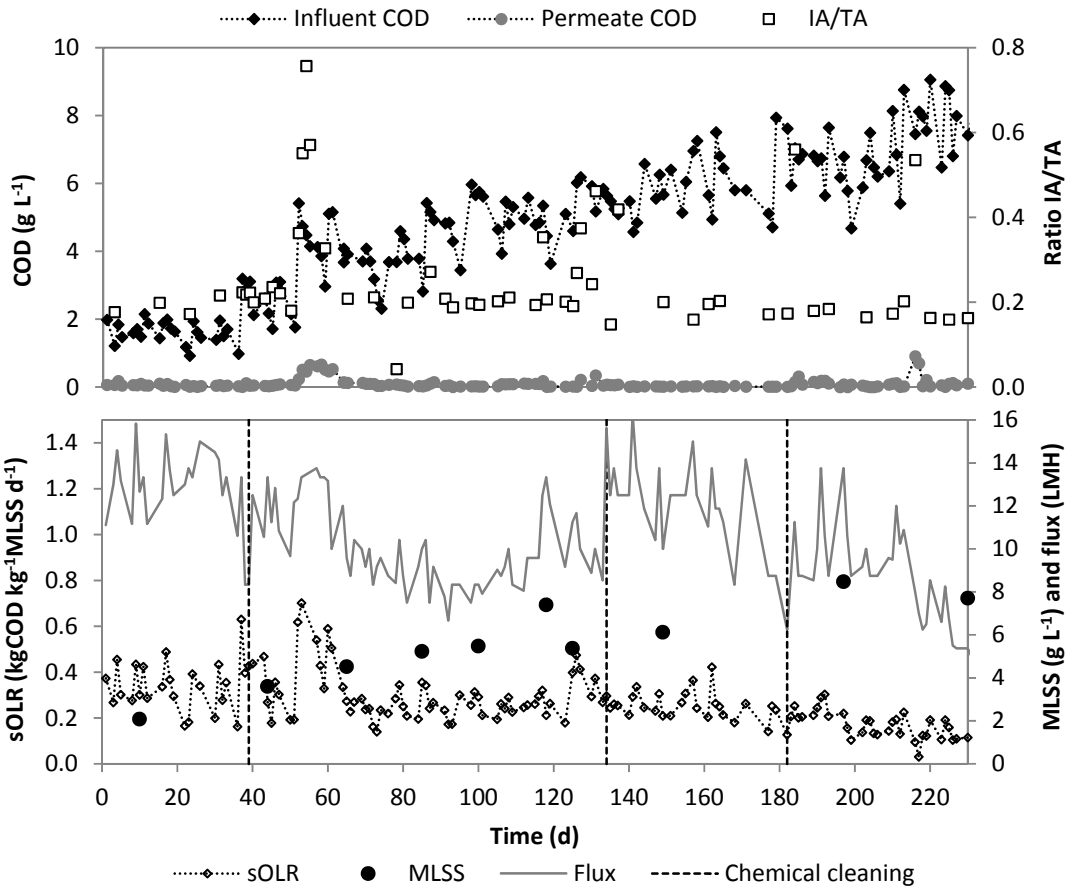


Figure 4.2. Influent and permeate COD concentration, ratio IA/TA, sOLR, flux and MLSS profile during Period I.

4.2.5. Period II - Operation of an AnMBR with synthetic fluctuating influent

A second experimental period was performed during 165 days applying different influent COD concentrations between 1 and 6 g L^{-1} to simulate the seasonal fluctuations of the winery wastewater. The operational parameters are shown in Table 4.2. Unlike the previous period where biomass was accumulated in the AnMBR, in Period II the MLSS was kept at 6.3±1.3 g L^{-1} with a SRT of 560 days. Due to the instabilities observed in the previous period due to a lack alkalinity, the amount of alkalinity added in the influent wastewater was maintained at 800 mg L^{-1}.

The sOLR during Period II was 0.22±0.09 kgCOD kg^{-1}MLSS d^{-1} achieving a COD removal efficiency of 95.7±3.7%. Despite the fluctuations applied in the influent COD shown in Figure 4.3, the ratio IA/TA was kept below 0.3 and the operation did not experiment any instability keeping a total alkalinity in the digester of 887±199 mg $CaCO_3$ L^{-1}. However, when influent COD sharply increased around day 150, COD removal efficiency decreased. It was observed a ratio IA/TA higher than 0.3, which meant that VFAs were accumulated in the AnMBR. At that moment, sOLR was 0.47 kgCOD kg^{-1}MLSS d^{-1} and the COD removal efficiency decreased to 63.6%. Stable operation was recovered immediately by decreasing the sOLR to values below 0.30 kgCOD kg^{-1}MLSS d^{-1}. In accordance with the results of Period I, when sOLR was around 0.30 kgCOD kg^{-1}MLSS d^{-1} stable operation was assured.

The crossflow velocity applied to the membrane unit was increased to 0.64 m s^{-1}, thus a higher flux was achieved that was on average 16.0±3.9 LMH. Hence, the HRT was reduced to 2.2±0.5 days. As shown in Figure 4.3, the flux at the beginning of the period was up to 24 LMH, which was close to the critical flux. It rapidly decreased to 20 LMH due to the cake layer on the membrane surface. With the crossflow velocity applied, the flux was maintained mostly between 15 and 20 LMH. Less fouling was observed because the higher crossflow velocity helped to remove the cake layer and to keep a more constant flux; thus a more constant sOLR. The system was more prone to suffer instabilities when changes in sOLR occurred, but it was not so affected by the fluctuations in influent COD between 1 and 6 g L^{-1}.

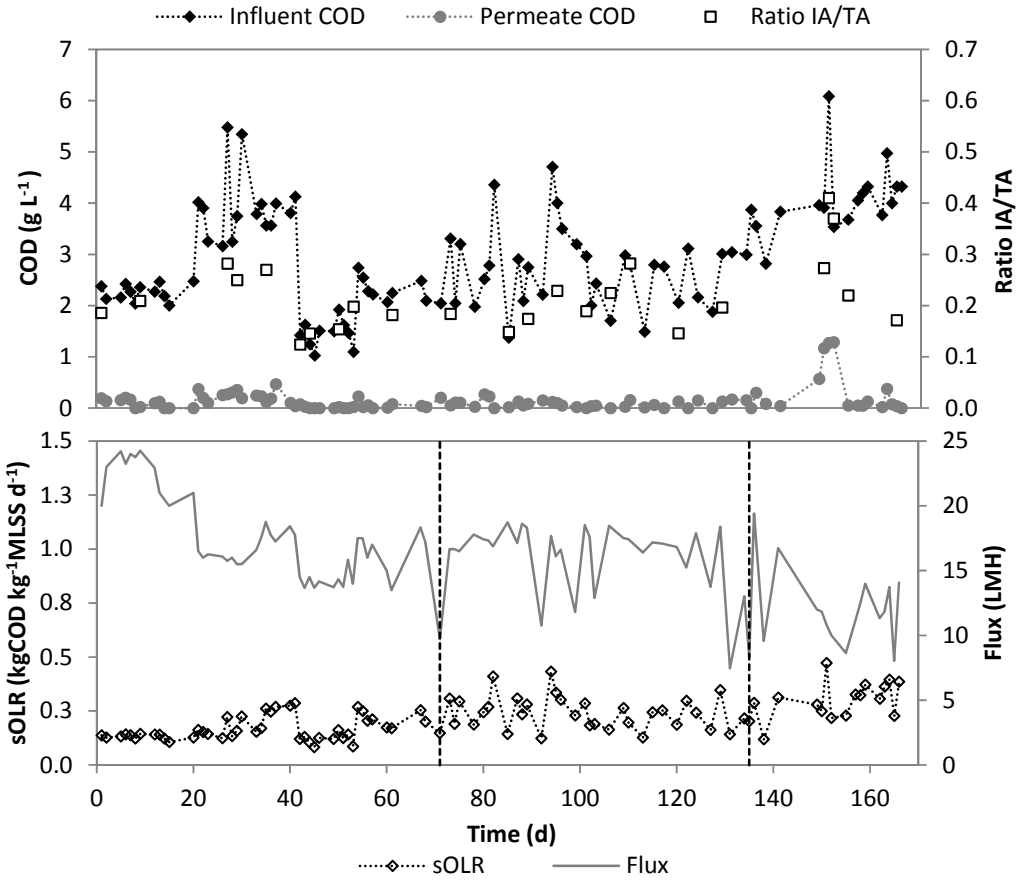


Figure 4.3. Influent and permeate COD concentration, ratio IA/TA, sOLR and flux profile during Period II.

4.2.6. Period III - Operation of an AnMBR with winery wastewater during vintage

A third experimentation period was performed with real winery wastewater collected during vintage. During the first 60 days of experimentation the winery wastewater was diluted and progressively increased its concentration to the original one. As shown in Figure 4.4, the ratio IA/TA was maintained at 0.18±0.03 for the first 100 days, coinciding with a low sOLR of 0.10±0.08 kgCOD kg^{-1}MLSS d^{-1}. Afterwards, when influent COD increased up to the original winery wastewater the ratio IA/TA increased to 0.33±0.07 and sOLR was 0.29±0.18 kgCOD kg^{-1}MLSS d^{-1} maintaining stable operation with a COD removal efficiency of 96.7±2.7%. A maximum sOLR of 0.74 kgCOD kg^{-1}MLSS d^{-1} was successfully treated. During this period, MLSS were maintained at 4.78±1.9 g L^{-1}. With a crossflow velocity of 0.64 m s^{-1} and a TMP pressure of 0.3 bar, a flux up to 40 LMH was reached. As shown in Figure 4.4, the

flux rapidly decreased below 20 LMH during the first days of operation after a chemical cleaning. A higher TMP pressure led to a higher flux. However, the operation over the critical flux implied a severe fouling. Hence, at a fixed TMP, flux decreased rapidly below the critical value. The sOLR profile had a similar behaviour as the flux profile, especially after day 100 when winery wastewater was fed directly.

Similar results were obtained treating real winery wastewater in terms of COD removal efficiency and biogas production that reached P_B=0.50±0.17 m^3_{biogas} $m^{-3}_{digester}$ d^{-1} with a 87.1±3.0% of methane. Despite the oscillations in the influent COD concentration, the stable operation was assured by keeping a sOLR around 0.30 kgCOD kg^{-1}MLSS d^{-1} and a ratio IA/TA below 0.40. The attempt to increase the flux through the membrane (up to 20.2±8.5 LMH) promoted higher flux decline of 0.5±0.33 LMH d^{-1}, and thus the necessity of periodic cleanings. Higher fluxes can also be achieved by reducing the amount of MLSS in the AnMBR, although the sOLR should be controlled in order not to exceed the system capacity.

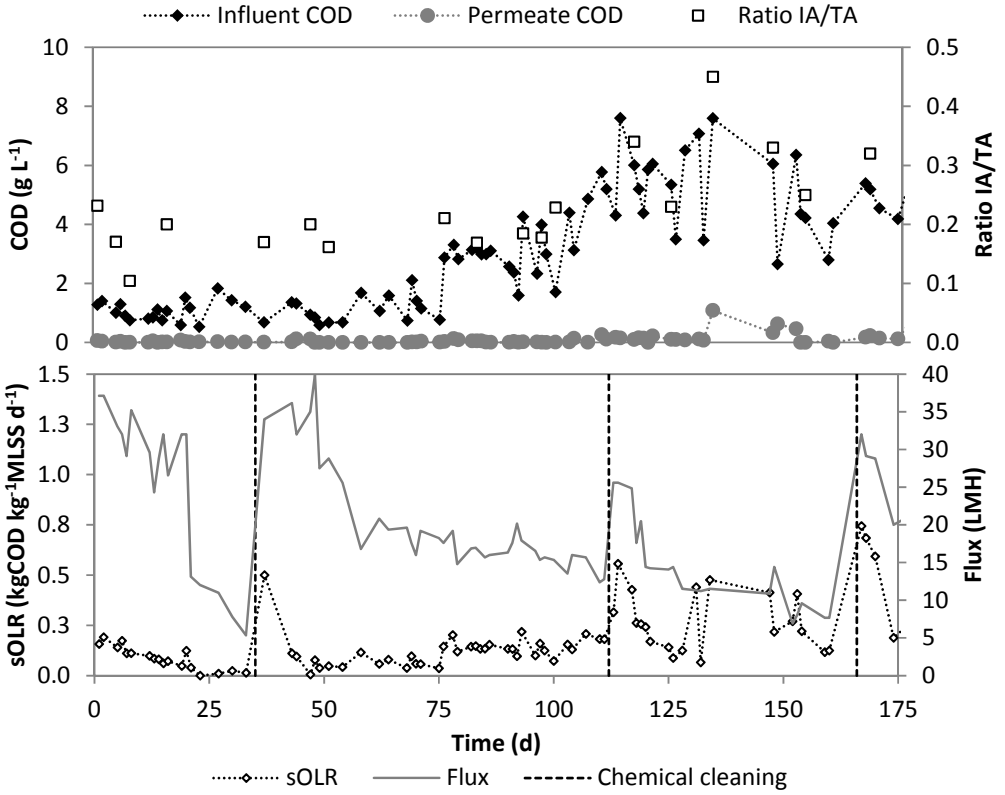


Figure 4.4. Influent and permeate COD concentration, ratio IA/TA, sOLR and flux profile during Period III.

4.2.7. Membrane fouling characterisation by SEM

The fouling on the membrane surface was analysed by scanning electron microscopy (SEM). Figure 4.5 shows the surface of the new membrane (a) and after 6 months of operation in Period III (b). It is noted that the surface was completely covered by a cake layer (Figure 4.5b), which was the main responsible for flux decline. During Period II and III higher fluxes were obtained because the cake layer was minimised by applying a higher crossflow velocity that helped to remove it. Because the membrane was made of PVDF (polyvinylidene flouride), the elements detected qualitatively in Figure 4.5a were C, O and F. In contrast, the cake layer (Figure 4.5b) contained mainly C and O, but also Si, Al, Fe, K and S due to the inorganic particles attached on it (Figure 4.5c and d). It can be stated that the majority of the cake layer was of organic origin made of sludge, although there were some salts attached on it, probably coming from winery wastewater.

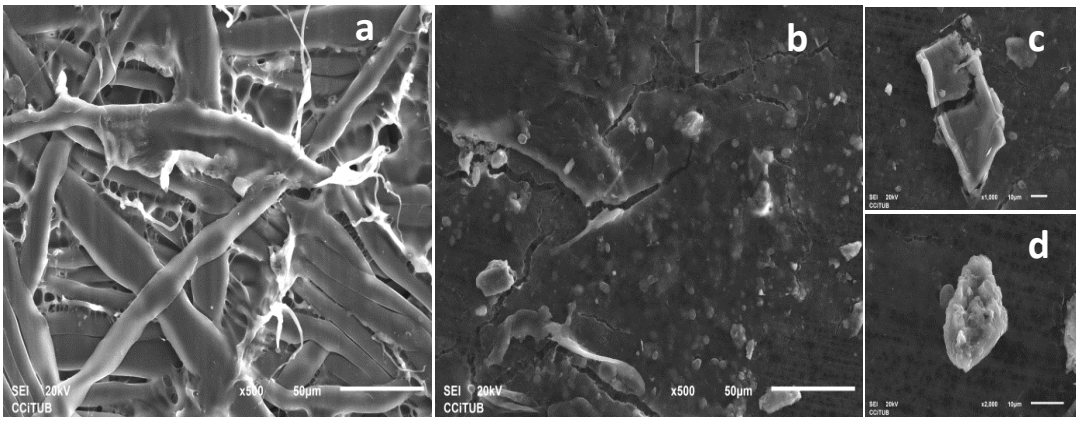


Figure 4.5. Scanning electron microscopy (SEM) of the new membrane (a) and the membrane after Period III (b). Inorganic salts attached in the cake layer made of Si, Al and K (c and d).

4.2.8. Evaluation of biomass acclimation by BMP tests

BMP tests were performed with three different wastewaters before the start-up of the AnMBR: two real winery wastewaters and a dilution of white wine simulating winery wastewater. Winery wastewaters were collected from two wineries located in Sant Sadurní d'Anoia and Pacs del Penedès (Barcelona). The main characteristics of the different substrates considered for the BMP test are shown in Table 4.3.

Table 4.3. Substrate characterisation for the BMP tests.

Wastewater	Parameters							
	COD_{total} (g L^{-1})	$COD_{soluble}$ (g L^{-1})	TS (g L^{-1})	VS (g L^{-1})	TSS (g L^{-1})	VSS (g L^{-1})	NH_4^+-N (mg L^{-1})	VFA (mg L^{-1})
WW1	5.91±0.03	1.23±0.03	6.55±0.02	3.02±0.04	2.46±0.09	1.92±0.02	12.4±0.5	2012±5
WW2	1.9±0.1	1.15±0.04	2.14±0.07	0.59±0.04	0.10±0.06	0.09±0.04	12.9±0.5	258±5
Synthetic WW	184±3	184±3	-	-	-	-	7.23±0.5	n.d.

The BMP test were carried out using anaerobic biomass coming from a sewage sludge anaerobic digester. The same biomass was used afterwards to inoculate the lab-scale AnMBR. The results of the BMP tests are summarised in Table 4.4. The three wastewaters tested showed similar profiles, achieving an average specific methane production (SMP) of 0.19 m^3 CH_4 kg^{-1}COD in just 4 days. The percentage of methane in the biogas obtained was 75±2%. Only WW1 reached a higher SMP of 0.23 m^3 CH_4 kg^{-1}COD after 20 days that was probably due to its higher content of solids and particulate organic matter that were degraded slower. These SMP values were low compared with other anaerobic systems reviewed in Mata-Alvarez et al. (2014). The inoculum used was not acclimatised to this easily biodegradable winery wastewater, thus it could be inhibited by an excess of substrate.

Table 4.4. SMA and SMP obtained during AnMBR operation

Inoculum	Wastewater	SMA (gCH_4-COD $gVSS^{-1}d^{-1}$)	SMP (after 20 d) (m^3 CH_4 kg^{-1}COD)	Biodegradation (%$COD_{removed}$)
Anaerobic digestion of sewage sludge	WW1	0.13	0.23	79.6
	WW2	0.06	0.19	72.7
	Synthetic WW	0.07	0.19	67.2
AnMBR Period I (100d operation)	Synthetic WW	0.28	0.28	80.3
AnMBR Period I (200d operation)	Synthetic WW	0.36	0.35	98.5
AnMBR Period III	Winery wastewater	0.22	0.34	97.4

The percentages of biodegradation were 79.6; 72.7 and 67.2 for WW1, WW2 and synthetic WW, respectively. Both real winery wastewaters resulted in a higher COD removal efficiency than the synthetic one, probably because they were partially fermented at the sampling point providing VFA. It is noted that WW1 had a significant amount of solids that were not present in WW2. In many cases, the winery wastewater is mixed with the black water produced by the winery itself, increasing nutrient and solid concentration. Since wineries are relatively small industries, the wastewater produced can differ from one facility to another. Moreover, VFA content is also quite variable depending on the fermentation grade at the sampling point. Because the COD in winery wastewater is easily biodegradable, composed mainly by glucose and ethanol, it can be rapidly fermented to acetate. For this reason, WW1 had higher VFA content compared with WW2 and the synthetic WW.

In order evaluate the biomass activity during the AnMBR operation, BMP tests were performed after 100 and 200 days during Period I (biomass was accumulated in the AnMBR). The same synthetic WW was used as substrate thus the methane production results are comparable. As shown in Table 4.4**¡Error! No se encuentra el origen de la referencia.**, COD removal efficiency increased from 67.2% to 80.3% (Day 100) and 98.5% (Day 200). Specific methanisation activity (SMA) also significantly increased from 0.07 gCH_4-COD $gVSS^{-1}$ d^{-1} to 0.28 gCH_4-COD $gVSS^{-1}$ d^{-1} (Day 100) and 0.36 gCH_4-COD $gVSS^{-1}$ d^{-1} (Day 200).

Finally, before feeding the AnMBR with winery wastewater from vintage season (Period III), another BMP test was performed. SMP and biodegradation achieved were similar to those from Period I (200 days), being 0.34 m^3CH_4 kg^{-1}COD and 97.4%. However, SMA was lower than in the previous tests of Period I. By using real wastewater SMA was 0.22 gCH_4-COD $gVSS^{-1}$ d^{-1}, while with synthetic wastewater 0.36 gCH_4-COD $gVSS^{-1}$ d^{-1} were reached. The use of a real winery wastewater that contained particulate organic matter, led to a decrease in the SMA although SMP obtained was similar. Because SMA was calculated from the slope of methane production during the first days, it mainly considered the methane produced by the easy biodegradable COD. Therefore, after 20 days the SMP reached the same value due to the complete degradation of the particulate COD.

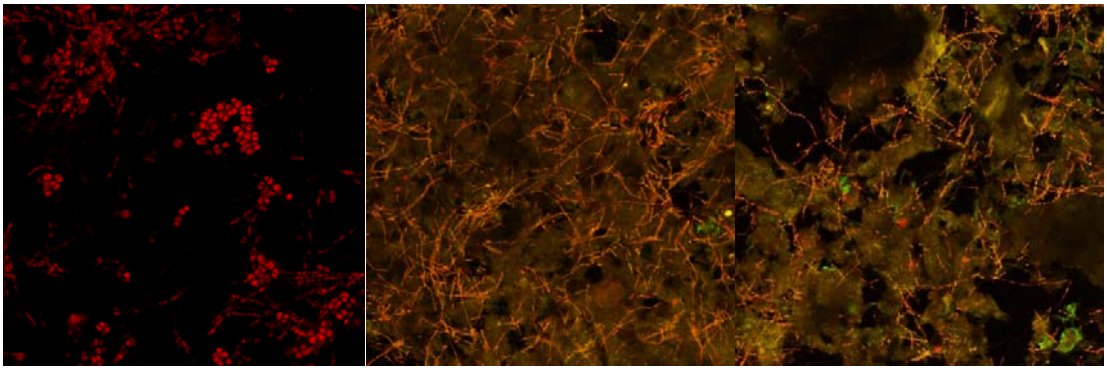
The substantial improvement in SMA, SMP and biodegradation was reflected in the AnMBR operation. During the first 60 days of Period I (shown in Figure 4.2), VFA were easily accumulated due to a low methanogenic activity and MLSS concentration. After months of operation, more stable conditions were achieved and higher influent COD concentration could be successfully treated.

4.2.9. Microorganism population

Biological population was determined by fluorescence in situ hybridization (FISH) following the procedure of Amann et al. (1990). In anaerobic digestion processes many bacteria species coexist, although the limiting step is usually the methanization, driven by archaea. For this reason the specific oligonucleotide probes used were: ARC915 for Archaea (Cy3); MX825 for Methanosaeta spp. (6-fam); MS821 for Methanosarcina (Cy3); MG1200b for Methanomicrobiales spp. (6-fam); and MB311 for Methanobacteriales (minus Methanothermus) (Cy3). Fluorescent signal were recorded with TCS-SP2 confocal laser scanning microscope (Leica, Germany) equipped with a DPSS 561nm laser for the detection of Cy3 fluorochrome (red) and an Ar ion laser for 6-fam fluorochrome (green). Two probes were applied in each sample, always combining a Cy3 probe with a 6-fam probe. The colocalization test was performed by ImageJ software determining Manders' coefficient as suggested by Bolte and Cordelières (2006). Manders' coefficient gives the correlation between pixels when overlapping two images (green and red). Therefore, M_1 corresponds to the proportion of pixels in green image that are positive in red image. Subsequently, M_2 corresponds to the proportion of pixels in red image that are positive in green image.

Samples during each experimental period were taken from the mixed liquor. In Period I, Methanosaeta spp. and Methanomicrobiales spp. were observed in the biomass (Figure 4.6a). Methanosaeta spp. had a filamentous appearance while Methanomicrobiales spp. were rounded. Although the specific probes MX825 and MG1200b occasioned fluorescence, the intensity of green fluorochrome (6-fam) was too low to perform the colocalization test. The percentage of each type of microorganism could not be quantified with precision but the existence of both species was clear. However, after 90 days of operation, Methanomicrobiales spp. were not observed. Hence, Methanosaeta spp. was grown at the expense of other methanogenic archaea.

In the following Period II and III, Methanosaeta spp. was the main methanogenic archaea present. It is clearly observed in Figure 4.6b that archaea had the filamentous shape. The overlapping of ARC915 and MX825 probes resulted in a colocalization calculated by Manders' coefficient of M1=0.95±0.07 and M2=0.97±0.05. Another sample was taken from the cake layer attached in the membrane surface achieving similar results of colocalization (M1=0.93±0.08 and M2=0.95±0.07; Figure 4.6c). It can be stated that the sludge attached in the membrane surface came from the mixed liquor and it was not biomass grown in the membrane itself.



(a) (b) (c)

Figure 4.6. FISH image of Archaea (ARC915) during Period I (a); overlapping of Archaea (ARC915) and Methanosaeta spp. (MX825) in the mixed liquor (b) and in the membrane surface (c) during Period III.

The operational conditions of the AnMBR favoured the acetotrophic path because the winery wastewater was easily degraded to acetate. Although Methanosarcina can grow with other substrates as formate or methanol and it has a higher growth rate, Methanosaeta spp. has a higher affinity for acetate specially at low acetate concentrations (Janssen, 2003). Due to low acetate concentration in the AnMBR biomass was enriched in Methanosaeta spp. rather than Methanosarcina. It should be considered that in Period I also Methanomicrobiales spp. were observed. The inoculum used to start up the AnMBR came from a sewage sludge digester in which Methanomicrobiales spp. appeared to be dominant in accordance with Kim et al. (2013). Since the SRT was very long and biomass tended to accumulate in the AnMBR, Methanomicrobiales spp. did not disappear until Period II when purges were carried out periodically in order to keep a fixed MLSS concentration.

The growth of Methanosaeta spp. was favoured as the only methanogenic species, which resulted in an improvement of the biomass affinity to the substrate. The evolution of the microorganism population can be related with the increase in biomass activity determined by the BMP tests. These tests highlight the importance of choosing a suitable inoculum when an anaerobic digester is started up.

4.3. CONCLUSIONS

- Winery wastewater was successfully treated by the AnMBR reaching a 96.7±2.7% of COD removal and a biogas production of 0.05±0.17 m^3_{biogas} $m^{-3}_{digester}$ d^{-1}.
- The SMP was on average 0.33±0.15 $m^{-3}CH_4$ $kg^{-1}COD$, with a high methane concentration in biogas of 87.1±3.0%.
- Instabilities were caused by sharp influent COD oscillations that were coped by the addition of alkalinity to keep a ratio IA/TA below 0.4; which was 1092±88 $mgCaCO_3$ L^{-1}.
- The stable operation was also favoured by the biomass acclimation. Because the AnMBR allowed the biomass selection and enrichment in methanogenic archaea, SMA increased from 0.07 to 0.36 gCH_4-COD $gVSS^{-1}$ d^{-1}.

5. Energetic aspects of the AnMBR technology compared with aerobic granulation

Abstract

The energy demand of the AnMBR was calculated considering the net energy production of a combined heat and power (CHP) unit, and the expenses of pumping, stirring, influent heating and heat losses. The energy production was calculated regarding the production of biogas obtained and the HRT at which the digester operated at lab-scale. It was concluded that only when influent COD was over 3.25 gCOD L^{-1} the energy balance was positive. Since winery wastewater suffers seasonal variations, to achieve a positive energy balance at mesophilic temperature is not always possible. During vintage, there is enough COD to cover energy expenses. However, the rest of the year COD concentration can be around 500 - 1000 mg L^{-1} so that the requirements would be higher than the energy recovered from biogas. Considering the upscaling of the AnMBR of the present study the submerged membrane configuration would be a more feasible option due to its lower operational costs, especially in winter season. Therefore, taking into account that a submerged membrane configuration requires around 0.3 kWh m^{-3}, the energy balance becomes positive when influent COD is over 460 mg L^{-1} which corresponds to a P_B=0.06 m^3_{biogas} $m^{-3}_{digester}$ d^{-1}. Hence, submerged AnMBR for winery wastewater treatment would be a suitable option at full-scale application.

Aerobic granulation was identified as a suitable technique to treat this kind of wastewater due to an excellent settleability, high biomass retention and good ability to handle with high organic loads and seasonal fluctuations. The energy demand was estimated to be in the range of 0.15 – 0.57 kWh m^{-3}, depending on the variation of influent COD (from 1.1 to 8 g L^{-1}) that was directly related to the cycle length. Hence, the higher COD concentration, the longer aeration step, and therefore the energy costs increased.

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- Comparison of aerobic granulation and anaerobic membrane bioreactor technologies for winery wastewater treatment. *6th IWA Specialized Conference, Winery 2013 – Viticulture and Winery wastes management*, Narbonne, France, 26th-30th May 2013.

And then <u>published</u> as:

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5.1. INTRODUCTION

The growing concern in the development of new intensive and compact technologies is due to the more and more stringent regulations regarding waste disposal and the aim of reducing energy and space requirements, particularly in industrial facilities as wineries. France, Italy and Spain are the most important world wine producers with more than the 80% of the European production. In 2012 the wine production of the above mentioned countries represented the 16.4%, 15.9% and 12.1% of the world production, respectively (last data found in *www.oiv.int* on 10/07/2013). Therefore, because wine making is an extended process in these countries, large volumes of winery wastewater are generated, mainly from washing operations. Historically, since wineries are a decentralised industry, wastewater was discharged into public sewers or evaporation ponds, often causing bad odours and polluting surface and groundwater. Nowadays, winery wastewater cannot be discharged into the environment harmlessly due to its particular characteristics: high biodegradable organic load, up to hundred times higher than domestic wastewaters, a significant seasonal variability of the organic load and acidic pH.

As wineries are relatively small industries with high volumes of wastewater to manage, compact and intensive technologies are considered to improve treatment efficiency as well as economic aspects. In the present work two advanced wastewater treatment technologies as aerobic granulation and membrane bioreactor are considered for the treatment of winery wastewater. Both technologies have already been tested for alcohol fermentation wastewaters (Kang et al., 2002; Wang et al., 2007), however there is a lack of data related to their application to winery wastewater treatment. By now, wineries in Spain generally treat their wastewater by means of conventional activated sludge systems. Hence, the comparison between different technologies of treatment enhancement may increase their possibilities of application.

On the one hand, aerobic granulation appears to be one of the most suitable techniques to treat this kind of wastewater due to its multiple advantages: an excellent granule settleability, high biomass retention and good ability to handle with shock loadings and inhibitory and toxic agents (Liu and Tay, 2004). Commonly, aerobic granules are cultivated in a sequencing batch reactor (SBR) at short hydraulic retention time (HRT), which allows the selection of biomass with good settling properties. Start-up periods can be very long, up to 300 days (Liu et al., 2010), although several strategies can be implemented to improve aggregation (addition of granular activated carbon, crushed granules, magnetic field, etc.). The key factor in aerobic granulation processes is to keep well aggregated and stable granules during operation, in order to minimise the effluent suspended solid (SS) concentration. There are several factors that affect granulation: substrate composition,

organic loading rate (OLR), shear force, settling time, HRT, volume exchange ratio, periodic starvation and reactor configuration (height/diameter ratio).

On the other hand, since winery wastewater is a high organic loaded effluent, energy generation from anaerobic digestion is worth considering, whose biogas production is expected to cover its energy requirements, otherwise the low cost of conventional aerobic treatments would be more feasible. Due to the characteristics of the organic matter present in winery wastewater (easy biodegradable and soluble) it is indispensable to immobilise biomass in order to avoid biomass washout. Conventional anaerobic digestion processes are well-known to achieve high organic matter removal efficiencies without oxygen requirement, low biomass production and energy generation from biogas (Metcalf and Eddy, 2003). AnMBR technology enables a wider range of anaerobic digestion possibilities. Therefore, it becomes a suitable process for the treatment of high and low loaded wastewaters, due to biomass retention that allows the uncoupling of the HRT and the solid retention time (SRT). It has been introduced in industrial applications since 1980s for the treatment of organic waste and industrial wastewater with high organic content from distilleries, septic tanks, food and paper industries, etc. (Liao et al., 2006a). Many studies treating industrial wastewater are summarized in the recent reviews about the developments and trends of AnMBR technology (Dereli et al., 2012; Lin et al., 2013). Several examples of wastewater treatment from food and brewery industry are presented in these reviews, although most of them worked with relatively constant influent characteristics. Only few studies deal with fluctuating conditions, for instance, Fuchs et al. (2003) and He et al. (2005) reported unwanted VFA accumulation treating food processing wastewater. Certainly, a sharp and unexpected increase in the organic load may cause a process destabilization. However, in the case of winery wastewater, the seasonal fluctuations, especially during vintage, can be foreseen to some extent based on historical data of each winery. Therefore, the preventive actions (pH and VFA control, alkalinity addition) should be intensified in this period.

The main goal of this work is to compare two different technologies for winery wastewater treatment in terms of energetic aspects: an aerobic Granular Sequencing Batch Reactor (GSBR) and an Anaerobic Membrane BioReactor (AnMBR).

5.2. RESULTS AND DISCUSSION

5.2.1. Experimental set-up

5.2.1.1. AnMBR

The characteristics of the AnMBR taken for the comparison are described in Chapter 4.

5.2.1.2. Aerobic Granulation in a GSBR

The set-up of the GSBR is described in detail by López-Palau et al. (2009). The GSBR was set-up as shown in section 3.1.4 with a working volume of 3L and a height/diameter (H/D) relation of 3. The reactor was equipped with a pH electrode and a dissolved oxygen probe and these parameters were monitored continuously. Air was introduced at the bottom side of the reactor using a porous stone connected to an air pump. The GSBR was operated in successive cycles of 4 hours length, consisting of four different phases: filling (5 min), aeration (230 min), settling (1 min) and effluent withdrawal (4 min). The short settling time acted as the selection parameter since only particles able to settle in one minute were retained in the reactor. The volume exchange ratio was 50% and therefore, the HRT was set at 8 h. The control system based on the oxidation reduction potential (ORP) slope and Feast-Famine strategy applied to optimise the GSBR operation can be found in López-Palau et al. (2012).

The GSBR was started-up with synthetic media, which was replaced later by real winery wastewater. In both situations, organic matter removal efficiencies higher than 91% were achieved (López-Palau et al., 2009). However, when COD was 7.5 g L^{-1}, which corresponded to an OLR of 22.5 kg COD m^{-3} d^{-1}, an immoderate biomass growth was observed and solid concentration in the effluent increased to 0.5 g VSS L^{-1}. Hence, the maximum OLR successfully applied to this system was 15 kg COD m^{-3} d^{-1}, corresponding to an influent COD concentration of 5 g L^{-1}.

When real winery wastewater was tested, high removal efficiencies were also obtained for a wide range of influent COD concentrations (from 1 to 6 g COD L^{-1}) at a predetermined and fixed cycle length of 4h (Figure 5.1). Initially, real wastewater was diluted to only 1 g COD L^{-1} in order to progressively adapt biomass to high concentrations, achieving COD removal efficiencies up to 95%. After 70 days of operation, when COD inlet was around 2.2 g COD L^{-1}, effluent COD did not exceed 90 mg COD L^{-1}. Afterwards, wastewater collected during the harvest period with 6 g COD L^{-1} was fed to the reactor. In order to avoid biomass inhibition, wastewater was again initially diluted and progressively concentrated. Firstly, biomass was not able to completely remove organic matter, but after only 10 days of operation,

degradation reached again 97%. During this period COD was increased until its real concentration, which corresponded to an OLR of 18 kg COD m^{-3} d^{-1}, obtaining an effluent COD concentration below 100 mg COD L^{-1}. Finally, on day 145, much diluted wastewater (around 1 g COD L^{-1}) was fed to the reactor and removal efficiencies did not show significant changes. Effluent SS concentrations between 20 and 40 mg L^{-1} were obtained during the whole operational period; however VSS in the reactor increased significantly from 4 g L^{-1} to 13 g L^{-1}.

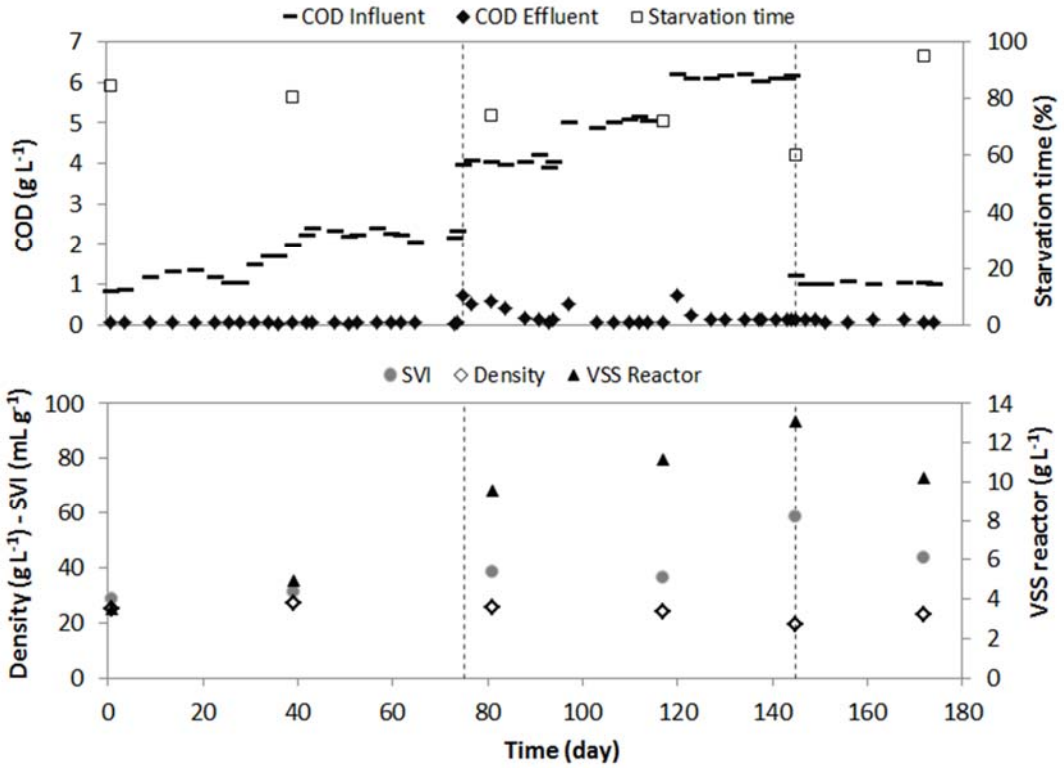

Figure 5.1. COD concentration, starvation time, density and VSS of the GSBR

While COD removal efficiency was slightly affected by the OLR, the percentage of starvation time over the whole aeration period significantly changed along operation. Despite the increasing biomass concentration (which would lead to longer famine periods), a progressive decrease of starvation time was monitored due to the increase of COD content. The effect of a shorter starvation time on biomass physical characteristics was noticeable around day 140, when a slow deterioration of biomass settling properties was observed (higher values of Sludge Volumetric Index (SVI_5) and lower granule density). Consequently, suspended solids in the effluent reached 65 mg SS L^{-1}.

In order to optimise GSBR operation, a control system based on ORP slope and Feast-Famine strategy was applied in order to adjust starvation time to the system requirements. Thus, HRT varied depending on influent COD concentration that led to a constant OLR between 2 and 3.2 kg COD m^{-3} d^{-1}. The control strategy permitted to modify cycle length taking into account that starvation time should be 67% according to the conclusions drawn by López-Palau et al. (2012).

5.2.2. Comparison of both systems

Concerning the capacity of organic matter removal of these systems, lab-scale experiments demonstrated that the GSBR can cope with considerably higher OLR (15 kg COD m^{-3} d^{-1}) than the AnMBR, as it is shown in **Table 5.1**. But when control system was used to optimise the GSBR, OLR applied was automatically adjusted at 2-3 kg COD m^{-3} d^{-1}. A clear advantage of the GSBR is its capacity to withstand sudden changes of influent COD concentration. Regardless of the control system application, it has been observed that GSBR could easily achieve a low effluent COD concentration after an abrupt change. However, higher OLR led to an unwanted increase of SS that negatively affect the effluent quality.

Table 5.1. Comparison between main features of AnMBR and GSBR

Parameter	AnMBR	GSBR	
		No control	DO and ORP control
OLR (kg COD m^{-3} d^{-1})	0.26-2.5	2.7-15	2.0-3.2
Influent COD (g L^{-1})	1.6-7.2	0.9-6.1	1.1-8.0
Effluent COD (mg L^{-1})	13-125	34-112	34-82
HRT (d)	3.5	0.33	0.21-0.83
Reactor VSS (g L^{-1})	2.1-8.5	4-13	4.9-5.6
Effluent SS (mg L^{-1})	n.d.	30	30
Turbidity (NTU)	1.7	-	-
Energy (kWh m^{-3})	Demand	Production	Demand
	7.9	6.6-30	0.15-0.57

Since anaerobic biomass growth is very slow, during the AnMBR start-up period 8.48 g VSS L^{-1} were reached inside the digester and the maximum OLR treated was 2.5 kg COD m^{-3} d^{-1}. Probably, higher OLR could be treated if methanogenic biomass had enough time to grow and acclimatise (up to 25 kg COD m^{-3} d^{-1} has been reported by Skouteris et al. (2012)). In addition, an increase of biomass would not affect the effluent

quality, even though higher degree of fouling may be observed. The AnMBR requires a longer start-up period and low influent variability, but it is expected to deal with an OLR as high as the GSBR.

High COD removal efficiencies was reached in both cases, independently of the OLR applied. Nevertheless, as winery wastewater has low nutrient content, some additions of nitrogen and phosphorus were required in order to allow biomass growth. Aerobic processes have higher nutrient requirement due to a higher biomass yield. In contrast, anaerobic biomass needs fewer nutrients, which is a positive point for the anaerobic winery wastewater treatment. The effluent quality is another important issue to bear in mind, not only because of the legal discharge requirements, but to water reuse, for instance, for irrigation or non-potable purposes. In this sense, AnMBR system is clearly preferred due to the total retention of SS by the membrane. With the GSBR, low SS values were obtained but they did not accomplish with the Spanish reuse legislation (RD 1620/2007).

5.2.3. Energy requirement estimation

In order to evaluate the potential application of these technologies, the energy requirements were estimated, regarding mainly operational costs of aeration, pumping, stirring, heating and/or filtration. Other costs related to maintenance, for instance, sludge management (of both the GSBR and the AnMBR), membrane cleaning and replacement, were not taken into account in this estimation.

To determine the energy requirement of the GSBR, it was firstly upscaled until a volume of 3 m^3 with a flow rate of 1.3 m^3 per cycle, as for Liu et al. (2005). The height to diameter ratio and the settling velocity were fixed, based on laboratory results, at 4 and 9.6 m h^{-1}. Hence, the air supply of the 3-m^3 SBR was estimated to be 32.6 m^3 h^{-1}. This value exceeds the oxygen requirement for COD removal, but a minimum velocity of 1.2 cm s^{-1} is needed to assure granules fluidisation (Tay et al., 2004). The energy requirement of the air diffusers and wastewater pumping can be calculated with Equations 5.1 and 5.2 (Metcalf and Eddy, 2003).

$$E_{req.}\ (kWh \cdot m^{-3}) = E_{aeration} + E_{pumping} = \frac{P_w \cdot t_{aeration}}{Q} + \frac{P_p \cdot t_{pumping}}{Q} \quad (5.1)$$

$$P_w = \frac{wRT}{29.7ne}\left[\frac{(p_2)^{0.283}}{p_1} - 1\right] \quad (5.2)$$

where $t_{aeration}$ and $t_{pumping}$ are the time of aeration and pumping (h d^{-1}); Q is the flow rate (m^3 d^{-1}); P_w is the power requirement (0.064 kW); w is flow of air ($4.3 \cdot 10^{-4}$ kg s^{-1}); R is the gas

constant (8.314 kJ $kmol^{-1}$ K^{-1}); T is the influent temperature (288.15 K); n is 0.283 for air; e is the efficiency (0.70); p_1 is the inlet pressure of the air blower (1 atm); p_2 is the outlet pressure (3 atm); P_p is the power requirement of pumping (0.37 kW – ref. SACI K5-T). Since the GSBR was operated with a variable cycle length depending on the influent COD concentration, the daily flow rate and times of aeration and pumping were automatically adjusted by the control system.

The energy demand was estimated to be in the range of 0.15 – 0.57 kWh m^{-3}, depending on the variation of influent COD (from 1.1 to 8 g L^{-1}) that was directly related to the cycle length. Hence, the higher COD concentration, the longer aeration step, and therefore the energy costs increased. Therefore, when influent COD was 1.1 g L^{-1}, the unknown variables of Equation 5.1, which are Q, $t_{aeration}$ and $t_{pumping'}$ were 19.2 m^3 d^{-1}; 22.8 h d^{-1}; and 3.7 h d^{-1}; respectively. In contrast, when influent COD was 8 g L^{-1}, these parameters were 3.04 m^3 d^{-1}; 23.80 h d^{-1}; and 0.59 h d^{-1}. Despite daily aeration and pumping times were similar, daily flow rate (Q) was significantly higher at low COD concentrations, therefore costs in terms of kWh m^{-3} resulted much lower.

The energy demand of an AnMBR was calculated considering the net energy production of a combined heat and power (CHP) unit, and the expenses of pumping, stirring, influent heating and heat losses. The energy production was calculated regarding the production of biogas obtained and the HRT at which the digester operated at lab-scale.

Taken the experimental biogas production (P_B) and HRT, the energy requirements of the AnMBR were calculated by Equation 5.3 and Equation 5.4, described in detail in Astals et al. (2012). Both equations consider the net energy production of a combined heat and power (CHP) unit, and the expenses of pumping, stirring, influent heating and heat losses. The energy production was calculated taking into account the P_B obtained in the experimental AnMBR at lab-scale. However, it can also be calculated depending on the influent COD. A term of energy consumption due to the membrane unit was included in the balance. Since lab-scale electric demand was unrealistic, this parameter was estimated to be 3.7 kWh m^{-3}, as Martin-Garcia et al. (2011) determined for an external membrane module.

$$E_{electricity}\ (kWh \cdot m^{-3}) = E_{CHPunit} - E_{pumping} - E_{stirring} - E_{membrane\ unit}$$

$$E_{electricity}\ (kWh \cdot m^{-3}) = [P_B \cdot HRT \cdot \xi \cdot \pi] - \theta - [HRT \cdot \omega] - E_{membrane\ unit} \qquad (5.3)$$

$$E_{electricity}\ (kWh \cdot m^{-3}) = \left[COD_{inf} \cdot SMP \cdot \xi \cdot \pi\right] - \theta - [HRT \cdot \omega] - E_{membrane\ unit}$$

$$E_{heat}\ (kWh \cdot m^{-3}) = E_{CHPunit} - E_{sludge\ heating}$$

$$E_{heat}\ (kWh \cdot m^{-3}) = [P_B \cdot HRT \cdot \xi \cdot \psi] - [\rho \cdot \gamma \cdot (T_d - T_{ss}) \cdot (1 - \varphi) \cdot (1 + \eta)] \quad (5.4)$$

$$E_{heat}\ (kWh \cdot m^{-3}) = \left[COD_{inf} \cdot SMP \cdot \xi \cdot \psi\right] - [\rho \cdot \gamma \cdot (T_d - T_{ss}) \cdot (1 - \varphi) \cdot (1 + \eta)]$$

where P_B is the specific biogas production ($m^3\ m^{-3}_{digester}\ d^{-1}$); HRT is the hydraulic retention time; COD_{inf} is the influent COD ($kgCOD\ L^{-1}$); SMP is the specific methane production ($m^3CH_4\ kg^{-1}COD$); ξ is the biogas heat capacity ($4.18 \cdot 10^4\ kJ\ m^{-3}$); π is the CHP efficiency for electricity (0.35); θ is the electrical requirement for pumping wastewater ($250\ kJ\ m^{-3}$); ω is the electrical requirement for stirring ($300\ kJ\ m^{-3}_{digester}\ d^{-1}$); ψ is the CHP efficiency for heating (0.55); ρ is the influent density ($10^3\ kg\ m^{-3}$); γ is the influent specific heat ($4.18\ kJ\ kg^{-1}\ °C^{-1}$); T_d is the digester temperature (35°C); T_{ss} is the influent temperature (15°C); ϕ is the energy recovered from the effluent (0.85); η is the heat loss (0.08). A factor of $2.78 \cdot 10^{-4}$ is applied to convert kJ to kWh.

A positive energy balance is essential for the settlement of the AnMBR technology as a feasible process. It is considered that the full-scale AnMBR would keep the HRT at 3.5 d, as for the lab-scale device. Hence, from the equations 5.3 and 5.4, the electricity (due to pumping and stirring) and heating requirements resulted to be 0.44 and 3.76 kWh m^{-3}, respectively. Since the membrane used during this study was an external crossflow module, it was estimated that its cost at full-scale would be 3.7 kWh m^{-3}. Hence, the total energy demand was calculated to be 7.9 kWh m^{-3}, as shown in **Table 5.1**. Taking into account that the methane production obtained in the lab-scale digester was 0.30 $LCH_4\ g^{-1}COD$, the energy production increased from 6.6 to 30 kWh m^{-3}, corresponding to a COD increase from 1.6 to 7.2 g L^{-1}. A positive energy balance was obtained when the influent COD was over 3.4 g L^{-1} which corresponded to a specific methane production (P_B) of 0.23 $m^3CH_4\ m^{-3}\ d^{-1}$.

The energy requirements calculated with the conditions applied in Period III (see Chapter 4, section 4.2.6) are shown in Equation 5.5 and 5.6. It was concluded that only when influent COD was over 3.25 gCOD L^{-1} the energy balance was positive. Therefore, at an influent COD concentration of 3.25 gCOD L^{-1} the electricity production was equivalent to the requirements and the energy balance became zero (Equation 5.5). However, the heat balance at these conditions (Equation 5.6) resulted positive, even considering the digester heating from 15°C to 35°C. The external membrane unit and the heating requirements were 3.70 kWh m^{-3} and 3.76 kWh m^{-3}, respectively. Both were the main contributors to the energy costs, the rest of the expenses (0.15 kWh m^{-3}) were due to the pumping and agitation.

$$E_{electricity} = \Big[[3.25\ kgCOD\ m^{-3} \cdot 0.33\ m^3_{CH4}\ kgCOD^{-1} \cdot 4.18 \cdot 10^4 kJ\ m^{-3}_{CH4} \cdot 0.35] - 250\ kJ\ m^{-3} - \big[2.26\ d \cdot 300\ kJ\ m^{-3} d^{-1}\big]\Big] \cdot 2.78 \cdot 10^{-4} kWh - 3.7\ kWh\ m^{-3} = 0.01\ kWh\ m^{-3} \quad (5.5)$$

$$E_{heat} = \Big[[3.25\ kgCOD\ m^{-3} \cdot 0.33\ m^3_{CH4}\ kgCOD^{-1} \cdot 4.18 \cdot 10^4 kJ\ m^{-3}_{CH4} \cdot 0.55] - \big[10^3 kg\ m^{-3} 4.18\ kJ\ kg^{-1}℃^{-1} \cdot (35℃ - 15℃) \cdot (1 - 0.85) \cdot (1 + 0.08)\big]\Big] \cdot 2.78 \cdot 10^{-4} kWh\ kJ^{-1} = 2.46\ kWh\ m^{-3} \quad (5.6)$$

Since winery wastewater suffers seasonal variations, to achieve a positive energy balance at mesophilic temperature is not always possible. During vintage, there is enough COD to cover energy expenses. However, the rest of the year COD concentration can be around 500 - 1000 mg L^{-1} so that the requirements would be higher than the energy recovered from biogas. From Equation 5.4 (heat balance), it can be calculated that only when influent COD is over 2.0 gCOD L^{-1} and P_B=0.13 m^3_{biogas} $m^{-3}_{digester}$ d^{-1} mesophilic conditions are feasible (the calculations were done taking into account that the wastewater was initially at 15ºC). Therefore, when the organic load is low, the operation of the AnMBR should be carried out at ambient temperature. The heating and electric efficiencies of CHP units are approximately 55% and 35%, respectively. Therefore, a positive balance in terms of electricity (Equation 5.3) would be the key point for the MBR application due to its high electric demand. Since an external membrane unit requires up to 3.70 kWh m^{-3} of electricity, the operation at ambient temperature does not improve the energy balance. In order to meet the electricity demand, the influent COD should be high and thus it would be heat in excess to work at higher temperatures.

A full-scale case study of a submerged AnMBR for municipal wastewater treatment was carried out by Ferrer et al. (2015). It was concluded that the overall energy demand of the AnMBR was 0.22 kWh m^{-3} obtaining 0.07 kWh m^{-3} of surplus. This study coincides with Martin-Garcia et al. (2011) that reported that submerged membrane units required around 0.3 kWh m^{-3}. Nevertheless, Xue et al. (2015) determined that sidestream MBR was better for leachate treatment because it experimented less fouling. The external membrane configuration is more practical at lab-scale because the equipment is mostly manually controlled. However, considering the upscaling of the AnMBR of the present study the submerged membrane configuration would be a more feasible option, especially in winter season. Therefore, taking into account that a submerged membrane configuration requires 0.3 kWh m^{-3}, the energy balance (Equation 5.3) becomes positive when influent COD is over

460 mg L^{-1} which corresponds to a P_B=0.06 m^3_{biogas} $m^{-3}_{digester}$ d^{-1}. Hence, submerged AnMBR for winery wastewater treatment would be a suitable option at full-scale application.

Comparing both technologies in terms of energy costs, it is clearly noticeable that the GSBR resulted in lower requirements, mainly due to the lack of heating and filtration units. The granular technology provided a huge increase of treatment capacity while achieving suitable effluent characteristics. However, energy demand increased at high COD content up to 0.57 kWh m^{-3}, although it does not suppose an excessive energy demand even when high organic loads should be treated. On the contrary, the AnMBR represented a significant energy cost regardless of the organic load, with a total amount of 7.9 kWh m^{-3}, thus high influent COD concentrations are desirable in order to maximise biogas generation. Nevertheless, it has been demonstrated that immersed membranes have lower energy costs, about 0.2-0.3 kWh m^{-3} (Ferrer et al., 2015; Martin-Garcia et al., 2011). Therefore, considering the possibility of AnMBR with immersed membrane configuration and the same biogas production of 0.30 L CH_4 g^{-1} COD, the minimum influent COD concentration that makes the AnMBR a feasible technology would be 2 g L^{-1} (P_B of 0.13 m^3 CH_4 m^{-3} d^{-1}) at mesophilic conditions and 0.46 g L^{-1} at ambient temperature.

5.2.4. Conclusions

The comparison of a GSBR and an AnMBR has resulted in several advantages and drawbacks of both technologies.

- The energy balance of the AnMBR revealed that the biogas produced could cover the electric requirements of a sidestream configuration during vintage season, when organic loads are high. However, in winter season when influent COD is lower, even operating at low temperature, the electricity demand could not be covered by the biogas production. Therefore, submerged membrane configuration could be taken into account for the upscaling of this system.
- Aerobic GSBR would be much efficient, especially thanks to its capacity to cope with high OLR and seasonal fluctuations. In spite of the aim of energy demand reduction, the GSBR will always represent a cost, while AnMBR is expected to become an energy production process.

6. Operation of an AnMBR for winery wastewater treatment at low temperature

Abstract

Winery wastewater was treated by means of an AnMBR at low temperatures of 25°C and 15°C simulating the conditions of winter season. Since the organic load of winery wastewater in winter is much lower than in summer (vintage season), the average organic loading rate (OLR) applied was 0.35 and 0.29 kgCOD $m^{-3}_{digester}$ d^{-1} *and the average chemical oxygen demand (COD) removal was 80% and 71% at 25°C and 15°C., respectively. As expected, the efficiency of the system was lower than in mesophilic conditions and higher amount of VFA were accumulated in the digester that promoted the enrichment of the biomass in Methanosarcina as the main methanogen observed.*

Due to the operation at low temperatures, more methane was dissolved in the permeate and low biogas production was obtained. Following Henry's law, it was determined that the methane lost dissolved in the liquid phase corresponded to a 6.7% and 10.2% at 25°C and 15°C, respectively.

Moreover, higher degree of fouling was observed despite the amount of suspended solids was lower. Frequent cleanings were necessary, although they were carried out without chemicals since the main resistance was due to the cake layer on the surface, thus a high crossflow velocity was enough to recover the initial flux.

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And then in preparation for publication as:

N. Basset, E. De Arana, A. Coll, J. Dosta, J. Mata-Álvarez. **Comparison of UASB-MBR and CSTR-MBR technolgies for winery wastewater treatment at low temperatures.**

6.1. INTRODUCTION

As concluded in Chapter 4 and 5, the AnMBR has shown a good performance on the removal of organic matter from winery wastewater at mesophilic temperature. However, a positive or neutral energy balance is essential for the AnMBR establishment (Pretel et al., 2014). Since the characteristics of the winery wastewater are variable along the year, the operation at low temperature is of interest in order to evaluate the feasibility of the treatment in winter season, when the organic load is low. In these conditions, the winery wastewater would approach the organic load of the urban wastewater. Several studies on the AnMBR for urban wastewater treatment have been summarised in section 1.2.4.

When anaerobic digestion is carried out at low temperature the kinetics of organic matter degradation, especially the hydrolysis of particulate organic matter, are slower (Lettinga et al., 2001). Due to total retention of solids in the AnMBR, an acceptable efficiency of particulate COD removal have been observed even in psychrophilic temperature (about 25°C), although it is reduced when temperature decreases in winter (Bandara et al., 2012). Hence, the SRT must be long enough to achieve high particulate organic matter removal. In addition, short HRT is preferable in order to minimize the bioreactor size. Nevertheless, these parameters are limited. A high SRT involves an increase of the biomass concentration and the production of extracellular polymeric substances (EPS) and soluble microbial products (SMP) (Huang et al., 2011), which have a direct effect on the membrane fouling. On the other hand, a short HRT also promotes production of EPS and SMP, particularly in the case of the AnMBR with external membrane module due to the high crossflow velocities that stress the biomass (Salazar-Peláez et al., 2011). Hence, a short HRT promotes the accumulation of soluble COD in permeate, reducing its quality (Baek et al., 2010).

The origin of the inoculum may have a significant impact during the start-up of the AnMBR at low temperature (Smith et al., 2012). However, according to Smith et al. (2012) studies, which compared mesophilic and psychrophilic inoculum, it was observed that after 275 days of operation at 15°C the microbial communities were similar, concluding that the communities were mainly formed by psychrotolerant mesophilic microorganisms. Even though the AnMBR works properly with a psychrophilic inoculum, its efficiency at higher temperatures should be considered since the temperature of wastewater may vary up to 20°C throughout the year. For this reason, a combination of both communities (psychrophilic and mesophilic) would be worth considering in order to deal with the seasonal variations.

The main goal of this chapter is to operate an anaerobic membrane bioreactor (AnMBR) fed with synthetic winery wastewater at low temperatures (15ºC and 25ºC) evaluating its removal efficiency and membrane performance, comparing it with the operation at mesophilic conditions. It should be taken into account that at low temperatures biogas tends to dissolve in the liquid phase, thus the loss of methane should be quantified in order to evaluate its impact on the overall biogas production. By means of biomethane potential (BMP) tests, the methane production and biomass activity are determined and compared with the continuous operation of the AnMBR. Since the inoculum came from mesophilic digester, the evolution of the microbial population is also of interest, especially when changes in temperature throughout the year are expected.

6.2. RESULTS AND DISCUSSION

6.2.1. Experimental set-up and substrate

The AnMBR was set-up as described in Chapter 4 and in section 3.1.1. Synthetic wastewater was used to feed the system prepared with around 1500 mgCOD L^{-1}, with diluted white wine (Artiga et al., 2005). NH_4Cl and K_2HPO_3 were added in accordance to the ratio COD/N/P of 800/5/1. Moreover, alkalinity was set at 1000 mg$CaCO_3$ L^{-1} by the addition of $NaHCO_3$ to keep the pH at neutral values.

The membrane module cleanings were performed with distilled water by applying a high crossflow velocity to remove the cake layer. Chemical cleanings were not carried out in this occasion because the main resistance was attributed to the cake layer.

6.2.2. AnMBR operation at low temperatures

The temperature of the AnMBR was reduced from 35°C (Chapter 4) to 25°C and then to 15°C, thus the inoculum was not acclimatised at these low temperatures. In fact, this would be the procedure in a real winery. In summer, the temperature of operation would be 35°C to cope with the high organic load and recover a significant amount of biogas from it. However, in winter, since the organic load is much lower, temperature would decrease progressively, because the biogas produced would not cover the heating requirements.

Table 6.1 summarises the main operational parameters and results obtained at the different temperatures. Many aspects should be highlighted when anaerobic digestion is carried out at low temperature. First of all, since the kinetics are slower (Lettinga et al., 2001), the risk of acidification is higher. As determined in Chapter 4, to keep a ratio IA/TA below 0.3 will assure a neutral pH, thus the synthetic feed was prepared with a minimum alkalinity of 1000 mg$CaCO_3$ L^{-1}. Compared with the results obtained at mesophilic temperature, VFA were

accumulated easier, obtaining 183±135 mg L^{-1} and 132±105 mg L^{-1} at 15°C and 25°C, respectively in the permeate. In order to reach acceptable removal efficiencies, the HRT was increased to 4.5 days. However, effluent COD was higher than the permitted limit due to the VFA accumulated.

Secondly, it should be taken into account that at low temperatures methane is dissolved in the liquid phase, thus part of the biogas production can be lost. Considering that the maximum methane production is 0.35 m^3CH_4 $kg^{-1}COD_{removed}$, which corresponds to the theoretical COD of methane, and the real production observed under mesophilic conditions (Chapter 4) that was 0.28±0.16 m^3CH_4 $kg^{-1}COD_{added}$; the specific methane production (SMP) was expected to be closer to these values. However, at 25°C biogas production observed was very low, 0.03 m^3CH_4 $kg^{-1}COD$, and at 15°C was not possible to determine. Similar results were obtained by Giménez et al. (2011) treating urban wastewater, they attributed the lack of biogas to the presence of sulphate reducing bacteria and also the loss of methane dissolved in the permeate. In the case of the synthetic winery wastewater, sulphate concentration was very low, thus the COD consumed to reduce sulphate would not be significant. Taking into account Henry's law, methane dissolved in the permeate can be calculated as Equation 6.1, where the pressure in the gas phase is related with the molar concentration in the liquid phase by Henry's constant. The temperature dependence of Henry's constant for methane dissolved in water can be calculated based on Van't Hoff equation, simplified in Equation 6.2 (Sander, 2015).

$$X_{CH_4}(mol\ m^{-3}) = P_{CH_4}(Pa) \cdot H\ (mol\ m^{-3} Pa^{-1}) \quad (6.1)$$

$$H(T) = H^* \cdot \exp\left(\frac{-\Delta H}{R} \cdot \left(\frac{1}{T} - \frac{1}{T^*}\right)\right) \quad (6.2)$$

where $H^*=1.4 \cdot 10^{-5}$ mol m^{-3} Pa^{-1}; $T^*=298.15$ K; $-\Delta H/R=1600$ K.

Hence, the amount of methane that can be lost in the permeate is 18.8 $mgCH_4$ L^{-1} (75.4 mgCOD L^{-1}) for 25°C, and 22.15 $mgCH_4$ L^{-1} (88.6 mgCOD L^{-1}) for 15°C. Considering the HRT applied, the rate of methane dissolved was 0.017 and 0.022 kgCOD $m^{-3}_{digester}$ d^{-1}, for 25°C and 15°C. Smith et al. (2013) observed a lost of 40-50% of methane dissolved operating at 16h of HRT and 15°C. Since the HRT was relatively high (4.4 d), the amount of methane lost in the liquid phase corresponded to a 6.7% and 10.2%, respectively. Nevertheless, this fact does not explain the lack of biogas production observed. Another possibility can be the overdesign of the headspace of the digester (being a 30% of the total volume), where the biogas production could be accumulated in by the pressure applied by the gas counter. Therefore, when biogas production was high as in Chapter 4, the gas counter worked

properly. However, when the biogas production was low because the OLR decreased, the pressure inside the headspace was not enough to overcome the liquid column of the gas counter. Samples from the inside of the headspace could be taken by increasing the working volume of the digester significantly, thus the gas accumulated in the headspace passed through the gas counter. In this way, the concentration of methane could be determined in both cases being 81% and 83%.

Regarding the membrane performance, a higher degree of fouling was perceived when lowering the temperature that caused the rapid decrease of flux. In order to maintain a similar flux, around 15 LMH, cleanings were required more often when the flux was below 10 LMH. The flux decline was 3.63 and 2.14 LMH d^{-1} at 15°C and 25°C, much higher than the 0.10 LMH d^{-1} measured at 35°C. Unlike in Chapter 4, where cleanings were carried out with chemicals following the manufacturer procedure, in this case cleanings were performed with distilled water at a high crossflow velocity of around 3 m s^{-1}. By applying only clear water to remove the cake layer, the flux afterwards increased significantly as shown in Figure 6.1 and Figure 6.2, and chemicals were not required at least at short term operation.

The suspended solids in the mixed liquor (MLSS) were much lower, around 2.7 gSS L^{-1}, than in Chapter 4 that were 6.3 gSS L^{-1}. However, the reduction in the MLSS concentration did not improve the filtration efficiency. Ng et al. (2006) determined that fouling was not controlled directly by MLSS but the SMP and EPS. Although SMP and EPS were not determined in this study, many references can be found stating that these polymeric substances increase fouling (Gao et al., 2010; Lin et al., 2014, 2009; Meng et al., 2009; Robles et al., 2013). Despite these studies revealed that at high temperatures the amount of SMP and EPS is higher, Stuckey (2012) stated that under any type of stress occasioned in an anaerobic digester can dramatically increase the SMP production that results in increasing fouling. Therefore, a part from the mechanical stress caused by pumping the mixed liquor through the membrane module, the oscillating OLR that suffered the winery wastewater may have an important contribution to the SMP release. At low temperatures the oscillations in OLR had a more significant effect, especially in terms of VFA accumulation, which led to changing conditions in the mixed liquor that stressed biomass.

Table 6.1. Operational parameters and results of each temperature (also including the results obtained in Chapter 4)

Operational parameters			
Temperature	**15°C**	**25°C**	**35°C**
Type of wastewater	Synthetic	Synthetic	Synthetic
pH	7.5±0.2	7.4±0.2	7.1±0.2
Alkalinity ($mgCaCO_3\ L^{-1}$)	915±71	898±179	887±199
MLSS ($g\ L^{-1}$)	2.74±0.34	2.69±1.16	6.30±1.33
HRT (d)	4.2±2.0	4.4±1.4	2.2±0.5
SRT (d)	565	435	560
COD influent ($g\ L^{-1}$)	1.10±0.30	1.41±0.39	2.92±1.05
COD effluent ($g\ L^{-1}$)	0.39±0.15	0.28±0.14	0.14±0.23
VFA effluent ($mg\ L^{-1}$)	183±135	132±105	114±120
%COD removal	71±9	80±9	96±4
OLR ($kgCOD\ m^{-3}_{digester}\ d^{-1}$)	0.29±0.21	0.35±0.19	1.32±0.51
sOLR ($kgCOD\ kg^{-1}MLSS\ d^{-1}$)	0.11±0.07	0.13±0.09	0.22±0.09
Membrane performance			
Flux (LMH)	13.8±6.8	12.2±4.4	16.0±3.9
Flux decline ($LMH\ d^{-1}$)	3.36±1.03	2.14±1.62	0.10±0.04
TMP (bar)	0.2	0.2	0.2
Crossflow velocity ($m\ s^{-1}$)	0.64	0.64	0.64
Biogas production			
P_B ($m^3_{biogas}\ m^{-3}_{digester}\ d^{-1}$)	-	0.007±0.002	0.42±0.26
$\%CH_4$ in biogas	81±1	83±3	85±1
SMP ($m^3CH_4\ kg^{-1}COD$)	-	0.03±0.01	0.28±0.16

The AnMBR was operated during 45 days at 25°C. The COD removal efficiency was on average 80±9%. Since winery wastewater contained easily biodegradable COD, the removal efficiency decreased due to occasional VFA accumulation, being on average 132±105 $mgVFA\ L^{-1}$. The alkalinity added to the system was enough to maintain a stable pH when high amount of VFA were accumulated. By keeping a ratio between intermediate and total alkalinity (IA/TA) below 0.3 the stable operation was assured. However, as shown in Figure 6.1, on day 30, VFA were accumulated up to 400 $mg\ L^{-1}$, thus the IA/TA ratio increased to 0.4 and the removal efficiency decreased to 59%.

The specific organic loading rate (sOLR) suffered huge variations because of the influent COD and the decrease in flux caused by the cake layer on the membrane surface. Hence, after a cleaning, the flux increased significantly and so the sOLR. For this reason, the sOLR slope presented a similar shape as the influent COD, although more abrupt oscillations were obtained as a result of the flux influence.

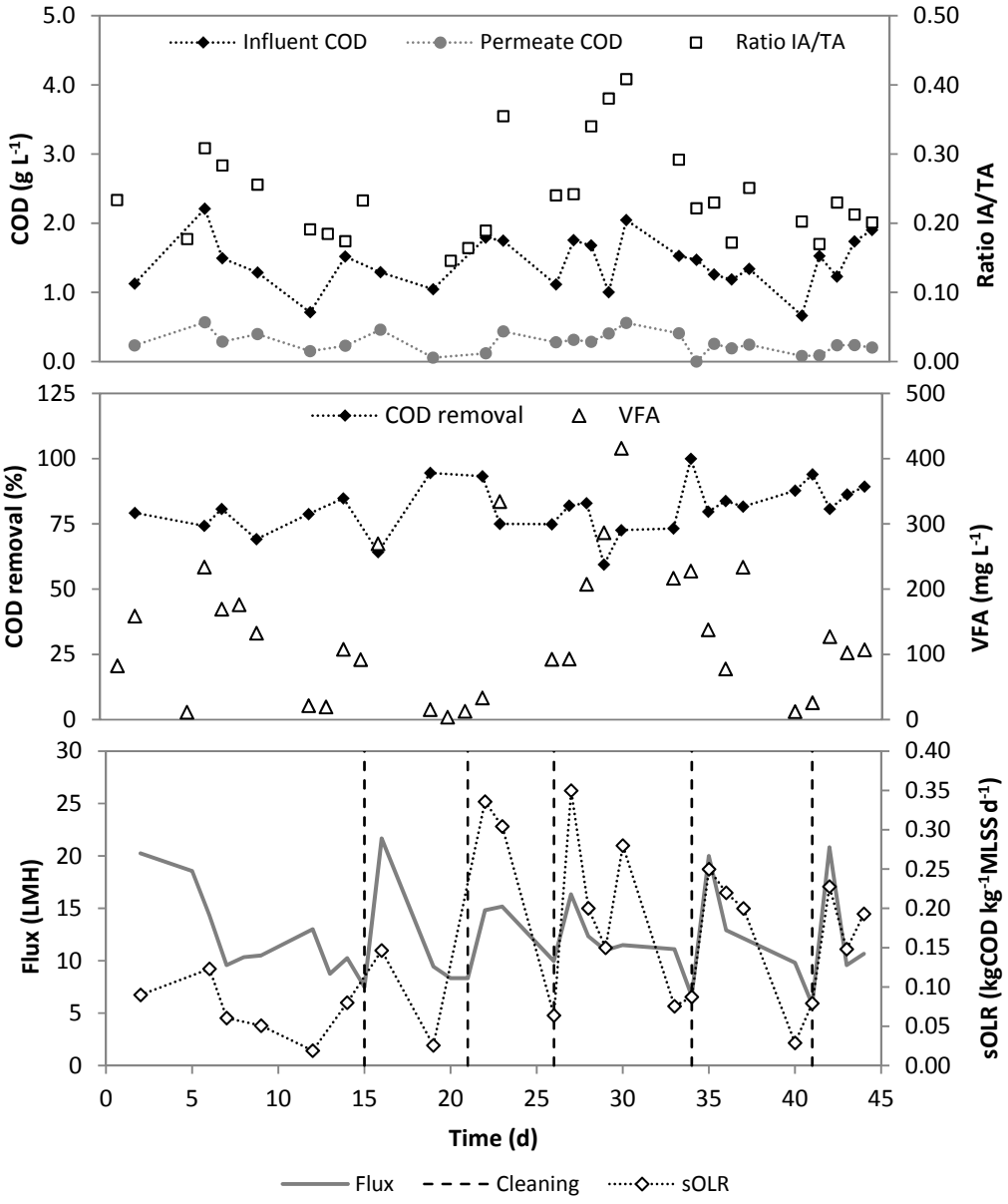


Figure 6.1. Evolution of the COD, ratio IA/TA, flux and sOLR of the AnMBR at 25°C

After the period at 25°C, the temperature was decreased to 15°C. In Figure 6.2, it can be observed that an acclimation period of around 15 days was required to achieve acceptable removal percentages. Since VFA were accumulated easily during this acclimation period, the influent COD was decreased to 500 mgCOD L^{-1}, and progressively increased to 1500 mgCOD L^{-1} from day 60 to 65. The average removal efficiency from day 65 on was 71±9%, mainly due to a higher amount of VFA accumulated. However, by keeping an alkalinity concentration in the AnMBR of 915±71 mg$CaCO_3$ L^{-1} the pH was maintained at neutral values and the ratio IA/TA below 0.3. Only during the first 15 days, the ratio IA/TA was between 0.35 and 0.4, fact that warned about the possible acidification and for this reason influent COD was decreased during those days.

On day 76, a peak of 386 mgVFA L^{-1} was observed because the membrane cleaning caused a huge increase in the flux up to 30 LMH and thus the sOLR reached 0.32 kgCOD kg^{-1}MLSS d^{-1}. However, the flux rapidly decreased due to the cake layer formed as well as VFA concentration, recovering a more stable operation.

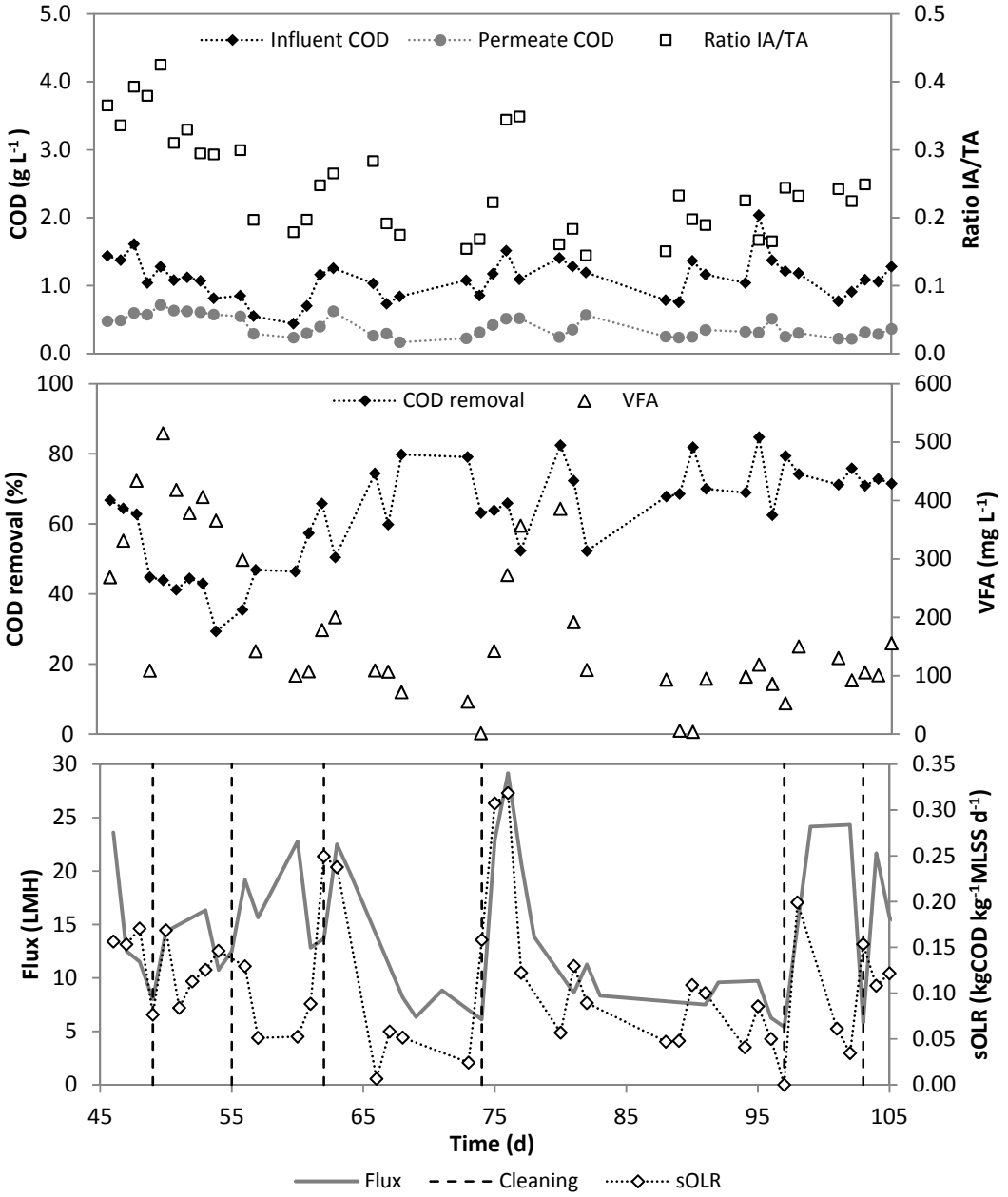


Figure 6.2. Evolution of the COD, ratio IA/TA, flux and sOLR of the AnMBR at at 15°C

6.2.3. Evaluation of biomass acclimation by BMP tests

Biomethane potential (BMP) tests were performed in order to evaluate biomass activity at different temperatures. In each case, the inoculum used was taken from the AnMBR after operating at the selected temperature for at least 30 days. The substrate used was synthetic winery wastewater prepared with diluted white wine with 5 gCOD L^{-1}, keeping a ratio $COD_{substrate}/COD_{inoculum}$ around 1. Nutrients were added as for the continuous AnMBR considering the requirements of COD:N:P of 800:5:1. Alkalinity of 1000 $mgCaCO_3$ L^{-1} was also supplied.

The specific methanogenic activity (SMA) and the specific methane production (SMP) obtained in the batch tests are presented in Table 4.4. The SMA was similar at 25°C and 35°C, although the SMP was much lower due to less biodegration and the loss of methane dissolved in the liquid phase. The biodegradation was determined to be 75% and the amount of methane dissolved in the liquid phase was 17.5 mgCOD L^{-1}, which represented a 1.2% of the production of methane.

At 15°C, the SMA and the SMP were notably lower, as expected due to the low temperature that promoted a poor methane production. Moreover, the biodegradation determined was low being 26%. However, the low biodegradation cannot be attributed to the sorption of methane in the liquid phase, because it was only a 3.6% (18.1 mgCOD L^{-1}). Probably the VFA generated during the batch test inhibited the methanogenic biomass.

Table 6.2. SMA and SMP obtained in the AnMBR at low temperature

Inoculum	Wastewater	SMA ($kgCH_4$-COD kg^{-1}SS d^{-1})	SMP (m^3 CH_4 kg^{-1}COD)	CH_4 in biogas
AnMBR at 15°C	Synthetic WW	0.14	0.09	66%
AnMBR at 25°C	Synthetic WW	0.35	0.26	77%
AnMBR at 35°C	Synthetic WW	0.36	0.35	81%

In Figure 6.3, the slopes of the different BMP tests are presented. It is clearly observed that the temperature favours the SMP and also the activity of the biomass. The SMA was calculated from the slope of the first days of SMP vs time, per amount of biomass (kgSS) added as inoculum. Despite the slope of the SMP at 25°C seemed much lower than at 35°C, the calculated SMA resulted in similar values.

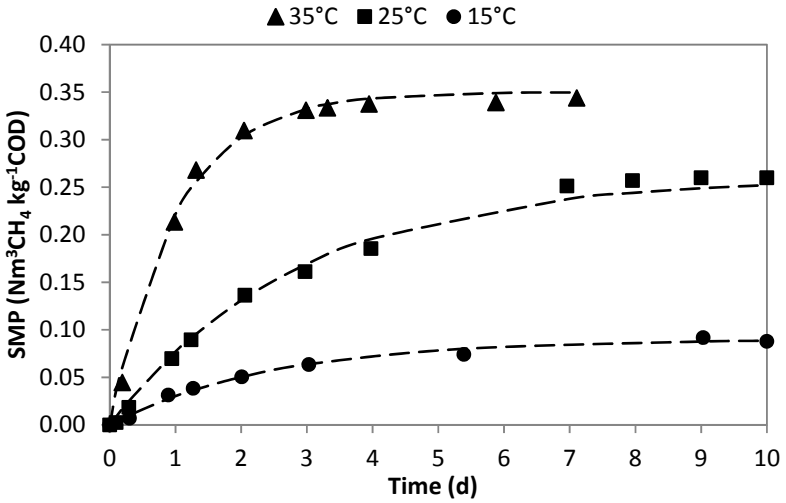


Figure 6.3. BMP test at different temperatures

Following the approach of Rincón et al. (2013) the slopes of the BMP test can be adjusted to a first-order kinetics, commonly applicable to easily biodegradable substrates. Hence the model would be as described in Equation 6.3, where B (m^3CH_4 $kg^{-1}COD$) is the methane production at each time (d) and B_{max} is the maximum methane produced observed in the test:

$$B = B_{max} \cdot [1 - e^{-k \cdot t}] \quad (6.3)$$

In order to determine *k* (d^{-1}), Equation 6.3 was adjusted to the SMP values found experimentally by least squares method (described in Equation 6.4), minimising the value of *E* that corresponds to the error between the calculated slope and the real values. B was calculated supposing an initial *k* and taking B_{max} as the maximum SMP observed in each case.

$$E = \sum (B - SMP)^2 \quad (6.4)$$

The kinetics constant obtained at 35°C was k_{35}=1.0 d^{-1} (E=7·10^{-4}), which was similar to the one obtained by Rincón et al. (2013) (k=0.91 d^{-1}) at the same temperature. At low temperature *k* was observed to be lower. At 25°C, it was k_{25}=0.35 d^{-1} (E=8·10^{-4}) and at 15°C it was k_{15}=0.41 d^{-1} (E=8·10^{-5}). Despite observed *k* was higher at low temperature; biodegradation observed was much lower probably due to VFA inhibition that stopped the methanisation reaction. If these kinetics constants were calculated considering that B_{max} should be 0.35 m^3CH_4 $kg^{-1}COD$ in any case, corresponding to a 100% biodegradation, the *k* obtained were k_{15}=0.04 d^{-1} (E=2·10^{-3}) and k_{25}=0.20 d^{-1} (E=1·10^{-3}). In these BMP tests at low

temperature, the first-order kinetics model was not the best option to describe the behaviour, since inhibitions by substrate should be considered in a more complex model.

Comparing the results of the BMP test with the performance of the continuous AnMBR, it can be noticed that the lack of SMP data in the AnMBR was related to an excessive headspace, where biogas could be accumulated. The SMA can be compared with the sOLR (based on COD removed) applied in the AnMBR, which was 0.11±0.08 kgCOD kg^{-1}SS d^{-1} and 0.07±0.05 kgCOD kg^{-1}SS d^{-1}, at 25°C and 15°C. These sOLR were lower than the SMA determined in the BMP test that were 0.35 and 0.14 kgCOD kg^{-1}SS d^{-1}. However, considering that the HRT of the AnMBR was 4 days, the SMA calculated as the slope of the SMP during the first 4 days, it resulted in 0.25 and 0.08 kgCOD kg^{-1}SS d^{-1}. Therefore, when temperature was 15°C, the sOLR applied was already the maximum activity that the biomass could maintain. In contrast, at 25°C the AnMBR could have coped with higher sOLR. In spite of this fact, since a significant amount of VFA was accumulated in the effluent, the AnMBR was operated under its maximum capacity in an attempt to improve COD removal efficiency.

6.2.4. Microorganism population

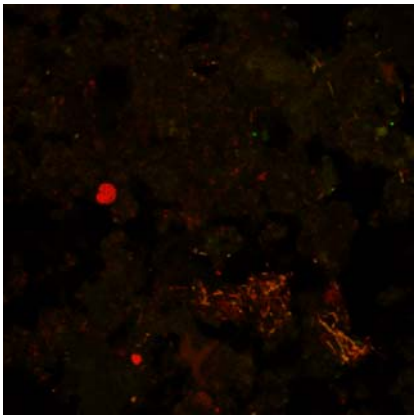
Biological population was determined by fluorescence in situ hybridization (FISH) following the procedure of Amann et al. (1990). The specific oligonucleotide probes used were: EUB338 for Bacteria (6-fam); ARC915 for Archaea (Cy3); MX825 for Methanosaeta spp. (6-fam); MS821 for Methanosarcina (Cy3); MG1200b for Methanomicrobiales spp. (6-fam); and MB311 for Methanobacteriales (minus Methanothermus) (Cy3).

Samples at each temperature were taken to determine the changes on the microbial population. At 25ºC, Methanosaeta spp. and Methanosarcina were detected, although the amount of Methanosaeta spp. was not as predominant as observed at 35ºC (section 4.2.9). In Figure 4.6a, the Archaea probe showed two different shapes of microorganism, rounded and elongated. The overlapping with Methanosaeta spp. revealed that the elongated ones corresponded to this specie of Archaea, marked in yellow. In Figure 4.6b, it is observed most of Bacteria in green colour, and in red colour Methanosarcina, which corresponded to the rounded Archaea were determined previously. The probes of Methanomicrobiales spp. and Methanobacteriales resulted negative. The microbial population observed at 15°C, was very similar. The only difference observed was the absence of Methanosaeta spp. (Figure 4.6c) and the only methanogenic specie determined was Methanosarcina (Figure 4.6d). The probes of Methanomicrobiales spp. and Methanobacteriales resulted negative.

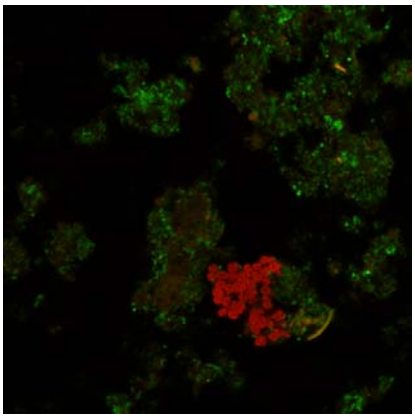
The mesophilic inoculum that contained mostly Methanosaeta spp. turned to Methanosarcina relatively fast, after 30 days at 25ºC both species were determined, and

after 80 days only Methanosarcina appeared. This fact is mainly because this specie is more tolerant to high acid concentrations, so the growth rate is higher than Methanosaeta spp. (Janssen, 2003). Despite Zhang et al. (2012) determined that Methanomicrobiales spp. played an important role in psychrophilic anaerobic digestion, the enrichment in acetotrophic methanogens can be attributed to the acetate concentration in the AnMBR. Smith et al. (2013) determined that a mesophilic inoculum can be used for psychrophilic anaerobic digestion. The development of mesophilic psychrotolerant populations may have a negative impact on the COD removal efficiency at low temperature, although their capacity to acclimatise to changes in temperatures rapidly is an advantage to ensure stable performance along the year.

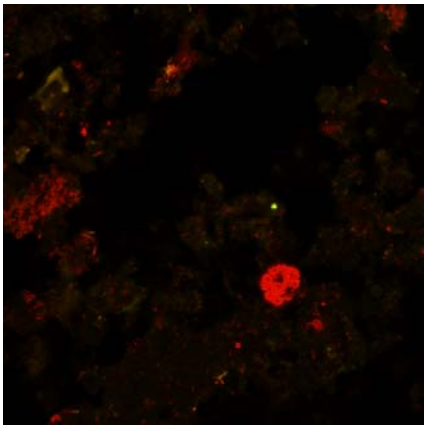
(a) Archea and Methanosaeta spp. (25°C)



(b) Bacteria and Methanosarcina (25°C)



(c) Archea and Methanosaeta spp. (15°C)



(d) Bacteria and Methanosarcina (15°C)

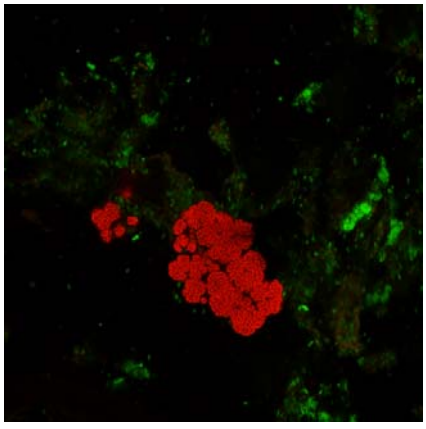


Figure 6.4. FISH image of Archaea (ARC915) and Methanosaeta spp. (MX825) (a) and Bacteria (EUB338) and Methanosarcina (MS821) at 25°C (b); and Archaea (ARC915) and Methanosaeta spp. (MX825) (c) Bacteria (EUB338) and Methanosarcina (MS821) at 15°C (d)

6.3. CONCLUSIONS

- Winery wastewater treatment at low temperatures resulted in a COD removal of 80% and 71% at 25°C and 15°C, respectively. Due to the VFA accumulation and methane retained in the liquid phase, the effluent COD not always accomplished the legal requirement. Therefore, a polishing post-treatment would be necessary to recover the methane and meet the legislation.
- A higher degree of fouling was observed compared with the mesophilic AnMBR despite the SS in the digester were lower. Although at lower temperatures less fouling was expected, the oscillations of organic load probably promoted SMP production that increased fouling.
- The methanogenic activity decreased at low temperatures as expected, although SMA obtained at 25°C was similar to the mesophilic one. However, at 15°C the activity decreased considerably. Comparing the load applied in the continuous AnMBR with the methanogenic activity observed in batch, the AnMBR was operating at its maximum capacity at 15°C. In contrast, at 25°C, the AnMBR could have coped with higher sOLR, although the COD removal efficiency would have decreased.
- The microbial population shifted from Methanosaeta spp. used as inoculum to Methanosarcina, because the higher amount of VFA in the AnMBR favoured the development of an acetotrophic methanogen with a higher growth rate under high acetate concentration.

7. Winery wastewater treatment by means of an UASB coupled with a membrane unit

Abstract

Winery wastewater was treated by means of an upflow anaerobic sludge blanket (UASB) at room temperature. An organic loading rate (OLR) up to 5.5±1.2 kgCOD m^{-3} d^{-1} was applied reaching an 84±9% of total chemical oxygen demand (COD) removal. A significant amount of suspended solids (SS) was observed in the UASB effluent that led to a high effluent COD over the discharge limits. For this reason, the UASB was coupled with a membrane unit (UASB-MBR) reaching higher COD removal efficiency of 92±4% with an effluent COD of 0.11±0.06 mg L^{-1} and free of suspended solids. However, compared with the membrane performance in the AnMBR at low temperatures described in Chapter 6, the membrane filtration was not significantly improved. The flux and the flux decline determined were only slightly better, although the solids in contact with the membrane unit were considerably lower.

Concerning biogas production, similar results of specific methane production (SMP) were obtained in both the UASB and the UASB-MBR, between 0.17 and 0.20 m^3CH_4 kg^{-1}COD. Nevertheless, higher SMPs were expected. Since the SMP could reach a maximum value of 0.35 m^3CH_4 $kg^{-1}COD_{removed}$ (according to calculated COD of methane), the observed SMP should be close to it, taking into account that the biodegradation efficiency was over 90% and the percentage of methane in biogas was 94.6±0.1%. The low temperature of operation promoted the sorption of methane in the liquid phase. By applying Henry's law, it can be calculated that an 8% of the methane production in terms of kgCOD $m^{-3}_{digester}$ d^{-1} was lost in the permeate.

This chapter is in preparation for publication as:

N. Basset, E. De Arana, A. Coll, J. Dosta, J. Mata-Álvarez. **Comparison of UASB-MBR and CSTR-MBR technolgies for winery wastewater treatment at low temperatures.**

7.1. INTRODUCTION

The invention of the upflow sludge anaerobic sludge blanket (UASB) reactor occurred in early 1970s by Lettinga (Lettinga et al., 1979). The major success of the UASB reactor was its ability to retain a high concentration of biomass due to formation of a thick dense sludge bed, which, in dependence on wastewater characteristics, may consist of well settleable methanogenic sludge granules. Formation of well settleable sludge allows the decoupling of hydraulic retention time (HRT) and sludge retention time (SRT) so that efficient treatment can be carried out at high organic loading rates (OLRs) with a significant decrease in reactor size (Lettinga et al., 2001).

With the growing application of anaerobic membrane bioreactors (AnMBR) for urban and industrial wastewater treatment, the UASB configuration has gained interest especially for wastewater treatment at low temperature (Ozgun et al., 2013). Despite AnMBR has several advantages in comparison with UASB, as the shorter start-up periods and the higher effluent quality, factors such as membrane fouling and high capital and operational costs may limit the application of the AnMBR.

The AnMBR configured as a continuous stirred tank reactor (CSTR) presents the advantage of well mixing that promotes a high hydrolysis rate thus increasing the substrate biomethane potential. However, the mixed liquor with high solid concentration is directly in contact with the membrane, favouring the solid attachment on its surface (Liao et al., 2006b). The interest in the evaluation and comparison the AnMBR (CSTR configuration) with other types of anaerobic digestion technologies lies on its incomplete COD removal at low temperatures, due to the significant VFA accumulation, and also the higher degree of fouling observed (presented in Chapter 6). The UASB appears as an interesting configuration due to the retention of biomass by means of good settling properties so that a higher amount of biomass could be retained and the mixed liquor that would be in contact with the membrane contains much less solid concentration. Several examples of UASB coupled with membrane filtration units are shown in Table 1.6 of section 1.2.4. Although the most of the UASB-MBR examples found are applied to urban wastewater treatment, its application to winery wastewater treatment at low temperature is of interest particularly in winter season when the organic load is low.

The main objective of this study is to operate an UASB for winery wastewater treatment evaluating its COD removal efficiency at low temperatures. The organic load will be increased progressively in order to examine the capacity the UASB and also the stability of the granular biomass. Afterwards, the UASB will be coupled with a membrane unit in order to improve the effluent quality, discussing the degree of fouling observed. Biomethane

potential tests (BMP) will be carried out to determine the activity of the granular biomass and compare it with the suspended biomass presented in Chapter 6. In addition, methanogenic population will be identified by means of FISH analysis.

7.2. RESULTS AND DISCUSSION

7.2.1. Experimental set-up and substrate

The UASB was set-up as described in section 3.1.2. It was a tubular reactor of 1.5 L made of glass, continuously fed by a peristaltic pump. The height to diameter ratio was H/D=3.5, which favoured the washout of the biomass with poor settling properties, while on the contrary granular biomass was kept inside the reactor. The UASB was inoculated with granular anaerobic biomass filling about 50% of the volume. The granular inoculum was taken from a potato industry (Verona, Italy) operating at room temperature; therefore the biomass was acclimatised to the low temperature and also to high organic loads. In order to improve biomass fluidisation an internal recirculation was required achieving an upflow velocity of 0.74 m h^{-1}. The biogas was collected in the upper part connected to an on-line measuring device (Ritter MGC-1).

Synthetic wastewater was used to feed the system prepared with around 1500 mgCOD L^{-1}, with diluted white wine (Artiga et al., 2005), NH_4Cl and K_2HPO_3 in accordance to the ratio COD/N/P of 800/5/1. In addition, alkalinity was added to keep the pH at neutral values. The amount of alkalinity supplied corresponded to the results obtained in Chapter 6, where at low temperatures VFA were accumulated easily and a minimum alkalinity of around 1000 mg$CaCO_3$ L^{-1} was required to avoid acidification.

7.2.2. Operation of an UASB for winery wastewater treatment

The UASB was operated for 107 days at room temperature. Three experimental periods can be differentiated depending on the OLR applied. Figure 7.1 shows the evolution of the main parameters, and they are summarised in Table 7.1. In the first period of 19 days, influent COD was relatively low, 0.87±0.30 gCOD L^{-1}, in order to acclimate the granular biomass to a new substrate. The removal efficiency was 91±4% and the effluent COD was 0.07±0.02 gCOD L^{-1}. Moreover, a HRT of 1 day was kept avoiding accumulation of volatile fatty acids (VFA), thus very low amount of VFA were measured in the effluent, 7.6±6.4 mgVFA L^{-1}. Since the amount of biomass in the UASB was very high, around 40 gSS L^{-1} of suspended solids (SS), compared with the AnMBR presented in Chapter 4 and 6; the specific organic loading rate (sOLR) was 0.015±0.009 kgCOD kg^{-1}MLSS d^{-1}. Therefore, UASB system did not experiment any instability by accumulation of VFA at low OLR of 0.6±0.4 kgCOD $m^{-3}_{digester}$ d^{-1}.

Afterwards, influent COD was progressively increased up to 1.55±0.24 gCOD L^{-1} on day 20 and the HRT was reduced to 14h. For this reason, the OLR suffered a sharp increase and the COD removal decreased to 47%. However, the system rapidly recovered a COD removal around 85%. From day 20 until day 55, marked in dash lines in Figure 7.1, an OLR of 2.6±0.8 kgCOD m^{-3} d^{-1} was supplied to the UASB achieving a COD removal of 75±10%. The changes in the OLR affected the effluent quality especially in terms of SS content. Although the feed was prepared synthetically, diluted white wine was degraded easily in the feeding tank. For this reason, feed was prepared every 2 or 3 days to keep as constant as possible the influent COD concentration. The COD in the effluent was higher than expected exceeding the standard limits (0.38±0.13 gCOD L^{-1}), due to the presence of SS that were on average 74±29 mg L^{-1}. However, if the COD removal was calculated based on the soluble COD (sCOD) that was 0.12±0.07 gCOD L^{-1} mainly composed by VFA, a 91±3% of removal was achieved.

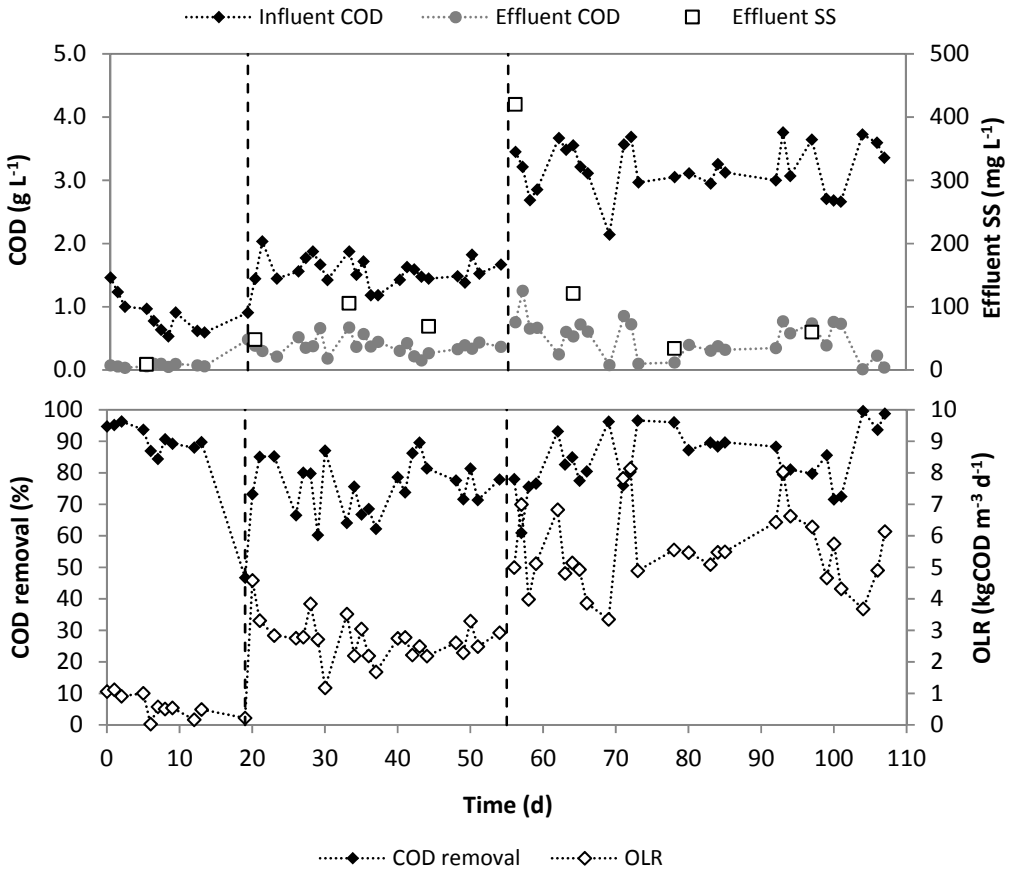


Figure 7.1. Evolution of COD, alkalinity and OLR in the UASB at room temperature

From day 55 on, the OLR was increased in order to evaluate the capacity of the UASB to cope with higher organic loads. The OLR applied was on average 5.5±1.2 kgCOD m^{-3} d^{-1}. It was observed that the granules were losing its aggregation capacity increasing the SS in the effluent significantly, up to 420 mgSS L^{-1}, shown in Figure 7.1. Since the HRT was relatively short, the disaggregated biomass was washed-out in few days, reducing the SS in the effluent to 34 mgSS L^{-1}. The average COD removal was 84±9%, which was higher than in the previous period basically because the influent COD was doubled (3.19±0.40 gCOD L^{-1}) but the effluent COD obtained was only slightly higher (0.49±0.30 gCOD L^{-1}). In fact, the COD removal in terms of sCOD was similar, being 92±5%. However, effluent sCOD was on average 0.26±0.22 gCOD L^{-1}, which was over the required limit.

Table 7.1. Main average operational parameters of the UASB

Operational parameters		Period I	Period II	Period III
OLR (kgCOD $m^{-3}_{digester}$ d^{-1})		0.6±0.4	2.6±0.8	5.5±1.2
sOLR (kgCOD kg^{-1}MLSS d^{-1})		0.015±0.009	0.06±0.02	0.15±0.03
Temperature		18±4	18±5	20±6
pH		7.7±0.2	7.5±0.3	7.4±0.6
Alkalinity (mg$CaCO_3$ L^{-1})		442±22	418±30	730±300
Ratio IA/TA		0.22±0.01	0.29±0.06	0.28±0.14
MLSS (g L^{-1})		40.3±4.2	41.5±6.9	36.5±1.0
HRT (h)		26±2	14±3	14±5
SRT (d)		790	790	790
COD influent (g L^{-1})		0.87±0.30	1.55±0.24	3.19±0.40
COD effluent (g L^{-1})		0.07±0.02	0.38±0.13	0.49±0.30
sCOD effluent (g L^{-1})		-	0.12±0.07	0.26±0.22
VFA effluent (mg L^{-1})	Acetic (HAc)	7.6±6.4	54±92 (56%)	153±68 (68%)
	Propionic (HPr)	-	13±17 (14%)	24±9 (11%)
	Butyric (HBu)	-	5±6 (5%)	22±12 (10%)
	C5-C7	-	24±26 (25%)	25± 10 (11%)
COD removal (%)		91±4	75±10	84±9
sCOD removal (%)		-	91±3	92±5
SS effluent (mg L^{-1})		9.1±2.8	74±29	159±125
Biogas production				
P_B (m^3_{biogas} $m^{-3}_{digester}$ d^{-1})		0.20±0.14	0.30±0.09	0.79±0.16
%CH_4 in biogas		97.0±1.2%	96.7±0.3%	93.9±0.6%
SMP (m^3CH_4 $kg^{-1}COD_{added}$)		0.28±0.13	0.20±0.04	0.17±0.03

Similar results were obtained by Keyser et al. (2003) treating winery wastewater in an UASB reactor with an OLR of 5 kgCOD $m^{-3}_{digester}$ d^{-1} and a HRT of 30 h. They suggested that the use of a granular sludge reduced considerably the start-up for the UASB, reaching high removal rates in few days. However, they never reached a COD removal efficiency over 86% and the biogas production was low; probably because the granules were not selective enough to treat a substrate as winery wastewater.

Regarding biogas production at low temperatures shown in Figure 7.2, during the first period biogas production (P_B) was 0.20±0.14 m^3_{biogas} $m^{-3}_{digester}$ d^{-1} and the specific methane production (SMP) achieved was 0.28±0.13 m^3CH_4 $kg^{-1}COD_{added}$, close to the expected one (0.35 m^3CH_4 $kg^{-1}COD_{removed}$). However, it was calculated following Henry's law (Chapter 6 - Equation 6.1) that 25.1 $mgCH_4$ L^{-1} (100.1 mgCOD L^{-1}) were dissolved in the liquid phase at 18°C and a 97% of methane in the biogas produced. Therefore, considering the OLR applied (0.6 kgCOD m^{-3} d^{-1}) and the biodegradation achieved (94%), a 15% of the removed OLR was lost as methane absorbed in the liquid phase. During the second and the third period, it was obtained a P_B of 0.30±0.09 m^3_{biogas} $m^{-3}_{digester}$ d^{-1} and 0.79±0.16 m^3_{biogas} $m^{-3}_{digester}$ d^{-1}, respectively. The concentration of methane in biogas was found to be 97% and 94%, respectively, much higher than the one obtained with the AnMBR described in Chapter 4 that was around 85%. Since methane has a higher desorption capacity than carbon dioxide, the biogas enrichment in methane was promoted by the short HRT. However, as discussed in Chapter 6, a short HRT leads to a higher loss of methane dissolved in the effluent. By applying Henry's law, at 20°C the amount of methane dissolved in the liquid phase, if the gas contains a 95% of methane, corresponds to 23.6 $mgCH_4$ L^{-1} (94.5 mgCOD L^{-1}). This concentration of methane that was lost in the effluent corresponded to a 7% of the overall P_B in Period II; and a 3% of the P_B in Period III. Since both OLR and thus P_B were higher than in period I, methane lost in the effluent was not as important as in the first period.

The solubilisation of methane in the liquid phase can play an important role affecting the feasibility of the process. Smith et al. (2013) observed that the 40-50% of methane was lost in the liquid phase, operating at 16h of HRT and 15°C treating urban wastewater. In our case, the amount of methane dissolved was not so significant because the OLRs were relatively high; although it should be taken into account not only for economic reasons, but environmental aspects. Dissolved methane can easily desorb when the liquid phase is in contact with the atmosphere, becoming a source of greenhouse gas emissions (Souza et al., 2011). Therefore, at low temperatures especially in winter, desorption post-treatment might be required.

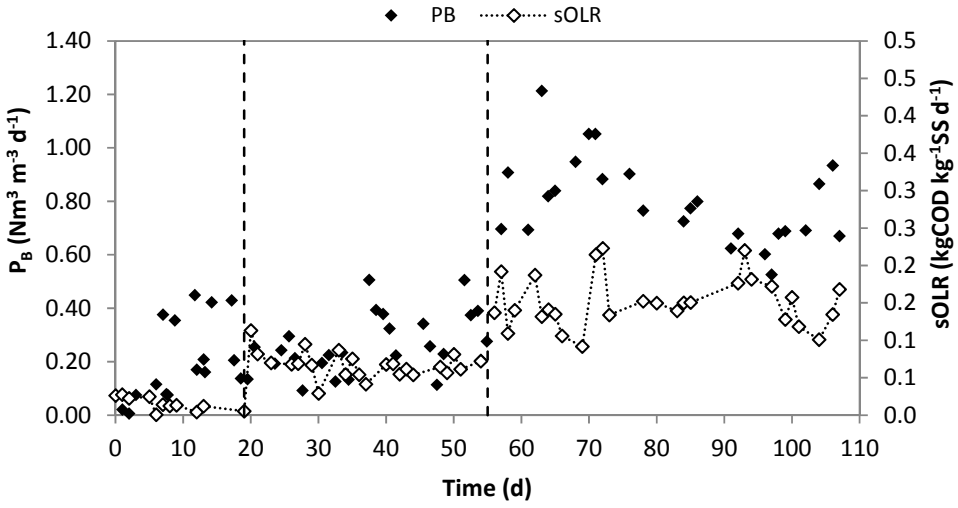


Figure 7.2. P_B and sOLR determined in the UASB operation

Not only had the OLR affected the stability of the process, but also the sOLR. As shown in Figure 7.2, during Period I the sOLR was very low 0.015±0.009 kgCOD kg^{-1}MLSS d^{-1}. At these values, the removal efficiency was high, thus VFA were not accumulated significantly. The same occurred in Period II, where sOLR was 0.06±0.02 kgCOD kg^{-1}MLSS d^{-1}, still low values. However, in the second period, the sOLR increased to 0.15±0.03 kgCOD kg^{-1}MLSS d^{-1}, reaching a maximum value around 0.22 kgCOD kg^{-1}MLSS d^{-1} on days 57, 62 and 72, corresponding to significant peaks of VFA. Therefore, by keeping low sOLR the peaks of VFA were softened obtaining more constant effluent parameters, as in the first period.

As determined in Chapter 4, a ratio between total alkalinity and intermediate alkalinity below IA/TA=0.30 assured a neutral pH. However, compared with the AnMBR described in Chapter 4, the requirements of alkalinity addition were higher in the UASB. The synthetic feed that was prepared with white wine and nutrients. It also contained 800 $mgCaCO_3$ L^{-1} in the first and second period and 1200 $mgCaCO_3$ L^{-1} in the third, reaching an alkalinity concentration in the UASB of 442, 418 and 730 $mgCaCO_3$ L^{-1}, respectively.

As presented in Figure 7.3, during the first and the second period the alkalinity added was enough to cope with the VFA accumulated keeping a ratio IA/TA around 0.30. However, with the increase of OLR in the third period, VFA were rapidly accumulated reaching 955 mgVFA L^{-1} (89% HAc; 4% HPr; 2% HBu; 5% C5-C7) on day 57 and a ratio IA/TA of 0.62. Therefore, alkalinity added was increased to keep this ratio below 0.40. Moreover, a sharp peak of OLR up to 7.8 kgCOD m^{-3} d^{-1} on day 71, also promoted an increase of ratio IA/TA to

0.46 due to the accumulation of 514 mgVFA L^{-1} (44% HAc; 20% HPr; 20% HBu; 16% C5-C7). These peaks of VFA, obtained in Period III, led to a ratio IA/TA over 0.40. These unstable conditions, when VFA were easily accumulated, also affected biogas production which decreased to P_B = 0.49 $m^3_b m^{-3}_{digester}$ d^{-1} on day 97, when 624 mgVFA L^{-1} and a ratio IA/TA=0.42 were determined.

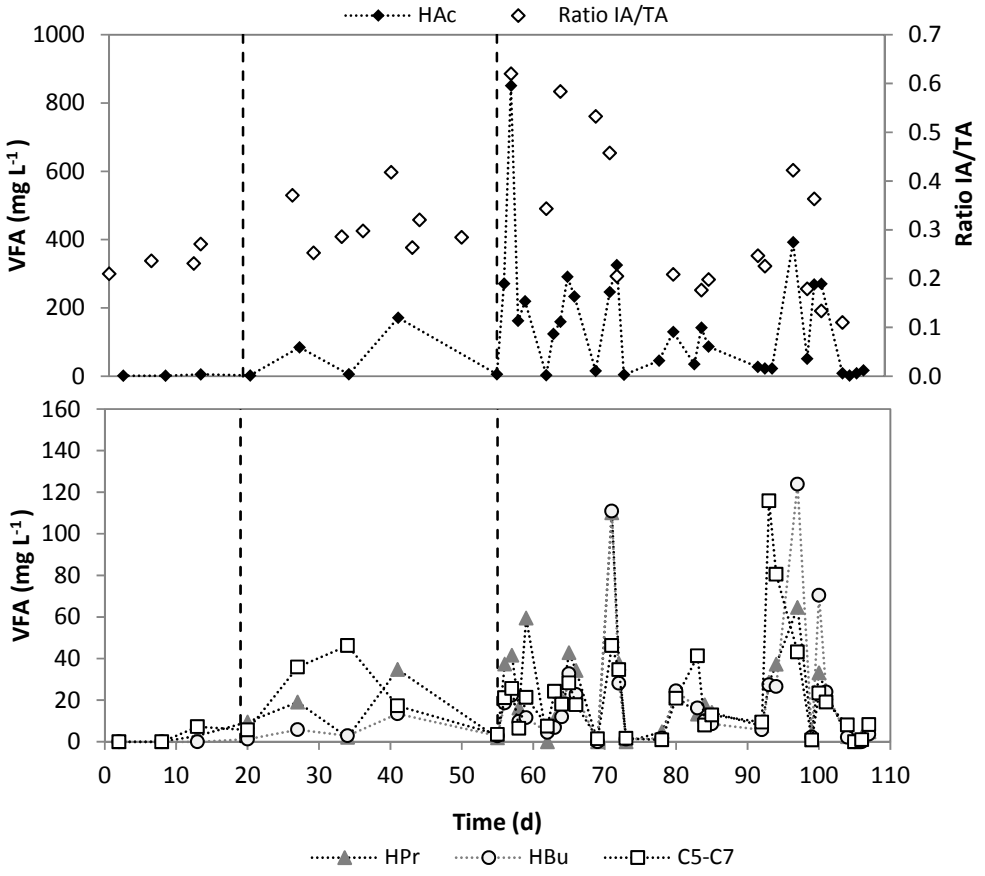


Figure 7.3. VFA and ratio IA/TA determined in the UASB operation

Compared with the AnMBR described in Chapter 6, UASB presented important advantages. Because a high amount of biomass was kept in the reactor, its capacity increased significantly. The UASB showed much more robustness, at least at short-term operation, than the AnMBR that was more prone to VFA accumulation, even at low OLRs. The application of an UASB to winery wastewater treatment might result at long-term operation in a disaggregation of the granules caused by the seasonal variability.

7.2.3. Operation of an UASB-MBR for winery wastewater treatment

The removal rates achieved in the UASB were high and the OLR applied was much higher than the OLR reached in the AnMBR described in Chapter 4. However, at high OLRs the discharge limits in terms of COD and SS were not accomplished and the amount of biomass in the USAB was decreasing due to the washout of the non-aggregated microorganisms. The variability of the winery wastewater is a clear drawback for anaerobic processes that are sensitive to OLR shocks (Dereli et al., 2012). Therefore, when the substrate characteristics negatively affect sludge granulation, a solid/liquid separation may favour the full biomass retention and so the slow growing organisms. For this reason, it was considered for this study the coupling of a membrane module to the UASB reactor, becoming an UASB-MBR.

Synthetic winery wastewater was fed to the UASB-MBR prepared with white wine and nutrients, achieving an influent average COD of 1.4±0.2 g L^{-1}. In Figure 7.4 the evolution of influent and permeate COD as well as the ratio IA/TA and VFA are shown. It should be noted that in the first 10 days COD removal was between 85% and 90% and the ratio IA/TA also slightly increased to 0.25, due to the presence of VFA in the reactor. In this case, the influent COD oscillations were much softer than in the previous experimental period described in section 7.2.2 (except on day 10 that decreased considerably). As a result, VFA accumulated were not so important, keeping a ratio IA/TA was always below 0.30. The behaviour of the permeate COD, which was on average 0.11±0.06 gCOD L^{-1}, was closely related to the influent COD, as observed in Figure 7.4. Especially when influent COD was over 1.5 gCOD L^{-1}, the permeate COD slightly exceeded the required limit of 0.125 gCOD L^{-1}. The average COD removal efficiency was 92±4%, as expected from the previous experiments with the UASB without membrane, where sCOD removal was over 90%.

Regarding VFA accumulation in Figure 7.4, during the first 10 days and then in particular on day 38, the highest peaks of VFA were obtained in the permeate. However, the amount of these VFAs was not so significant, being as maximum around 100 mg L^{-1}. Compared with the VFA accumulated in the UASB of section 7.2.2, in this case the risk of acidification was very low. For this reason, the ratio IA/TA was always around 0.2 indicating enough buffer capacity to cope the acids in the reactor.

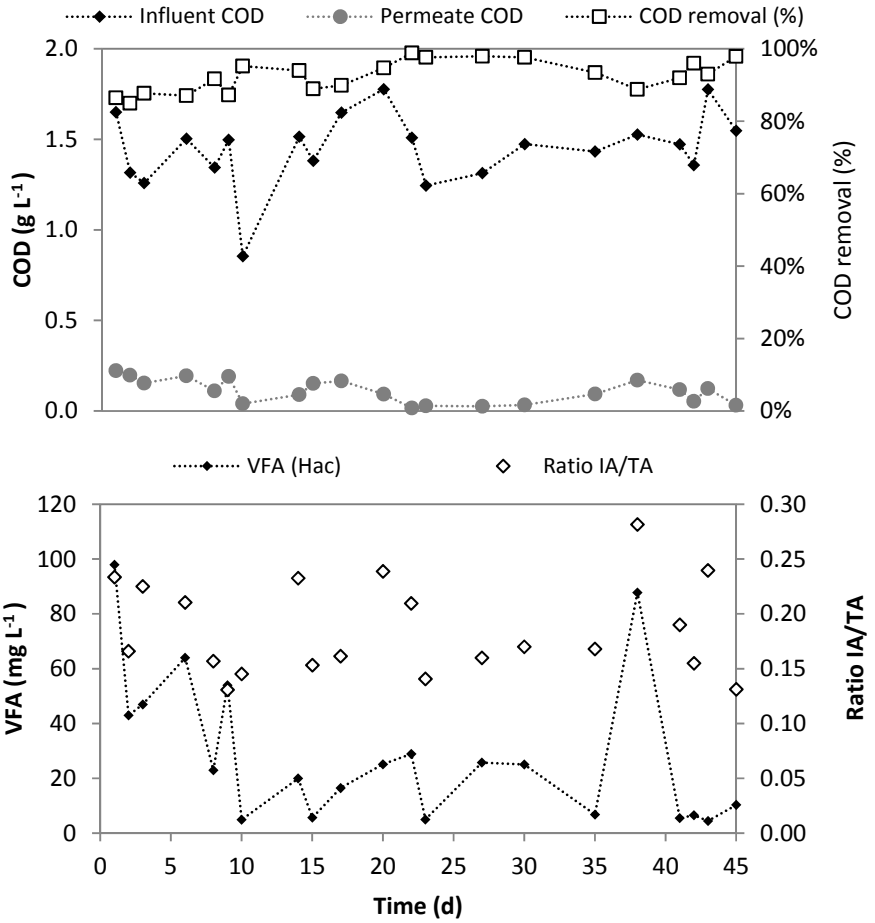


Figure 7.4. COD, VFA and ratio IA/TA observed during UASB-MBR operation

The main operational parameters and results obtained in the UASB-MBR are summarised in Table 7.2. The OLR achieved was in this case much lower than in the UASB without membrane, because it depended on the permeate flux, being 1.6±0.5 kgCOD $m^{-3}_{digester}$ d^{-1}. In this occasion, it was not observed any instability during the experimental period caused by VFA accumulated. Operating at a low sOLR of 0.052±0.016 kgCOD kg^{-1}MLSS d^{-1}, VFA were kept at 15.5±8.8 mg L^{-1} of acetic acid. The alkalinity supplied in the feed was the same as in the previous section 7.2.2. However, much less VFA were produced so that the alkalinity in the reactor was 954±126 mg$CaCO_3$ L^{-1} and the pH was 8.1±0.3.

Table 7.2. Main operational parameters of the UASB-MBR

Operational parameters of the UASB-MBR	
OLR (kgCOD $m^{-3}_{digester}$ d^{-1})	1.6±0.5
sOLR (kgCOD kg^{-1}MLSS d^{-1})	0.052±0.016
Temperature	22±5
pH	8.1±0.3
Alkalinity (mg$CaCO_3$ L^{-1})	954±126
Ratio IA/TA	0.19±0.04
MLSS (g L^{-1})	31.7±0.2
HRT (h)	19±3
SRT (d)	790
COD influent (g L^{-1})	1.4±0.2
COD permeate (g L^{-1})	0.11±0.06
VFA permeate (mg L^{-1})	15.5±8.8
COD removal (%)	92±4
Membrane filtration	
Flux (LMH)	7.5±2.3
Flux decline (LMH d^{-1})	1.3±0.8
TMP (bar)	0.3
Crossflow velocity (m s^{-1})	0.05
Biogas production	
P_B (m^3_{biogas} $m^{-3}_{digester}$ d^{-1})	0.24±0.1
%CH_4 in biogas	94.6±0.1%
SMP (m^3CH_4 $kg^{-1}COD_{added}$)	0.17±0.06

The most significant achievement in the UASB-MBR compared with the UASB of section 7.2.2 is the effluent quality. Due to the total retention of biomass in the reactor, the effluent was free of SS improving at the same time the overall COD removal. In fact, the COD removal reached in the UASB-MBR was similar to the sCOD removal of the UASB, over 90%. (Table 7.1). Obviously, the membrane unit was expected to improve the system efficiency. However, compared with the membrane performance in the AnMBR at low temperatures described in Chapter 6 - Table 6.1., the membrane filtration was not significantly improved. The flux and the flux decline determined were only slightly better 1.3±0.8 LMH d^{-1} (Table 7.2) compared with 2.14±1.62 LMH d^{-1} (Table 6.1), although the solids in contact with the membrane unit were lower. A probable reason for this lack of improvement could be that the crossflow velocity in the membrane module was lower than in the AnMBR (0.05 vs 0.64

m s^{-1}). Since the retentate was directly connected to the UASB reactor, the flow rate was limited to the upflow velocity required in the UASB, around 1 m h^{-1}. Therefore, the membrane module was not operated at its optimal conditions to reduce the cake layer formed. Hence, membrane cleanings were carried out every 3 or 4 days when flux decreased below 5 LMH. In Figure 7.5 it is presented the flux and the OLR applied. It is clearly observed that the OLR, which was on average 1.6±0.5 kgCOD $m^{-3}_{digester}$ d^{-1}, tended to oscillate as the flux did leading to a moderate accumulation of VFA (shown in Figure 7.4) when OLR were high. For instance on days 1, 6, 9 and 38 higher VFA concentrations were measured in the permeate.

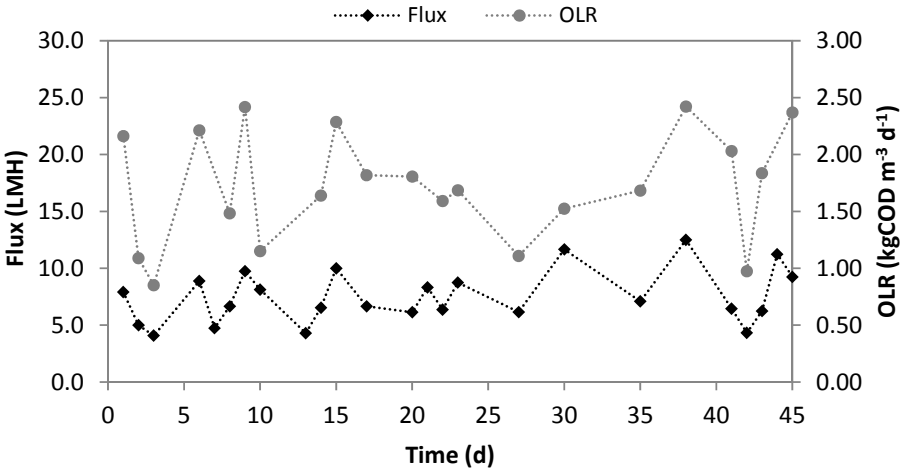


Figure 7.5. Flux and OLR observed during UASB-MBR operation

Biogas production obtained varied in accordance with the sOLR applied, as shown in Figure 7.6, achieveing an average P_B of 0.24±0.1. Similar results of specific methane production (SMP) were obtained in Periods II and III of the UASB and the UASB-MBR between 0.17 and 0.20 m^3CH_4 kg^{-1}COD (Table 7.1 and Table 7.2). Nevertheless, higher SMP were expected, as 0.28 m^3CH_4 kg^{-1}COD obtained in Period I (Table 7.1). Since the SMP could reach a maximum value of 0.35 m^3CH_4 kg^{-1}COD, the observed SMP should be close to it taking into account that the biodegradation efficiency was over 90% and the percentage of methane in biogas was 94.6±0.1%. Once more, the low temperature of operation promoted the sorption of methane in the liquid phase. By applying Henry's law considering the operational parameters (HRT, OLR, COD removal, methane percentage), it can be calculated that an 8% of the methane production in terms of kgCOD $m^{-3}_{digester}$ d^{-1} was lost in the permeate of the UASB-MBR.

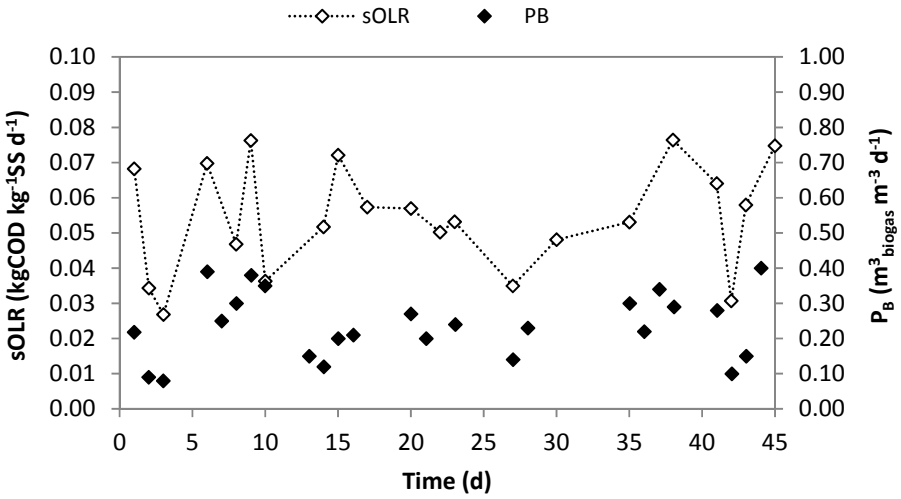


Figure 7.6. sOLR and biogas production during UASB-MBR operation

A biomethane potential (BMP) test was carried out at 25°C to evaluate the specific methanogenic activity and also the maximum SMP that can be achieved for winery wastewater treatment. The inoculum was taken from the UASB just after Period II. It can be observed in Figure 7.7 that the maximum SMP was reached in 4 days corresponding to 0.18 m^3CH_4 $kg^{-1}COD$, which was close to the SMP determined in the continuous reactor. Despite the SMP was lower than the one determined for suspended biomass in Chapter 6 that was 0.26 m^3CH_4 $kg^{-1}COD$, the SMA was 2.1 gCH_4-COD $g^{-1}VSS$ d^{-1}, which was significantly higher than the one observed for suspended biomass of 0.35 gCH_4-COD $g^{-1}VSS$ d^{-1}. Therefore, granular biomass produced biogas at a higher rate. However, it was not capable to degrade all the supplied COD. The slope of the SMP obtained can be adjusted to a first-order kinetics as done in Chapter 6 – Equation 6.3 and 6.4. Considering that B_{max} was 0.18 m^3CH_4 $kg^{-1}COD$, the kinetics constant was k = 0.77 d^{-1} (E=2·10^{-5}) much higher than the one obtained for the suspended biomass at low temperature (k_{25}=0.35 d^{-1}).

Since the BMP test was prepared with an initial COD concentration of 3.25 $gCOD\ L^{-1}$, the lack of biodegradation can be attributed to an inhibition of substrate. During Period I, the SMP reached (0.28 m^3CH_4 $kg^{-1}COD$) was almost the maximum expected because VFA concentration in the mixed liquor was very low. In contrast when VFA were present in the mixed liquor, during Period II and III and also in the BMP with suspended biomass at low temperature in Chapter 6, SMP was lower than expected. For this reason, the hypothesis to explain the low SMP observed could be because of VFA accumulation promoted the inhibition of methanogenic biomass.

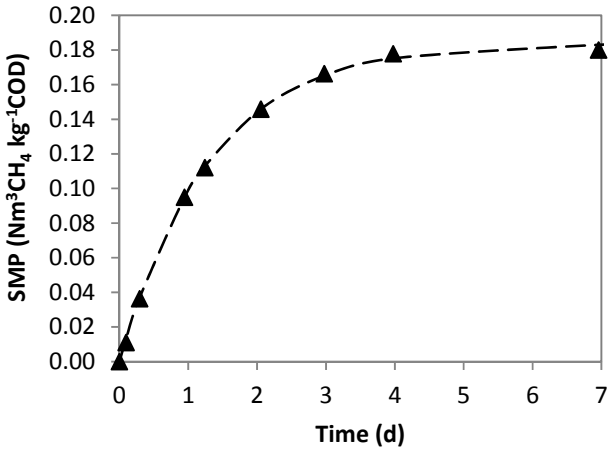


Figure 7.7. SMP determined during a BMP test

7.2.4. Microbial population

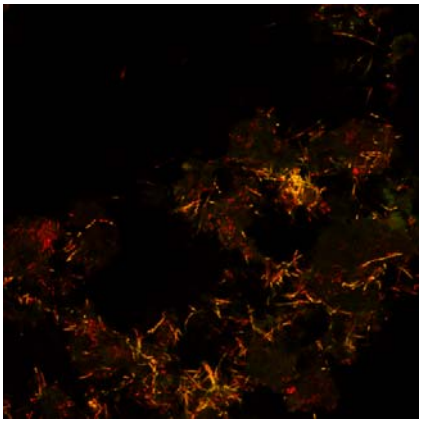
Biological population was determined by fluorescence in situ hybridization (FISH) following the procedure of Amann et al. (1990). The specific oligonucleotide probes used were: EUB338 for Bacteria (6-fam); ARC915 for Archaea (Cy3); MX825 for Methanosaeta spp. (6-fam); MS821 for Methanosarcina (Cy3); MG1200b for Methanomicrobiales spp. (6-fam); and MB311 for Methanobacteriales (minus Methanothermus) (Cy3).

The samples analysed were taken in the UASB after Period II, and afterwards in the UASB-MBR system. No differences were observed in terms of species detected in both reactors. It is shown in Figure 4.6a and Figure 4.6c that the main methanogenic archaea identified was Methanosaeta spp. Compared with the results shown in Chapter 4 and 6 related to suspended biomass in an AnMBR, Methanosaeta spp. was found to be dominant at 35ºC and at low VFA concentration in the mixed liquor; whereas Methanosarcina appeared at low temperatures, 25ºC and 15ºC, coinciding with a high amount of VFA. Surprisingly, although VFA concentration was quite high in the UASB, Methanosarcina was not detected.

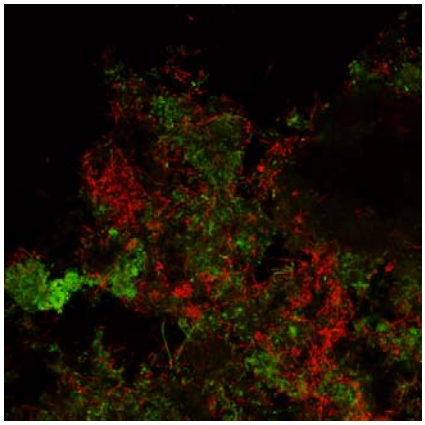
Granular biomass has the particularity that different microorganisms can coexist in the same granule at different layers (see Chapter 1 – section 1.3.2.2). Methanogenic archaea may be located in the inner layers, therefore, even if VFA concentration in the mixed liquor is high, they are less affected because the diffusion of the substrate inside the granule would limit the direct contact (Liu and Tay, 2004).

Regarding Figure 4.6b and d, it can be observed that the amount of archaea seemed to decrease in the UASB-MBR compared to the amount bacteria. Although the membrane unit was installed in order to avoid the possible loss of methanogenic microorganisms, the stress caused by the upflow velocity and the pumping through the membrane module may cause the decrease of archaea. However, further research should done about these aspects to draw more consistent conclusions.

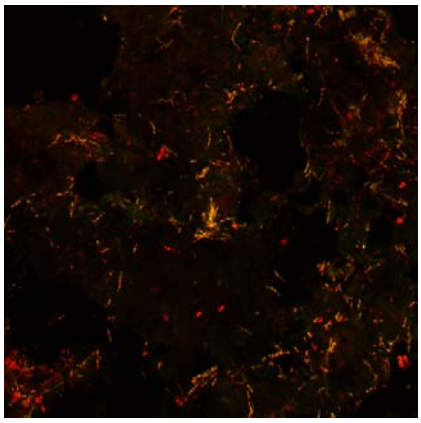
(a) Archaea and Methanosaeta spp. (UASB)



(b) Archaea and Bacteria (UASB)



(c) Archaea and Methanosaeta spp. (UASB-MBR)



(d) Archaea and Bacteria (UASB-MBR)

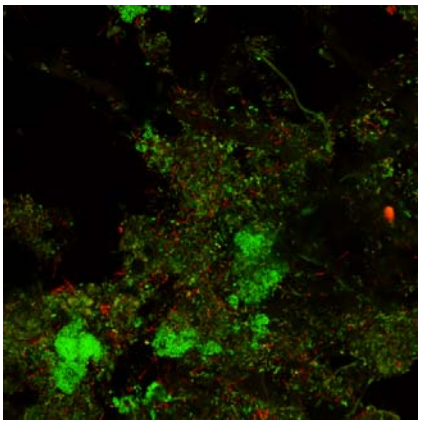


Figure 7.8. FISH image of overlapping of Archaea (ARC915) and Methanosaeta spp. (MX825) (a) and overlapping of Archaea (ARC915) and Bacteria (EUB338) in the UASB (b); and overlapping of Archaea (ARC915) and Methanosaeta spp. (MX825) (c) and overlapping of Archaea (ARC915) and Bacteria (EUB338) in the UASB-MBR (d)

7.3. CONCLUSIONS

- Higher OLR were successfully treated in the UASB in comparison with the AnMBR described in Chapter 4, mainly due to the presence of a higher amount of biomass in the reactor, thus the sOLR was kept lower.
- The changes in OLR negatively affected the granules stability, increasing the SS in the effluent and washing-out the non-aggregated biomass.
- Coupling the UASB to a membrane unit allowed the accomplishment of legal requirements of the effluent, especially at high OLRs. However, the cost that a membrane unit supposes may not be justified in the case of a granular technology as the UASB. The UASB by itself has enough capacity of solid retention, thus the strategy to reduce the effluent COD would be focused on maintaining the granule stability.
- The activity of the granular biomass determined by batch test was higher than the activity of the suspended biomass. Nevertheless, the methane production was lower, probably due to the inhibition by substrate.
- The main methanogenic archaea identified was Methanosaeta spp. However, the proportion of archaea compared to bacteria seemed to decrease in the UASB-MBR. Further research should be done in this aspect to draw more consistent conclusions about the microbial population.

8. Integrating the selection of PHA storing biomass and nitrogen removal via nitrite treating UASB effluent

Abstract

A novel scheme was developed for the treatment of municipal wastewater; consisting in an upflow anaerobic sludge blanket (UASB) reactor followed by a short cut sequencing batch reactor (scSBR) in the main water line. Nitritation/denitritation was integrated with the selection of polyhydroxyalkanoates (PHA) storing biomass. An aerobic-feast and anoxic-famine regime was adopted. Denitritation was driven by internally stored PHA. Biowaste fermented liquid was applied as carbon source in the feast regime. The sequencing batch reactor was operated at a nitrogen loading rate of 0.075 kgN·m^{-3}·d^{-1}. The average nitrogen removal was 83%. In the presence of volatile fatty acids (VFAs) and low dissolved oxygen (DO=1.5 mg L^{-1}), the specific ammonium uptake rate (sAUR) was low (0.01-0.41mgN·gVSS^{-1}·h^{-1}). VFA depletion was followed by an increase of sAUR up to 3.16 mgN·$gVSS^{-1}$·h^{-1}. PHA stored reached its maximum at the time VFA were depleted, and it progressively decreased under aerobic conditions when nitritation took place. After achieving an acceptable ammonia removal of 93%, there was enough available PHA for the subsequent denitritation, reaching a maximum nitrite removal of 98%. The PHA accumulation capacity was evaluated in fed batch tests. The maximum PHA content was 10.6% (gPHA $gTSS^{-1}$) after 10 h of accumulation when biowaste fermented liquid (C/N/P= 100/4.5/0.42) was applied. Nitrogen removal limits could be successfully met while PHA-storing biomass was selected. Although higher PHA yields can be achieved under complete aerobic conditions, this novel scheme presents an added value due to the integration of the PHA production in the nitritation/denitritation process.

This chapter was presented as oral communication in:

- Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line. *2nd IWA Specialized International Conference "Ecotechnologies for Wastewater Treatment (EcoSTP2014)",* Verona, Italy, 23rd – 25th June 2014.

In preparation for publication as:

N. Basset, E. Katsou, N. Frison, S. Malamis, J. Dosta, F. Fatone (2016). **Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line**

8.1. INTRODUCTION

The treatment of domestic and municipal wastewater by UASB technology is an interesting option that can solve both waste and wastewater management issues at a decentralized level. In decentralized communities, the separation of black and grey water and the treatment of the more concentrated black water by the upflow anaerobic sludge blanket (UASB) followed by suitable post treatment for nutrients recovery/removal is a sustainable treatment option.

The integration of PHA production within a WWTP at full scale is of real added value and within the scope of the current work. The selection of PHA storing biomass through the feast and famine regime using biowaste derived carbon source is investigated. In this study, PHA is produced using the mixed culture of activated sludge and real wastewater as substrate. Thus, wastewater is treated and the amount of waste sludge that is generated in the WWTP can be reduced. In the examined mechanism, the last biological step is that of maximizing the PHA content within the sludge (accumulation step). The sludge that is rich in bacteria that are able to store PHA is collected at the end of the famine period. It is placed in a batch reactor and subjected to consecutive spiking with excess organic carbon.

Microorganisms produce PHA as an energy and carbon reserve that can be used when food is limited (like fat is produced in humans). More than 300 different microorganisms that synthesize PHA have been isolated (Dias et al., 2006). Nevertheless, the feasibility of PHA production with less expensive mixed microbial cultures has been demonstrated (Morgan et al., 2010; Morgan-Sagastume et al., 2014; Salehizadeh and Van Loosdrecht, 2004).

Concerning the mechanism of their production, an aerobic feast and famine regime is the most appropriate scheme for the selection of PHA storing biomass (Jiang et al., 2009). The feast and famine regime creates favourable conditions for microorganisms capable of storing volatile fatty acids (VFAs) as PHA. The VFAs are taken up very fast by PHA accumulating bacteria during the feast phase and can be utilized to gain a competitive advantage during the famine phase (Dionisi et al., 2006; Dircks et al., 2001). The carbon source that is added can be fermented waste, since it is rich in VFAs and can be produced at low cost within the wastewater treatment plant (WWTP) (Katsou et al., 2015, 2014).

The selection of PHA storing biomass is usually conducted in a dedicated reactor. The use of cheap substrates, such as organic waste, has been investigated by many researchers for the selection, enrichment and growth of PHA storing biomass as a way to reduce the production cost of PHA (Albuquerque et al., 2010; Khosravi-Darani et al., 2013; Koller et al., 2010).

Another point that the current study addresses is related with the feasibility of simultaneous nitrogen removal using the internally stored PHA. Nitritation-denitritation (N/DN) can be a sustainable alternative for reducing the energy requirements of the WWTPs (Méndez et al., 2010). The nitrite pathway not only improves the total nitrogen removal efficiency but also reduces the aeration costs compared to the conventional activated sludge process (Ma et al., 2009).

A novel scheme was tested for the treatment of municipal wastewater by applying an anaerobic step, in an attempt to produce some VFA from municipal wastewater, followed by the via nitrite pathway to remove nitrogen and integrating the PHA storing biomass selection Figure 8.1. An external carbon source was required for nitrogen removal, since municipal wastewater could not provide enough available VFA. The external carbon source was added during the aerobic phase. An anoxic phase was introduced after the aeration for nutrient removal. The post-anoxic denitrification via nitrite was performed without the addition of carbon source. In this innovative scheme, the via nitrite nitrogen removal was integrated with the selection of PHA storing biomass in the main wastewater treatment line. This was accomplished by adopting a feast and famine regime.

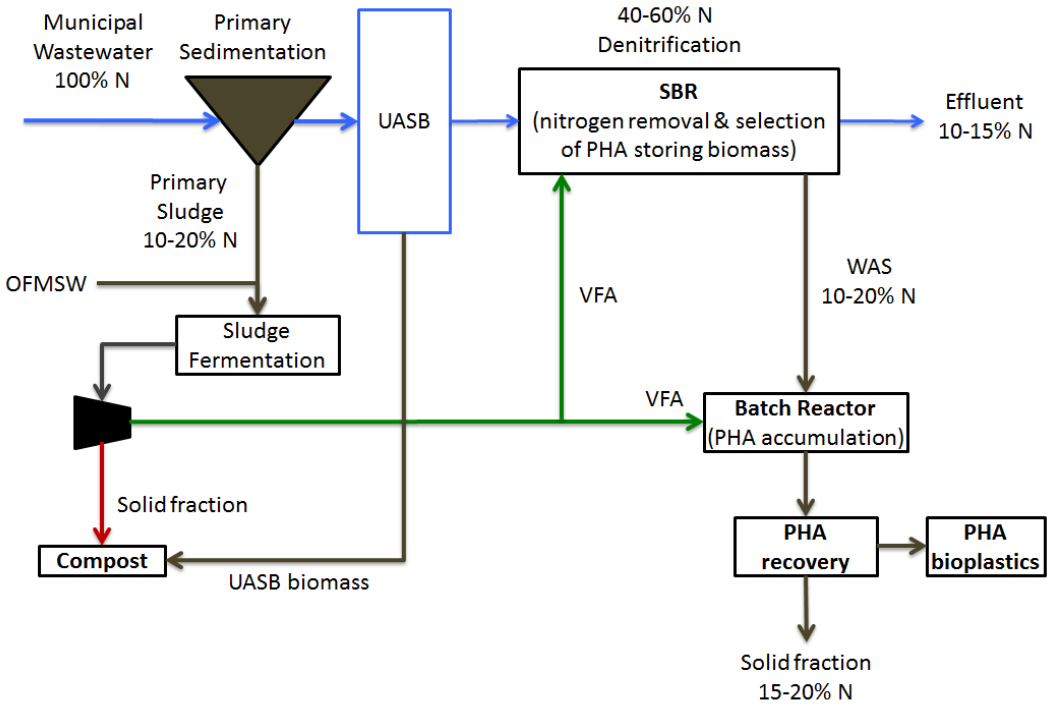


Figure 8.1. Integration of nitrogen removal and PHA production in the main water line

The main concept was to achieve and demonstrate denitritation driven by storage compounds. The carbon sources chosen for this purpose were the organic fraction of municipal solid waste fermentation liquid (OFMSW FL) and the primary sludge and OFMSW fermentation liquid (PS & OFMSW FL). OFMSW FL can be easily obtained from the separate collection of municipal waste, widely applied in Europe. In contrast, PS & OFMSW FL was selected in order to simulate the cases where food waste disposers (FWDs) are applied for the management of municipal wastewater together with household organic waste (Iacovidou et al., 2012). In these cases where regular separate collection of food waste from households cannot be practiced, the PS (obtained from FWDs) is actually the mixture of the PS from municipal wastewater and OFMSW. The novel scheme will be examined in terms of energy aspects comparatively with the conventional process of separate wastewater treatment for nitrogen removal and the selection of PHA storing biomass. The main objective is to achieve an acceptable nitrogen removal and to evaluate the potential for PHA production by means of accumulations tests.

8.2. RESULTS AND DISCUSSION

8.2.1. Substrate and carbon sources characteristics

Two types of external carbon sources were tested in the process; OFMSW FL and PS & OFMSW FL. The OFMSW FL was produced in a 3 L – fermenter at a controlled temperature of 37°C and pH of 5.0. The OFMSW was previously grinded and diluted with SBR effluent up to 6% TS (total solids). The fermenter organic loading rate (OLR) was 20 kgTVS m^{-3} d^{-1} (total volatile solids) and the hydraulic retention time (HRT) was 3 days. For the production of PS & OFMSW FL the following procedure was applied: PS was mixed with OFMSW in a fermenter of 10 L at room temperature (around 20°C) with a ratio of 0.8 g OFMSW L^{-1} PS and at HRT of 8.7 days. The separation of the liquid and solid stream was performed by centrifugation.

Wastewater that was collected from the municipal WWTP of Verona (Italy) was fed into the SBR. The feast conditions were established in aerobic environment, by feeding the reactor with VFAs. The feast conditions were established in aerobic environment, by feeding the reactor with fermentation liquid. The oxidation of ammonium to nitrite occurred during feast and famine, aerobic conditions, while denitritation occurred under famine, anoxic conditions. The selection of PHA storing biomass took place under the established feast and famine regime. In situ and ex situ biomass activity tests were carried out to evaluate the rate of nitrogen removal and VFA uptake. The main characteristics of the wastewater and of the two different types of carbon source that were used in the current work are summarised in **Table 8.1**.

Table 8.1. Characteristics of wastewater and carbon source used in the examined periods

Parameter	Wastewater	OFMSW FL	PS & OFMSW FL
pH	7.21 ± 0.26	5.00 ± 0.52	5.21 ± 0.12
Chemical Oxygen Demand - COD ($mg \cdot L^{-1}$)	363 ± 68	21497 ± 1535	10500 ± 177
Soluble COD - sCOD ($mg \cdot L^{-1}$)	212 ± 69	18075 ± 248	1230 ± 57
Total Kjeldahl nitrogen – TKN ($mg \cdot L^{-1}$)	115 ± 14	959 ± 4	580 ± 5
Phosphate – PO_4-P ($mg \cdot L^{-1}$)	3.7 ± 1.1	90.8 ± 1.1	122.3 ± 1.8
Acetic acid – HAc ($mgCOD \cdot L^{-1}$)	133 ± 56	2717 ± 1082	1584 ± 285
Propionic acid – HPr ($mgCOD \cdot L^{-1}$)	29 ± 16	560 ± 256	766 ± 231
Butyric acid – HBu ($mgCOD \cdot L^{-1}$)	-	2790 ± 1391	445 ± 125
C5-C7 ($mgCOD \cdot L^{-1}$)	-	1365 ± 700	372 ± 109
COD/N/P		100/4.5/0.42	100/5.5/1.2

8.2.2. Anaerobic digestion of municipal wastewater by an UASB

The UASB (described in section 3.1.3) was fed continuously with municipal wastewater, which characteristics are described in Table 8.1. The influent flow rate was 40 L d^{-1}, thus the HRT was kept at 10h. The working temperature was 21±3°C. Biogas production (P_B) was 0.30±0.02 $m^3\ m^{-3}_{digester}\ d^{-1}$ with a 60% of methane, thus specific methane production (SMP) was 0.28±0.06 $m^3CH_4\ kg^{-1}COD$. The characteristics of the effluent obtained are summarised in

Table **8.2**.

Table 8.2. Characteristics of UASB effluent

Parameter	Wastewater
pH	7.26 ± 0.26
Chemical Oxygen Demand - COD ($mg\ L^{-1}$)	66.8 ± 33.9
Soluble COD - sCOD ($mg\ L^{-1}$)	38.6 ± 21.5
Total Kjeldahl nitrogen – TKN ($mg\ L^{-1}$)	94.2 ± 30.6
NH_4^+-N ($mg\ L^{-1}$)	50.6 ± 15.6
Phosphate – PO_4-P ($mg\ L^{-1}$)	5.2 ± 1.7
Acetic acid – HAc ($mgCOD\ L^{-1}$)	11.3 ± 7.2
Propionic acid – HPr ($mgCOD\ L^{-1}$)	1.2 ± 0.8
Butyric acid – HBu ($mgCOD\ L^{-1}$)	-
C5-C7 ($mgCOD\ L^{-1}$)	-

An 82% of COD removal was achieved in the UASB reactor. However, the presence of sulphates can reduce the expected methane production due to the presence of sulphate-reducing bacteria. Influent and effluent sulphate concentration were 25±19 mg L^{-1} and 14±10 mg L^{-1}, respectively. Sulphate-reducing bacteria consume 1.5 gCOD $g^{-1}SO_4^{2-}$ (Gerardi, 2003) to produce H_2S. The ratio COD/sulphate observed in the municipal wastewater was around 12. When this ratio is over 3, the presence of sulphate-reducing bacteria does not affect the methanisation reaction. Nevertheless, it should be taken into account that the biogas produced would contain some amount of H_2S that must be removed to preserve the equipment.

UASB granules were characterised in terms of COD, TKN, sludge volumetric index (SVI), etc., shown in Table 8.3. The granular biomass is characterised by a high settling capacity, thus the SVI did not change significantly in 5 and 30 minutes. The distribution of the biomass in the UASB was determined by measuring the suspended solids (SS) in three points. It was clearly observed that in the bottom part there were the most concentrated granules and the upper part was almost clarified. The upflow velocity of 0.98 m h^{-1} could keep the biomass enough fluidised to achieve a good mixing in the bottom part of the UASB while the upper part served as a clarifier to obtain an effluent with low SS.

Table 8.3. UASB granules characteristics

	Parameter	Average
UASB granules	COD (g L^{-1}TSS)	14.4±0.5
	TKN (mg g^{-1} TSS)	32±15
	P (mg g^{-1} TSS)	10.6±4.0
	SVI_5 (mL g^{-1}TSS)	25.3
	SVI_{30} (mL g^{-1}TSS)	23.2
	gCOD/gTSS	0.71
	gVSS/gTSS	0.77
	gCOD/gVSS	0.92
UASB upper point	TSS (g L^{-1})	0.8±0.2
	VSS (g L^{-1})	0.5±0.1
UASB middle point	TSS (g L^{-1})	17.7±0.8
	VSS (g L^{-1})	14.0±0.7
UASB bottom point	TSS (g L^{-1})	38.9±7.5
	VSS (g L^{-1})	30.2±6.2

Biomethane potential (BMP) tests were performed to determine the activity of the UASB granules. Two kinds of substrates were used: municipal wastewater and acetate. The specific methanogenic activity (SMA) observed was 0.86 and 1.1 gCH_4-COD $gVSS^{-1}$ d^{-1}, respectively, with a COD removal efficiency higher than 98% in both tests. A higher SMA was obtained with acetate as substrate since it is easily biodegradable. In contrast, municipal wastewater contained particulate COD that was more difficult to degrade. Nevertheless, the values of SMA obtained indicated that the granules had a higher activity compared with the those reported by Chong et al. (2012), between 0.25 and 1.0 gCH_4-COD $gVSS^{-1}$ d^{-1}, and also the results obtained with suspended biomass in Chapter 4 of 0.35 gCH_4-COD $gVSS^{-1}$ d^{-1}.

8.2.3. SBR operational strategy

In the examined process, the oxidation of ammonia to nitrite occurred simultaneously with PHA accumulation in the same reactor (described in section 3.1.3). The SBR operation was divided into two periods of 45 and 30 days based on the applied carbon source. The strategy applied consisted in the simultaneous PHA storing biomass selection and N/DN process driven by the stored PHA by adding carbon source during aerobic phase for PHA storage.

- Period I: OFSMW FL was added at the beginning of the aerobic phase, while denitritation was driven by the stored PHA during the anoxic phase. The DO during the aerobic reaction phase was set at 1.5-2.5 mg L^{-1}. The time of the aerobic phase was 130 min and then it was increased to 160 min, while anoxic phase lasted 40 min.
- Period II: PS & OFMSW FL was used as carbon source in the beginning of the aerobic reaction phase of 160 min, while denitritation was driven by the stored PHA during the anoxic phase that was increased to 50 min. The DO was maintained at 2.0-3.0 mg L^{-1}.

8.2.4. Via nitrite N/DN in a short-cut SBR driven by the stored PHA

The approach of the novel system described in Figure 8.1 consisted of a short-cut SBR (scSBR), where N/DN via nitrite took place with the simultaneous selection of PHA accumulating biomass. The feasibility of this process was examined focusing on accomplishing an adequate N/DN rate and evaluating its potential for PHA production. In order to promote the PHA storage, feast and famine conditions must be assured. The addition of an external carbon source was necessary due to a lack of VFA in the influent wastewater. Municipal wastewater contained a certain amount of COD that could be used for PHA storage, although it was not enough to reach a complete denitritation that required around 2.5 $gCOD \cdot g^{-1}N$. The carbon source added under aerobic conditions favoured the PHA storage, which was consumed in the subsequent famine phase removing nitrite. In the scSBR, feast phase corresponded to the time VFA were present in the system under aerobic

conditions, while famine phase corresponded to the time after VFA depletion when nitritation (aerobic) and denitritation (anoxic) occurred.

The scSBR was operated in cycles of aerobic and anoxic periods of 130 min and 40 min. Since the carbon source was added at the beginning of the aerobic phase and the DO was low (1.5 $mg \cdot L^{-1}$), nitritation suffered a lag phase matching with the time VFA were removed. Hence, it resulted in an inefficient ammonia removal of 67.6 ± 14.3% and the effluent contained 18.5 ± 9.5 $mgNH_4\text{-}N \cdot L^{-1}$. In order to improve nitritation rate, the aerobic phase length was set at 160 min. Afterwards complete nitritation was achieved although a lag phase of about 10 min was still present.

The results obtained with two different carbon sources are shown in Table 8.4. In Period I (OFMSW FL), the overall nitrogen removal was 83.0±13.7%, with a volumetric nitrogen loading rate of vNLR=0.075 ± 0.027 $kgN \cdot m^{-3} \cdot d^{-1}$. Complete nitritation was achieved obtaining an ammonium removal of 93.4 ± 5.25% and a specific ammonium uptake rate, after VFA depletion, of sAUR=3.16 ± 0.11 $mgNH4\text{-}N \cdot (gVSS \cdot h)^{-1}$. The subsequent nitrite removal achieved was on average 74.0 ± 22.9% and the specific nitrite uptake rate was sNUR=8.44 ± 1.80 $mgNO_2\text{-}N \cdot (gVSS \cdot h)^{-1}$, much higher than the endogenous one that is around 1 $mgNO_2\text{-}N \cdot (gVSS \cdot h)^{-1}$. Despite the average nitrite removal was not as high as expected to meet the limits, the removal rate was improving during Period I. After 30 days of operation complete denitritation and a 98.3% of nitrogen removal was achieved.

In Period II, where PS & OFMSW FL was used as carbon source, an 83.2 ± 3.4% of nitrogen removal was reached at a vNLR=0.080 ± 0.017 $kgN \cdot m^{-3} \cdot d^{-1}$. Ammonium removal was 88.0 ± 1.4%, which was slightly lower than in Period I. The lag phase caused by the presence of carbon to oxidise under low DO concentrations was longer, of around 40 min. It was probably due to the presence of a considerable amount of non-VFA COD (Table 8.1) that competed for oxygen as well. After this lag phase sAUR was 4.39 ± 0.78 $mgNH_4\text{-}N \cdot (gVSS \cdot h)^{-1}$, similar to the one obtained in the previous period. In an attempt to improve denitritation rate, the anoxic phase length was increased to 50 min. Hence, nitrite removal was increased to 83.7 ± 16.8% at a similar sNUR of 9.36 ± 2.3 $mgNO_2\text{-}N \cdot (gVSS \cdot h)^{-1}$.

The volumetric organic loading rate - vOLR was 0.20 ± 0.02 and 0.26 ± 0.04 $kgCOD \cdot m^{-3} \cdot d^{-1}$ for Period I and II, respectively. COD removal increased from 51% to 63% in Period I due to the increase in the length of the aerobic phase from 130 min to 160 min that helped the removal of the remaining non-VFA COD. In both periods, the effluent COD obtained was below the limits.

Table 8.4. Operational results of the different experimental periods

Parameter	Period I	Period II
Type of carbon source	OFMSW FL	PS & OFMSW FL
vNLR influent ($kgN \cdot m^{-3} \cdot d^{-1}$)	0.075 ± 0.027	0.080 ± 0.017
vOLR ($kgCOD \cdot m^{-3} \cdot d^{-1}$)	0.20 ± 0.02	0.26 ± 0.04
SS ($g \cdot L^{-1}$)	2.86 ± 0.19	2.31 ± 0.22
VSS ($g \cdot L^{-1}$)	2.60 ± 0.08	2.18 ± 0.18
F/M ($kgCOD \cdot kgVSS^{-1}$)	0.02 ± 0.004	0.03 ± 0.01
HRT (h)	16.3 ± 1.2	15.8 ± 2.0
SRT (d)	25	25
Feast / Famine ratio (based on VFA depletion length)	0.08 ± 0.04	0.09 ± 0.07
C/N	2.4 ± 0.4	2.3 ± 0.4
NH_4-N influent ($mg \cdot L^{-1}$)	49.93 ± 14.90	51.65 ± 13.22
NO_2^--N after aerobic phase	20.5 ± 3.9	15.9 ± 0.51
N effluent ($mg \cdot L^{-1}$)	8.15 ± 6.64	8.73 ± 2.71
NH_4-N effluent ($mg \cdot L^{-1}$)	2.96 ± 1.85	6.17 ± 0.89
NO_2-N effluent ($mg \cdot L^{-1}$)	5.20 ± 5.01	2.57 ± 2.35
% NH_4-N removal	93.4 ± 5.25	88.0 ± 1.4
% NO_2-N removal	74.0 ± 22.9	83.7 ± 16.8
% N removal	83.0 ± 13.72	83.2 ± 3.4
sAUR ($mgNH_4\text{-}N \cdot (gVSS \cdot h)^{-1}$) (after VFA depletion)	3.16 ± 0.11	4.39 ± 0.78
sNUR ($mgNO_2\text{-}N \cdot (gVSS \cdot h)^{-1}$)	8.44 ± 1.80	9.36 ± 2.3
-qVFA ($mgCOD \cdot (gVSS \cdot h)^{-1}$)	61.6 ± 25.8	53.2 ± 20.9
sCOD influent ($mg \cdot L^{-1}$)	88.0 ± 28.3	92.0 ± 22.6
sCOD effluent ($mg \cdot L^{-1}$)	29.4 ± 9.0	25.5 ± 3.5
%COD removal	63.1 ± 22.2	70.9 ± 11.0

This strategy can be applied in the main wastewater treatment line by assuring an efficient DN. A good fermentation (reaching high VFA concentration) and the subsequent liquid/solid separation would help to reduce the non-VFA COD minimising the competition for oxygen. Complete denitritation was achieved driven by stored PHA due to the operation under feast and famine conditions. Feast and famine ratio is an important parameter to assure the selection of PHA storing biomass (Morgan-Sagastume et al., 2010b). Feast and famine ratios

applied in the scSBR, 0.08 and 0.09, were adequate to favour the storage yield instead of the growth (Albuquerque et al., 2010).

8.2.5. Simultaneous N/DN and PHA storing biomass selection using OFMSW FL as carbon source - Period I

The feasibility of the examined process was firstly evaluated by performing ex situ tests. The results are presented in **Figure 8.2**. It should be noted that the amount of nitrogen and VFA in the ex-situ test were higher than the concentrations in the scSBR. OFMSW FL was added at a C/N=2.5 and DO=3.5 $mg \cdot L^{-1}$. The sAUR before and after the VFA depletion (30 min) was 6.94 and 10.92 $mgNH_4\text{-}N \cdot (gVSS \cdot h)^{-1}$, respectively. The oxygen requirement was higher when VFAs were present in the system; the specific oxygen uptake rate (sOUR) reached 104 $mgO_2 \cdot (gVSS \cdot h)^{-1}$ and it decreased to 25 $mgO_2 \cdot (gVSS \cdot h)^{-1}$. Several authors reported the similar phenomenon that acetate was consumed fast at the beginning of the cycle and stored as internal compounds (Carucci et al., 2001; Chen et al., 2013; Dircks et al., 2001). At the end of the feast phase, the PHA content was 3.8% (wt.) that was consumed in the subsequent anoxic phase reaching a sNUR=8.36 $mgNO_2\text{-}N \cdot (gVSS \cdot h)^{-1}$ and complete denitritation. However, the overall nitrogen removal was only 41% due to poor nitritation.

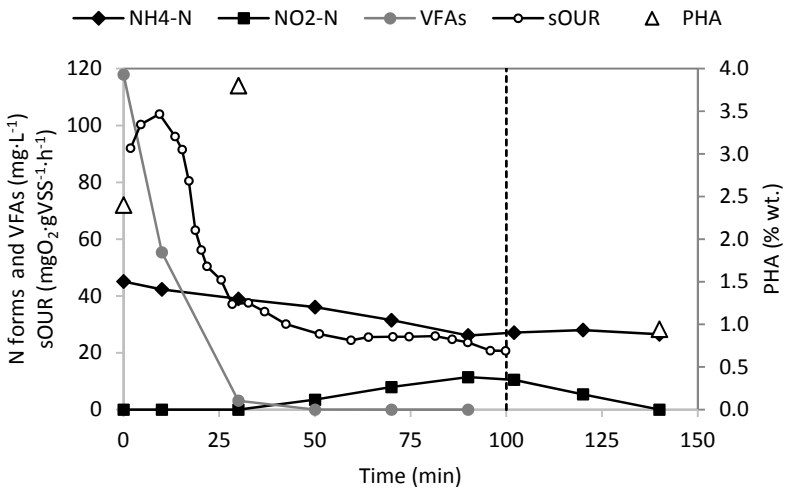
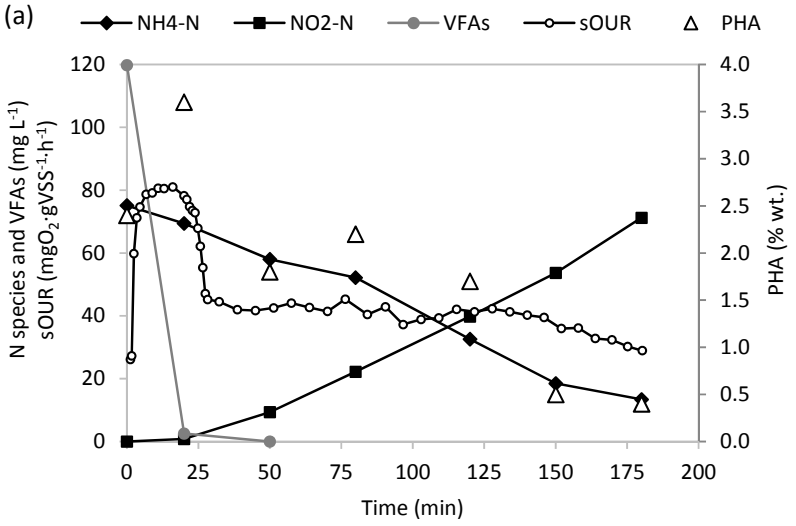


Figure 8.2. Ex-situ test with OFMSW FL achieving N/DN driven by stored PHA

In order to determine the impact of the length of the aerobic phase on the PHA availability for denitrification, aeration was extended up to 180 min. The maximum sOUR observed in **Figure 8.3**a was 81 $mgO_2 \cdot (gVSS \cdot h)^{-1}$, coinciding with VFA oxidation, and decreased to 40 $mgO_2 \cdot (gVSS \cdot h)^{-1}$ afterwards. The sAUR during the VFA consumption was 6.6 $mgNH_4$-

$N \cdot (gVSS \cdot h)^{-1}$, and after it increased up to 8.1 $mgNH_4\text{-}N \cdot (gVSS \cdot h)^{-1}$. The carbon source affected nitritation decreasing the sAUR at the beginning of the test, even at high DO concentrations, because heterotrophic biomass has a higher affinity for oxygen than autotrophic nitrifiers (Third et al., 2003). The same test was carried out without carbon source (**Figure 8.3**b) obtaining a sAUR of 7.8 $mgNH_4\text{-}N \cdot (gVSS \cdot h)^{-1}$ and a sOUR around 40 $mgO_2 \cdot (gVSS \cdot h)^{-1}$. However, when ammonium concentration tended to decrease below 20 $mg \cdot L^{-1}$, the sOUR also decreased to around 30 $mgO_2 \cdot (gVSS \cdot h)^{-1}$ probably due to lower affinity to the substrate.

The presence of carbon source resulted in a lag phase of 30 min. During this lag phase, stored PHA reached a maximum value of 3.6%. As shown in **Figure 8.3**, the PHA content progressively decreased to 1.7% within 120 min, while in 150 min PHA were almost completely consumed under aerobic conditions. Therefore, ammonium oxidation should be enough fast to assure the availability of PHA for the subsequent denitritation. Similar results were obtained by Chen et al. (2013), who determined that the key point in controlling the post-anoxic denitrification driven by PHB was the aeration time, concluding that the appropriate aeration time was 150 min.



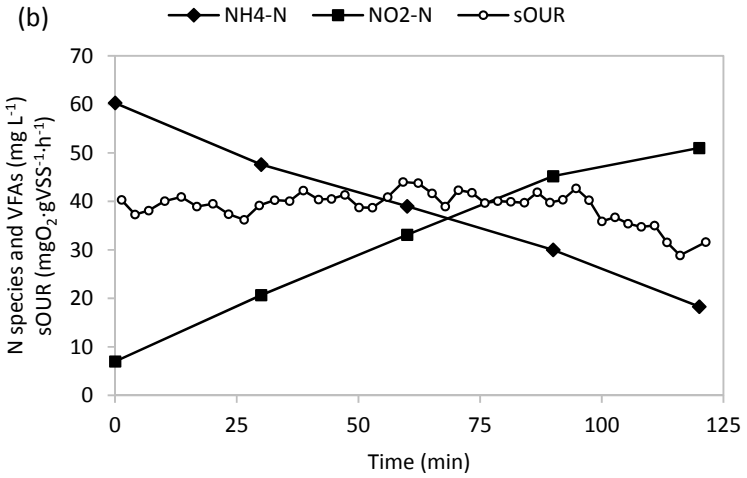


Figure 8.3. Ex-situ test with OFMSW FL (a) and without carbon source (b) under complete aerobic conditions

During Period I, the percentages of nitrogen removal improved until complete N/DN driven by the stored PHA was achieved. In particular, nitrite removal increased from 57% to 98% in 40 days of operation. The cycle analysis of an in-situ test is shown in **Figure 8.4**. Since lower amount of VFA were added compared with the ex-situ test, the lag phase was observed for only 10 min. After this lag phase the sAUR obtained was 3.3 $mgNH_4\text{-}N\cdot(gVSS\cdot h)^{-1}$, much lower than in the ex-situ tests shown in **Figure 8.3** because the initial amount of ammonium was considerably lower in the scSBR. Aerobic conditions were maintained for 160 min until nitritation was almost completed. PHA was accumulated to a maximum quantity of 5.5% coinciding with the depletion of VFA. The amount of PHA decreased during nitritation due to the lack of available COD. However, after complete nitritation, there was enough PHA to carry out the denitritation step with a sNUR of 9.36 $mgNO_2\text{-}N\cdot(gVSS\cdot h)^{-1}$. The overall nitrogen removal was 98% obtaining an effluent with only 0.8 $mgNH_4\text{-}N\cdot L^{-1}$. Similar results were obtained by Morgan-Sagastume et al. (2010b) that reached a 4% of PHA stored using fermented sludge as carbon source. However, after the optimisation of the SBR under complete aerobic conditions and a high OLR of 6 $kgCOD\cdot m^{-3}\cdot d^{-1}$, they obtained around 20% of PHA.

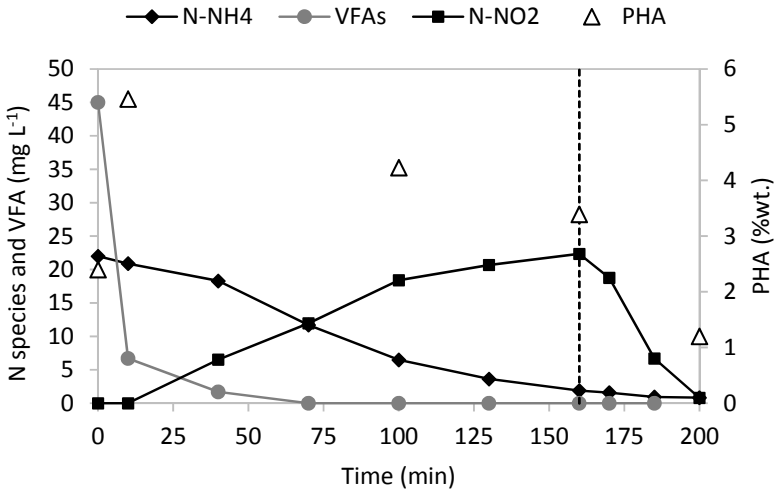


Figure 8.4. In-situ test with OFMSW FL achieving N/DN driven by stored PHA

The ratio feast and famine was calculated based on the time of VFA removal, thus feast lasted 40 min and famine 160 min obtaining a ratio of 0.25. PHA storing biomass selection was favoured by applying feast and famine ratios below 0.6 (Albuquerque et al., 2010), thus in this case the selection was promoted by the adequate transient conditions.

8.2.6. Simultaneous N/DN and PHA storing biomass selection using PS & OFMSW FL as carbon source - Period II

The pilot scale scSBR was operated with PS & OFMSW FL as carbon source (Period II). A cycle profile is shown in **Figure 8.5**. It can be noted that nitritation started after 40 min lag phase, when both VFAs and a major part of the sCOD were depleted. Since biomass was rich in PHA storing bacteria, PHA was rapidly accumulated up to 6.2%; and it was progressively consumed before the anoxic phase up to 2.3%, which was enough to complete the subsequent denitritation. The profile of sOUR indicated that sCOD was oxidised during the first 40 min, although PHA stored did not increased. Therefore, non-VFA COD did not to contribute to PHA storage. Albuquerque et al. (2010) demonstrated that the residual sCOD favoured the growth of non-storing biomass that consumed this COD fraction during famine phase, resulting in a loss of PHA storage capacity.

The feast and famine ratio calculated based on VFA profile resulted in 0.05, considering that in 10 min VFA were consumed followed by 190 min under famine conditions. Nevertheless, the non-VFA COD present in the system resulted in a higher sOUR around 40 $mgO_2 \cdot (gVSS \cdot h)^-$

[1] due to its oxidation. After that, sOUR decreased to 10 $mgO_2 \cdot (gVSS \cdot h)^{-1}$ corresponding to the nitritation demand. Calculating the feast and famine ratio based on sCOD it resulted 0.25, which was also adequate to favour the selection of biomass.

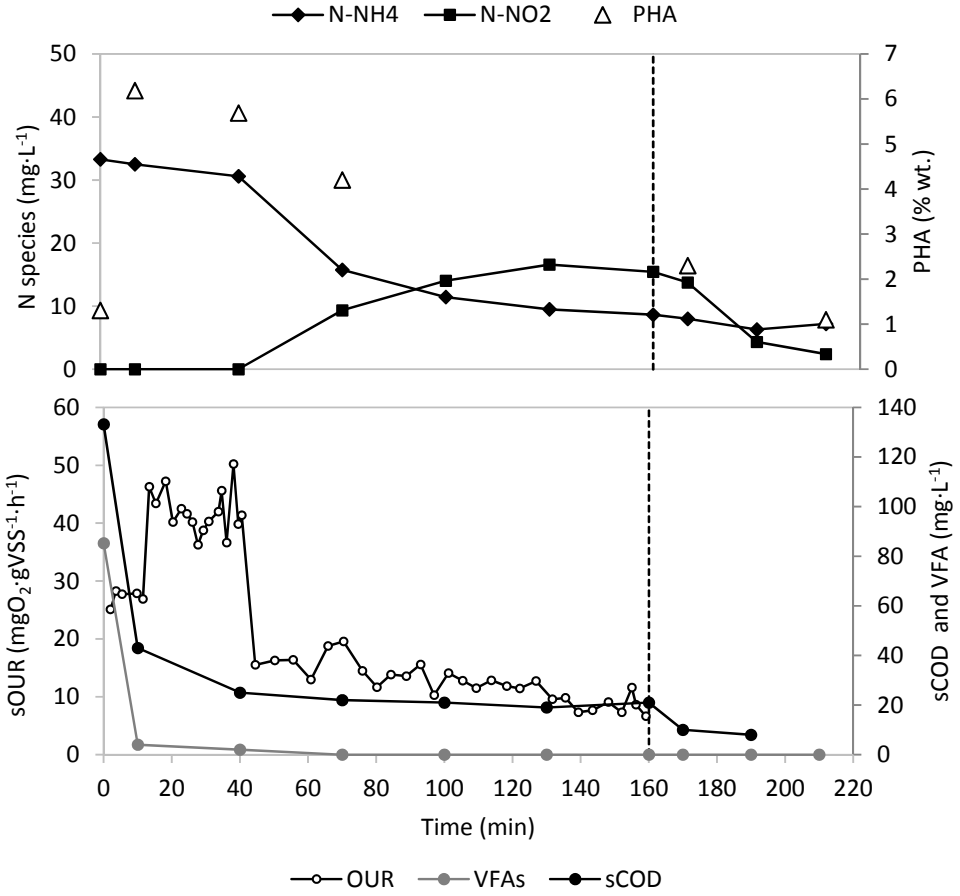


Figure 8.5. In-situ test with PS & OFMSW FL achieving N/DN driven by stored PHA

During the cycle presented in **Figure 8.5**, the overall nitrogen removal was 84%, obtaining an effluent with 7.2 $mgNH_4\text{-}N \cdot L^{-1}$ and 2.4 $mgNO_2\text{-}N \cdot L^{-1}$. The sAUR observed during the first 40 min was 1.7 $mgNH_4\text{-}N \cdot (gVSS \cdot h)^{-1}$ and right after it increased to 3.4 $mgNH_4\text{-}N \cdot (gVSS \cdot h)^{-1}$. In this Period II, the removal of ammonium was 88%, lower than in Period I (93%). Nitritation was clearly affected by the presence of non-VFA COD that promoted a longer lag phase, thus ammonium was not completely oxidised as in the previous period. However, an increase of the aerobic phase length would lead to a loss of PHA available for the anoxic phase. The nitrite removal was 84% with a sNUR of 8.38 $mgNO_2\text{-}N \cdot (gVSS \cdot h)^{-1}$.

8.2.7. Energy demand for the innovative and conventional treatment scheme

The energy requirements of the SBR applying the aerobic (feast) – anoxic (famine) scheme were estimated and compared with the typical aerobic feast and famine regime for the selection of the PHA storing biomass (Table 8.5). The innovative process that was examined in the current study – system 1, exhibited 25% higher air demand compared to system 2, where only nitrogen removal is performed in the SBR. However, in 'system 1' only one stage reactor is required for both processes that take place in two separate reactors in case 2 (nitrogen removal) and case 3 (selection of PHA storing biomass). Thus, 'our proposed scheme' results in ≈50% less air demand compared to the conventional process that is widely applied. Additionally, the selection of PHA storing biomass under feast (aerobic) / famine (anoxic) conditions requires less air compared to the typical feast / famine regime carried out under continuous aerobic conditions.

Table 8.5. Air demand for alternative schemes applied for N removal and the selection of PHA storing biomass within the WWTP

Parameter	System 1 'Our process' Nitrogen removal and PHA selection in one single reactor[a]	System 2 SBR for nitrogen removal nitritation/denitritation[b]	System 3 SBR for the selection of PHA storing biomass[c]
Time of aerobic period/ cycle (h)	2.67	2.67	3.5
Time of feast aerobic/cycle (h)	0.4-0.5	-	-
Aerobic feast period / cycle (%)	10 – 12	-	10 – 15
Time of anoxic phase (h)	0.83	0.83	-
MLVSS (g L^{-1})	2.5	2.5	2.5
Energy (kWh $m^{-3}d^{-1}$)	**0.42**	**0.32**	**0.56**

[a]**System 1:** Our process: SBR for nitrogen removal with nitritation and denitritation using the PHA produced from the aerobic feast phase.

[b]**System 2:** SBR for nitrogen removal with nitritation/denitritation. The carbon source is fed in the beginning of the anoxic phase.

[c]**System 3:** SBR for the selection of PHA storing biomass. The SBR operates with the feast and famine regime during aerobic conditions.

8.2.8. PHA accumulation with different carbon sources

The PHA accumulation capacity of the biomass, which had been previously selected in the pilot scale SBR, was evaluated in lab-scale accumulation batch tests that were performed with the use of OFMSW FL and PS & OFMSW FL as carbon sources. The efficiency of PHA accumulation is higher when the media and the carbon source are free of nutrients that promote the biomass growth. The fermented liquids contained significant concentration of nutrients. For this reason, the COD/N/P ratio was considered. The OFMSW FL and the PS & OFMSW FL had a COD/N/P ratio of 100/4.5/0.42 and 100/5.5/1.2, respectively.

During the accumulation test sOUR, VFA and PHA were monitored. When sOUR decreased, around 1 $g \cdot L^{-1}$ of carbon source was spiked. After 10 h of accumulation with OFMSW FL, the stored PHA was 10.6 (%wt.). The VFA depletion rate was $-qVFA=88.64$ $mgCOD \cdot mgCOD^{-1} \cdot h^{-1}$ and the PHA storing rate was $qPHA=19.87$ $mgCOD \cdot mgCOD^{-1} \cdot h^{-1}$. The PHA yield based on VFA consumed was $Y_{PHA/VFA}=0.22$ ($mgCOD \cdot mg^{-1}COD$). The total amount of oxygen consumed can be calculated by the area under the sOUR slope, obtaining an oxidation yield of $Y_{OX/VFA}=0.42$. The remaining of VFAs contributed to biomass growth; thus the growth yield was $Y_{X/VFA}=0.35$. However, the same yields can be calculated based on total COD removed, resulting in $Y_{PHA/COD}=0.08$, $Y_{OX/COD}=0.15$ and $Y_{X/COD}=0.78$. Those yields were lower because not all the COD was in form of VFA, the ratio VFA/COD was 0.35, thus the non-VFA COD was found to mainly contribute to the biomass growth, as shown in Figure 8.6a.

In the case of PS & OFMSW FL, the yields based on total COD were $Y_{PHA/COD}=0.003$, $Y_{OX/COD}=0.047$ and $Y_{X/COD}=0.95$. The ratio of VFA/COD was 0.30, thus the yields based on VFA were $Y_{PHA/VFA}=0.011$, which was lower than the yield obtained using OFMSW FL, $Y_{OX/VFA}=0.16$ and $Y_{X/VFA}=0.83$. In the case of PS & OFMSW, the contribution to growth was much more significant (Figure 8.6b), due to the higher nutrient content and the lower VFA/COD ratio. After 10 h of accumulation with PS & OFMSW FL, the stored PHA was 8.6 (%wt.). The VFA depletion rate was $-qVFA=94.1$ $mgCOD \cdot mgCOD^{-1} \cdot h^{-1}$ and the PHA storing rate was $qPHA=10.51$ $mgCOD \cdot mgCOD^{-1} \cdot h^{-1}$.

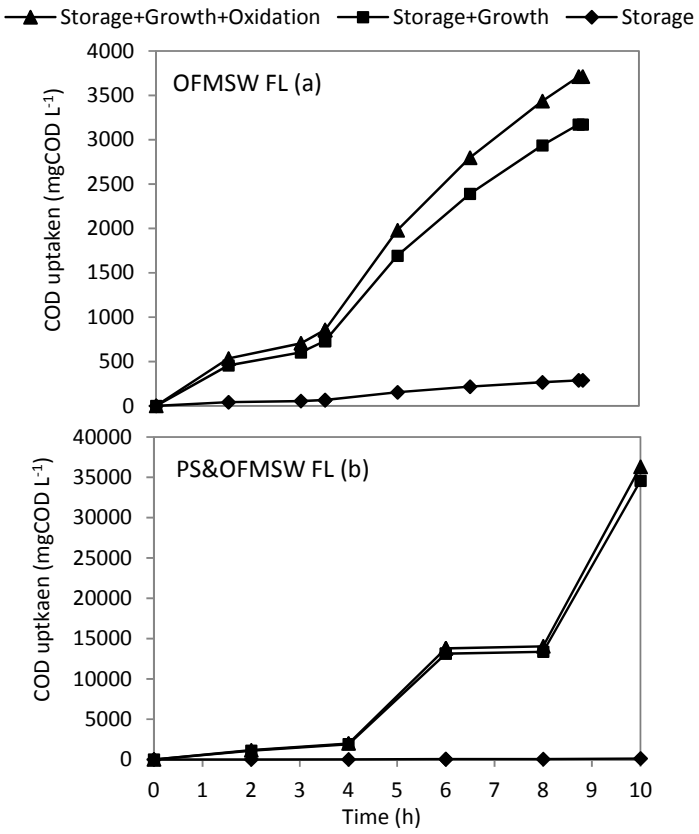


Figure 8.6. Contribution of PHA storage, growth and oxidation based on total COD of (a) OFMSW FL and (b) PS & OFMSW FL

8.2.9. The role of the carbon source

The carbon source played an important role in the scSBR performance as well as in the accumulation batch. Several studies have determined the drawbacks of using fermented wastes for PHA production attributed to the presence of nutrients and non-VFA COD. The non-VFA COD can contribute to the growth of non-PHA-storing biomass; as extra substrate for PHA storing organisms during famine phase; and/or as storage substrate but in terms of polysaccharides (Morgan-Sagastume et al., 2010b).

When OFMSW FL was used in the scSBR, nitritation suffered a lag phase due to the oxidation of VFA. As it is shown in **Figure 8.4**, the sAUR increased when VFA are depleted, but a clear effect of the non-VFA COD was not detected. In contrast, when PS & OFMSW FL was used, nitritation started when the major part of the COD was removed after 40 min

(**Figure 8.5**) and not only when VFA were depleted after 10 min. This fact was also confirmed by the sOUR profile, which reached the maximum values during the first 40 min corresponding to the COD removal. Afterwards, sOUR decreased to the values related to nitritation oxygen demand.

The PHA storing rate (qPHA) obtained in the scSBR was 9.77 mg $COD \cdot gCOD^{-1} \cdot h^{-1}$ with OFMSW FL as carbon source. However, when the mixture of PS & OFMSW FL was added in Period II, qPHA slightly decreased to 7.73 mg $COD \cdot gCOD^{-1} \cdot h^{-1}$. Higher rates were obtained in the accumulation batch, although it was higher when OFMSW FL was used (qPHA=19.87 $mgCOD \cdot mgCOD^{-1} \cdot h^{-1}$) than with PS & OFMSW FL (qPHA=10.51 $mgCOD \cdot mgCOD^{-1} \cdot h^{-1}$). The higher content of non-VFA COD in the PS & OFMSW FL promoted not only lower storage yields but lower storage rates.

Despite the carbon affected the storage yield and promoted longer aeration periods to achieve acceptable nitritation, denitritation efficiency and especially sNUR was not affected by the carbon source. If there was enough PHA, denitritation was completed with sNUR around 9.4 $mgNO_2\text{-}N \cdot (gVSS \cdot h)^{-1}$ in both periods.

In the accumulation tests the biomass growth was favoured over PHA storage, even when the biomass had the required storing capacity. Although the fermentation liquid was obtained by centrifugation, the solid amount was 5.5 $gTS \cdot kg^{-1}$ OFMSW FL and 18 $gTS \cdot kg^{-1}$ PS & OFMSW FL. During the batch accumulation tests, the amount of fermentation liquid added corresponded to the 50% of the initial volume. Therefore, the effect of the particulate organic matter was more significant than in the scSBR performance, which led to a loss of PHA accumulation capacity. PHA storage yields could be potentially improved with a more efficient solid-liquid separation after the fermentation process. Percentages up to 52% of PHA can be reached within only 4h batch test, using centrifuged fermented sludge as carbon source with less than 1 $gTSS \cdot L^{-1}$ (Morgan-Sagastume et al., 2014). Therefore, the reduction of the non-VFA COD would result in an increase of the PHA storage yield.

8.3. CONCLUSIONS

- A novel process was developed that integrates the selection of PHA storing biomass, and nitrogen removal via nitrite in one stage SBR treating municipal wastewater. Denitrification was accomplished through the internally stored PHA. The rates were higher than the ones attributed to endogenous respiration, but lower than the respective sNUR accomplished with the addition of fermentation liquid during the anoxic reaction phase.
- The increased competition between the heterotrophic and autotrophic bacteria in the aerobic phase for the DO in the presence of VFA is a drawback of the examined scheme, resulting in low sAUR.
- Comparing the efficiency of the examined carbon sources, the use of OFMSW FL resulted in enhanced PHA accumulation. However, the presence of non-VFA COD results in a loss in the PHA accumulation capacity of sludge, both in the SBR and in the batch accumulation step.
- Although higher PHA yields can be achieved under complete aerobic conditions, this novel scheme presents an added value due to the integration of the PHA production in the nitritation/denitritation process.

9. Start-up and operation of a two-step PN– Anammox SBR for reject water treatment

Abstract

A two-step partial nitritation (PN)/Anammox process was carried out at lab-scale conditions to treat reject water of a municipal WWTP. PN was achieved in a granular SBR obtaining an effluent with a NH_4^+-N/NO_2^--N molar ratio around 1.0. The microbial characterization of this reactor revealed a predominance of Betaproteobacteria, with a member of Nitrosomonas as the main autotrophic ammonium oxidizing bacteria (AOB). Nitrite oxidizing bacteria (NOB) were under the detection limit of 16S rRNA gene pyrosequencing, indicating their effective inhibition. The effluent of the PN reactor was fed to an Anammox SBR where stable operation was achieved with an observed NH_4^+-N:NO_2^--N:NO_3^--N stoichiometry of 1:1.25:0.14. The slight deviation to the theoretical stoichiometry could be attributed to the presence of heterotrophic biomass in the Anammox reactor (mainly members of Chlorobi and Chloroflexi). Planctomycetes accounted for 7.9% of the global community, being members of Brocadia the main anaerobic ammonium oxidizer detected.

This chapter was presented as a poster communication in:

- Nitrogen removal of sewage sludge anaerobic digestion supernatant by partial nitritation/Anammox. *2nd IWA Specialized International Conference "Ecotechnologies for Wastewater Treatment (EcoSTP2014)",* Verona, Italy, 23rd – 25th June 2014.

And then submitted for publication as:

J. Dosta, J. Vila, I. Sancho, N. Basset, M. Grifoll, J. Mata-Álvarez (2015). T**wo-step partial nitritation/Anammox process in granulation reactors: start-up operation and microbial characterization.**

9.1. INTRODUCTION

The supernatant of anaerobically digested sludge (reject water) contains the 10-30% of the total nitrogen load in a flow, and it is usually returned to the head of the sewage treatment works (Fux and Siegrist, 2004). A feasible treatment of this highly ammonium loaded wastewater with very low biodegradable COD is the Anammox (ANaerobic AMMonium OXidation) process, combined with a previous step of partial nitritation (PN) where total ammonium nitrogen should be oxidized for about 50% to NO_2^--N. The Anammox process converts ammonium together with nitrite (electron acceptor) directly to dinitrogen gas under anoxic conditions in the absence of any organic carbon source. If catabolism and anabolism of Anammox biomass are considered, the overall stoichiometric consumed ammonium:consumed nitrite:produced nitrate on molar basis is 1:1.32:0.26 (Lotti et al., 2014; Van Dongen et al., 2001). When compared to conventional biological nitrogen removal process, the PN - Anammox process avoids the requirement of organic carbon source to denitrify, produces about 85% less of sludge and allows saving around 60% of the oxygen supply, thus reducing energy requirements (Fux and Siegrist, 2004). Anammox biomass can be inhibited by nitrite concentration, temperature, pH, visible light exposure, dissolved oxygen (DO), organic matter, among many others (Jin et al., 2012). Therefore, careful control of the Anammox process is required since it becomes easily unstable.

The PN – Anammox process can be performed in 2-stage or single-stage reactors. In the 2-stage reactors configuration the processes can be optimized individually, leading to a lower risk for Anammox bacteria to be overgrown by heterotrophs and/or to be inhibited by dissolved oxygen. However, the 1-stage configuration allows important savings in equipment, lower risk of NO_2^--N inhibition and produces less N_2O emissions (Desloover et al., 2012; Lotti et al., 2014; Third et al., 2001).

The microbial communities dealing with ammonium oxidation have been described for both, independent PN (Ahn et al., 2011; Ganigué et al., 2009; Qiao et al., 2010) and Anammox reactors (Cho et al., 2010; Li et al., 2009; Park et al., 2010; Qiao et al., 2008), and single-stage PN - Anammox processes (Cho et al., 2011; Vlaeminck et al., 2009). In the partial nitritation process, members of *Nitrosomonas* are generally present, often being predominant components of the community and working under a wide range of NH_4^+-N concentrations (Ganigué et al., 2009; Qiao et al., 2010). Regarding the microbial communities present in Anammox reactors, their study has been mainly addressed using synthetic wastewater, and generally reveals a coexistence of members of *Chlorobi*, *Cloroflexi* and *Bacteroidetes* accompanying a number of *Planctomycetes*, including Anammox bacteria (Cho et al., 2010; Qiao et al., 2008). However, the microbial community

composition of two-stage PN-Anammox reactors treating actual supernatant of anaerobically digested sludge has been scarcely addressed (Yamamoto et al., 2011). In addition, the detection of Anammox bacteria is generally based on the utilization of selective detection tools, such as *Planctomycetes*-specific primers or probes, precluding establishing their actual preponderance within the global microbial community. The utilization of high throughput sequencing of 16S rRNA gene libraries allows now obtaining a whole view of the microbial community in a single analysis.

In this study we aim to evaluate the viability of a coupled two-step partial nitritation plus Anammox process using granular biomass in SBRs for the treatment of the supernatant from anaerobic digestion of sewage sludge. For a better understanding of the underlying biological processes, the microbial communities present in both reactors are also characterized in depth by means of direct observation of the granules by FISH and the application of 16S rRNA gene fingerprinting and high throughput sequencing methods.

9.2. RESULTS AND DISCUSSION

9.2.1. Experimental set-up and substrate

The study of the partial nitritation of the supernatant from anaerobic digestion of sewage sludge was carried out in a lab-scale granular sequencing batch reactor (described in section 3.1.4). Operating temperature was controlled by a heating system (RM6 Lauda). A Programmable Logic Controller (PLC, LOGO SIEMENS 230RC) was usually used to control the length of the different stages of the operational cycles. This reactor was equipped with a pH electrode and ORP electrodes (Crison pH 28) and a dissolved oxygen probe (Oxi 340i, WTW). The 4-20 mA signals of the probes were collected and logged on a PC equipped with the Advantech AdamView software package. Fill and discharge stages were performed by peristaltic pumps. The air flow inside the reactor was supplied by several air blowers (Rena Air 300) connected to a sparging system.

The Anammox process was carried out in a lab-scale SBR of 5 L (described in section 3.1.4), since it has been reported that good mixing and high nitrogen elimination rates can be easily achieved in sequencing batch reactors with suspended or granulated biomass (Strous et al., 1998). The operating temperature was maintained at 33ºC using a thermostatic bath (Haake DC 30). Mixing was provided by means of a mechanical stirrer (IKA Werke RW 16 basic) working at about 120 rpm. Fill and draw stages were performed by two peristaltic pumps (Ismatec Reglo and P-Selecta Percom N-M, respectively). The reactor was continuously flushed with nitrogen to maintain anaerobic conditions and to give some excess pressure to the reactor in order to avoid the entrance of oxygen. Neoprene tubing

and connections were used in order to minimize the diffusion of oxygen. Moreover, reactor was covered in order to avoid light incidence. Operational cycles were controlled with a PLC Siemens 230-RC.

The supernatant from anaerobic digestion of sewage sludge (sludge reject water) was collected in a municipal wastewater treatment plant of the Barcelona Metropolitan Area and stored at 5ºC until its use. Table 9.1 shows the main characteristics of this wastewater.

Table 9.1. Reject water characteristics

Parameter	Value	Units
NH_4^+-N	0.74±0.23	g NH_4^+-N L^{-1}
NO_2^--N	n.d.	g NO_2^--N L^{-1}
NO_3^--N	n.d.	g NO_3^--N L^{-1}
Soluble COD	0.34±0.08	g COD L^{-1}
pH	7.8±0.2	-
TS	2.76±0.12	g TS L^{-1}
TSS	0.04±0.01	g TSS L^{-1}
VSS	0.08±0.02	g VSS L^{-1}
Alkalinity/NH_4^+-N	1.08±0.12	mole HCO_3^- (mole NH_4^+-N)$^{-1}$

The PN reactor was inoculated with active granular biomass from a laboratory scale SBR treating reject water at steady state (S López-Palau et al., 2011). The Anammox reactor was inoculated with granular biomass from an Anammox pilot plant in the Netherlands that was preserved at 5ºC until its use. To start up the process, the reactor was fed with synthetic wastewater having the composition described by Van de Graaf et al. (1996). Once PN was stable and the nitrogen load in the effluent was suitable to biomass according to the results obtained in specific Anammox activity test, the reactor was used to treat the effluent from the PN process.

9.2.2. Partial nitritation step

The partial nitritation reactor was set up with 4.1 g VSS/L of nitrifying granules of the reactor described in López-Palau et al. (2011). At the beginning of this study, reject water was diluted to reach approximately 500 mg NH_4^+-N L^{-1}. Then, the concentration in the feed was progressively increased until undiluted reject water was treated (from day 60 on). Figure 9.1 shows the evolution of influent and effluent concentrations of NH_4^+-N, NO_2^--N and NO_3^--N, during the whole operational period. As further discussed in López-Palau et al.

(2011), nitrite route was achieved due to free ammonia concentration during the operational cycle. Ammonium oxidation to nitrite remained at 50±6.4% thanks to the alkalinity to NH_4^+-N molar ratio of the reject water, therefore the effluent obtained was suitable for a subsequent Anammox step. However, the DO concentration in the obtained effluent, which was around 6.5 mg O_2 L^{-1}, was removed by flushing nitrogen and the pH, which was below 6.0, was regulated with NaOH near neutrality (7.2-7.4), before treating the PN effluent in the Anammox reactor.

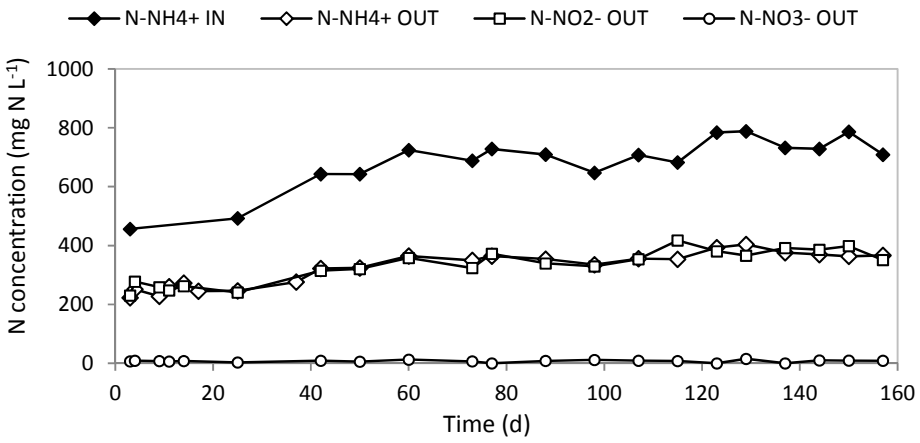


Figure 9.1. Evolution of nitrogen concentration in PN reactor.

When treating undiluted reject water under steady state conditions, pH decreased during the nitritation reaction and the ORP increased during this period of time up to approximately 440 mV. When a pH value near 6.0 was reached, nitritation activity ceased which lead to a rise of the DO concentration. This rise was located at 2.7 hours from the beginning of the cycle, therefore a maximal theoretical ammonium uptake rate of 3.1 kgN m^{-3} d^{-1} could be reached.

9.2.3. Anammox operation

Figure 9.2 shows the applied and removed nitrogen loading rate (NLR), the effluent nitrogen composition and the comparison of specific NLR and the maximum SAA of the Anammox reactor for the whole experimental period. To avoid the inhibition of Anammox biomass, the following criteria were adopted in the operation of the Anammox reactor: the specific NLR should not exceed the maximum SAA and NO_2^--N should not be detected in the effluent. When these criteria were not met, the influent was diluted and then, progressively concentrated.

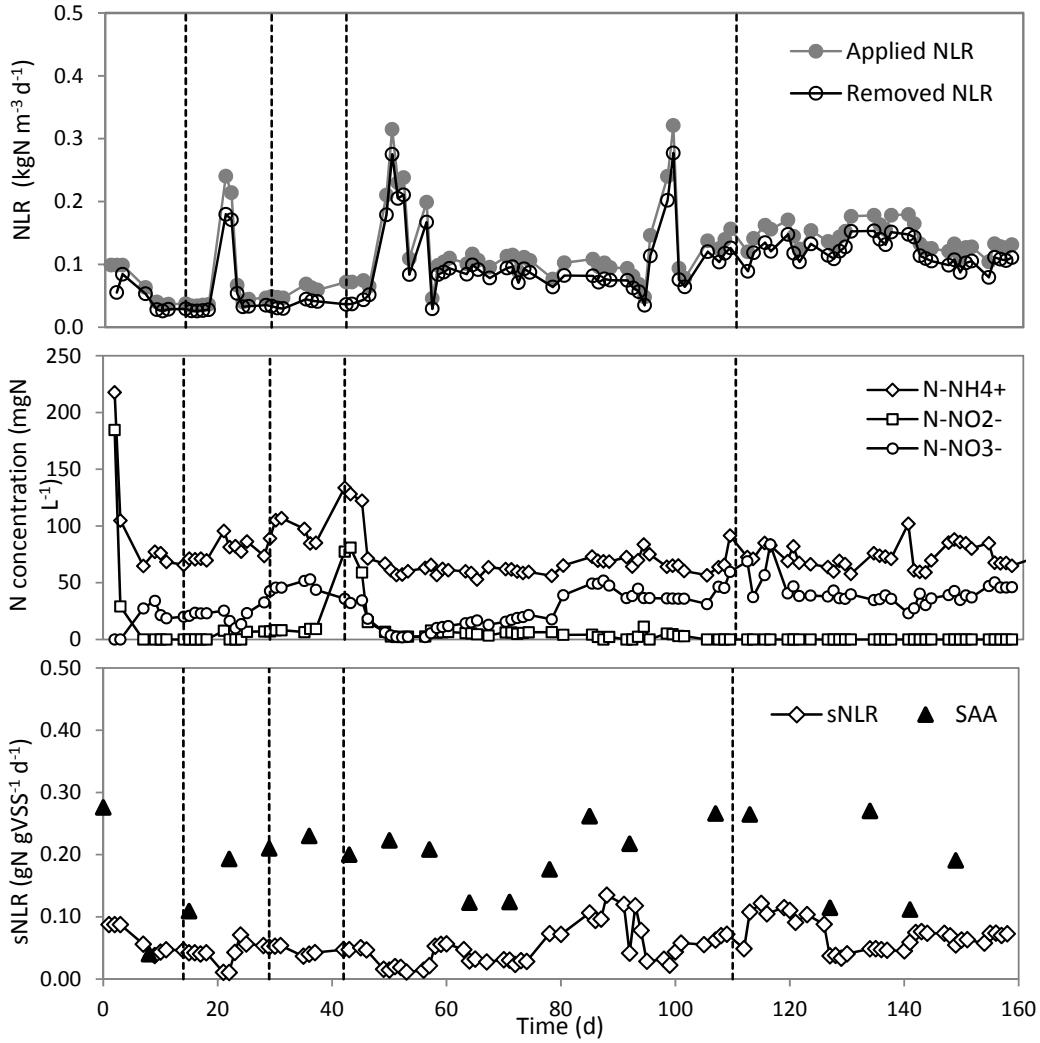


Figure 9.2. Applied and removed nitrogen loading rate (NLR) (a), the effluent nitrogen composition (b) and comparison of specific NLR and the maximum SAA (c) of the Anammox reactor for the whole experimental period (P1: with synthetic wastewater, P2 and P4: with diluted PN effluent; P3 and P%: with undiluted PN effluent).

In a first stage (1-13 days), the Anammox reactor was started up with synthetic wastewater having the composition described by Van de Graaf et al. (1996) with a NH_4^+-N concentration of 500 mg NH_4^+-N L^{-1}. At day 11, the influent NH_4^+-N concentration was reduced to 200 mg NH_4^+-N L^{-1} since the initial one was too high according to the activity of biomass. At day 14, synthetic wastewater was substituted by diluted PN effluent in order to maintain the influent NH_4^+-N concentration and it was gradually increased up to 370 mg NH_4^+-N L^{-1}.

During days 28 to 38, the observed Anammox stoichiometry (consumed NH_4^+-N:consumed NO_2-N:produced NO_3^--N) was 1:1.32:0.16 , which is a ratio very close to the theoretical Anammox stoichiometry reported by Van Dongen et al. (2001).

However, on day 39, a failure of the heating system lead to a decrease in the working temperature, which negatively affected Anammox performance and promote the rise of NO_2^-concentrations up to 81 mg NO_2^--N L^{-1}. The influent was then diluted to reach 100 mg NH_4^+-N L^{-1} and NO_2^- concentrations in the effluent were not detected. However, from day 40, Anammox granules evolved from reddish to brownish color and the formation of nitrate decreased up to concentration values around 1.5 mg NO_3^- -N L^{-1}, indicating an observed stoichiometry of NH_4^+-N:NO_2^--N:NO_3^--N far from the theoretical one. This could be explained by the co-existence of heterotrophic bacteria inside the reactor, carrying out conventional denitrification, using biodegradable COD from the decay of some Anammox bacteria.

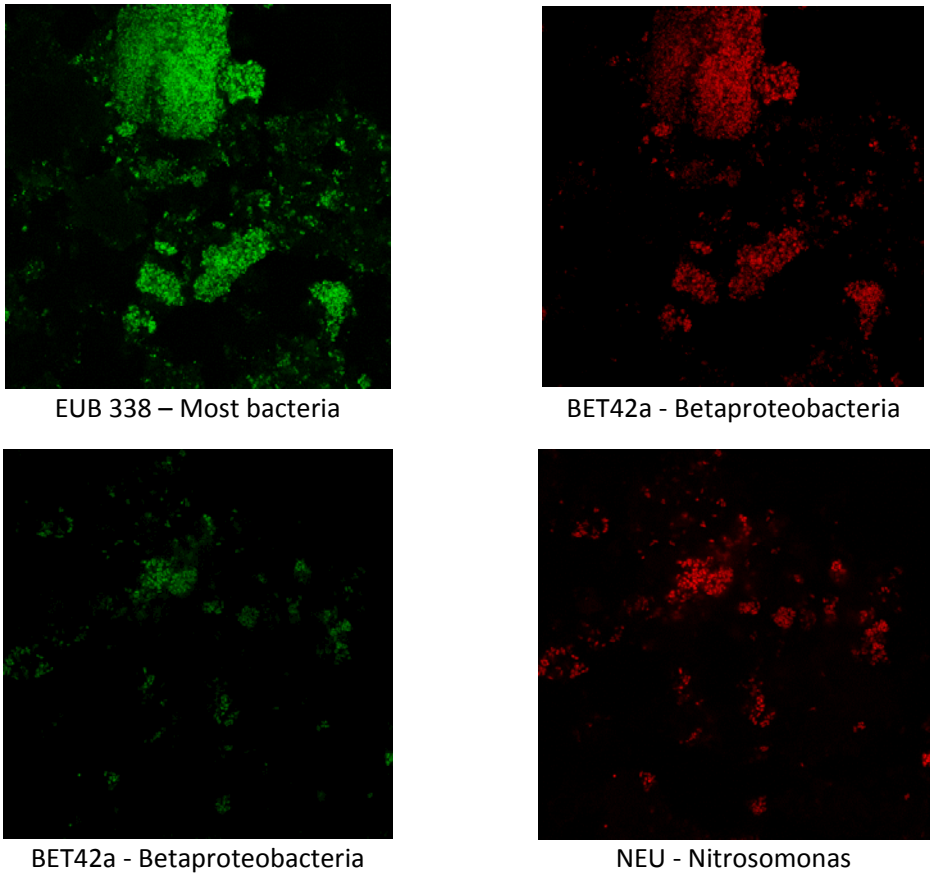
From day 56, nitrate profile started to increase again. The Anammox process efficiency was slowly recovered. Nitrogen loading rate was modulated based on SAA and effluent NO_2^--N concentration. From day 78 on, the filling time was shortened to 40 min in order to promote a higher Anammox activity due to higher initial NH_4^+-N and NO_2^--N concentrations (López et al., 2008). At day 110, undiluted PN effluent (≈370 mg NH_4^+-N L^{-1}) was treated in the Anammox reactor obtaining a steady performance and reaching a constant value around 40 mg NO_3^- -N L^{-1} in the effluent, resulting in a NH_4^+-N:NO_2^--N:NO_3^--N stoichiometry of 1:1.25:0.14 which is close to the theoretical one. As observed in Figure 9.2c, the SAA was in the range of the values reported in literature (a. Dapena-Mora et al., 2007), suggesting the potential for the nitrogen loading rate increase with time. Globally, the combined PN and Anammox treatment lead to a maximum NH_4^+-N removal efficiency up to 91.9 % and to a total nitrogen removal up to 88.1%.

9.2.4. Fluorescent In Situ Hybridization (FISH) analysis

Fluorescence in Situ Hybridisation (FISH) using a set of fluorescent-labelled 16 rRNA-targeted probes was applied according to the procedure described by Amann et al. (1990). The granules from the PN reactor were analysed using the following specific oligonucleotide probes: EUB338, specific for most bacteria; ALF1b, specific for *Alphaproteobacteria*, some *Deltaproteobacteria* and *Spirochaetes*; BET42a, specific for *Betaproteobacteria*; GAM42a, specific for *Gammaproteobacteria*; NEU, specific for *Nitrosomonas spp.*; and NSV443, specific for *Nitrosospira spp*. Probes BET42a, GAM42a and NEU were used in a 1:1 ratio together with their specific probe competitors. For Anammox biomass, FISH analysis was hampered by inefficient sample fixation and, as reported by other authors, the high levels of autofluorescence and strong clustering of cells in the granules (Quan et al., 2008). Details on

oligonucleotide probes are available at probeBase (http://www.microbial-ecology.net/probebase/).

FISH analyses were carried out in order to study the presence of specific taxonomic groups of interest. For the PN reactor, probes were applied in hierarchical order from more general (class level) to more specific ones (genus level). A first analysis was performed for different classes of *Proteobacteria* (*Alpha, Beta* and *Gamma* sub groups) showing the predominance of members of the *Betaproteobacteria* and a minor representation of *Alphaproteobacteria* (**Figure 9.3**a). *Gammaproteobacteria* were not detected. Considering these results, specific probes for the detection of the betaproteobacterial ammonium oxidizing bacteria *Nitrosomonas* spp. and *Nitrosospira* spp. were tested, being the members of *Nitrosomonas* (**Figure 9.3**b) the main autotrophic group in this reactor.



EUB 338 – Most bacteria

BET42a - Betaproteobacteria

BET42a - Betaproteobacteria

NEU - Nitrosomonas

Figure 9.3. FISH analysis of two representative samples (a and b) corresponding to the PN granules using probes targeting total *Eubacteria* (EUB338), *Betaproteobacteria* (BET42a) and members of *Nitrosomonas* (NEU).

9.2.5. PCR-DGGE (polymerase chain reaction - denaturing gradient gel electrophoresis) analysis

After 148 days of operation, samples from both the PN and Anammox reactors were taken to analyse the microbial community structure of these systems. The DGGE fingerprints from the three replicates of each reactor showed nearly identical profiles, with a total of 11 and 8 major bands being detected in the PN and Anammox reactors, respectively (

Figure 9.4), which was consistent with the presence of highly homogeneous microbial communities. It is interesting to note that none of the bands present in the PN reactor was detected in the Anammox SBR, indicating the effective biomass retention of the initial nitrification step.

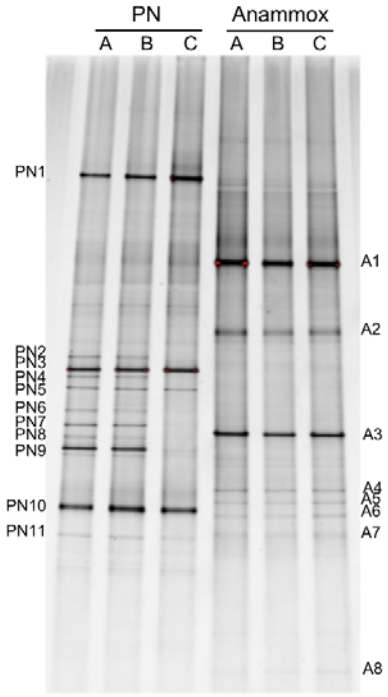


Figure 9.4. DGGE analysis of the eubacterial microbial community present in three replicate samples (A, B and C) of the PN and Anammox reactors

Sequence analysis of the recovered bands from PN reactor revealed that the microbial community present was clearly dominated by members of the *Betaproteobacteria* (5 out of 7 sequenced bands). Band PN1, one of the predominant bands in the profiles, corresponded

to members of *Nitrosomonas* closely related to *N. eutropha*, a well-known AOB. This, together to the fact that NOB such as *Nitrobacter* and *Nitrospira* were absent, confirms that the microbial community present was consistent with that expected in a partial nitrification reactor. The remaining *Betaproteobacteria* detected were assigned to the genus *Achromobacter* (bands PN2, PN7 and PN9) and *Comamonas* (PN3), both associated to heterotrophic ammonium oxidation (Chen and Ni, 2011; Kundu et al., 2012). In addition, the closest relative to the detected members of *Comamonas* was *C. badia*, a floc-forming bacterium isolated from sewage sludge with aggregating capacity (Tago and Yokota, 2004), suggesting their role in granule formation.

DGGE profiles of the anaerobic ammonium-oxidizing reactor showed four major bands that could be assigned to members of *Chlorobi* (*Ignavibacterium*, A1), candidate division TM7 (A2), *Chloroflexi* (fam, *Anaerolineaceae*, A3) and *Acidobacteria* (A7). Interestingly, no bands related to Anammox bacteria could be detected, which could be attributed to their low relative abundance. In fact, when conventional eubacterial PCR primers are used, it is estimated that phylotypes that constitute ≤9% of a complex bacterial community may be under the limit of detection of this technique (Straub and Buchholz-Cleven, 1998), suggesting the need for a higher resolution technique to gain further insight in the rare microbial diversity.

9.2.6. Analysis of microbial community structure by 16S rDNA pyrosequencing

Barcoded pyrosequencing was applied to analyse in depth hardly represented components of both reactors. A subdivision into operational taxonomic units (OTUs) sharing 97% identity was performed and a total of 681 different OTUs were detected, 418 and 389 in the PN and Anammox reactors, respectively. More than 80% of those phylotypes were only distantly related to previously identified species (<97% of sequence similarity), and the majority of them (69.3%) were scarcely represented (<0.1% of abundance).

Within the PN reactor members of fifteen different bacterial phyla were detected: *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes*, *Proteobacteria* (*Alpha*, *Beta*, *Delta* and *Gamma* subdivisions), *Spirochaetes*, *Tenericutes*, *Verrucomicrobia* Despite the number of phyla and richness observed, the microbial community was clearly dominated by the members of *Proteobacteria* (89.7±1.6%), especially from the *Beta* (55.1±5.6%) and *Gamma* (28.3±4.3%) subgroups (**Figure 9.5**). The predominance of *Betaproteobacteria* is in accordance with the results from the DGGE analysis, being members of *Nitrosomonas* (18.3%), *Achromobacter* (17.9%) and *Comamonas* (13.9%) the main members of the group. *Nitrosomonas* was the major autotrophic AOB detected in the analysis, and only a few representatives of the

gammaproteobacterium *Nitrosococcus* (0.2%) were present in the PN libraries. This clear preponderance of members of *Nitrosomonas* within the autotrophic AOB could be explained, besides its high tolerance for O_2 and NO_2, by its high growth rate at high ammonia concentrations that outcompete other AOB less efficient in ammonium utilization, such as *Nitrosospira* or *Nitrosococcus* (Schramm et al., 2000). The *Gamma* subgroup of *Proteobacteria* was clearly dominated by different members of a unique genus, *Rhodanobacter* that accounted for about 25% of the total abundance. The high prevalence of the members of this genus was unexpected, as members of this group have not been described as ammonium oxidizers. However the description of several denitrifying strains within the genus, and the maximum similarity of the phylotypes detected here with denitrifiers isolated elsewhere (Green et al., 2012), suggests that their reducing activity could be favoured in the oxygen limiting concentrations achieved inside the granules of the PN reactor. Interestingly, even with this higher resolution technique NOB were not detected.

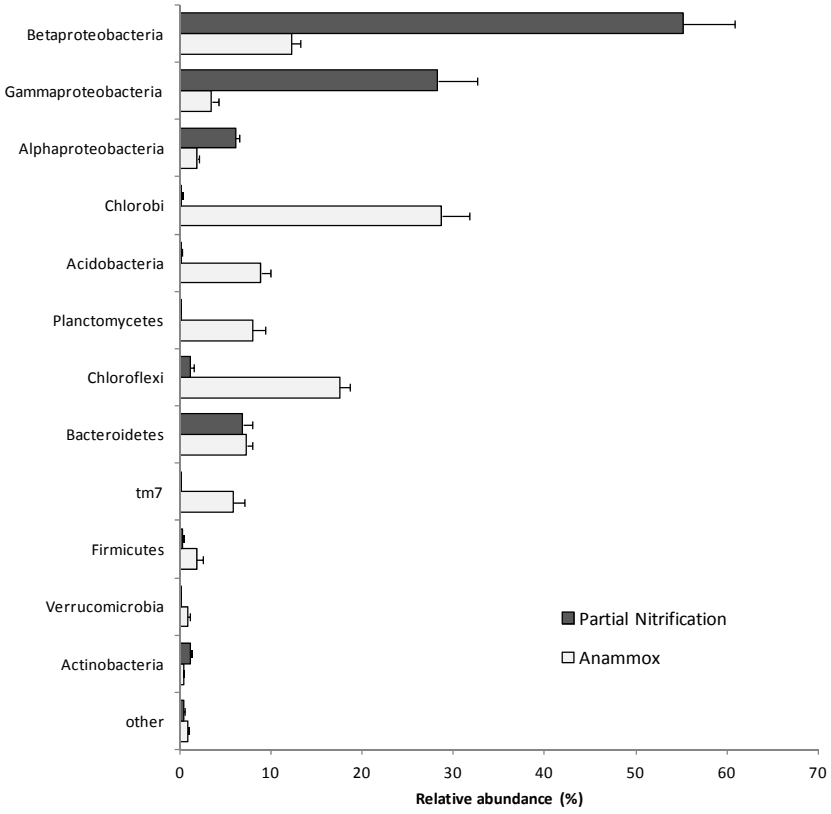


Figure 9.5. Relative abundance of the major phylotypes (>0.5%) detected in the 16S rRNA gene libraries analysed by tag-encoded pyrosequencing.

The microbial community composition from the Anammox biomass was completely different from that observed in the PN reactor (**Figure 9.5**). Interestingly, a directed analysis targeting the most relevant genus of the previous PN step revealed that they were nearly absent in the Anammox reactor, accounting for less than 2% of 16S rRNA gene libraries of the Anammox community [*Achromobacter* (0.07%), *Comamonas* (0.37%), *Nitrosomonas* (1.1%) and *Rhodanobacter* (0.07%)] and mainly corresponding to different phylotypes, thus confirming the effectiveness of the granular configuration in retaining PN biomass. Twenty four different phyla were detected including *Acidobacteria*, *Actinobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria* (*Alpha*, *Beta*, *Delta* and *Gamma* subdivisions), *Spirochaetes*, *Tenericutes*, *Thermotogae* and *Verrucomicrobia*. In contrast with PN reactor, no single phylum predominated, with several groups showing relatively high abundances: *Chlorobi* (28.7±3.0%), *Chloroflexi* (17.5±1.1%), *Proteobacteria* (19.9±2.7%), *Acidobacteria* (8.9±1.0%), *Bacteroidetes* (7.3±0.6%), candidate division tm7 (5.8±1.2%) and *Planctomycetes* (7.9±1.4%). Their presence here was consistent with previous observations from other Anammox reactors, were members of these phyla were predominant (Cho et al., 2010; Kindaichi et al., 2012; Li et al., 2009; Park et al., 2010). In fact, sequence comparison of the ten most abundant phylotypes, accounting for more than half of the microbial community composition (56.9±3.3%), identified their closest relatives (97-100%) with sequences retrieved from other Anammox communities. For example, the members of *Chlorobi* were closely related (99.8%) to an uncultured *Ignavibacterium* from an autotrophic nitrogen removal granular sludge bed reactor (Wang et al., 2012), while the members of *Chloroflexi* showed their maximum similarity (99.5%) with uncultured members of *Anaerolinea* detected in a swine wastewater activated sludge facility with anammox activity (Yamagishi et al., 2013). Their presence in Anammox communities has been attributed to heterotrophic growth, often scavenging organic matter from dead Anammox biomass (Kindaichi et al., 2012), and to their structural contribution in granule and biofilm formation due to their filamentous morphology (Cho et al., 2011; Yamada et al., 2005).

Regarding the anaerobic ammonium oxidizing bacteria, 24 phylotypes within the phylum *Planctomycetes*, accounting for a total 7.9% of relative abundance, could be detected by means of high throughput sequencing methods. Within the phylum *Planctomycetes*, Anammox bacteria are allocated in the deep phylogenetic branch of the order *Brocadiales* that have been described for their ability to anaerobically oxidize ammonium in different environments (Jetten et al., 2009; Kuenen, 2008). To unequivocally identify those OTUs

belonging to the Anammox group, the sequences corresponding to the 24 phylotypes related to *Planctomycetes* were phylogenetically analysed with reference sequences of different members of this phylum (**Figure 9.6**). Most of the sequences clustered with members of *Phycisphaera*, *Pirellula* and *Rhodopirellula*. However, 5 phylotypes (accounting for a 1.4% of the total microbial community in the reactor) clustered within the Anammox group. The most abundantly represented, accounting for 93.3% of the Anammox-like sequences detected, were closely related with members of the candidate genus *Brocadia*, a recognized anammoxidant (Kartal et al., 2008; Strous et al., 1999; Third et al., 2001), thus confirming their main role in the ammonium oxidizing activity observed in the reactor.

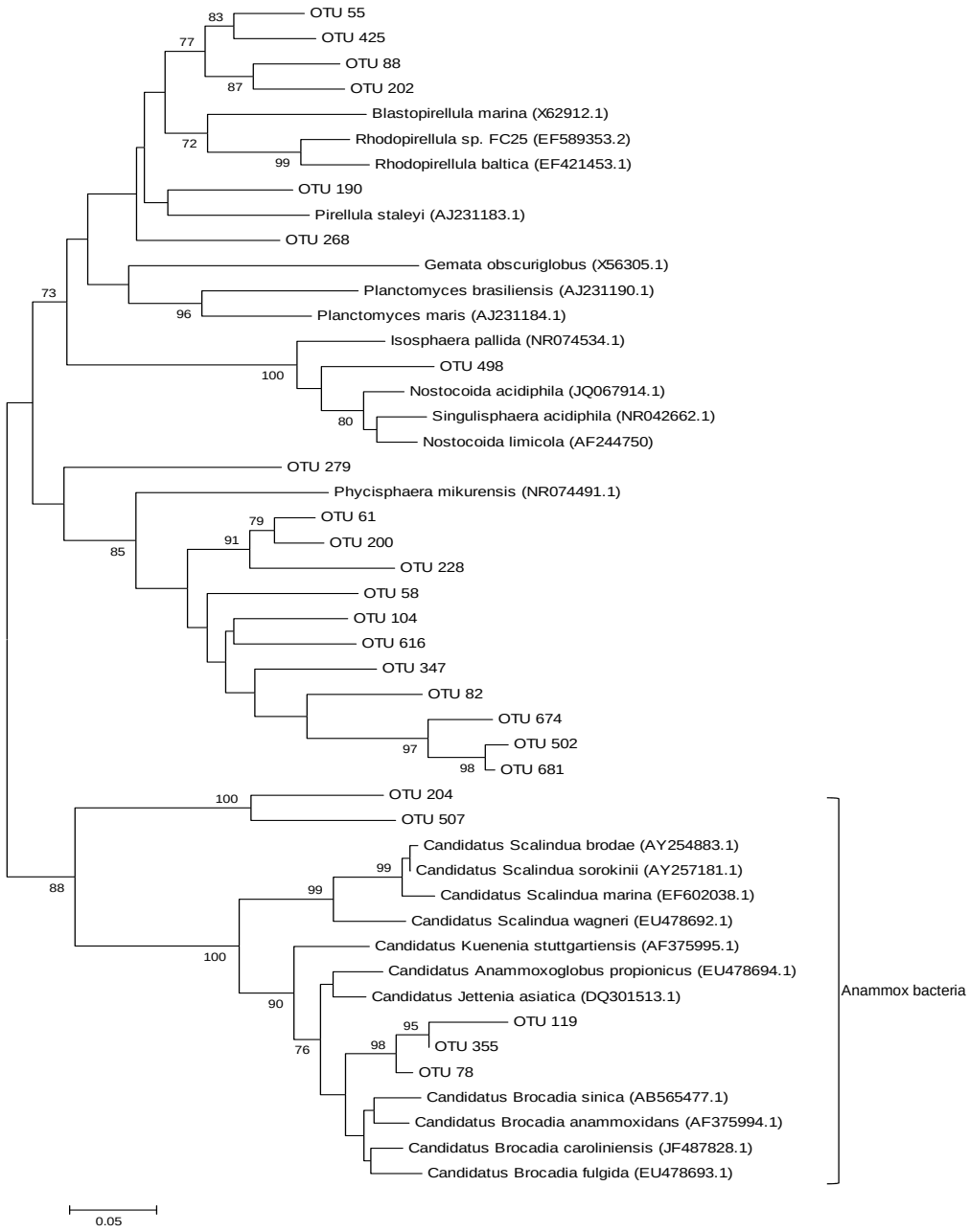


Figure 9.6. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic affiliation of the 24 OTUs corresponding to *Planctomycetales* detected in the tag encoded 16S rRNA gene pyrosequencing libraries from the Anammox reactor.

9.3. CONCLUSIONS

- The supernatant form anaerobic digestion of sewage sludge was successfully treated using a PN - Anammox system in two separated SBRs.
- The microbial characterisation of the PN SBR revealed a member of *N. eutropha* as the main AOB, and confirmed complete NOB inhibition.
- The effluent from the PN process served as substrate for an Anammox SBR operating with a stoichiometric relation of 1:1.25:0.14, close to the theoretical one.
- Members of the candidate genus *Brocadia*, identified as the key players in Anammox activity, were detected with low abundance, revealing a bulk of accompanying microbiota whose actual role needs further research.

10. Conclusions and recommendations

10.1. CONCLUSIONS

In this study, the application of anaerobic systems to obtain biogas and bioplastics from the organic matter and the subsequent biological nitrogen removal step has been tested in order to contribute to the knowledge of more sustainable treatment processes.

The main conclusions extracted from this work are compiled in this section:

Chapter 4: Start-up and operation of an AnMBR for winery wastewater treatment at mesophilic temperature

- Winery wastewater was successfully treated by the AnMBR reaching a 96.7±2.7% of COD removal and a biogas production of 0.05±0.17 $m^3_{biogas}\ m^{-3}_{digester}\ d^{-1}$.
- The SMP was on average 0.33±0.15 $m^{-3}CH_4\ kg^{-1}$COD, with a high methane concentration in biogas of 87.1±3.0%.
- Instabilities were caused by sharp influent COD oscillations that were coped by the addition of alkalinity to keep a ratio IA/TA below 0.4; which was 1092±88 $mgCaCO_3\ L^{-1}$.
- The stable operation was also favoured by the biomass acclimation. Because the AnMBR allowed the biomass selection and enrichment in methanogenic archaea, SMA increased from 0.07 to 0.36 gCH_4-COD $gVSS^{-1}\ d^{-1}$.

Chapter 5: Energetic aspects of the AnMBR technology compared with aerobic granulation

- The energy balance of the AnMBR revealed that the biogas produced could cover the electric requirements of a sidestream configuration during vintage season, when organic loads are high. However, in winter season when influent COD is lower, even operating at low temperature, the electricity demand could not be covered by the biogas production. Therefore, submerged membrane configuration could be taken into account for the upscaling of this system.
- Aerobic GSBR would be much efficient, especially thanks to its capacity to cope with high OLR and seasonal fluctuations. In spite of the aim of energy demand reduction, the GSBR will always represent a cost, while AnMBR is expected to become an energy production process.

Chapter 6: Operation of an AnMBR for winery wastewater treatment at low temperature

- Winery wastewater treatment at low temperatures resulted in a COD removal of 80% and 71% at 25°C and 15°C, respectively. Due to the VFA accumulation and methane retained in the liquid phase, the effluent COD not always accomplished the legal requirement. Therefore, a polishing post-treatment would be necessary to recover the methane and meet the legislation.
- A higher degree of fouling was observed compared with the mesophilic AnMBR despite the SS in the digester were lower. Although at lower temperatures less fouling was expected, the oscillations of organic load probably promoted SMP production that increased fouling.
- The methanogenic activity decreased at low temperatures as expected, although SMA obtained at 25°C was similar to the mesophilic one. However, at 15°C the activity decreased considerably. Comparing the load applied in the continuous AnMBR with the methanogenic activity observed in batch, the AnMBR was operating at its maximum capacity at 15°C. In contrast, at 25°C, the AnMBR could have coped with higher sOLR, although the COD removal efficiency would have decreased.
- The microbial population shifted from Methanosaeta spp. used as inoculum to Methanosarcina, because the higher amount of VFA in the AnMBR favoured the development of an acetotrophic methanogen with a higher growth rate under high acetate concentration.

Chapter 7: Winery wastewater treatment by means of an UASB and an UASB-MBR

- Higher OLR were successfully treated in the UASB in comparison with the AnMBR described in Chapter 4, mainly due to the presence of a higher amount of biomass in the reactor, thus the sOLR was kept lower.
- The changes in OLR negatively affected the granules stability, increasing the SS in the effluent and washing-out the non-aggregated biomass.
- Coupling the UASB to a membrane unit allowed the accomplishment of legal requirements of the effluent, especially at high OLRs. However, the cost that a membrane unit supposes may not be justified in the case of a granular technology as the UASB. The UASB by itself has enough capacity of solid retention, thus the strategy to reduce the effluent COD would be focused on maintaining the granule stability.

- The activity of the granular biomass determined by batch test was higher than the activity of the suspended biomass. Nevertheless, the methane production was lower, probably due to the inhibition by substrate.
- The main methanogenic archaea identified was Methanosaeta spp. However, the proportion of archaea compared to bacteria seemed to decrease in the UASB-MBR. Further research should be done in this aspect to draw more consistent conclusions about the microbial population.

Chapter 8: Integrating the selection of PHA storing biomass and nitrogen removal via nitrite treating UASB effluent

- A novel process was developed that integrates the selection of PHA storing biomass, and nitrogen removal via nitrite in one stage SBR treating municipal wastewater. Denitrification was accomplished through the internally stored PHA. The rates were higher than the ones attributed to endogenous respiration, but lower than the respective sNUR accomplished with the addition of fermentation liquid during the anoxic reaction phase.
- The increased competition between the heterotrophic and autotrophic bacteria in the aerobic phase for the DO in the presence of VFA is a drawback of the examined scheme, resulting in low sAUR.
- Comparing the efficiency of the examined carbon sources, the use of OFMSW FL resulted in enhanced PHA accumulation. However, the presence of non-VFA COD results in a loss in the PHA accumulation capacity of sludge, both in the SBR and in the batch accumulation step.
- Although higher PHA yields can be achieved under complete aerobic conditions, this novel scheme presents an added value due to the integration of the PHA production in the nitritation/denitritation process.

Chapter 9: Start-up and operation of a two-step partial nitritation – Anammox SBR for reject water treatment

- The supernatant form anaerobic digestion of sewage sludge was successfully treated using a PN - Anammox system in two separated SBRs.
- The microbial characterisation of the PN SBR revealed a member of *N. eutropha* as the main AOB, and confirmed complete NOB inhibition.
- The effluent from the PN process served as substrate for an Anammox SBR operating with a stoichiometric relation of 1:1.25:0.14, close to the theoretical one.

- Members of the candidate genus *Brocadia*, identified as the key players in Anammox activity, were detected with low abundance, revealing a bulk of accompanying microbiota whose actual role needs further research.

10.2. RECOMMENDATIONS

For further research, the following recommendations are proposed:

Regarding AnMBR (CSTR and UASB type) for winery wastewater treatment:

- The implementation of an AnMBR for winery wastewater treatment at full-scale must be preceded by an exhaustive economical study. The use of a membrane system would be justified if the effluent obtained needs to be reused.
- In order to improve the capacity of the AnMBR, higher amount of biomass should be retained in the digester. However, it would represent a higher degree of fouling. The advances in membrane technology must be considered to apply the most viable configuration.
- The operation at low/ambient temperatures needs further research, especially in terms of microbiology, because the slow growing anaerobic microorganisms would be subjected to seasonal changes of temperatures and organic load. Therefore, the most adequate inoculum should be identified to reduce the start-up periods and, moreover, the procedure to cope these temperature variations also needs to be determined.
- The use of a conventional UASB for industrial wastewaters has been widely studied and has a high treatment capacity. However, since winery wastewater suffers huge seasonal oscillations, the application of UASB-MBR systems increased the effluent quality in terms of suspended solids and organic matter. Further research should be focused on the real necessity to couple a membrane unit in an UASB, since the operational costs increased significantly.
- The operation of anaerobic processes at low temperature leads to a loss of methane dissolved in the liquid phase. Therefore, a post-treatment should be considered to recover it for economic and environmental reasons. Moreover, it can be used as a carbon source for a subsequent nitritation/denitritation step if required.

Regarding N/DN via nitrite integrated with PHA production:

- Further research should be focused on the process stability, especially at long term operation subjected to seasonal variations. Microbial population identification is also of great interest to determine the most adequate microorganisms that achieve denitritation driven by the stored compounds and apply the conditions to favour its growth.
- Many parameters should be optimised to achieve a good nitritation/denitritation rates as aeration time, SRT, DO, feast and famine ratio, etc.
- Other carbon sources can be applied for this purpose, although they should be rich in VFA with low nutrient content. Using fermented biowastes, a good solid/liquid separation must be assure to increase the storage yield. Winery wastewater can be adequate for PHA production since it has low nutrient content and low solids.
- The limiting step for the application of PHA production in a WWTP is the high cost of the extraction technologies, which are only feasible when PHA percentages are over 90% of dry matter. For this reason, more economic extraction techniques should be studied.

Regarding PN-Anammox process:

- Partial nitritation-Anammox is a cost-effective process that reduces considerably the operational costs of nitrogen removal. However, the implementation at full-scale has some drawback as the presence of organic matter, low temperatures, variations in nitrogen loads, etc. All of these parameters should studied and optimise in order to assure stable operation, since Anammox bacteria are very sensitive to changes and the start-up or recovery periods are very long.
- The application of a partial nitritation-Anammox system is also an interesting after the anaerobic treatment of municipal wastewater. In this way, COD present in urban wastewater is recovered as biogas, and nitrogen can be removed at a very low cost. However, the main drawback of this scheme is the necessity to operate at low temperatures that significantly affect the kinetics of both processes.

Publications and congress communications

PUBLICATIONS

N. Basset, J. Dosta, J. Mata-Álvarez (2011) Biorreactores de membrana: Evolución y perspectivas. *Tecnología del agua* 328, 28-40. ISSN 0211-8173.

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N. Basset, S. López-Palau, J.Dosta, J. Mata-Álvarez (2014) Comparison of aerobic granulation and anaerobic membrane biorreactor technologies for winery wastewater treatment. *Water Science and Technology* 69 (2), 320-327.

N. Basset , E. Santos, J. Dosta, J. Mata-Álvarez. Start-up and operation of an AnMBR for winery wastewater treatment. *Submitted.*

Dosta, J, Vila, Sancho, I., Basset, N., Grifoll, M., Mata-Álvarez, J. Two-step partial nitritation/anammox process in granulation reactors: start-up operation and microbial characterization. *Submitted.*

N. Basset , E. Katsou, S. Malamis, N. Frison, J. Dosta, F. Fatone. Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line. *Submitted.*

N. Basset, E. De Arana, A. Coll, J. Dosta, J. Mata-Álvarez. Comparison of UASB and AnMBR technolgies for winery wastewater treatment at low temperatures. *In preparation.*

CONGRESS COMMUNICATIONS

Platform presentations

N. Basset, S. López-Palau, J. Dosta, J. Mata-Álvarez (2013) Comparison of aerobic granulation and anaerobic membrane bioreactor technologies for winery wastewater treatment. *6th IWA Specialized Conference, Winery 2013 – Viticulture and Winery wastes management*, Narbonne, France, May 26-30th, 2013.

N. Basset, J. Dosta, J. Mata-Álvarez (2013) Start-up of an AnMBR for winery wastewater treatment. *13th World Congress on anaerobic Digestion,* Santiago de Compostela, Spain, 25-28th June 2013.

N. Basset; E. Santos; J. Dosta; A. Guastalli; J. Mata-Álvarez (2014) Operación de un Bioreactor de Membranas Anaeróbico (BRM-An) para el tratamiento de agua residual vitivinícola. *XI Reunión de la Mesa Española de Tratamiento de Aguas (META),* Alicante, Spain, 18th – 20th June 2014.

N. Basset; A. Jelic; E. Katsou; F. Fatone (2014) Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line *2nd IWA Specialized International Conference "Ecotechnologies for Wastewater Treatment (EcoSTP2014)"*, Verona, Italy, 23rd – 25th June 2014.

N. Basset; E. Santos; J. Dosta; A. Guastalli; J. Mata-Álvarez (2014) La microfiltración para el tratamiento anaerobio de aguas industriales vinícolas. *Tecnología de membranas, 1ª Jornadas monográficas investigación e industria.* Madrid 17-18 Septiembre 2014.

Poster presentations

N. Basset, J. Dosta, J. Mata-Álvarez (2012) Preliminary assessment of AnMBR for winery wastewater. *IWA International Conference, EcoTechnologies for Wastewater Treatment (EcoSTP)*, Santiago de Compostela, Spain, 25th-27th June 2012.

N. Basset, I. Sancho, J. Dosta, J. Mata-Álvarez (2014) Nitrogen removal of sewage sludge anaerobic digestion supernatant by partial nitritation/Anammox. 2nd IWA Specialized International Conference "Ecotechnologies for Wastewater Treatment (EcoSTP2014)", Verona, Italy, 23rd – 25th June 2014.

N. Basset; E. Santos; J. Dosta; A. Guastalli; J. Mata-Álvarez (2014) Operation of an Anaerobic Membrane Bioreactor (An-MBR) for winery wastewater treatment. *13th Mediterranean Congress of Chemical Engineering,* Barcelona 30th September - 2nd October 2014.

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Notation

Symbol	Description	Units
ABS	Absorbance	-
Anammox	Anaerobic Ammonium Oxidation	-
AnMBR	Anaerobic Membrane Bioreactor	-
AOB	Ammonium Oxidizing Bacteria	-
APHA	American Public Health Association	-
BMP	Biomethane Potential	-
BOD	Biological Oxygen Demand	g O_2 L^{-1}
C/N	Carbon/Nitrogen	-
CANON	Completely Autotrophic Nitrogen Removal over Nitrite	-
CAS	Conventional Activated Sludge	-
CHP	Combined Heat and Power unit	-
COD	Chemical Oxygen Demand	g O_2 L^{-1}
CSTR	Completely Stirred Tank Reactor	-
DEMON	Deammonification	-
DGGE	Denaturing Gradient Gel Electrophoresis	-
DO	Dissolved Oxygen	mg L^{-1}
DOC	Dissolved Organic Carbon	g C L^{-1}
DOM	Dissolved Organic Matter	-
EGSB	Expanded Granular Sludge Blanket	-
EPS	Extracellular Polymeric Substances	-
F/M	Feed to Microorganisms ratio	-
FA	Free ammonia	mg NH_3 L^{-1}
FID	Flame Ionization Detector	-
FISH	Fluorescence in Situ Hybridization	-
FL	Fermentation Liquid	-
FNA	Free nitrous acid	mg HNO_2 L^{-1}

Symbol	Description	Units
FS	Flat sheet	-
FWD	Food Waste Disposer	-
GAO	Glycogen Accumulating Organisms	-
GC	Gas Chromatography	-
GSBR	Granular Sequencing Batch Reactor	-
H/D	Height to Diameter Ratio	-
HB	Hydroxybutyrate	-
HF	Hollow fibre	-
HRT	Hydraulic Retention Time	d
HV	Hydroxyvalerate	-
IA/TA	Intermediate Alkalinity to Total Alkalinity ratio	-
iMBR	Immersed Membrane Bioreactor	-
MBR	Membrane Bioreactor	-
MF	Microfiltration	-
MLSS	Mixed Liquor Suspended Solids	$g\ L^{-1}$
N/DN	Nitrification/Denitrification	-
NLR	Nitrogen Loading Rate	$kg\ N\ m^{-3}\ d^{-1}$
NOB	Nitrite Oxidizing Bateria	-
NTU	Nephelometric Turbidity Unit	-
OFMSW	Organic Fraction of Municipal Solid Waste	-
OLAND	Oxygen-Limited Autotrophic Nitrification Denitrification	-
OLR	Organic Loading Rate	$kg\ COD\ m^{-3}\ d^{-1}$
ORP	Oxidation Reduction Potential	mV
OTU	Operational Taxonomic Units	-
PAO	Polyphosphate Accumulating Organisms	-
P_B	Biogas Production	$m^3\ m^{-3}_{digester}\ d^{-1}$
PCR	Ploymerase Chain Reaction	-

Symbol	Description	Units
PHA	Polyhydroxyalkanoate	-
PHB	Polyhydroxybutyrate	-
PHV	Polyhydroxyvalerate	-
PLC	Programmable Logic Controller	-
PN	Partial nitritation	-
PS	Primary Sludge	-
PVDF	Polyvinylidene Fluoride	-
qPHA	Polyhydroxyalkanoate production rate	$mgCOD\ gVSS^{-1}\ h^{-1}$
-qPHA	Polyhydroxyalkanoate depletion rate	$mgCOD\ gVSS^{-1}\ h^{-1}$
-qVFA	Volatile Fatty Acids depletion rate	$mgCOD\ gVSS^{-1}\ h^{-1}$
SAA	Specific Anammox Activity	$gN\ (gVSS\ d)^{-1}$
sAUR	Specific Ammonium Uptake Rate	$gN\ (gVSS\ h)^{-1}$
SBR	Sequencing Batch Reactor	-
sCOD	Soluble Chemical Oxygen Demand	$g\ O_2\ L^{-1}$
scSBR	Short-cut Sequencing Batch Reactor	-
SEM	Scanning Electron Microscope	-
SHARON	Single reactor system for High activity Ammonium Removal Over Nitrite	-
SMA	Specific methanogenic activity	$gCH_4\text{-}COD\ gVSS^{-1}\ d^{-1}$
sMBR	Sidestream Membrane Bioreactor	-
SMP	Specific methane production	$m^3CH_4\ kg^{-1}COD$
SMP	Soluble Microbial Products	-
sNLR	Specific Nitrogen Loading Rate	$kgN\ (kgVSS\ d)^{-1}$
sNUR	Specific Nitrite Uptake Rate	$gN\ (gVSS\ h)^{-2}$
sOLR	Specific Organic Loading Rate	$kgCOD\ (kgVSS\ d)^{-1}$
SRT	Sludge Retention Time	d
SS	Suspended Solids	$g\ L^{-1}$
SVI	Sludge Volume Index	$mL\ g^{-1}SS$

Symbol	Description	Units
TCD	Thermal Conductivity Detector	-
TKN	Total Kjeldahl Nitrogen	$gN\ L^{-1}$
TMP	Transmembrane Pressure	bar
TN	Total Nitrogen	$gN\ L^{-1}$
TOC	Total Organic Carbon	$gC\ L^{-1}$
TP	Total Phosphorus	$gP\ L^{-1}$
TS	Total Solids	$g\ L^{-1}$
TSS	Total Suspended Solids	$g\ L^{-1}$
TVS	Total Volatile Solids	$g\ L^{-1}$
UASB	Upflow Anaerobic Sludge Blanket	-
UF	Ultrafiltration	-
VFA	Volatile Fatty Acids	$mgVFA\ L^{-1}$
VSS	Volatile Suspended Solids	$g\ L^{-1}$
WW	Wastewater	-
WWTP	Wastewater Treatment Plant	-
$Y_{OX/COD}$	Oxidation yield based on total COD	-
$Y_{OX/VFA}$	Oxidation yield based on VFA	-
$Y_{PHA/COD}$	PHA yield based on total COD	-
$Y_{PHA/VFA}$	PHA yield based on VFA	-
$Y_{X/COD}$	Growth yield based on total COD	-
$Y_{X/VFA}$	Growth yield based on VFA	-

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Resum en català

Un dels reptes de les estacions depuradores d'aigües residuals (EDAR) és aconseguir una elevada eficiència d'eliminació de contaminants amb menys demanda energètica, atenent als requeriments legals, cada dia més restrictius. A més a més, l'afany de recuperació de recursos a partir dels residus promou el desenvolupament de noves tecnologies pel tractament d'aigües residuals valoritzant-ne la matèria orgànica i els nutrients que conté.

La digestió anaeròbia és un procés pel qual la matèria orgànica es transforma en una font d'energia, com és el biogàs. Actualment, la digestió anaeròbia s'aplica a residus com els fangs de depuradora, purins, etc. En aquesta tesi, s'ha estudiat la digestió anaeròbia per al tractament d'aigües residuals amb poca càrrega orgànica, utilitzant bioreactors de membrana anaeròbics (BRM-An) per tal de retenir la biomassa en el digestor. S'ha tractat aigua residual procedent de la indústria vitivinícola, caracteritzada per un alt contingut en matèria orgànica i pobre en nutrients, avaluant la flexibilitat del BRM-An a oscil·lacions de càrrega orgànica, així com la seva viabilitat en termes energètics.

Pel que fa a l'aigua residual municipal, la digestió anaeròbia requereix de post-tractaments d'eliminació de nutrients. En aquesta tesi, s'han estudiat dos processos d'eliminació de nitrogen: nitrificació/desnitrificació (N/DN) via nitrit i nitrificació parcial – Anammox (de l'anglès anaerobic ammonium oxidation). Ambdós possibilitats presenten un estalvi considerable en comparació amb la N/DN convencional.

És ben sabut que la N/DN via nitrit suposa una reducció de les necessitats d'aireig i matèria orgànica. En el present treball s'ha estudiat la integració de l'eliminació de nitrogen via nitrit amb la producció de polihidroxialcanoats (PHA), bioplàstics d'alt interès comercial que s'acumulen a l'interior de les cèl·lules donades les condicions adients (sacietat – fam).

Per altra banda, la nitrificació parcial – Anammox és un dels sistemes menys costosos per a l'eliminació de nitrogen d'aigües residuals. S'ha estudiat la seva aplicació per al tractament del centrat de la digestió anaeròbia de fangs de depuradora, que conté el 30% de la càrrega de nitrogen d'una EDAR.

Tots els estudis s'han portat a terme a escala de laboratori i s'han obtingut conclusions satisfactòries en al majoria de casos, arribant a la conclusió que valoritzant la matèria orgànica present en les aigües residuals com una font de recursos i reduint el cost dels sistemes d'eliminació de nutrients, les EDAR esdevindran processos molt més sostenibles.

1. INTRODUCCIÓ

Actualment, la preservació de recursos hidràulics així com la regeneració i reutilització de les aigües residuals s'estan convertint en una tasca necessària de la nostra societat. El motiu principal és l'escassetat d'aigua, fet afavorit per l'augment de població i d'activitats industrials. Les estacions depuradores d'aigües residuals (EDAR) són, per tant, un element inevitable que suposa un elevat cost per a la societat. Les noves tecnologies i estratègies d'operació permeten reduir aquests costos, sobretot energètics, i fins i tot obtenir beneficis dels subproductes generats, a fi de convertir la depuració d'aigües en un procés molt més sostenible.

1.1. Incrementant la capacitat de tractament i la reutilització d'aigua mitjançant la tecnologia de membranes

Durant els darrers anys la tecnologia de bioreactors de membrana (BRM) s'ha desenvolupat i aplicat en el camp del tractament d'aigües degut als avantatges que aporta en el procés biològic i en la qualitat de l'efluent obtingut. Un BRM consisteix en la combinació d'un reactor biològic i un sistema de separació sòlid-líquid per membranes, habitualment de micro- o ultrafiltració (0.01µm – 1µm). A la Taula 1 es resumeixen els principals avantatges i inconvenients de la tecnologia.

Taula 1. Avantatges i inconvenients de la tecnologia BRM (Lawrence K. Wang et al., 2009b).

Avantatges	Inconvenients
- Alta qualitat de l'efluent per reutilització - Instal·lació compacta i disseny modular - Separació del temps de retenció cel·lular i temps de retenció hidràulic - Completa separació sòlid-líquid independentment de la capacitat de sedimentació de la biomassa - Menor producció de fangs i major concentració de biomassa en el reactor - Operació simple i flexible	- Alt consum energètic degut a l'aireig o a la velocitat tangencial necessaris per reduir l'embrutiment de la membrana - Neteges de manteniment periòdiques

Existeixen dues possibles configuracions de reactors BRM: de membrana submergida o externa. A finals del segle XX i principis del XXI, la membrana externa era la més utilitzada, però tenint en compte la capacitat dels BRM instal·lats, els sistemes de membrana submergida predominen, sobretot en el camp del tractament d'aigües urbanes. Els tipus de membrana més comercialitzats són: placa plana i fibra buida per membranes submergides, i tubular per membranes externes (Judd, 2011).

1.1.1. Bioreactor de membrana anaeròbic

Els BRM aeròbics representen la majoria dels BRM instal·lats. No obstant això, l'interès en el BRM-An està augmentant degut als avantatges de la combinació d'un digestor anaeròbic amb la filtració per membrana. El BRM-An elimina la matèria orgànica biodegradable de manera eficient i sense necessitat d'oxigen, baixa producció de biomassa, la generació d'energia a partir de biogàs i ofereix molts avantatges sobre altres processos anaeròbics que han de funcionar a alts temps de retenció hidràulics (TRH) per permetre el creixement de microorganismes metanogènics.

En general, per a les aigües residuals municipals amb menors concentracions de DQO biodegradable, temperatures més baixes i requisits d'eliminació de nutrients, els processos aeròbics són preferibles actualment. En canvi, per a les aigües residuals industrials amb molt més altes concentracions de DQO biodegradable i temperatures elevades, els processos anaeròbics poden resulten més viables.

La digestió anaeròbia és un procés bioquímic que, en absència d'oxigen, la matèria orgànica biodegradable es descompon en biogàs. La conversió de la matèria orgànica en biogàs és un procés que implica diverses reaccions, tant en sèrie com en paral·lel, i diferents grups de microorganismes (bactèries i arquees). El procés de digestió anaeròbia es pot subdividir en les següents quatre fases Figura 1:

1. Hidròlisi: la matèria orgànica complexa i no dissolta es descompon en molècules orgàniques solubles i simples que poden passar a través de les parets cel·lulars i membranes de les bactèries fermentatives.
2. Fermentació o acidogènesi: els compostos dissolts presents en les cèl·lules de bactèries fermentatives es converteixen en compostos simples (àcids grassos volàtils, alcohols, àcid làctic, CO_2, H_2, NH_3 i H_2S).
3. Acetogènesi: els productes de fermentació es converteixen en acetat, hidrogen i diòxid de carboni pel que es coneix com a bactèries acetogèniques.
4. Metanogènesi: Acetat i hidrogen/diòxid de carboni es converteixen en metà i CO_2 per arquees metanogèniques.

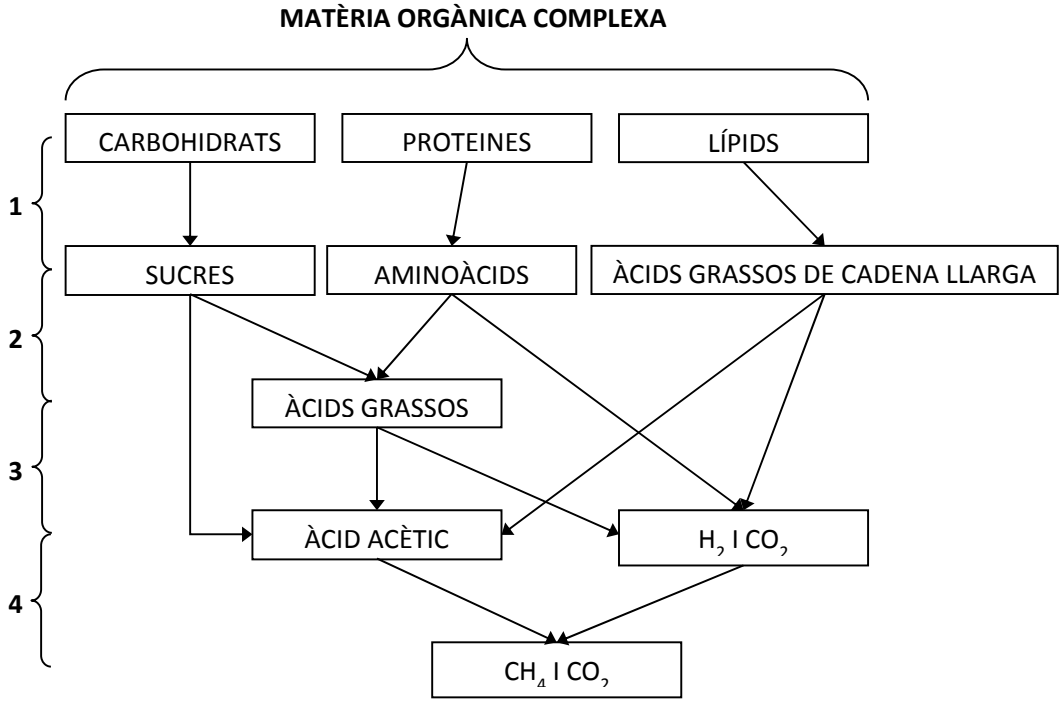


Figura 1. Camí de reacció de la digestió anaeròbia

Les possibles configuracions BRM-An són anàlogues als de BRM aeròbic (Judd, 2011). No obstant això, mentre que les instal·lacions de BRM aeròbics han optat clarament per la configuració de membrana submergida, per a BRM-An encara no hi ha una tendència clara. Consultant la literatura recent, les aplicacions de membranes submergides han augmentat degut a l'experiència positiva en el BRM aeròbic (Dereli et al., 2012; Ferrer et al., 2015; Smith et al., 2012). En aquests casos, el biogàs produït s'utilitza com una eina per reduir l'embrutiment de la membrana, en lloc d'aireig (Akram and Stuckey, 2008). No obstant això, si no s'obté suficient biogàs, ha de ser comprimit per a ser recirculat, per tant, el cost energètic de la BRM-An podria no ser menor que un BRM aeròbic.

El BRM-An és una alternativa a altres tipus de reactors d'alta capacitat per a la digestió anaeròbica, com ara el UASB (de l'anglès upflow anaerobic sludge blanket), el EGSB (de l'anglès expanded granular sludge blanket) i el IC (de l'anglès internal circulation); basats en la tecnologia de biopel·lícules i gràmuls, aplicats generalment per al tractament d'aigües industrials amb alta càrrega orgànica (Van Lier, 2008). Encara que aquestes tecnologies poden fer front a càrregues més altes, de fins a 40 kgDQO m^{-3} d^{-1} (Liao et al., 2006), un dels avantatges del BRM-An és la retenció total de la biomassa, independentment de les seves propietats d'agregació o sedimentació. Per tant, quan les característiques de l'aigua residual

afecten negativament les biopel·lícules o la formació de grànuls (per exemple, alt contingut de sòlids, alta temperatura, toxicitat, alta salinitat, canvis dràstics en les càrregues orgàniques o TRH), la retenció de la biomassa de creixement lent capaç de aclimatar-se a les condicions extremes és crucial (Dereli et al., 2012).

1.1.2. Aspectes energètics de la tecnologia BRM

La competitivitat de la tecnologia BRM es veu amenaçada pel baix cost d'operació dels tractaments convencionals. Per aquesta raó, cal considerar els avenços en la tecnologia i les seves aplicacions a gran escala. A la Taula 2 es mostren exemples dels costos d'energia d'algunes EDAR equipades amb BRM. Cal assenyalar que el requisit d'energia d'un BRM es pot reduir significativament amb un procés híbrid (Verrecht et al., 2010), doncs les plantes BRM estan sobredimensionades, dissenyades per tractar el cabal màxim de la planta. En canvi, el BRM híbrid està dissenyat per tractar un flux constant, de manera que el sistema convencional de fangs actius s'utilitzaria únicament per les sobrecàrregues de flux. També cal destacar que els sistemes de membranes externes consumeixen molt més que els de membrana submergida. Per aquest motiu, els sistemes de membrana externa s'utilitzen generalment pel tractament d'aigües molt carregades o per BRM-An on la producció de biogàs compensi la demanda energètica.

Taula 2. Consum energètic d'algunes EDAR amb BRM

Tecnologia	Consum ($kWh \cdot m^{-3}$)	Capacitat o cabal tractat	Autors
BRM tubular	1-4	-	(Cornel and Krause, 2006)
BRM placa plana	0.8-1.2	10,600 p.e.	(Mulder, 2009)
BRM fibra buida	0.64	28,000 p.e.	(Fenu et al., 2010b)
Fangs actius convencionals	0.19	18,000 p.e.	
BRM fibra buida	0.5-1.8	20,851 m^3d^{-1}	(Verrecht et al., 2010)
Híbrid BRM-convencional	0.4	20,851 m^3d^{-1}	
BRM fibra buida	0.3	-	(Martin et al., 2011)
BRM tubular	3.7	-	

1.2. Reduint els costos d'operació de l'eliminació biològica de nitrogen

L'eliminació de nitrogen convencional suposa un alt cost degut les necessitats d'oxigen i matèria orgànica per nitrificar i desnitrificar. Concretament es requereixen entre 4.2 i 4.5 gO_2 $(gN)^{-1}$ per oxidar l'amoni a nitrat, i 3.7 gDQO $(gN)^{-1}$ per reduir el nitrat a nitrogen gas.

Segons l'esquema representat en la Figura 2, la N/DN convencional implica oxidar el nitrogen de -3 a +6 per llavors reduir-lo a 0. Existeixen diferents estratègies mpe rtal de reduir els costos d'oxidar i reducció. Per exemple, en la N/DN via nitrit, l'amoni (-3) s'oxida només a nitrit (+3) i, per tant, la quantitat matèria orgànica per reduir-lo a nitrogen (0) és menor. Per altra banda, el procés Anammox consisteix en oxidar parcialment l'amoni a nitrit i juntament amb l'amoni restant s'obté nitrogen gas, reduint encara més les necessitats d'oxigen i carboni.

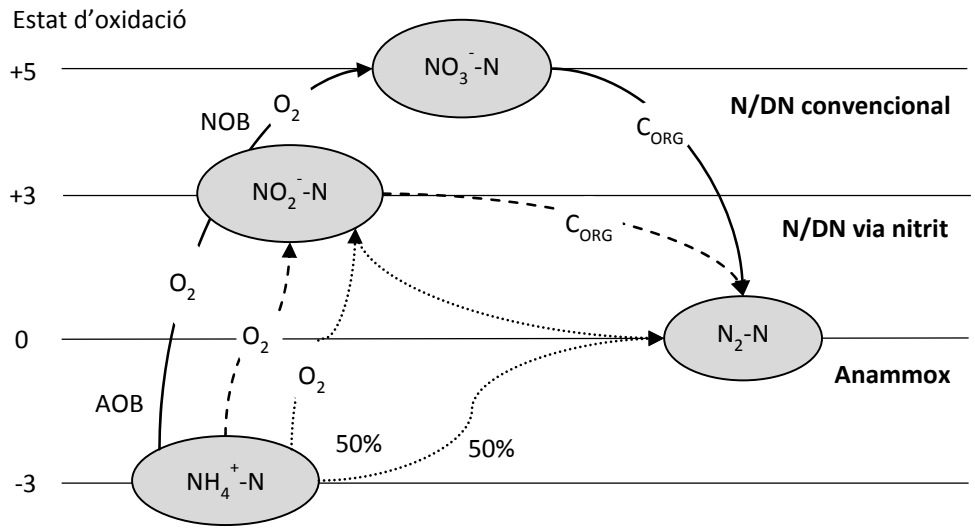


Figura 2. Estats d'oxidació del nitrogen durant la nitrificació/desnitrificació

1.2.1. Nitrificació/Desnitrificació via nitrit

La N/DN via nitrit és una opció interessant ja suposa una reducció significativa de les necessitats del procés, sent 3.4 gO_2 $(gN)^{-1}$ i 2.3 gDQO $(gN)^{-1}$. En comparació amb el procés de N/DN convencional, s'aconsegueix reduir l'aireig un 25% i la font de carboni un 40%, a més de produir un 30% menys de fangs (Gu et al., 2012).

Per tal d'aconseguir aturar la reacció de nitrificació, cal promoure la producció de nitrit i inhibir l'oxidació cap a nitrat. Els tres factors que afecten principalment la nitritació són (Lawrence K. Wang et al., 2009a):

Taxa de creixement específica. El creixement de les bactèries amoni-oxidants (AOB) i les nitrit-oxidants (NOB) varien en relació a la temperatura, de manera que per sobre de 20°C es maximitzen aquestes diferències afavorint el creixement de les AOB (Hellinga et al., 1998). La combinació del control de la temperatura i un temps de retenció cel·lular reduït resulta en una bona estratègia per aconseguir un procés de nitrificació parcial estable.

Oxigen dissolt. Com que l'oxigen és un cosubstrat de la reacció de nitrificació, la seva concentració té un efecte important sobre el procés de nitrificació. L'afinitat de les bactèries NOB és molt menor que la de les bactèries AOB (Wiesmann, 1994) i, per tant, una baixa concentració d'oxigen afavoreix la nitrificació via nitrit.

Concentracions d'amoníac i d'àcid nitrós. Nombrosos estudis han atribuït efectes inhibitoris a elevades concentracions d'amoníac lliure i d'àcid nitrós (Anthonisen et al., 1976). De fet, l'amoníac lliure és considerat com un factor afavoridor per al creixement de les bactèries AOB, i no exclusivament com un inhibidor de les NOB. Varis autors conclouen que elevades concentracions de nitrogen amoniacal a l'influent (>500 mg L^{-1}) afavoreixen la nitrificació via nitrit (Yamamoto et al., 2011).

1.2.2. Nitrificació Parcial-Anammox

El procés Anammox és un procés innovador, ambientalment sostenible i econòmicament viable que té un elevat potencial d'eliminació de nitrogen d'aigües residuals amb una baixa relació C/N (Fernández, 2010). Com indica el seu nom, consisteix en l'oxidació anaeròbia de l'amoni, utilitzant el nitrit com acceptor d'electrons. Aquest procés, però, requereix un pas previ de nitrificació parcial via nitrit, mitjançant el qual només el 50% del nitrogen amoniacal contingut en l'aigua residual és oxidat a nitrit. El ràtio molar alcalinitat/amoni controla la quantitat d'amoni nitrificada, ja que 1 mol d'amoni requereix dos mols d'alcalinitat per ser oxidat. La combinació d'aquest procés de nitrificació parcial i el procés Anammox dóna lloc a l'Equació 1.

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (1)$$

Com mostra l'estequiometria, es tracta d'un procés completament autotròfic, i, per tant, no és necessària l'aportació externa d'una font de carboni orgànic. A més, el subministrament d'oxigen es redueix en un 65% i la producció de fangs és menor.

1.2.3. Aspectes econòmics de l'eliminació biològica de nitrogen

Els costos dels diferents processos de N/DN descrits s'han estimat seguint les directrius de Fernández (2010), actualitzant els preus al mercat actual. A la Taula 3 es mostren els costos estimats per a dos escenaris: sense necessitat de font de carboni i amb necessitat de font de carboni per a la desnitrificació.

Taula 3. Estimació de costos dels diferents processos de N/DN

Tecnologia	O_2	Electricitat		Metanol		Gestió fangs		Cost total
	kg	kWh	€	kg MeOH	€	kg	€	€
N/DN convencional	4.3	1.19	0.18	-	-	1.00	0.14	0.32
N/DN convencional carboni extern	4.3	1.19	0.18	2.5	1.00	1.00	0.14	1.32
N/DN via nitrit	3.4	0.94	0.14	-	-	0.60	0.08	0.22
N/DN via nitrit carboni extern	3.4	0.94	0.14	1.5	0.60	0.60	0.08	0.82
N parcial /Anammox	2.0	0.56	0.08	-	-	0.15	0.02	0.10

Clarament s'observa que el sistema Anammox és el més econòmic, ja que és un sistema autotròfic. La necessitat de font de carboni, calculada en base al cost del metanol comercial, implica més d'un 70% més de cost, per tant, utilitzar fonts de carboni de fàcil accés i poc costoses (per exemple, residus orgànics d'altres indústries) implicaria una reducció molt important. En comparació amb els resultats obtinguts per Fernández (2010), caldria destacar que tot i que el preu de l'electricitat quasi bé s'ha doblat en cinc anys de 0.09 € kWh^{-1} to 0.15 € kWh^{-1}, l'eficiència dels sistemes d'aireig ha augmentat tant significativament que el cost total és menor.

1.3. Producció de bioplàstics en una estació depuradora d'aigües residuals

A part de la reducció de la demanda energètica d'una EDAR aplicant tecnologies com la digestió anaeròbia o l'Anammox, la possibilitat de valoritzar la matèria orgànica per produir bioplàstics s'ha convertit en una de les tendències més recents en el camp del tractament d'aigües residuals.

Nombroses bactèries són capaces d'acumular compostos en el seu interior (Figura 3), com el greix en els humans, si se les sotmet a condicions transitòries de sacietat i fam (Jiang et al., 2012). Un grup d'aquests compostos són els polihidroxialcanoats (PHA), tipus de polièsters amb propietats similars a les del plàstics derivats del petroli.

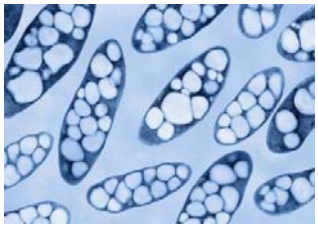


Figura 3. PHA acumulat en E. coli (Quan and Tian, 2009)

Malgrat els nombrosos avantatges de l'ús de plàstics biodegradables, la comercialització de PHA té grans limitacions. L'alt cost de producció de PHA és el principal inconvenient, sobretot per l'etapa d'extracció del PHA de les cèl·lules. Tenint en compte el preu de la majoria dels plàstics derivats del petroli, com ara polietilè i polipropilè, estan per sota d'1 € kg^{-1} (Chee et al., 2010). Per tant, actualment el PHA no pot competir actualment amb l'enorme producció de plàstics no biodegradables.

La producció de PHA a partir de biomassa amb diversitat d'espècies consisteix en una primera fase de selecció dels microorganismes, de manera que, aplicant cicles de sacietat i fam, s'afavoreix el creixement d'aquelles espècies que tenen la capacitat d'acumular PHA durant la fase de sacietat i utilitzar-lo per sobreviure en la fase de fam. Seguidament en la fase d'acumulació, es pren la biomassa prèviament seleccionada i se li addiciona contínuament matèria orgànica (preferiblement rica en àcids grassos volàtils (AGV) i pobre en nutrients) per tal de que els microorganismes acumulin el màxim de PHA possible. La fase de selecció de la biomassa acumuladora de PHA es pot integrar amb la nitrificació/desnitrificació, doncs en ambdós casos s'apliquen condicions de sacietat i fam. En la Figura 4 es mostra l'esquema de la integració de la N/DN amb l'acumulació de PHA. D'aquesta manera, en una primera fase aeròbica, l'amoni s'oxida alhora que la matèria orgànica s'acumula en forma de PHA. A continuació, en fase anòxica i manca de matèria orgànica (fam), el PHA acumulat s'utilitza com a font de carboni per a la desnitrificació.

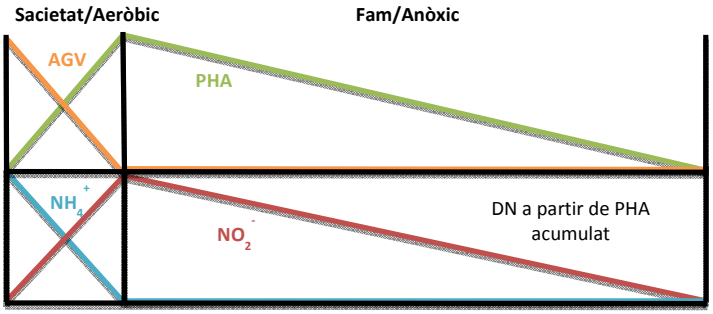


Figura 4. N/DN via nitrit mitjançant PHA com a font de carboni

2. OBJECTIUS

En aquesta tesi, s'han emprat diferents sistemes per a la recuperació de recursos de les aigües residuals. En primer lloc s'ha estudiat la recuperació de la matèria orgànica en forma de biogàs mitjançant digestió anaeròbia, i posteriorment en forma de bioplàstic en l'etapa d'eliminació de nutrients. A més a més, s'han aplicat processos biològics d'eliminació de nitrogen com la via nitrit i l'Anammox que suposen una reducció de costos operacionals important.

Els objectius específics d'aquest treball, que alhora defineixen l'estructura de la tesi són:

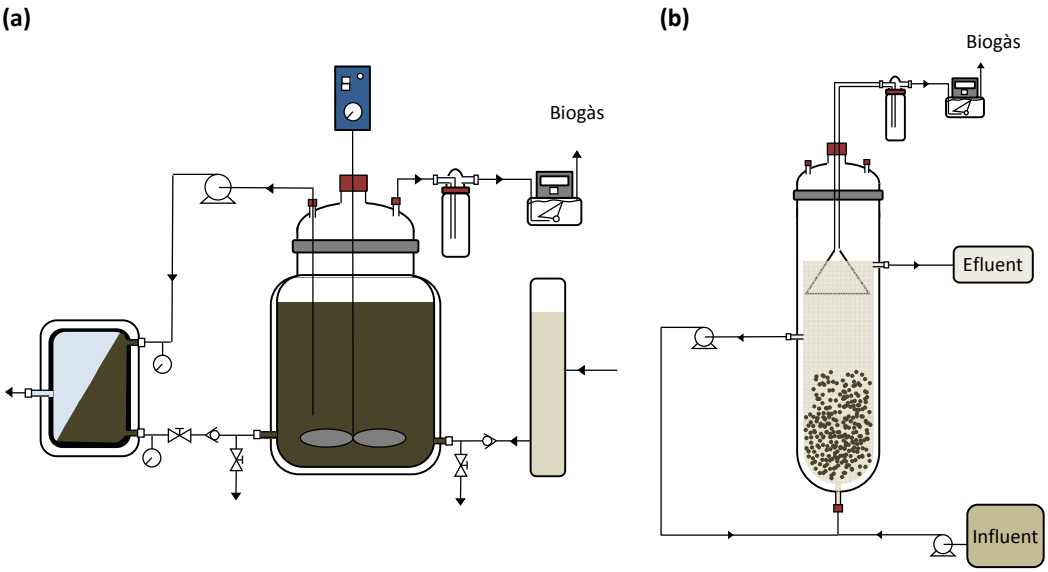
- Posar en marxa i operar un bioreactor de membrana anaeròbic (BRM-An) per al tractament d'aigua residual vitivinícola a diferents càrregues orgàniques avaluant la flexibilitat del sistema (Capítol 4).
- Estimar els requeriments energètics del BRM-An determinant la seva possible viabilitat en comparació amb un tractament aerobi (Capítol 5).
- Estudiar l'eficiència i l'activitat de la biomassa anaeròbia operant el BRM-An a baixa temperatura (Capítol 6).
- Posar en marxa i operar un reactor tipus UASB (de l'anglès upflow anaerobic sludge blanket), avaluant-ne la seva capacitat per tractar aigua residual vitivinícola a temperatura ambient en comparació amb el BRM-An (Capítol 7).
- Estudiar el sistema de nitrificació/desnitrificació via nitrit, utilitzant el bioplàstic acumulat en la biomassa com a font de carboni per al tractament de l'efluent de la digestió anaeròbia (UASB) d'aigua residual urbana (Capítol 8).
- Posar en marxa i optimitzar un sistema de nitrificació parcial / Anammox per al tractament del centrat de la digestió anaeròbia de fangs de depuradora, determinant-ne la població microbiana (Capítol 9).

3. MATERIALS I MÈTODES

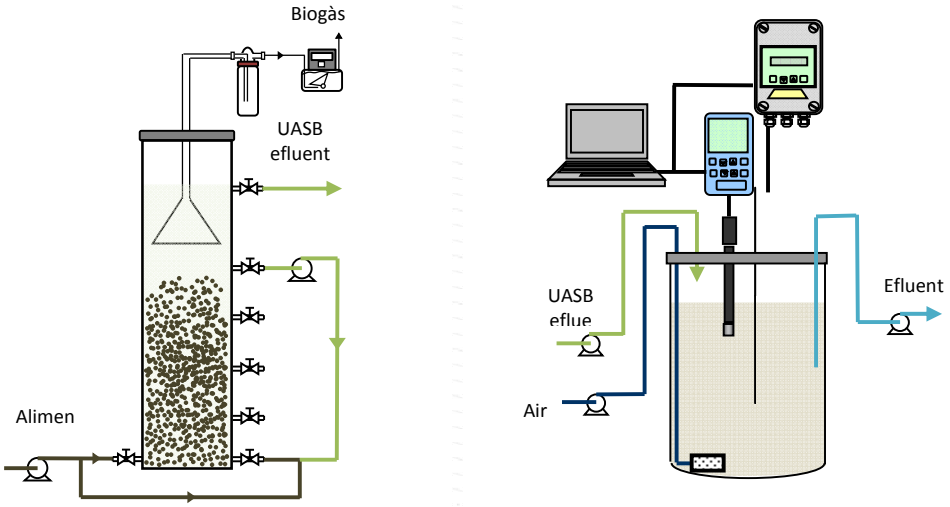
A continuació es detallen les instal·lacions i tècniques emprades per satisfer els objectius proposats.

3.1. Reactors biològics

S'han utilitzat quatre instal·lacions diferents esquematitzades a la Figura 5: (a) un BRM-An de 5L amb un sistema de membrana externa i (b) un reactor UASB de 1.5L per al tractament d'aigua residual vitivinícola; (c) un reactor UASB de 16L i un SBR (de l'anglès sequencing batch reactor) de 25L per a l'eliminació de matèria orgànica i nutrients d'aigua residual urbana; i (d) un reactor SBR de 3L per a la nitrificació parcial del centrat de la digestió anaeròbia de fangs de depuradora i un SBR de 5L per al posterior procés Anammox.



(c)



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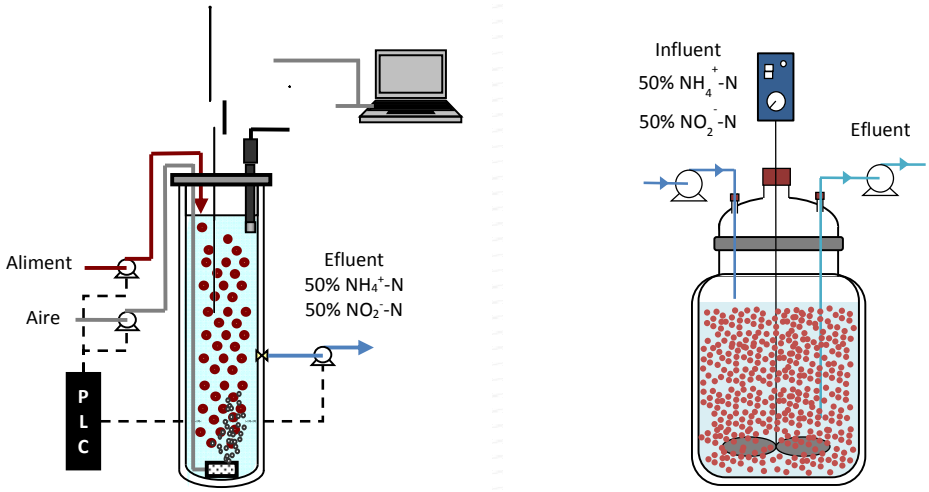


Figura 5. Esquemes dels reactors emprats: (a) AnMBR i (b) UASB per aigua residual vitivinícola; (c) UASB+SBR per aigua residual urbana i (d) SBR+Anammox per centrat de la digestió anaeròbia de fangs de depuradora

3.2. Mètodes analítics

Els paràmetres analitzats i els mètodes analítics emprats es recullen a la Taula 4.

Taula 4. Mètodes analítics

Paràmetre	**Mètode**
ST i SV	Mètodes de referències 2540B i 2540E*
SS i SSV	Mètodes de referències 2540D i 2540E*
DQO	Mètode de referència 5220C*
Amoni	Elèctrode selectiu Crison, Micro pH2002 model (mètode 4500-NH_3D*) Cromatografia iònica (Metrohm. Columna Metrosep C4 – 150/4.0 mm)
Nitrit i nitrat	Cromatografia iònica (Metrohm. Metrosep A Supp 4 250/4.0 mm)
Alcalinitat	Titració (pH-Burette 24)
AGV	Cromatografia de gasos amb detector FID
Metà i diòxid de carboni	Cromatografia de gasos amb detector TCD
Poblacions bacterianes	Hibridació fluorescent in Situ (FISH) (Amann, 1995)
Estudi de la membrana	Microscòpia Electrònica de Rastreig (SEM)
Potencial de metanització	Mètode segons Angelidaki et al. (2009)
Determinació de PHA	Mètode segons Lanham et al. (2013)
Test d'activitat nitrificant i d'acumulació de PHA	Seguiment de les espècies de N a diferents condicions mitjançant un respiròmetre (MARTINA, SPESS)
Test d'activitat Anammox	Mesura de l'increment de la pressió deguda a la producció de nitrogen en vials tancats sota condicions d'absència d'oxigen (Dapena-Mora et al., 2007)

* *Standard Methods for the Examination of Water and Wastewater (APHA, 2005)*

4. RESULTATS I DISCUSSIÓ

En aquest apartat s'inclourà una petita descripció de cada procés estudiat i se'n destacaran les conclusions més importants.

4.1. Bioreactor de membrana anaeròbic (BRM-An) per al tractament d'aigua residual vitivinícola

L'aigua residual vitivinícola es caracteritza per un alt contingut en matèria orgànica biodegradable i baix en nutrients. L'aplicació d'un procés anaeròbic per tal de valoritzar la matèria orgànica transformant-la en biogàs, pot contribuir a reduir significativament la demanda energètica del tractament. A més la manca de nutrients juga a favor dels tractaments anaeròbics, que requereixen una relació C/N/P d'aproximadament 800/5/1 (Moletta, 2005), mentre que en els tractament aeròbics el ràtio necessari és 100/5/1. No obstant això, l'aigua residual vitivinícola varia molt segons l'estació, de manera que durant el mesos d'estiu, quan es fa la verema, el volum a tractar i la càrrega orgànica és molt elevada i pot arribar als 10 gDQO L^{-1}. En canvi, a l'hivern, la concentració pot oscil·lar entre 0.5 i 1 gDQO L^{-1}. Això és un inconvenient per a l'aplicació de la digestió anaeròbia convencional, a causa de la escassa flexibilitat que força a operar a llargs temps de retenció hidràulics (TRH) per evitar el rentat de la biomassa. Per tant, les tecnologies d'immobilització de biomassa (com ara bioreactors de membrana, filtres biològics, llits fluïditzats, granulació...) permeten separar el TRH del temps de retenció cel·lular (TRC).

El BRM-An utilitzat en aquest estudi (Figura 5a) es va inocular amb biomassa mesofílica. el cabal d'aliment variava en funció del permeat obtingut, de manera que a mesura que la membrana s'embrutava el cabal disminuïa.

Es diferencien tres períodes d'operació a 35°C. En el primer període, es va alimentar el BRM-An amb aigua sintètica preparada amb vi blanc diluït i els nutrients necessaris en forma de NH_4Cl i KH_2PO_4, a més a més, es va addicionar $NaHCO_3$ per aportar alcalinitat suficient al sistema. La quantitat d'alcalinitat s'ajustava per mantenir un ràtio alcalinitat intermèdia (AI) respecte alcalinitat total (AT) inferior a 0.3, ja que es va observar que un ràtio superior donava lloc a una situació d'acumulació d'àcids en el reactor perillosa. En aquest primer període es va augmentar progressivament la concentració de DQO de l'influent fins a 9 gDQO L^{-1} arribant a tractar una càrrega de 2.5 kgDQO m^{-3} d^{-1}. El percentatge d'eliminació de DQO va ser del 96±3% i la producció de metà de 0.30±0.02 m^3CH_4 kg^{-1}DQO. Tot i que la càrrega orgànica no fos gaire alta en comparació amb altres exemple de sistemes anaeròbics intensius, la càrrega específica sí que era considerable amb un màxim de 0.70 kgDQO gSS^{-3} d^{-1}.

En un segon període experimental, es va alimentar el BRM-An amb aigua sintètica oscil·lant la seva concentració entre 1 i 6 gDQO L^{-1}. La quantitat d'alcalinitat necessària per compensar les variacions de DQO en l'aliment es va augmentar fins a 800 $mgCaCO_3$ L^{-1}.

Finalment, en un tercer període, es va alimentar aigua residual vitivinícola procedent de la verema incrementant progressivament la seva concentració fins a 5 gDQO L^{-1}. El percentatge d'eliminació de DQO es va mantenir elevat a 97±2%, tot i observar una concentració d'àcids més elevada en el reactor, cosa que va implicar una major addició d'alcalinitat (1000 $mgCaCO_3$ L^{-1}). La producció de biogàs obtinguda era de 0.50±0.17 m^3_{biogas} $m^{-3}_{digestor}$ d^{-1} amb una composició del 87% en metà. L'elevat percentatge de metà en el biogàs es veu afavorit per un TRH curt, al voltant de 2.3 dies.

Pel que fa a la membrana, a l'inici de l'experimentació es va calcular que el flux crític era de 23 LMH. Per tant, operar el BRM-An amb un flux per sobre del crític implicaria un embrutiment sever de la membrana disminuint-ne ràpidament el flux. Durant l'experimentació, es va augmentar el flux de treball, sent 10; 15 i 20 LMH en cada un dels períodes, per aconseguir una major capacitat de tractament. Al augmentar el flux mig, l'embrutiment de la membrana també augmentava, observant una pèrdua de flux de 0.07; 0.10 i 0.54 LMH d^{-1} provocant que les neteges de la membrana siguin més freqüents.

Per altra banda, es va determinar mitjançant el model de resistències a la filtració basat en la llei de Darcy, que la principal resistència es trobava en la formació de una capa a la superfície de la membrana, i que l'obturació dels porus només implicava un 1% de la resistència total observada. Després de mesos d'operació, es va determinar mitjançant microscòpia electrònica de rastreig que la capa de sòlids formada a la superfície de la membrana era principalment d'origen orgànic (Figura 6).

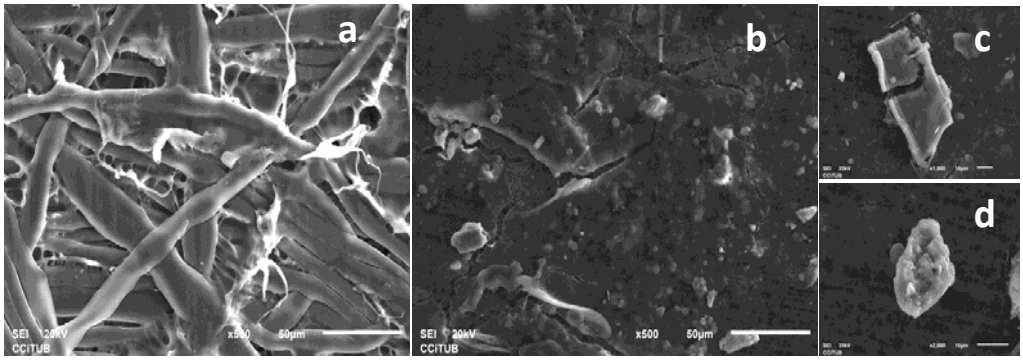
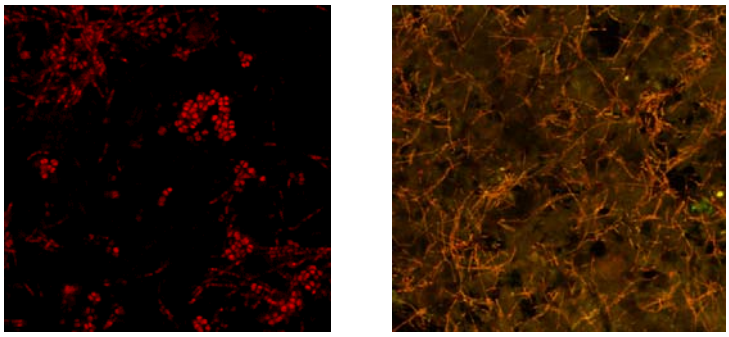


Figura 6. Microscòpia electrònica de rastreig d'una membrana nova (a) i una membrana després d'un període experimental (b).

Mitjançant testos de biometanització, es va observar un increment de l'activitat metanogènica de la biomassa. L'inòcul procedent de la digestió anaeròbia de fangs d'EDAR, tenia una activitat de 0.07 gCH_4-DQO $gVSS^{-1}d^{-1}$, mentre que al cap de 100 dies i 200 dies d'operació corresponents als dos primer períodes, aquesta va augmentar fins a 0.28 i 0.36 gCH_4-DQO $gVSS^{-1}d^{-1}$, respectivament. Aquest canvi substancial també es va observar en la població d'arquees metanogèniques. Es va observar que l'inòcul utilitzat contenia Methanomicrobiales spp., comuns en les EDAR, però que amb el temps es va enriquir en Methanosaeta spp. degut a una major afinitat amb el substrat (Figura 7).



(a) (b)

Figura 7. Resultat del FISH per a la sonda d'arquees (ARC915) durant el primer període (a); superposició de la sonda d'arquees (ARC915) i Methanosaeta spp. (MX825) (b).

4.2. Aspectes energètics de la tecnologia BRM-An en comparació amb un tractament aerobi basat en biomassa granular

L'estimació energètica del BRM-An (Equacions 1 i 2) es va dur a terme considerant la producció neta d'energia procedent d'una unitat CHP (de l'anglès combined heat and power) i les despeses de bombeig, agitació i calefacció (Astals et al., 2012). A més, es va afegir el terme de demanda energètica corresponent a la filtració de la membrana, que segons Martin et al. (2011) seria de 3.7 kWh m^{-3} per una membrana externa.

$$E_{electricitat}\ (kWh \cdot m^{-3}) = E_{CHP} - E_{bombeig} - E_{agitació} - E_{membrana}$$
$$E_{electricitat}\ (kWh \cdot m^{-3}) = [P_B \cdot TRH \cdot \xi \cdot \pi] - \theta - [TRH \cdot \omega] - E_{membrana} \quad (1)$$

$$E_{calor}\ (kWh \cdot m^{-3}) = E_{CHP} - E_{calefacció}$$
$$E_{calor}\ (kWh \cdot m^{-3}) = [P_B \cdot TRH \cdot \xi \cdot \psi] - [\rho \cdot \gamma \cdot (T_d - T_{ss}) \cdot (1 - \varphi) \cdot (1 + \eta)] \quad (2)$$

on P_B és la producció de biogàs; TRH=3.5d; ξ=4.18·10^4 kJ m^{-3}; π=0.35; θ=250 kJ m^{-3}; ω=300 kJ $m^{-3}_{digester}$ d^{-1}; ψ=0.55); ρ=10^3 kg m^{-3}; γ=4.18 kJ kg^{-1} °C^{-1}); T_d=35°C; T_{ss}=15°C; ϕ=0.85; i η=0.08.

Segons els balanços proposats, la producció de biogàs (P_B) mínima que fa que el balanç d'energia elèctrica sigui positiu és de 0.23 m^3 CH_4 m^{-3} d^{-1}, que es correspon a una concentració de DQO de 3.4 gDQO L^{-1}. Per a la mateixa P_B, el balanç de calor resulta en excés, ja que les unitats CHP o microturbina sempre són més eficients per produir calor que electricitat.

Per tal de reduir demanda energètica i poder obtenir un balanç positiu quan la càrrega orgànica és menor, és necessari treballar a temperatura ambient. Tot i així, com que la demanda elèctrica és tant alta degut a la filtració per membrana, aquesta aproximació no aportaria cap estalvi. Per aquest motiu, caldria modificar el sistema BRM per tal de minimitzar la demanda elèctrica. Diversos estudis de BRM a escala pilot han demostrat que les configuracions de membrana submergida requereixen entre 0.2 i 0.3 kWh m^{-3} (Ferrer et al., 2015; Martin et al., 2011), tot i necessitant recircular el biogàs per crear turbulències a la superfície de la membrana. Considerant que l'aplicació a gran escala del sistema estudiant es configuraria com un BRM-An de membrana submergida operant a temperatura ambient, el balanç d'energia esdevindria positiu per a concentracions de DQO superiors a 460 mg L^{-1} i P_B de 0.06 m^3 CH_4 m^{-3} d^{-1}.

Per altra banda, s'han calculat els requeriments energètics d'un SBR granular per al tractament del mateix residu descrit a López-Palau et al. (2012). El principal cost energètic d'operació del SBR és degut a l'aireig i al bombeig. Tenint en compte que els temps d'aireig i bombeig varien en funció de la càrrega orgànica (a més càrrega més temps d'aireig es necessita per eliminar major DQO), les equacions emprades pel càlcul són:

$$E_{req.}\ (kWh \cdot m^{-3}) = E_{aireig} + E_{bombeig} = \frac{P_w \cdot t_{aireig}}{Q} + \frac{P_p \cdot t_{bomeig}}{Q} \quad (3)$$

$$P_w = \frac{wRT}{29.7ne}\left[\frac{(p_2)^{0.283}}{p_1} - 1\right] \quad (4)$$

on t_{aireig} i $t_{bombeig}$ són variables (h d^{-1}); Q és el cabal diari (m^3 d^{-1}); P_w=0.064 kW; w=4.3·10^{-4} kg s^{-1}; R=8.314 kJ $kmol^{-1}$ K^{-1}; T=288.15 K; n=0.283; e=0.70; p_1=1 atm; p_2=3 atm; P_p=0.37 kW.

Per tant, tenint en compte que el reactor SBR es va operar satisfactòriament amb concentracions d'entre 1 i 8 gDQO L^{-1}, la demanda energètica oscil·la entre 0.15 i 0.57 kWh m^{-3}. Comparant ambdós tecnologies, cal destacar que el SBR aeròbic implica menys demanda energètica, tot i que en qualsevol cas sempre implicarà una despesa. El punt clau dels processos anaeròbics, tot i que consumir més energia i ser sistemes més complexos, és que la producció de biogàs compensi la despesa. Segons els càlculs realitzats, el BRM-An seria viable únicament amb la configuració de membrana submergida.

4.3. Bioreactor de membrana anaeròbic (BRM-An) per al tractament d'aigua residual vitivinícola a baixa temperatura

Degut a les variacions estacionals que l'aigua residual vitivinícola pateix al llarg de l'any, el BRM-An descrit en l'apartat anterior es va operar a baixa temperatura, concretament a 25°C i després a 15°C, simulant les condicions que s'obtindrien a l'hivern. La temperatura del BRM-An es va reduir de 35°C a 25°C i posteriorment a 15°C, de manera que es va utilitzar un inòcul mesofílic format únicament per Methanosaeta spp.

Quan la digestió anaeròbia es duu a terme a baixa temperatura, cal considerar diversos aspectes importants. Primer de tot, ja que la cinètica és més lenta (Lettinga et al., 2001), el risc d'acidificació és major.Cal afegir un mínim d'alcalinitat al sistema per tal d'assegurar un pH neutre en cas d'acumulació d'àcids. El paràmetre que alerta sobre la manca d'alcalinitat és el ràtio entre alcalinitat intermèdia i alcalinitat total (AI/TA). De manera que en condicions estables, aquest ràtio és menor a 0.30 i quan augmenta significa que s'estan acumulant àcids al medi. En comparació amb els resultats obtinguts a temperatura mesofílica, es van acumular AGV més fàcilment obtenint en el permeat 183±135 mgAGV L^{-1} i 132±105 mgAGV L^{-1} a 15°C i 25°C, respectivament. Per tal d'assolir eficiències d'eliminació de DQO acceptables, el TRH es va augmentar fins a 4.5 dies. No obstant això, la DQO de l'efluent va ser més gran que el límit permès a causa dels AGV acumulats, sent de 0.39 gDQO L^{-1} i 0.28 gDQO L^{-1}. D'aquesta manera, l'eliminació de DQO va ser del 71% a 15°C i del 80% a 25°C.

En segon lloc, cal tenir en compte que el metà es dissol a la fase líquida més fàcilment a baixa temperatura, de manera que part de la producció de biogàs es pot perdre per aquesta via. Mitjançant els balanços de matèria corresponents, es va calcular que la producció de metà esperada havia de ser al voltant de 0.30 m^3CH_4 kg^{-1}DQO, tal i com es va observar a temperatura mesofílica. No obstant, a 25°C es va obtenir una producció molt baixa de 0.03 m^3CH_4 kg^{-1}DQO i a 15°C no es va poder determinar. A partir de la llei de Henry, es va calcular que es perdia un 6.7% i un 10.2% de la càrrega orgànica en forma de metà dissolt en el permeat. Tot i així, la quantitat de biogàs dissolt en el permeat no justifica la baixa producció observada. Un altre aspecte que pot influir en la mesura de la producció de biogàs és el volum buit del reactor, sent en aquest cas d'un 30% del volum total. D'aquesta manera és possible que en cas d'una producció baixa de biogàs, aquest es quedi acumulat en la part superior del reactor sense ser capaç de superar la pressió que exerceix el comptador de biogàs. El percentatge de metà en el BRM-An es va poder determinar agafant mostra de la part superior del digestor, obtenint un 81% i un 83% de metà.

Pel que fa al mòdul de membranes, es va observar un major grau d'embrutiment que en condicions mesofíliques. Com a conseqüència, les neteges eren cada vegada més freqüents ja que el flux decreixia a 3.63 i 2.14 LMH d^{-1} per a 15°C i 25°C, tot i que la quantitat de SS en el reactor era menor, de 2.7 g L^{-1}.

Es va avaluar l'activitat de la biomassa mitjançant testos del potencial de biometanització. Es obtenir que per 25°C l'activitat metanogènica era similar a l'obtinguda a 35°C, 0.35 gCH_4-DQO g^{-1}SS d^{-1}. No obstant, la producció de metà era menor, de 0.26 m^3CH_4 kg^{-1}DQO, degut a una menor biodegradació del substrat. A 15°C tant l'activitat com la producció van disminuir significativament fins a 0.14 gCH_4-DQO g^{-1}SS d^{-1} i 0.09 m^3CH_4 kg^{-1}DQO.

Pel que fa a la població microbiana, es va observar que, tot i partint d'un inòcul mesofílic on l'espècie metanogènica principal era la Methanosaeta spp., amb la disminució de temperatura que va provocar una major acumulació d'àcids es va desenvolupar la Methanosarcina. Aquest canvi va ocórrer relativament ràpid, al cap de 30 dies d'operació a 25°C les dues espècies eren presents en el reactor i al cap de 80 dies ja només es va observar la Methanosarcina. Les Methanosarcina són més tolerants a la presència d'àcids que les Methanosaeta spp., motiu pel qual el seu creixement es veu afavorit en les condicions del BRM-An a baixa temperatura.

4.4. Tractament d'aigua residual vitivinícola mitjançant un UASB amb membrana externa

Els reactors UASB (de l'anglès Upflow Anaerobic Sludge Blanket) van sorigr als anys 70 i s'han estat usant pel tractament d'aigües residuals d'alta càrrega orgànica gràcies a la seva capacitat d'acumular grans quantitats de biomassa en forma de grànuls. Degut al creixent interès en l'ús del BRM-An per al tractament d'aigües menys carregades, el reactor tipus UASB resulta una opció interessant per tal de reduir l'embrutiment de la membrana ja que la quantitat de sòlids en contacte directe amb la membrana és més reduïda.

En aquest estudi es va utilitzar un reactor UASB convencional a temperatura ambient, tal i com es mostra a la Figura 5b, i després se li va acoblar el mòdul de membrana utilitzat pel BRM-An, descrit anteriorment.

El reactor UASB es va operar durant 107 dies diferenciant-ne dos períodes en funció de la càrrega orgànica aplicada, 2.6 i 5.5 kgDQO m^{-3} d^{-1}. Durant ambdós períodes, no es van observar inestabilitats degudes a l'acumulació d'AGV ja que la càrrega orgànica específica era baixa gràcies a la gran quantitat de biomassa en el reactor (41.5 gSS L^{-1}). Tot i així, l'eficiència d'eliminació de DQO no era tant alta com s'esperava, 75% i 84%, respectivament.

Si bé en el període de més alta càrrega es va observar més quantitat d'AGV a l'efluent, la DQO total era elevada a causa dels sòlids que es perdien. Les oscil·lacions de la càrrega aplicada afavorien la desagregació dels grànuls fent que la biomassa amb poca capacitat de sedimentació no fos retinguda.

Per aquest motiu, es va acoblar el mòdul de membranes al UASB, convertint-se en un BRM-UASB. D'aquesta manera, l'eliminació de DQO es va incrementar fins a un 92%, obtenint un efluent lliure de SS i amb un DQO inferior a 125 mg L^{-1}, complint el requeriments legals. Comparant la producció de biogàs del UASB i el BRM-UASB, no es van observar diferències significatives, obtenint una producció de 0.17 m^3CH_4 kg^{-1}DQO i un alt percentatge de metà en el biogàs (95%).

En comparació amb el BRM-An a baixa temperatura, el BRM-UASB presenta una sèrie d'avantatges. La possibilitat de retenir una major quantitat de biomassa permet una major capacitat de tractament i assegura l'estabilitat del procés. No obstant això, no es va observar una millora en la filtració tot i que l'efluent del UASB contenia molts menys sòlids. Amb les variacions de càrrega, els grànuls mica en mica perdien la seva estructura que a la llarga es convertirien en biomassa suspesa.

Mitjançant testos del potencial de biometanització, es va determinar que la biomassa granular del UASB tenia molta més activitat que la biomassa suspesa del BRM-An, 2.1 gCH_4-DQO g^{-1}SS d^{-1}respecte 0.35 gCH_4-DQO g^{-1}SS d^{-1}. Per contra, el potencial de producció de biogàs obtingut era menor, 0.18 m^3CH_4 kg^{-1}DQO respecte 0.26 m^3CH_4 kg^{-1}DQO, possiblement per una falta de difusió del substrat en l'estructura granular.

4.5. Tractament d'aigua residual urbana mitjançant un UASB acoblat a un SBR de nitrificació/desnitrificació via nitrit

Un nou esquema aplicat al tractament d'aigua residual urbana es mostra a la Figura 8. Consisteix en una primera etapa anaeròbica mitjançant un UASB seguit d'una etapa d'eliminació de nitrogen a partir d'un reactor SBR, on es duu a terme la nitrificació/desnitrificació via nitrit. Degut a que el sistema d'eliminació de nitrogen via nitrit requereix de condicions canviants aeròbiques/anòxiques i d'un subministrament de font de carboni per desnitrificar, en aquest capítol s'ha enfocat cap a la seva possible integració amb la producció de PHA.

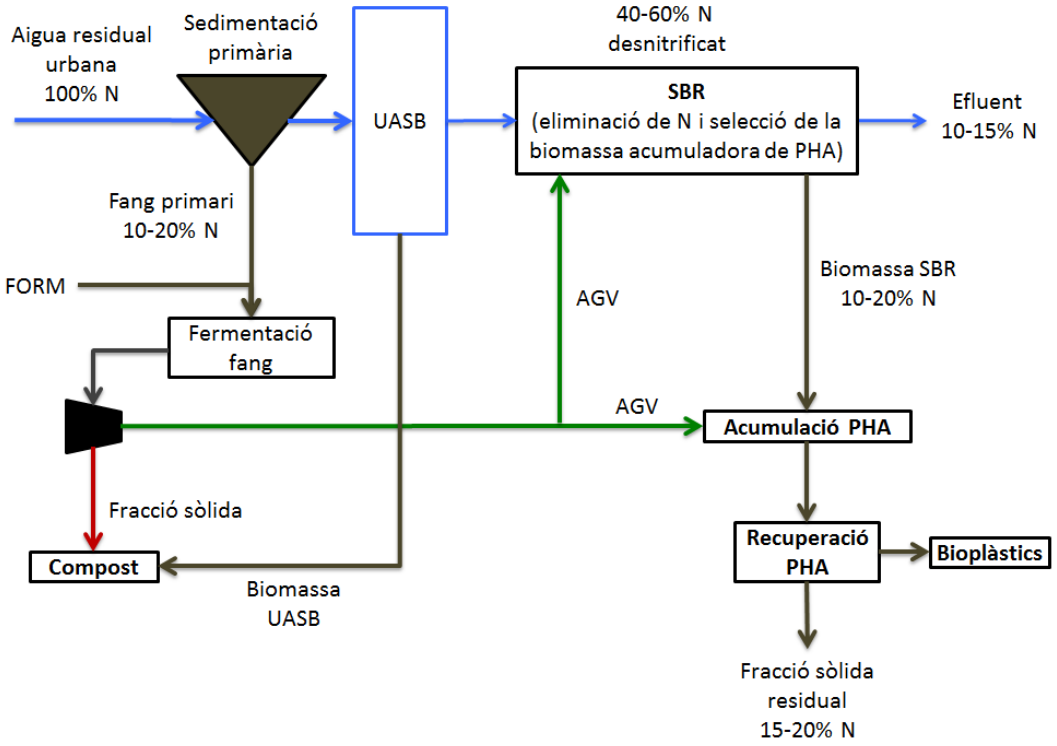


Figura 8. Integració dels processos d'eliminació de nitrogen i producció de PHA

El reactor UASB es va operar en condicions fixes per tal d'obtenir un efluent estable per alimentar el SBR. Es va inocular amb biomassa anaeròbia granular ocupant aproximadament la meitat del seu volum. És important mantenir una bona velocitat ascensional, al voltant de 1 m h^{-1}, per la qual cosa va ser necessari afegir una recirculació interna tal i com es mostra a la Figura 5. Les condicions d'operació del UASB eren un cabal de 40 L d^{-1} amb un TRH de 10h i a una temperatura de 21±3°C. La producció de biogàs va ser de P_B=0.30±0.02 $m^3\,m^{-3}\,d^{-1}$ amb un 60% de metà, i per tant una producció específica de 0.28±0.06 $m^3CH_4\ kg^{-1}$COD. L'eliminació de DQO aconseguida va ser del 82%, de manera que l'efluent conté un ràtio C/N baix.

Per tal de poder dur a terme una correcta N/DN via nitrit es requereix un ràtio C/N de 2.5, i per aquest motiu es van estudiar diferents fonts de matèria orgànica econòmicament viables. S'han considerat dues fonts de carboni per a l'acumulació de PHA en el SBR de N/DN: el líquid de fermentació de la fracció orgànica del residu municipal (FORM) i de la mescla entre FORM i fang primari. Ambdós residus contenien gran quantitat d'AGV, de manera que s'ajustava la dosi segons l'amoni present a l'efluent de l'UASB. A la Figura 9 es

mostra un exemple del cicle del SBR. En una primera fase aeròbica, els AGV es degraden ràpidament mentre s'acumula PHA en la biomassa. Després en la fase anòxica, el PHA acumulat s'utilitza per desnitrificar.

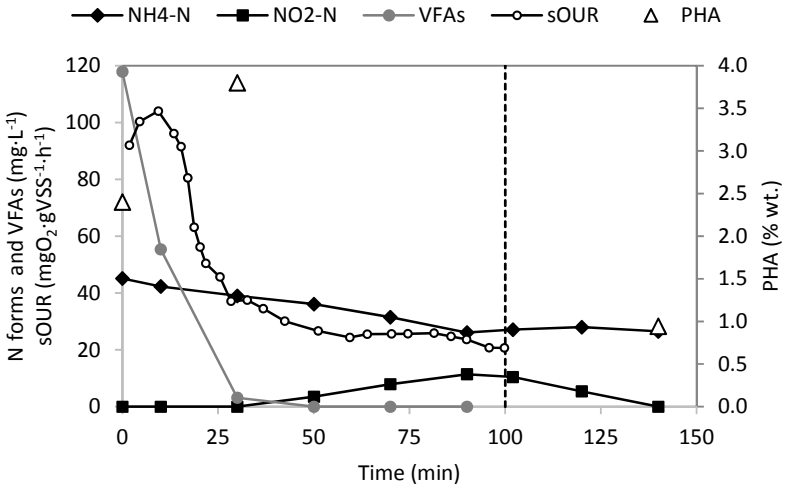


Figura 9. Fase de selecció de la biomassa acumuladora de PHA amb eliminació de nitrogen.

Degut als nivells d'oxigen baixos, necessaris per mantenir la N/DN via nitrit, la presència d'AGV afectava significativament la velocitat de nitrificació. De manera que un excés d'AGV resultava en una nitrificació escassa, i pel contrari, una deficiència d'AGV resultava en una desnitrificació escassa.

Pel que fa a la fase d'acumulació, es va obtenir al cap de 10h d'alimentar segons demanda, un 10.6% i un 8.6% de PHA utilitzant FORM i la mescla de fang primari i FORM, respectivament.

Degut als nutrients que contenen aquestes fonts de carboni, gran part de la DQO afegida s'utilitza pel creixement de la biomassa i no per l'acumulació de PHA. A més a més, la DQO que no es troba en forma d'AGV, no contribueix a la producció de PHA, fet que també promou el creixement de la biomassa. Per aquests motius, és preferible que la font de matèria orgànica contingui el màxim d'AGV i el mínim de nutrients i matèria particulada. No obstant això, cal tenir en compte el cost d'aquesta font de carboni, ja que per tal d'integrar la producció de PHA en una EDAR no pot suposar un increment significatiu en els costos d'operació, sinó un benefici afegit de la depuració d'aigües.

4.6. Nitrificació parcial – Anammox per al tractament de centrat de la digestió anaeròbia de fangs de depuradora

El centrat de la digestió anaeròbia de fangs de depuradora es caracteritza per un alt contingut d'amoni i molt baix en DQO, i per un ràtio molar alcalinitat/amoni al voltant de 1. Aquestes característiques afavoreixen el sistema de nitrificació parcial – Anammox, ja que l'escassa alcalinitat frena la reacció de nitrificació obtenint un efluent amb 50% amoni i 50% nitrit, ideal per a les bactèries Anammox.

Els reactors emprats per aquest procés (Figura 5c) consistien en SBR operant en cicles. En el primer pas de nitrificació parcial, la biomassa ja estava aclimatada per aquest tipus d'aigua i es van mantenir les condicions d'operació segons López-Palau et al. (2011) per obtenir un efluent estable.

El reactor Anammox es va inocular amb biomassa procedent d'una planta pilot holandesa. Es va posar en marxa el reactor amb aigua sintètica ajustant-ne la concentració fins que a la sortida no s'observés nitrit per tal d'evitar la inhibició de la biomassa, molt sensible a altes concentracions de nitrit. Paral·lelament a l'operació del reactor SBR, es duien a terme testos d'activitat Anammox determinant la SAA (de l'anglès specific Anammox activity). Calia procurar que la càrrega del reactor (sNLR de l'anglès specific nitrogen loading rate) no superés l'activitat de la biomassa per evitar la seva fallida. A la Figura 10 es mostra l'evolució de la SAA i la sNLR per a diferents períodes.

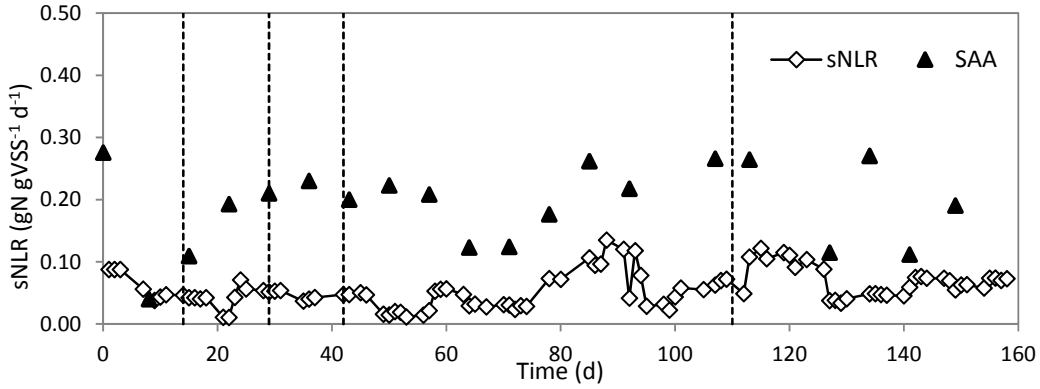


Figura 10. SAA i sNLR del reactor Anammox (P1: aigua sintética; P2: efluent diluït del SBR de nitrificació; P3: efluent del SBR de nitrificació; P4: efluent diluït del SBR de nitrificació)

Globalment es va aconseguir una eliminació d'amoni del 92% i de nitrit del 88%. No obstant això, es va observar que l'aspecte de la biomassa havia canviat de vermell a marronós i que

l'estequiometria NH_4^+-N:NO_2^--N:NO_3^--N observada era 1:1.25:0.14, lleugerament diferent de la teòrica (1:1.32:0.26). Aquest fet pot indicar que no només hi ha Anammox en el reactor sinó que com que l'aigua tractada conté certa DQO, hi podria haver simultàniament desnitrificació heterotròfica.

Anàlisis de PCR-DGGE (de l'anglès polymerase chain reaction - denaturing gradient gel electrophoresis) es van dur a terme per tal d'identificar la població microbiana present en el reactors. Es va determinar clarament que la biomassa de nitrificació parcial no es trobava ni interferia en el reactor Anammox i que, per tant, la retenció dels grànuls nitrificants era efectiva. Pel que fa a la biomassa Anammox, La tècnica DGGE no va detectar cap tipus de bactèria Anammox, degut probablement a la poca abundància relativa, que si és de <9% es trobaria per sota del límit de detecció del mètode.

Llavors, pel tal d'identificar algun tipus de bactèria Anammox, es va utilitzar el mètode de piroseqüència 16S rADN, més acurat que el DGGE. Es va determinar l'abundància relativa de la població microbiana tant del reactor Anammox, com el de nitrificació parcial (Figura 11).

Es van seleccionar les seqüències relacionades amb les *Planctomycetes* (fílum on es troben les espècies Anammox). Es va determinar que un 1.4% de la població microbiana formava part del grup de bactèries Anammox i que el 93.3% d'aquestes són del gènere *Brocadia.* Aquest gènere seria el responsable de la reacció Anammox, tot i que el rol de la resta de població microbiana necessitaria de més estudi.

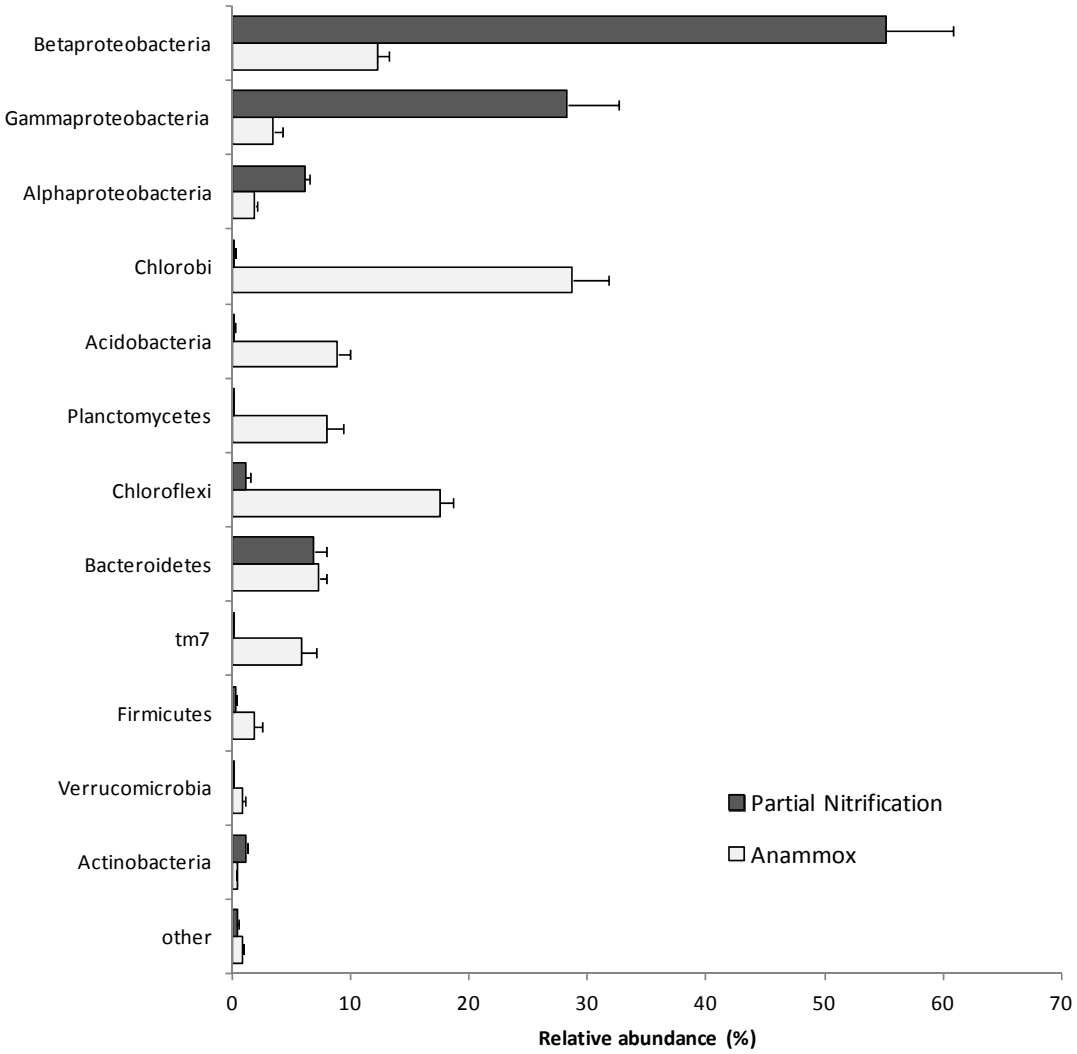


Figura 11. Abundància relativa dels majors fílums detectats (>0.5%) mitjançant la piroseqüència 16S rARN

5. CONCLUSIONS I RECOMANACIONS

5.1. Conclusions

Pel que fa als capítols 4 i 5 sobre el BRM-An pel tractament mesofílic d'aigua residual vitivinícola:

- Es va depurar satisfactòriament l'aigua residual vitivinícola aconseguint un 97% d'eliminació de DQO i una producció de biogàs de 0.05±0.17 $m^3_{biogàs}$ $m^{-3}_{digestor}$ d^{-1}.
- La producció de metà va ser de 0.33±0.15 $m^{-3}CH_4$ kg^{-1}DQO, amb una elevada concentració en el biogàs de 87% afavorit per un TRH curt.
- Les inestabilitats produïdes per les oscil·lacions en la concentració de DQO en l'influent es van controlar mitjançant l'addició de suficient alcalinitat per mantenir un ràtio AI/AT inferior a 0.4, corresponent a una alcalinitat mitja en el reactor al voltant de 1000 $mgCaCO_3$ L^{-1}.
- L'estabilitat del procés també es va veure afavorida per l'aclimatació de la biomassa. Degut a que el BRM-An permetia la selecció de la biomassa amb característiques més afines al substrat, l'activitat va augmentar de 0.07 a 0.36 gCH_4-DQO $gVSS^{-1}$ d^{-1}.
- El balanç energètic del BRM-An va revelar que el biogàs produït era suficient per compensar la demanda energètica de la membrana externa només quan la càrrega orgànica era alta, és a dir, durant la verema. Per a que el sistema BRM-An sigui viable tot l'any, la configuració més adequada a gran escala seria la membrana submergida.

Pel que fa capítol 6 sobre l'operació del BRM-An a baixa temperatura:

- A baixa temperatura l'eficiència d'eliminació de DQO era menor, d'un 71% i un 80% a 15°C i a 25°C, respectivament. Degut a l'acumulació d'àcids en el reactor i també al metà dissolt, la DQO de l'efluent no sempre complia els requisits d'abocament, per tant caldria considerar un post-tractament de recuperació de metà per tal de polir l'efluent obtingut.
- En comparació amb el BRM-An a temperatura mesofílica, es va observar un major embrutiment de la membrana tot i treballant a menors concentracions de sòlids en el reactor. Degut a que la major resistència es devia als sòlids dipositats a la superfície, les neteges es duien a terme només amb aigua destil·lada aplicant una velocitat de flux creuat alta, arrossegant la capa de sòlids i recuperant el flux inicial.
- L'activitat de la biomassa es va veure reduïda a mesura que disminuïa la temperatura. Tot i així, l'activitat a 25°C era similar a l'obtinguda a 35°C.

- Respecta a la població microbiana, es va observar que partint d'un inòcul mesòfil format principalment per Methanosaeta spp. es va anar enriquint en Methanosarcina, molt més tolerant a la presència d'àcids en el medi.

Pel que fa al capítol 7 sobre el UASB i el BRM-UASB pel tractament psicrofílic d'aigua residual vitivinícola:

- La capacitat de tractament del reactor BRM-UASB era major que el del BRM-An degut a una major capacitat de retenció de la biomassa.
- Les oscil·lacions típiques d'aquesta aigua residual afavorien la desagregació de la biomassa granular obtenint un efluent amb una DQO per sobre el límit legal.
- Acoblant la membrana al UASB convencional, BRM-UASB, s'obtenia un efluent amb les condicions necessàries per l'abocament.
- L'activitat de la biomassa granular era molt superior que la de la biomassa suspesa, però per contra el potencial de biometanització era inferior, possiblement per una manca de difusió del substrat en els grànuls.
- El cost que suposa la implementació d'un sistema de membranes en el cas d'un reactor UASB, que per sí sol reté la biomassa, pot ser difícil de justificar. Reduint la càrrega per tal d'evitar la desagregació dels grànuls i minimitzar els sòlids en l'efluent seria una estratègia menys costosa.

Pel que fa al capítol 8 sobre el sistema UASB – SBR via nitrit integrat a la producció de PHA en la principal línia d'aigües d'una EDAR:

- Un nou esquema per al tractament d'aigua residual urbana es va desenvolupar integrant la producció de PHA i l'eliminació de nitrogen en una sola etapa, utilitzant el PHA acumulat com a font de carboni per a la desnitrificació.
- L'addició del líquid de fermentació en la fase aeròbica va promoure la competició per l'oxigen dissolt entre les bactèries heterotròfiques i autotròfiques, afectant significativament a la velocitat de nitrificació.
- Comparant l'eficiència de les dues fonts de carboni utilitzades, el líquid de la fermentació de la FORM va donar lloc a una major acumulació de PHA, possiblement pel fet de que el seu contingut en DQO lentament biodegradable era menor.

Pel que fa al capítol 9 sobre el procés Anammox pel tractament del centrat de la digestió anaeròbia:

- El centrat de la digestió anaeròbia va ser tractat satisfactòriament mitjançant el procés de nitrificació parcial – Anammox en dues etapes.
- A diferència de l'esperat teòricament, l'estequiometria de la reacció Anammox observada era 1:1.25:0.14, probablement degut a la presència de certa DQO que dóna lloc a la desnitrificació heterotròfica convencional.
- La caracterització microbiana va determinar que la principal AOB és *N. eutropha* confirmant la completa inhibició de les NOB.
- Es va identificar el gènere *Brocadia* com el principal responsable de la reacció Anammox, però amb una abundància relativa molt baixa (1.4%). El paper de la resta de població microbiana identificada en el reactor Anammox necessita de major estudi per determinar-ne la seva funció.

5.2. Recomanacions

- Per consolidar l'aplicació d'un sistema BRM-An pel tractament d'aigua residual vitivinícola caldria un estudi econòmic exhaustiu. Generalment, l'aplicació d'un sistema de membranes és viable si l'aigua obtinguda es reutilitza, d'altra banda difícilment és econòmicament factible.
- Un estudi microbiològic a fons podria determinar les condicions òptimes per tal de que els processos anaerobis a temperatura ambient patissin el mínim de repercussions davant els canvis estacionals tant de temperatura com de càrrega orgànica.
- A baixa temperatura el metà dissolt suposa una pèrdua de rendiment i una amenaça pel medi ambient. Caldria considerar post-tractaments per tal de recuperar-lo o utilitzar-lo com a font de carboni, per exemple, per una posterior nitrificació/desnitrificació.
- La producció de PHA en una EDAR és un valor afegit valuós, ja que encara que el rendiment no sigui alt s'aconsegueix mitjançant fonts de carboni econòmiques. No obstant, el procés d'extracció és el factor limitant ja que suposo un cost molt alt i només és factible si el percentatge de PHA a les cèl·lules és per sobre del 90%. Per aquest motiu, cal investigar altres tècniques d'extracció més econòmiques per obtenir bioplàstics de la biomassa.

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