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Master Thesis

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LAB-SCALE ANAEROBIC DYNAMIC MEMBRANE BIOREACTOR WORKING UNDER PSYCHROPHILIC CONDITIONS: TOWARDS A SUSTAINABLE SOLUTION FOR LOW- STRENGTH MUNICIPAL WASTEWATER TREATMENT

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A Ludovica,
a cui 'la tesi dei batteri'
era da subito piaciuta

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A tutti grazie per avermi accompagnato verso questo nuovo inizio.

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SECTION I:

My Thesis and Me

I began thinking about the topic of my Master thesis in spring 2014. I had just come back from Germany, were I studied one semester thanks to the Erasmus program. The experience in Dresden was a turning point in my life. It was a challenging period, which made me more conscious about my strong points but my weaknesses, too. Anyway, I had not clear plans for my future. For this reason, I took into considerations several different themes, as I thought that the topic of the thesis might have an influence on my work opportunities and, consequently, on my life. The way towards this thesis was not therefore straight, but it brought me back to one of my "first loves", which was Wastewater Treatment.

When Dr. Luca Alibardi and Dr. Alessandro Spagni suggested me this topic, I knew very little about Membrane Bioreactors and even nothing about Dynamic Membranes technology. Anyway, it made me curious, so I began the work with enthusiasm.

The first meeting with my co-supervisors took place on July 1st. They explained me what I was asked to do. We set together a precise schedule for the whole experiment and the goals of the work. The next day we were ready to start. Nearly all the parts of the setup had been already prepared, as a similar experiment had been performed the previous year. Anyway, all pieces were lying in a box and everything had to be installed again. Even though I had never been good at "men's stuffs", I learned not only what a hex-key is, but also how to use it. We worked hard and in few days Andy- the name of the setup, which stands for Anaerobic Dynamic membrane reactor- was ready for the startup. Figure 1 is a picture of me at work, opening the membrane support. Andy's setup is represented in Figure 2.

Figure 1: Me at work during setup preparation.

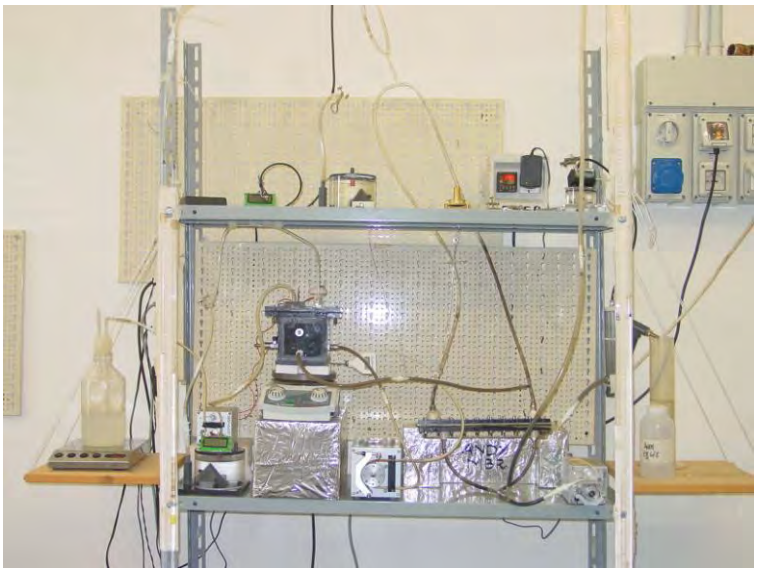

Figure 2: Andy setup.

On the contrary, I did not feel so ready. The first weeks where in fact quite tiring, as I had to learn a lot in very few time and I felt inadequate. I had to understand how the system was actually working and how to manage the different daily problems occurring. My co-supervisors, together with the technician Dr. Annalisa Sandon, taught me how to move in the chemistry lab and how to perform the periodical analysis on the effluent, on the mixed liquor and on the biogas extracted from the system. My experiences in a lab were in fact pretty limited and I barely knew how to hold a pipette. The issue was not simply learning the standard procedures. I had to understand how to find out the meaning of the results, how to reach a balance between accuracy in the measurement and useless superabundance, the number of significant digits in the results, the adequate frequency of the analyses. During the first weeks I used to spend a lot of time for the analyses, as I was clumsy and I had often to repeat the same measurements because, for example, the sample dilution performed was not sufficient for reading. Anyway, with the time I sped up and organized my time better. In this sense, I have to thank my fellows, too, as they helped me a lot. We supported each other as a team and enjoyed working together, in order to optimize our time and have good time.

During the first two months, Andy showed several problems. It was subjected to clogging and the startup turned out to be pretty long. It happened very often that, as we arrived to the lab in the morning, we found the reactor empty because all biomass had been washed out from the system during the night. Consequently, new sludge had to be inoculated. An example of the consequences of clogging on the system is represented in Figure 3. Figure 4 reports a picture of sludge seed.

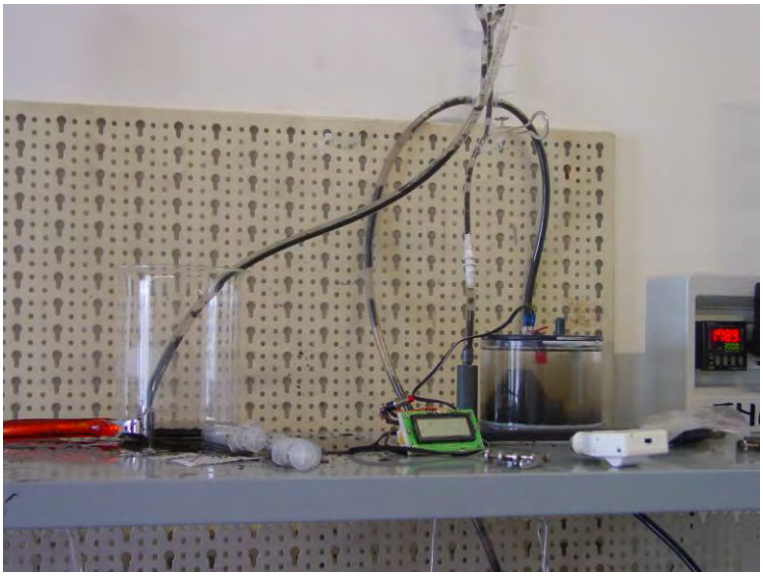

Figure 3: An example of solids washout induced by system clogging. Sludge went up the pressure gauge and dropped into the gas meter connected to the membrane support.

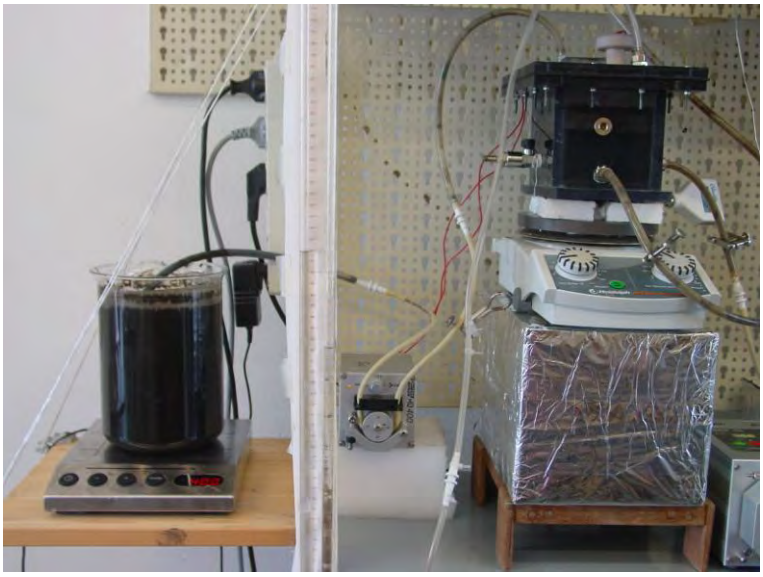

Figure 4: new sludge inoculum.

We had to open the system several times and restart it. Even though no outstanding results were achieved during the first period, all these problems allowed us to understand better the influence of operating conditions on the system and, in particular, on biomass acclimation. Meanwhile, an issue to tackle was the improvement of some analytical procedure we were applying for effluent and mixed liquor characterization. In fact, Dr. Alibardi and Dr. Spagni were interested in applying the spectrophotometric method to COD concentration determination.

Spectrophotometry had been already largely used in the lab for, as an example, phosphorous or ammonium ion analysis. Anyway, it had not been tested for COD, yet, even though this method is reported in Standards Methods. The interest in spectrophotometric COD analysis rose mainly from the needs of reducing sample volumes used. Moreover, this method is quick and involves less reagents consumption and waste production. Anyway, no calibration curves had been already set in the spectrophotometer, so Dr. Spagni taught me how to prepare one by means of defined-concentration samples. As a sort of exercise, we made the first trial with ammonium concentration, so that it was possible to compare the results with the curve already set in the apparatus. After that, we moved on to COD. During the first two months I prepared three different calibration curves, focusing on different COD concentration ranges, in order to find out which one fitted more for the characteristics of our effluent. We tested several ways to prepare the blank and the samples, as they showed the tendency of turning turbid. It took some weeks but we managed polishing the procedure up we obtained reliable results. Some pictures about the spectrophotometric COD analyses are reported in Figure 5.

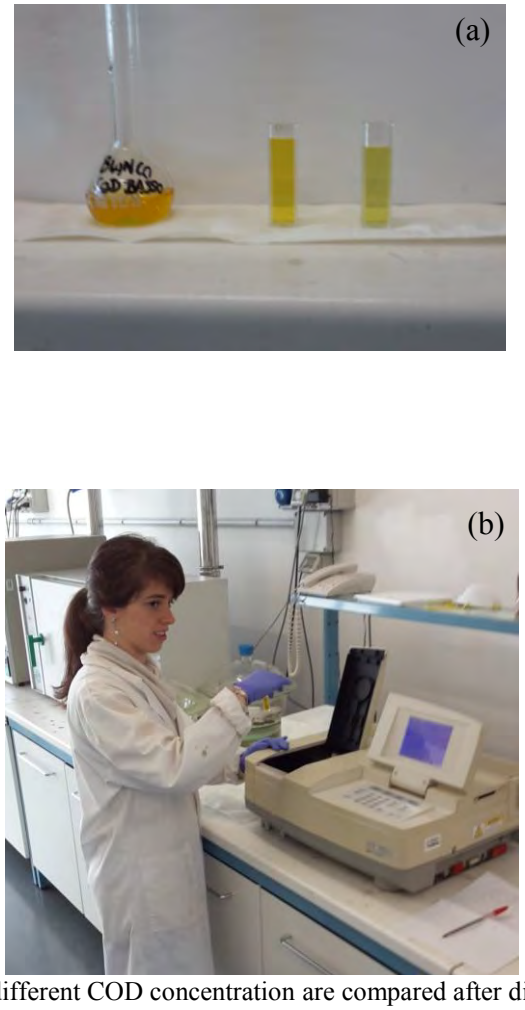

Figure 5: (a) Sample with different COD concentration are compared after digestion with the blank and (b) spectrophotometric reading of the samples.

The system reached stable conditions at the end of August. The results achieved in term of wastewater treatment and fluxes applied were promising. Moreover, our suppositions about the effective role of dynamic membrane in organic matter oxidation seemed to be valid.

I felt more acquainted to both the analyses and the system management. Anyway, when Dr. Alibardi expressed his intentions to settle temporarily in the UK, I was worried, as I was afraid of not being able to run the system alone. During the first days after his departures, I felt pretty lost. In fact, Dr. Alibardi had been always in the lab and I had rarely taken decisions alone. Anyway, I decided to catch the opportunity to show to myself that I was able to do it all alone and to apply everything I had learned the months before. Anyway, I kept having daily contacts with both my co-supervisors. Skype and WhatsApp turned out to play a fundamental role in the positive ending of this research project.

The system was shut down in the second week of November. It was sad to me to take Andy apart, as it had become a sort of figure in my daily life in the lab. Moreover, putting Andy again into the box made me realizing that this peculiar period of my life was going to end.

Overall, I am really satisfied about the work for my thesis. This experience was very formative and gave me the opportunity to work with nice and competent people. Moreover, I got very interested in the topic of dynamic membrane and I would be pleased to have the possibility to keep working in this field.

SECTION II:

Scientific paper

1. Introduction

The main advantages of anaerobic biological treatment applied to waste and wastewater were already outlined in 1960s (McCarty, 1964). Low biomass growth yield was remarked under anaerobic conditions with respect to aerobic ones. This leads to a lower production of excess sludge to be disposed off, lower nutrients requirement, and higher degree of organics stabilization, as a smaller portion of biodegradable waste is converted into biomass. Moreover, anaerobic plants may potentially work under neutral or positive energy balance, as aeration is not required and methane-rich biogas is generated as end product. As reported recently by Smith *et al.* (2013), nearly 100% of biodegradable COD in the influent stream may be theoretically converted into methane.

Anaerobic treatment started to be considered as a feasible solution for municipal wastewater only in 1970s, when new attractive technologies (i.e. anaerobic filters, extended and fluidized beds, Upflow Anaerobic Sludge Blanket and Expanded Granular Sludge Bed) were implemented. The sudden interest in this research field might be seen as a possible solution for the energy requirement reduction induced by the economic and political global situation of those years (Seghezzo *et al.*, 1998). Among these configurations, UASB is the most studied one. In fact, even though it has been mainly applied to the treatment of high-strength industrial wastewater, full-scale UASBs fed with domestic stream have been successfully implemented (Seghezzo *et al.*, 1998; Chong *et al.*, 2012)

Good efficiency in organic matter removal has been reported at temperature higher than 12°C and in case a good contact between biomass and organics was provided (i.e. high organic load, granulation). Due to working temperature constrictions, UASB is likely to be a sustainable technology for tropical areas but its successful applicability to low-strength municipal wastewater in temperate countries is currently under evaluation and this sector is still marginal in the overall wastewater industry. Conventional Activated Sludge (CAS) is on the whole the most applied solution for secondary wastewater treatment (i.e. organic matter removal) despite of impressive drawbacks such as high costs for sludge handling and aeration (Tchobanoglous *et al*., 2004).

Nowadays, there is an increasing interest in overcoming the main concerning points of anaerobic treatment, in order to make this process feasible for its application even at cold climate and/or for low-strength wastewater. The first issue to deal with is a correct handling of Sludge Retention Time (SRT) and Hydraulic Retention Time (HRT). On one hand, because of the low energy yields of anaerobic microorganisms, satisfying COD removal can be achieved only by assuring long SRT within the system. On the other hand, the HRT should be kept low to reduce

the volume of tanks, i.e. capital costs and environmental footprint, especially when working at low volumetric loading rates. As a result, decoupling HRT and SRT is a key issue in developing efficient anaerobic technologies (Stuckey, 2012; Smith *et al.*, 2013). In this sense, a proper solution may be given by Membrane Bioreactors (MBRs). In MBRs, dissolved and suspended organic particles are biologically removed, while solid-liquid separation is usually performed by micro- and ultra filtration (Judd, 2006). Forward osmosis was also recently applied in few studies (Chen *et al.*, 2014). MBRs were developed in 1990s for industrial applications and are now largely used in full-scale industrial and municipal treatment plants especially in Europe and North America, where MBR market is growing fast (Kraemer *et al.*, 2012; Skouteris *et al.*, 2012; Singhania *et al.*, 2012). An important characteristic of MBRs is the high solids retention capability, which assures mixed-liquor suspended solids concentration even higher than 8-10 g L^{-1} (Kraemer *et al.*, 2012). Consequently, outstanding quality effluent in term of COD, solids and pathogens may be achieved, even though problems regarding gas transfer and filterability may occur. Moreover, the nearly total biomass retention provides a good control of SRT value in case MBRs are designed to work under anaerobic conditions (Kraemer *et al.*, 2012, Smith *et al.*, 2012). Anaerobic MBRs (AnMBRs) have been recently developed from aerobic ones, as they move towards a sustainable solution for water treatment allowing for energy and resources recovery (Kraemer *et al.*, 2012; Smith *et al.*, 2012). Theoretically, AnMBRs show the same plus points of the other anaerobic treatments, i.e. positive energy balance, low sludge production, possibility of nutrients recycle (Stuckey, 2012). Skouteris *et al.* (2012) and Casu *et al.* (2012) reported that AnMBRs may assure the required solid retention as well as granule- and biofilm technologies, so that high Mixed Liquor Suspended Solids (MLSS) concentration can be achieved even in case some factors reduce the attachment or granulation propensity of biomass (e.g. high suspended solid concentration in the effluent, salinity, high temperature). Moreover, membrane is a good option for liquid-solid separation in anaerobic systems because of the low flocculation capacity of anaerobic sludge (Jeison *et al.*, 2008).

However, some critical issues should be tackle preliminary to a wide large-scale application of AnMBRs. Firstly, both anaerobic and aerobic MBR technology requires high capital and operating expenditure, even though the cost of membrane modules is currently dropping and effective strategies in reducing fouling (i.e. maintaining adequate fluxes at relatively low pressure) may be applied (Kraemer *et al.*, 2012).

A low-cost feasible solution may be the use of so-called Dynamic (or Secondary) Membranes for liquid-solid separation. A Dynamic Membrane (DM) is composed of suspended solids originally present in the filter solution. These particles attach onto a macroporous support material and

create a layer (i.e. a membrane), which entraps foulants and act itself as a rejection medium. In other words, the main drawback of conventional membrane (i.e. the fouling layer) is what now determines the rejection properties of the system and it is actually desired (Chu *et al.*, 2014). Moreover, this membrane reduces fouling propensity of the underlying support material, so that modules replacement is no longer necessary. In case the secondary membrane induces an excessive increase in Trans Membrane Pressure (TMP), this layer can be easily scoured off and re-form quickly (Ma *et al.*, 2013; Ersahin *et al.*, 2012; Jaison *et al.*, 2008; Zhang *et al.*, 2010). The replacement of conventional membranes with dynamic ones would reduce significantly the expenditure involved in MBRs, as cheap material (e.g. nylon mesh, woven and non-woven fabrics) could be use as support and no high-pressure are required to perform filtration.

The concept of Dynamic Membrane was originally developed in aerobic bioreactors, as activated sludge shows a flocculated structure and it is likely to be separated even by relatively large-pore media. The studies performed from mid 1990s on proved that DMBRs could achieve solids and pollutants removal similar to conventional MBRs even at low TMP. Anyway, at the initial stages of filtration, higher solids concentration in the effluent may be detached, as the DM has not built up yet (Ersahin *et al.*, 2012). One of the first studies in this sector was performed by Kiso *et al.* (2000), who proved the overall treatment performance of a submerged filtration aerobic bioreactor equipped with a 100 μm mesh at fluxes over 20 $Lm^{-2}h^{-1}$. The Authors report that the sludge accumulated on the mesh, resulting in good suspended solids rejection. The rejection capacity of the mesh increased with time (i.e. with the development of sludge layer on the mesh surface) up to an asymptotic value. Moreover, the Authors suggest that biochemical reactions may occur within or on this sludge layer, leading to a higher COD and/or nutrients removal. On overall, BOD removal efficiency of the system was as high as 99%. Dynamic Membrane technology was then applied to anaerobic systems, even though formation mechanisms of the cake layer may be different from those in aerobic systems, as the nature of foulants is likely to change according to operating conditions (Judd, 2006). Ho *et al.* (2007) successfully run an AnMBR equipped with a 10 μm mesh working at 25°C. Fluxes of 4-12 $Lm^{-2}h^{-1}$ were obtained by keeping TMP value in the range 0.07-0.2 bar. The Authors observed that a thin sludge layer deposited quickly on the mesh, acting as a DM. Mixed Liquor Volatile Suspended Solids (MLVSS) concentration up to 10 gL^{-1} were kept in the reactor, so that the system performance resulted to be comparable with granular sludge technology. Jaison *et al.* (2008) were among the firsts who intentionally achieved the formation of dynamic membranes in submerged and side-stream AnMBRs under mesophilic and thermophilic conditions. Even though biomass retention up to 99% was obtained, the membrane showed an unstable behavior and high resistance. In fact,

fluxes of 10 $Lm^{-2}h^{-1}$ could be obtained by applying TMP nearly ten times higher than values usually applied in conventional MBRs.

Characteristics of support material are recognized as key issues in DM formation and efficiency. In particular, porosity is likely to affect sludge attachment on the support surface, rejection properties and overall membrane resistance. A balance should be found to allow proper fluxes without compromise membrane formation. Even though porosity up to 500 μm was applied in aerobic systems, lower porosity is likely to be required under anaerobic conditions, as anaerobic sludge does not exhibit a flocculated structure. In the first studies performed by Ho *et al.* (2007) and Jaison *et al.* (2008) on AnMBRs, meshes with pore size of 12-20 μm were used. By means of filtration experiments, Jaison *et al.* (2008) showed the impossibility of building up a DM on support coarser than 60-70 μm. In other studies, dynamic filtration was tested with porosity of 30, 61,70, 80, and 90 μm (Li *et al.*, 2011; Li *et al.*, 2012; Loderer *et al.*, 2013; Ma *et al.*, 2013; Zhang *et al.*, 2010). Anyway, the upper limit resulting from the experiments by Jaison *et al.* (2008) has not been explicitly validated yet. On the contrary, Alibardi *et al.* (2014) recently achieved to build up a DM on a large pore-sized mesh (200 μm) by properly managing hydrodynamic conditions. Fluxes of 1.0-7.2 $Lm^{-2}h^{-1}$ were achieved, in accordance with the results obtained before using mesh with lower porosity. This result may lead to an optimization of AnDMBR technology by reducing energy demand and investment and management expenditure.

Besides high cost of membrane modules, the second significant obstacle in the spread of anaerobic bioreactor using both conventional and dynamic membranes arises from working temperature limitations. Temperature affects sludge properties, microbial kinetic (i.e. effluent quality) and cake layer formation in bioreactors. Consequently, membrane fouling and DM build-up are likely to be influenced by temperature conditions (Gao *et al.*, 2012). Temperature is therefore a key parameter in the overall performance of bioreactors. Most anaerobic systems are designed to work under mesophilic conditions because of slow growth rate of biomass (Stuckey *et al.*, 2012). Anyway, the effluent heating process up to 35°C affects negatively the energy balance of the system in case the stream is colder. In developed countries, domestic wastewater show an average temperature of about 15°C and noteworthy seasonal variations (Smith *et al.*, 2013). Moreover, household wastewater is characterized by low-strength (i.e. COD equal or lower than 1 gL^{-1}) and/or high flow rate, so that the methane recovered from the system may not be sufficient to reach mesophilic conditions and extra energy input would be required. These drawbacks may be overcome by working at ambient temperature (Bandara *et al.*, 2011; Smith *et al.*, 2012; Stuckey, 2012; Martinez-Sosa *et al.*, 2011; Gimènez *et al.*, 2012). In addition, reactors

without heating device would be characterized by simpler technology and easier maintenance (Stuckey, 2012). Despite the feasibility of performing anaerobic digestion at low temperature was already suggested by McCarty (1964), further studies should be performed before a widespread implementation of full-scale anaerobic reactors without heating loop in temperate regions (Gimènez *et al.*, 2012a; Smith *et al.*, 2013).

When working at psychrophilic conditions (< 20°C), an important aspect of concern is linked to microbial kinetic. More specifically, lower temperatures decrease reaction and hydrolysis rates and increase half-rate constant for methanogens, leading to lower COD removal and methane production. This problem could be overcome by lengthening SRT, so the use of MBRs may help in improving anaerobic systems efficiency. In fact, as stated above, this technology allows decoupling of HRT and SRT and assures nearly total biomass retention (Smith *et al.*, 2013).

Another noteworthy issue is the inverse relationship between gas solubility and temperature. As a result, a considerable fraction of methane produced turns out to be dissolved in the effluent when working under psychrophilic conditions. Gimènez *et al.* (2012b) report that the methane lost in the effluent increases of 25% by shifting from mesophilic conditions to ambient temperature. Recovery strategies (e.g. stripping, degassing membrane) should be therefore applied to maximize energy efficiency of the system and reduce the release of greenhouse gases into the environment, as methane has a global warming potential 25 times higher than carbon dioxide (Smith *et al.*, 2012). Methane recovery can be enhanced by applying low HRT, as carbon dioxide is nearly 12 times more soluble than methane so that it can be easily washed out from the system and biogas turns out to be characterized by a higher fraction of methane. In this sense, better efficiency can be obtained with membrane technology, as they are can be run at HRT values even down to three hours (Stuckey, 2012).

Another issue of concern of working at psychrophilic conditions is the changes in bacterial communities occurring in the reactor during the start-up of the system. This point is relevant especially considering that inoculum is usually performed with sludge taken from mesophilic plants, UASBs or even aerobic systems (Martinez-Sosa *et al*., 2011; Smith *et al*., 2010), i.e. systems run under different operating conditions. Anaerobic fermentation at psychrophilic temperatures is likely to be performed mainly by mesophilic microbial consortia acclimatized at low temperature, even though truly psychrophilic methanogens have been detached in low-temperature systems (Stuckey, 2012; Kashyap *et al*., 2003). Biomass may therefore require a longer start-up to become psychrotolerant. Stuckey (2012) reported that an increase in Extracellular Polymeric Substances (EPSs) production was detected in case microorganisms are experimenting suffering conditions, leading to an increase in membrane fouling propensity.

Consequently, the behavior of the system during microbial acclimation is likely to be unstable and the overall efficiency low (Martinez-Sosa *et al.*, 2011). Despite of these drawbacks, some studies suggest that the development of psychrotolerant mesophilic biomass instead of truly psychrophiles would lead to a better long-term stability of the system in case of temperature increase (e.g. seasonal fluctuations). In fact, true psychrophiles would die at temperature around 20°C. On the contrary, acclimatized mesophilic communities would increase their kinetic improving COD removal. Anyway, the response of the microorganisms to further decrease in temperature is not clear yet (Smith *et al*., 2013; Kashyap *et al.*, 2003).

Besides drawbacks linked to dissolved methane in the effluent and biomass acclimation, membrane technology is likely to help overcoming the main drawbacks of the application of anaerobic digestion at low temperature, only few studies analyzed AnMBR performance at psycrophilic conditions and even less pointed out the application of DMs at ambient temperature. A interesting study was performed by Martinez-Sosa *et al*. (2011). A submerged AnMBR treating low-strength municipal wastewater was successfully run at 35, 28, and 20°C. Stable fluxes up to 7 L m^{-2} h^{-1} were reached. COD removal efficiencies close to 90% were observed even under psychrophilic conditions, despite a drop occurred when temperature was turned down. On the whole, the work proved the feasibility of applying AnMBRs at psychrophilic temperature. Anyway, the system stability proved to be affected by temperature variations, which may be a drawback when working without temperature control. Even lower temperatures was investigated by Smith *et al.* (2013). The Authors assessed the long-term performance of a lab-scale AnMBR treating low-strength municipal wastewater at 15°C, obtaining COD removal up to 92%. Noteworthy differences in Mixed Liquor and effluent soluble COD concentration were observed. As a result, the fouling layer on the membrane was supposed to be responsible not only of physical solid-liquid separation but also of biological degradation, in accordance with what observed by Kiso *et al.* (2000) in aerobic systems. These reactions are likely to take place in DMs, too, as they are made up with foulants. As a consequence, the application of DMs in MBRs under psycrophilic conditions may help overcoming the decrease in COD removal resulting from the reduction in microbial kinetic. Anyway, discordant results are present in literature. Zhang *et al*. (2010) studied the development and structure of a DM in an AnMBR run at 10-15°C. The system had a low COD removal (barely 60%) and no significant improvement was observed after the DM built up. The Authors therefore suggested that the role of DM was limited to solid separation. On the contrary, Ersahin *et al.* (2014) recently obtained soluble COD removal by the DM of more than 60% and overall COD removal of 99%. Anyway, the study was

performed under optimal conditions, i.e. mesophilic temperature and high organic matter concentration in the inlet.

Further works should be performed to test DM formation and behavior under different operating conditions (e.g. temperature) and configurations (e.g. support material porosity). More generally, few works on AnDMBRs are available in literature and further studies are required to evaluate the possible application of this technology on full-scale wastewater treatment plants (Ersahin *et al.*, 2012).

The aim of the present work is the development of an AnDMBR system equipped with a coarse filtration mesh (200 μm) for the treatment of low-strength municipal wastewater at ambient temperature, and the assessment of the COD removal capacity of the dynamic membrane itself and its contribution to the overall efficiency of the system.

2. Materials and methods

2.1 Experimental setup

The study was performed by means of a bench-scale Anaerobic Dynamic Membrane Bioreactor (AnDMBR) equipped with an external cross-flow filtration unit. The experimental setup is schematically reported in Figure 2.1.

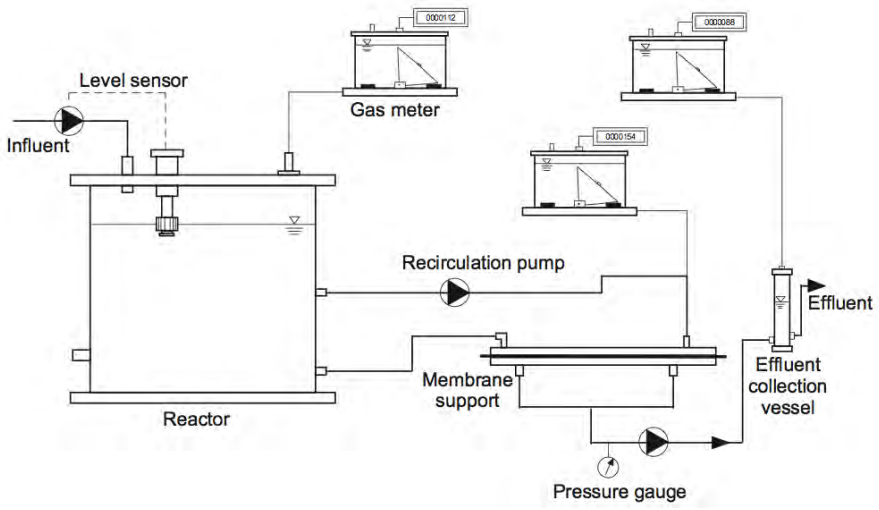

Figure 2.1: schematic diagram of the experimental setup

The anaerobic reactor was made of PVC. It had total volume of 898 mL (W x H x D: 9.5 x 10.5 x 9 cm) and a working volume of 684 mL. The level of the mixed liquor inside was kept constant by using a level sensor connected to the influent pump. The filtration support was made of PVC. Initially, it had an inner volume of 48 mL (W x H x D: 20 x 1.2 x 2 cm). On day 17 since the beginning of the experiment, the height of the device was increased up to 1.5 cm to reduce clogging. A monofilament woven mesh made of polyamide/nylon (SaatiMil PA 7, Saati s.p.a., Italy) with openings of 200 µm, thread diameter of 120 µm, mesh count of 31/cm and 39% opening area (data from the supplier) was inserted in the central longitudinal part of a filtration support. The filtration area was of 40 cm^2 (L x W: 20 x 2 cm). Considering the reactor, the membrane support and the pipes used to connect the reactor and the filtration support, the total working volume of the system is 745 mL.

The feed was provided by a peristaltic pump (Watson Marlow 401U/D1, Falmouth, Cornwall, UK) controlled by the level sensor inside the reactor. Sludge mixing was provided by a magnetic stirrer (Heidolph, Hei-Standard). The circulation of the mixed liquor along the mesh surface and

the return of the retentate into the reactor were provided by a second peristaltic pump (Watson Marlow 505S, Falmouth, Cornwall, UK) applying a cross flow velocity of nearly 10 mh^{-1}. A third peristaltic pump (Watson Marlow 401U/DM3, Falmouth, Cornwall, UK) was installed to extract the effluent out of the system and provide the necessary TMP across the membrane for the filtration to occur. An airtight vessel of approximately 100 mL was installed down-flow the third pump to assess the presence of oversaturated biogas which may be released in the effluent. The effluent flowed out this vessel into a collection tank.

TMP was measured by means of two U- tube pressure gauge filled with water and placed up- and down- flow the filtration support, respectively.

The biogas production by the reactor and by the membrane was monitored by using two home-made wet-tip gas meters. On day 92, a third gas-meter was installed down- flow the membrane support in order to assess the production of biogas as a result of microbiological reactions which might occur within the pipes connecting the filtration support and the pump and/or in the sludge flocs attached to the lower side of the membrane. A forth gas meter was connected to the effluent collection vessel. All the gas meters had an opening for the assessment of gas composition.

2.2 Inoculum

The reactor was seeded with anaerobic sludge taken from a full- scale mesophilic anaerobic digester treating the excess sludge of the municipal wastewater treatment plant of Ca' Nordio (Padova, Italy). The seed had a concentration of TSS and VSS equal to 12 gTSS L^{-1} and 6 gVSS L^{-1}, respectively.

During the experiment, three additional sludge *inocula* were performed to compensate for biomass washout induced by clogging of the filtration support. The first additional seed (day 17) was performed by means of the same sludge used in the startup phase. Instead, the sludge used for the second additional inoculum (day 59) had a concentration of 37 gTSS L^{-1} and 17 gVSS L^{-1}. For the third inoculum (day 119) it was decided to reintroduce into the reactor the sludge accumulated in the vessel down-flow the exiting pump and extracted the previous days. The sludge used for the forth further inoculum (day 125) had a concentration of 10 gTSS L^{-1} and 6 gVSS L^{-1}.

2.3 Feed

The reactor was fed with synthetic wastewater, which simulated municipal wastewater. The feed had a concentration of approximately 900 mgCOD L^{-1}_{feed}. It comprised sucrose (45% of total COD), powder milk (10%) and corn starch (45%) as organic matter.

The followings chemicals were added to ensure alkalinity, macro- and micronutrients: $NaHCO_3$ (830 mg L^{-1}_{feed}), NH_4Cl (50 mgN L^{-1}_{feed}), KH_2PO_4 (10 mgP L^{-1}_{feed}), $FeCl_3 \cdot 6H_2O$ (2.1 mgFe L^{-1}_{feed}), $CaCl_2 \cdot 2H_2O$ (8.2 mgCa L^{-1}_{feed}), $MgCl_2 \cdot 6H_2O$ (2.4 mgMg L^{-1}_{feed}), $Na_2MoO_4 \cdot 2H_2O$ (0.22 mgMo L^{-1}_{feed}), $ZnSO_4 \cdot 7H_2O$ (0.23 mgZn L^{-1}_{feed}), $CuSO_4 \cdot 5H_2O$ (0.128 mgCu L^{-1}_{feed}), $NiCl_2 \cdot 6H_2O$ (0.1 mgNi L^{-1}_{feed}), H_3BO_4 (0.007 mgB L^{-1}_{feed}). All the compounds were dissolved in tap water.

Beside NH_4Cl, powder milk is an extra source of nitrogen, as this substrate is rich in proteins. According to the information provided by the producer, this further contribution to the overall inlet TKN concentration is around 18 mgN $g_{powder\ milk}^{-1}$.

Even though COD concentration was kept constant, changes in feed composition turned out to be necessary along the experiment. From day 27 to day 31 the system was fed with 1 gCOD L^{-1}_{feed} as acetic acid to stimulate methanogenic bacteria. During the holiday break from day 38 to day 59, COD was given by sucrose, entirely, as this substrate is readily available for microorganisms. Because of the tendency of starch to settle on the bottom of the inlet tank, after the holiday break it was decided to invert the amount of starch and milk in the feed solution. As a consequence, from day 59 on, sucrose, corn starch and powdered milk accounted for 45%, 10% and 45% of the total inlet COD, respectively.

From day 18 to day 62, the concentration of NH_4Cl in the feed was doubled (i.e. 100 mgN L^{-1}_{feed}) as a response to a decrease in ammonia concentration in the effluent. Similarly, from day 104 on, the concentration of KH_2PO_4 was gradually increased up to 20 mgP L^{-1}_{feed}.

2.4 Operating parameters

During the study, the feed concentration was kept constant. On the contrary, the HRT was intentionally varied to evaluate the performance of the systems under different conditions.

The system was implemented with a HRT of 5 d. On day 15, the HRT was halved and worked at a HRT of 2.5 d for three weeks. Before the holiday break, on day 38 the value was increased again up to 5 d. The system worked under this condition until day 62, when the HRT was decreased to 2 d. After three weeks (day 83), the value was reduced to 1 d. After two other

weeks (day 97), the HRT was further halved. The system worked at HRT of 0.5 d until day 121, when it was reduced to 0.25 d.

The changing in HRT values along the experiment induced variations in OLR and fluxes applied to the membrane. These values varied in the range 0.2 - 2 kgCOD m^{-3} d^{-1} and 1.4 – 15.5 L m^{-2} h^{-2}, respectively.

Since temperature was not controlled, the system worked at ambient temperature in the range 23.8 – 28.9 °C.

2.5 Analytical methods

To evaluate the performance of the system, periodical analysis on the effluent, mixed liquor and biogas were performed. On the effluent, the following parameters were analyzed: total COD, filterable COD, soluble COD, total (TSS) and volatile suspended solids (VSS), pH, alkalinity,, $NH_4^+$and total P concentration. On the mixed liquor, the following parameters were analyzed: total COD, filterable COD, soluble COD, TSS, VSS, pH.

Sample filtration was performed using Whatman GF/C filters . To determine soluble COD, 0.2 µm syringe filters were used. COD, TSS, VSS, pH, alkalinity, NH_4^+- and total P- concentration were measured according to Standard Methods (APHA, 2005).

Biogas composition was measured by a micro-gas chromatograph (Varian 490-GC) equipped with a 10 m MS5A column and 10 m PPU column, using argon as carrier gas and a thermal conductivity detector.

2.6 COD mass balance

A COD mass balance was established in the system run at HRT values of 2 d (day 63 – 83), 1 d (day 84 – 97), 0.5 d (day 98 - 121), and 0.25 d (day 122 -129).

The systems borders, inputs and outputs applied in COD mass balance are represented schematically in Figure 2.2.

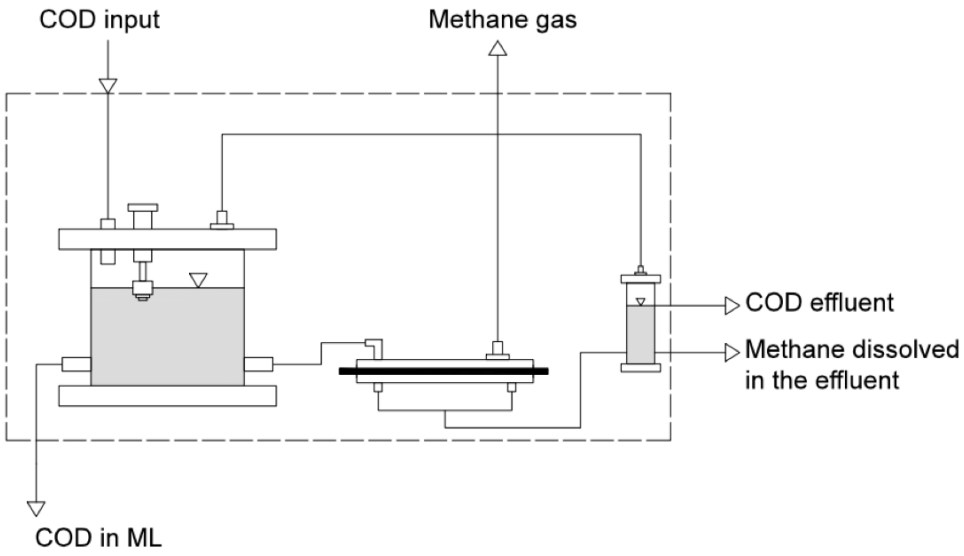

Figure 2.2: Schematic drawing of the system used for COD mass balance. The dotted line represents the system boundary. The boundary is sketched by means of dotted line. The arrows represent input terms (i.e. inlet COD) and output terms (COD as methane gas, effluent COD, methane dissolved in the liquid phase, and COD in the Mixed Liquor lost with analyses).

Input COD is the feed solution. Output terms are: (1) total COD exiting the system with the effluent, (2) COD in ML extracted for the periodical analysis, (3) methane gas, and (4) methane dissolved in the effluent. All terms are expressed as mass (mgCOD) and are referred to the whole period during which the system was kept at a certain HRT value. Effluent COD (1) was calculated considering the daily flow rate and total effluent COD concentration. The COD wasted during ML characterization is given by the reactor biomass concentration as VSS and the filterable COD (1.2 μm filtration was considered). In the calculation of the biomass COD, an averaged chemical formula of microorganism ($C_5H_7O_2N$) was used, so that the oxygen equivalent of the biomass is approximately 1.42 gCOD $gVSS^{-1}$ (Tchobanoglous *et al*., 2003). The methane gas (3) was computed by considering daily biogas production, biogas composition and the molar volume of gas at the temperature registered on average. The transformation factor of 64 gCOD $molCH_4^{-1}$ was applied (Tchobanoglous *et al*., 2003).

The mass of methane dissolved in the liquid phase $m_{(aq)}^{CH_4}$ (4) was estimated by means of the method proposed by Giménez *et al*. (2012a), which assumes equilibrium conditions between phases:

$$m_{(aq)}^{CH_4} = \frac{M^W \cdot P \cdot y^{CH_4} \cdot MW^{CH_4} \cdot V_L}{H^{CH_4}(T) - P \cdot y^{CH_4}}$$

where M^W is the molarity of pure water (55.56 mol L^{-1}), P is the working pressure (atm), y^{CH_4} is methane molar fraction in the biogas, MW^{CH_4} is the molecular weight of methane (16 g mol^{-1}), V_L is the total volume of effluent (L), and $H^{CH_4}(T)$ is the time- dependent Henry's constant for pure water. The latter constant can be calculated with the formula:

$$H^{CH_4}(T) = 10^{\left(\frac{-675.74}{T}+6.88\right)}$$

where the T is the working temperature (K).

The observed methane yield coefficient $Y_{obs}^{CH_4}$ (L_{CH_4} $kgCOD^{-1}$) was calculated as Giménez *et al.* (2012b):

$$Y_{obs}^{CH_4} = \frac{V_{obs}^{CH_4}}{COD_{rem}}$$

where $V_{obs}^{CH_4}$ is the volume of methane recovered with the biogas (L_{CH_4}) and COD_{rem} (kgCOD) is the COD removed in the system, calculated as the difference between the inlet COD, the COD in the effluent and the COD wasted with the ML analysis.

The methane yield coefficient was then calculated again ($\widehat{Y}_{obs}^{CH_4}$) by taking into account also the methane dissolved in the liquid phase under equilibrium conditions.

2.7 Membrane COD removal efficiency experiments

A short-term experiment was performed after the system was shut down on day 129, to evaluate the contribution of the well-formed dynamic membrane to COD removal. The experiment was carried out by means of the setup used in the operating phase. The reactor was opened, emptied from the Mixed Liquor and filled with the inlet solution. The level of the inlet solution was kept constant by the sensor connected with the peristaltic pump. During this preliminary phase, the support was isolated to avoid damages to the membrane. The system was set in motion again and run for four days. During the first 72 hours the HRT was approximately 1 d, then it was reduced to 0.5 d and kept constant until the end of the experiment. The removal efficiency of the membrane was determined by the analysis of the effluent and the feed solution in the reactor. The first effluent sample was taken after 18 hours from the starting. Other five effluent samples were then taken. The feed solution in the reactor was analyzed at the beginning and at the end of the test. On the samples, the following parameters were analyzed: total COD, filterable COD,

TSS, and VSS. Other operating parameters monitored regularly where TMP and biogas production. Biogas composition was measured at the end of the experiment. The analytical methods used are reported in Paragraph 2.5.

3. Results and discussion

3.1 System startup

During the first two months of experiment, the system showed an unstable behavior. Clogging problems occurred in the membrane support, leading to the frequent presence of sludge in the pipes used to measure TMP and the not correct operation of the sludge recirculation system. For this reason, after fifteen days since the starting (day 17), it was necessary to open the membrane support. The upper part of the device resulted to be clogged by a dense sludge layer attached onto the mesh. The clogged device after the opening is reported in Figure 3.1.

Figure 3.1: the upper part of the membrane support on day 17. On the left it is possible to notice the sludge clogging the device.

The support was cleaned and an additional height of 0.5 cm was added before the system re-starting to reduce clogging propensity. Sludge characterization of the material scoured off showed a water content of 82%. The VS/TS was nearly 45%. This value can be compared with the VSS/TSS of the mixed liquor, by assuming a good correlation between VSS and VS. In fact, no solids enter the system through the inlet stream and the only contribution to the overall solids content is given by biomass (i.e. VSS). The VSS/TSS ratio of the mixed liquor up to that day was equal to approximately 60%. As a result, non-volatile solids showed a major propensity than volatile solids to attach onto the membrane. A second and a third membrane clogging occurred

on day 27 and day 45, obstructing the recirculation system and making the mixed liquor being partially washed out through the TMP-measuring device. A picture of the pressure gauge filled with sludge because of clogging is reported in Figure 3.2.

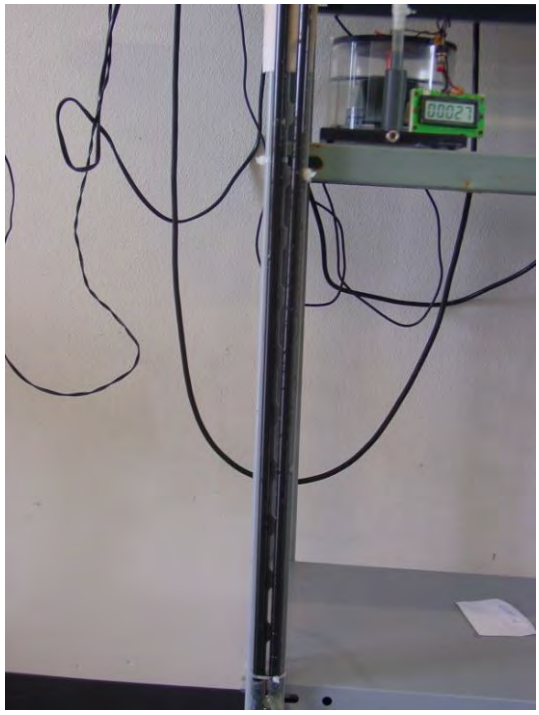

Figure 3.2: Pressure gauge filled with sludge as result of clogging problems occurred on the system on day 27.

In both cases, the cross-flow velocity on the mesh was temporarily increased to try to remove bigger sludge flocs. Anyway, on day 45 it was also necessary to clean the support device with tap water to wash away bigger particles. During this operation, the support was not opened to avoid excessive damages to the membrane under formation. On the whole, propensity to clogging was observed in the system until day 65.

The biogas production was negligible until day 63 and consequently the pressure in the reactor was low or even negative. Some hypotheses were therefore evaluated to understand this behavior. A possible explanation may be the tendency of some inlet components (i.e. starch and powder milk) to settle down in the feed tank, reducing the COD concentration effectively entering the reactor. A stirring device was therefore inserted in the inlet tank on day 34. Nevertheless, biogas production did not increase significantly and kept low even during the holiday break (day 38-59), when the system was fed with only-sucrose solution, which does not show settling propensity. Anyway, it may be worth noticing that during the holiday break the HRT was increased from 2.5 d up to 5 d, so that the OLR decreased from 0.4 to 0.2 kgCOD m^{-3} d^{-1}. Another explanation to the low biogas production might be the occurrence of aerobic organic

matter removal due to the residual presence of oxygen in the reactor. Anyway, oxygen is normally quickly reduced so this hypothesis is not likely to be valid on a medium time scale. A more plausible hypothesis may be the necessity for mesophilic biomass to acclimate to lower and fluctuating temperature, which leads to decrease in microbial kinetic. In fact, as reported in Paragraph 2.2, the system was seeded with sludge taken from an anaerobic digestor working at 35°C, i.e. nearly ten degrees higher than the average temperature registered during the experimental time (25.8°C). Moreover, the temperature was not constant because of seasonal and daily fluctuations and the absence of heating/cooling loop. These different and not constant operating conditions are likely to have induced a stress condition to the biomass, leading to a slower kinetic. This would explain why the biogas production was low even when the system was fed with a solution of acetic acid (i.e. readily available COD) to stimulate methanogens (day 27-31).

Stuckey (2012) reported that stress conditions to microbial culture can increase EPSs production and, consequently, membrane fouling. The clogging propensity showed by the system in the startup may be therefore explained as a consequence of a stress condition of microorganisms not acclimatized yet. Accordingly, it should be noticed that the initial biomass inoculum and the additional seeds performed within the first two months (day 17 and 59, respectively) were performed by means of sludge collected the same day or few days before. After the *inocula*, clogging problems occurred. On the contrary, for the third inoculum (day 119) it was decided to use the biomass washed out from the system and accumulated in the vessel down-flow the exiting pump. The sludge used for the forth further inoculum (day 125), which had a lower solids concentration, had been stored for approximately three weeks at ambient temperature before the use. In these both cases, the biomass was likely to be acclimatized to the new conditions, so no significant clogging problems were detached.

3.2 Transmembrane Pressure and Fluxes

The TMP developed during the study and the applied HRTs are reported in Figure 3.3. The fluxes across the membrane are reported in Figure 3.4, together with the applied HRTs.

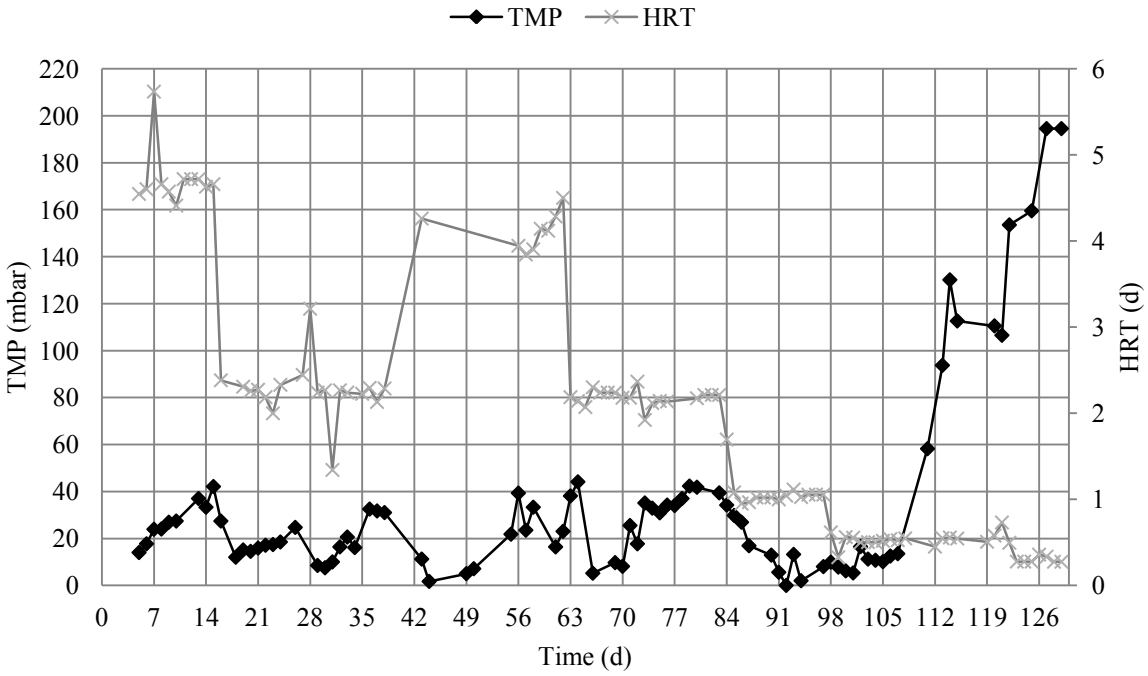

Figure 3.3: TMP pattern and applied HRT during to the study.

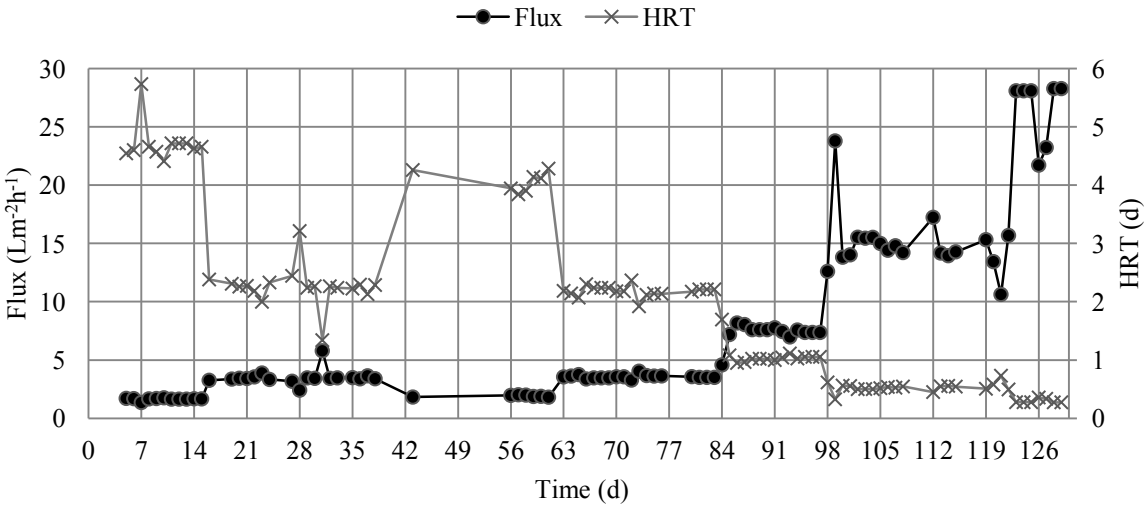

Figure 3.4: HRT and resulting membrane fluxes during the study.

The system was implemented with a relatively high HRT of 5 d in order to enhance biomass acclimation. On day 15, the HRT was halved and worked at a HRT of 2.5 d for three weeks. Before the holiday break, on day 38 the value was increased again up to 5 d. The system worked under this condition until day 62. Because of HRT variations, the fluxes applied across the membrane during the first two months ranged between 1.4 and 3.6 L m^{-2} h^{-1}. A higher value of nearly 6 L m^{-2} h^{-1} was registered on day 31 as the HRT was downed to 1 d to increase biogas

production. As the system showed a more stable behavior with respect to the previous phase, on day 63 the L m^{-2} h^{-1} was downed to 2 d and kept nearly constant for three weeks. On day 83, the HRT value was further halved and the system worked at HRT of 1 d for two weeks. In these two periods, the flux was equal to 3.6 and 7.8 L m^{-2} h^{-1}, respectively. No significant fluctuations were observed. On day 97, the HRT was reduced to 0.5 d. In the following 3 three weeks, the system showed less stable behavior and the flux across the membrane ranged significantly between 10.6 and 23.8 L m^{-2} h^{-1}. Nevertheless, on day 122, the HRT was reduced to 0.25 d to test the system under critical operating conditions. Consequently, the flux was comprised between 21.7 and 28.3 L m^{-2} h^{-1}.

For what concerned TMP, an up and downtrend was observed during the first two months. The TMP started to increase from values close to zero and in approximately two weeks reached values between 30 and 45 mbar. The gradual increase was followed by a sharper decrease down to values around zero. Two days after the HRT was reduced to 2 d, the TMP started to increase steeply up to the value of 35 mbar (day 73). The TMP kept around the value of 35 mbar for two weeks. On day 87 the TMP started to decrease even though the pressure in the reactor had slightly increased because of biogas production. The TMP showed a decreasing trend until day 91. This drop might be caused by gas in the pipes down-flow the membrane. This gas might be produced by biomass accumulated in the pipes or in the lower part of the membrane support or it might be the result of volatilization of compounds dissolved in the liquid phase. Anyway, the visual inspection performed after the system was shut down revealed that the biomass in the pipes was negligible. On the contrary, there was sludge attached onto the lower surface of the mesh, so it is more likely that the gas had been produced during reactions occurring in this cake layer. On day 92 the TMP was nearly zero. A gas meter was therefore installed down-flow the membrane to avoid excessive pressure drop and evaluate gas composition. The TMP started to increase slightly up to about 10 mbar and kept stable around this value until day 106. From day 107, the TMP increased sharply up to values of one order of magnitude higher than those reported in the previous phases. On day 114, the TMP was equal to 130 mbar. After this peak, it reduced to 110 mbar. The TMP started to increase again on day 119 and at the end of the experiment it reached values of nearly 200 mbar.

A clear correlation between HRT applied and TMP developed during experiment cannot be seen, even though a more unstable behavior was observed at HRT of 0.5 d or lower.

On overall, the TMP showed an unstable trend. It is likely to assume that the cake layer acting as a medium filter formed gradually on the mesh, inducing an increase in filtration resistance. The TMP therefore increased up to a critical value. At this value, the pressure applied induced the

partial break of the cake layer, which resulted in a fall in transmembrane resistance. The cake started then to build up again even quicker, probably because of the presence of an irreversible fouling layer. Ersahin *et al*. (2012) report that a gel layer composed of EPS and other particles tends to form under the cake layer. These particles are stickily attached on the mesh and cannot be not easily removed, so they are likely to enhance the re-building of the cake layer after scouring. The visual inspection performed on the support mesh after the system shut down seems to confirm the formation of this underlying gel layer. The mesh after sludge scouring performed after the system shutdown is represented in Figure 3.5.

Figure 3.5: Image of the support mesh after cake layer scouring. The biofilm layer developed on the mesh can be noticed.

Jeison *et al*. (2008) already reported that MBRs equipped Dynamic Membranes show a quite unstable TMP behavior. Moreover, the TMP trend is considerably different from that observed by Alibardi *et al*. (2014), which operated with the same setup and mesh porosity but under different operating conditions in term of temperature and OLR. As a result, operating conditions are likely to greatly affect dynamic membrane formation.

Anyway, it has to be stressed that the high porosity of the support mesh (200 μm) allowed achieving stable fluxes up to 15 L m^{-2} h^{-1} at TMP values lower than 50 mbar and HRT down to 0.5 d. Similar findings (flux of 4-12 L m^{-2} h^{-1} at TMP of 70-200 mbar and HRT of 18 h) were obtained by Ho *et al*. (2007) working under similar conditions in term of OLR and temperature and similar configuration (i.e. AnMBR equipped with external membrane unit). Anyway, the

Authors used a 10 µm-porous medium, i.e. one of order of magnitude of the porosity used in the present experiment. Higher fluxes of 60 µm were obtained by Ma *et al*. (2013) at similar TMP and HRT values, even though a less course Dacron mesh (61 µm) was installed.
The results obtained are close to those reported by Alibardi *et al*. (2014). In fact, the Authors achieved fluxes between 1 and 7.2 L m^{-2} h^{-1} at TMP lower than 200 mbar by means of a 200 µm-porous mesh. Anyway, HRT values lower than 1 d were not tested in that study.

3.3 Wastewater treatment and Solids retention

As the inlet COD concentration was kept constant during the experiment, the OLR value ranged between 0.2 and 3.6 kgCOD m^{-3} d^{-1} according to the HRT applied to the system.
The concentration of total, filterable and soluble COD detached in the effluent is reported in Figure 3.6 along with the HRT applied. The COD removal efficiency is reported in Figure 3.7. It has been decided to distinguish between the total, the filtrable and the soluble COD removal. The first parameter has been calculated by considering the total COD in the effluent (i.e. taking into account also the solids discharged). The total effluent COD has been calculated by summing the filterable COD to the COD of VSS in the effluent. Under the assumption that VSS concentration is an indicator of biomass and considering the average composition of microbial cells, the factor of 1.42 gCOD gVSS $^{-1}$ was therefore applied (Tchobanoglous *et al.*, 2004). On the contrary, the biological COD removal refers to the filterable and soluble COD, only. Under the hypothesis that the amount of filterable and soluble compounds released by the biomass is negligible, filterable and soluble COD removals describe the efficiency of the system in degrading inlet organic matter.

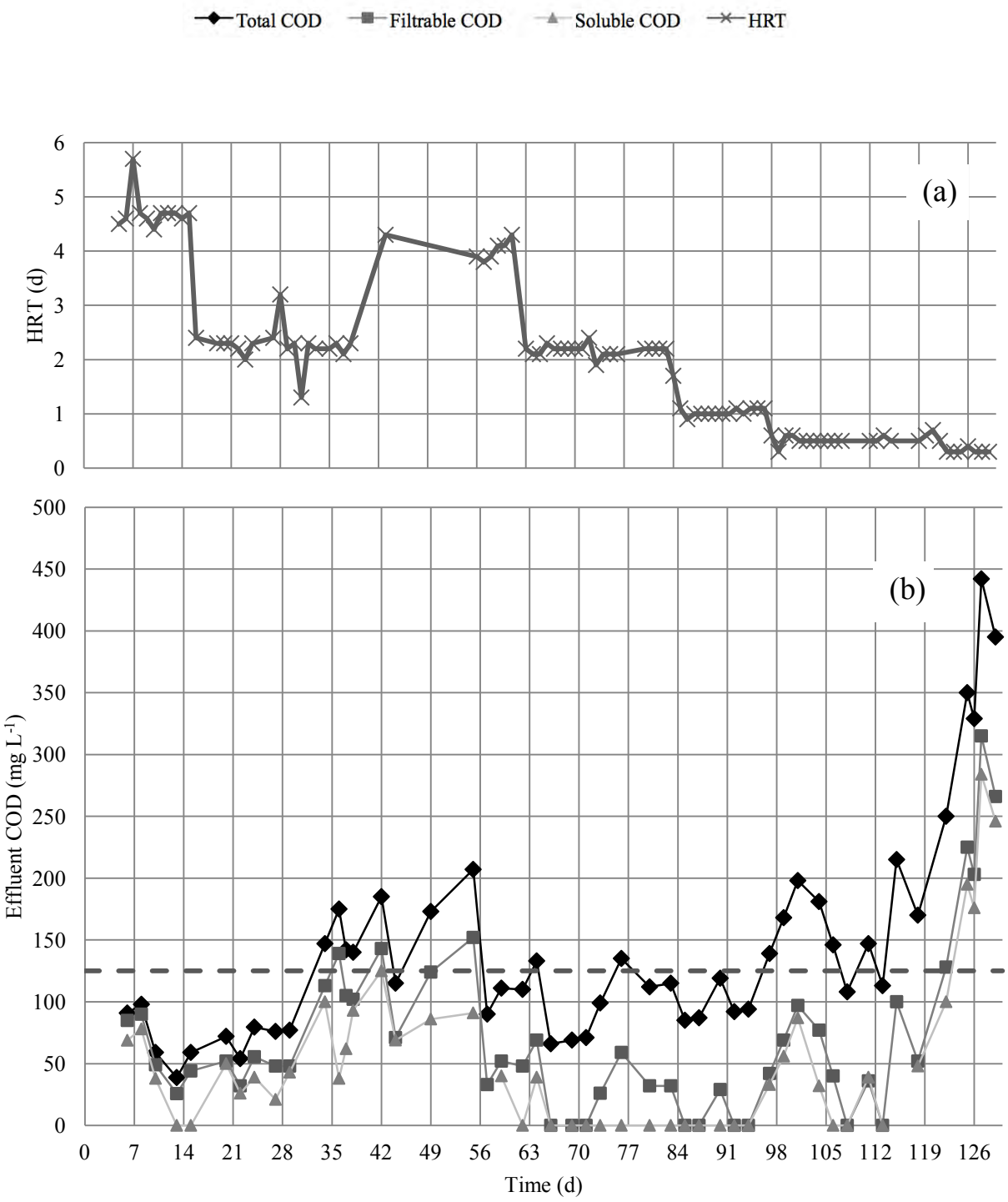

Figure 3.6: (a) HRT and (b) total, filterable, and soluble COD in the effluent during the experiment. Horizontal dotted line indicates the COD discharge limit on superfitial water bodies fixed by Italian legislation (D.Leg 152/2006).

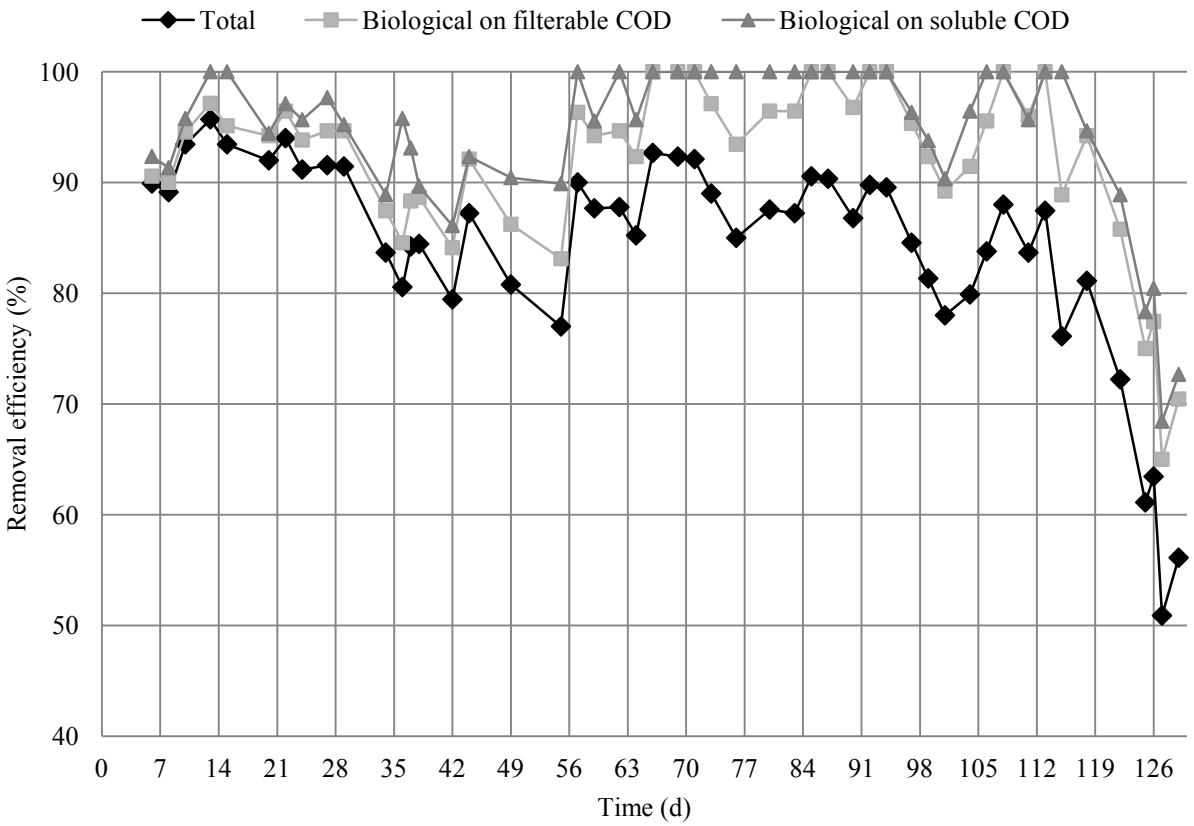

Figure 3.7: Removal efficiency of total, filterable and soluble COD performed by the system during the experiment.

According to Figure 3.6 and Figure 3.7, the total COD concentration in the effluent kept below 100 mg L^{-1} during the first month of operation. The total COD removal efficiency was therefore higher than 90%. Anyway, these impressive results might be due to an underestimation of the effective VSS concentration in the effluent, as no reliable data about the solids accumulated in the vessel down-flow the membrane were available. Moreover, as the inlet solution was originally not stirred and some COD sources tended to settle down onto the feed bottle, it is likely that the COD concentration effectively entering the system in this first phase was lower than the theoretical value of 900 mg L^{-1}. In fact, after a stirrer was placed in the feed tank on day 34, higher COD concentration values were observed in the outlet. During the second operation month (HRT of 2.5 and 5 d), the total and the filterable effluent COD staid in the ranges 107-115 mg L^{-1} and 70-140 mg L^{-1}, respectively. The filterable COD was lower than 125 mg L^{-1}. In this period, the average total COD removal efficiency was 82%. The removal efficiency of filterable and soluble COD was equal to 87% and 91%, respectively. On day 57, the total COD dropped of about 50% from 207 to 90 mg L^{-1}. Filterable and soluble COD concentration showed a similar pattern. During the following two months, when the system was run at HRT of 2 and 1 d, a very

high efficiency was reached. Filterable and soluble COD concentration kept lower than 75 mg L^{-1} showing removal efficiencies close to 100%. Similarly, the average total COD removal efficiency was nearly 90% and only two points (day 64 and day 76) slightly overcame the values of 125 mg L^{-1}. When the HRT was reduced to 0.5 d on day 98, the total COD in the effluent began to rise gradually up to nearly 200 mg L^{-1} (day 101). Similarly, soluble and filterable COD concentration increased from nearly zero to about 100 mg L^{-1} and then lowered again down to values close to zero. On the contrary, total COD kept values close to 150 mg L^{-1} because increasing solids content in the effluent. When run at 0.25 d, the removal efficiency of the system collapsed quickly because of the low solids retention capability of the system at such a low HRT. In the last week, the total COD reached values close to 450 mg L^{-1}, while soluble and filterable COD peaked at about 300 mg L^{-1}. Consequently, both total and biological removal efficiency dropped to values lower than 70%.

On overall, the system showed an average removal efficiency of 84% 92%, and 95% for total, filterable, and soluble COD, respectively. Outstanding performance was therefore observed, even though significant variations occurred. The COD removal achieved was slightly higher than that observed in the previous study performed by Alibardi *et al*. (2014) by means of the same setup. The Authors reports a COD removal ranging between 65% and 92% and an average value of 75%. It should be also considered that mesophilic temperature conditions were applied in the previous study and sucrose was the only COD-source in the inlet. On the contrary, the present work was performed at ambient temperature and slightly slower biodegradable components (i.e. powder milk and starch) were used in the feed. Moreover, the COD concentration had been lowered of more than 5 times with respect to the previous study in order to simulate a real MWW stream. In the light of these considerations, the results achieved are impressive. Here below, these findings are compared with those reported in other studies on AnDMBRs. Ersahin *et al*. (2014) achieved higher COD removal of 99% but working at 35°C. Ma *et al*. (2013) obtained lower efficiency (74%) but treating real MWW. The results of the present study are similar to those reported by Ho *et al*. (2007) treating low-strength synthetic wastewater at 25°C. Anyway, a low-porous mesh (10 μm) was used as support material, so that it may be assumed that the solids retention capacity of the system was higher. In term of COD removal, the system may be compared with conventional AnMBRs, whose COD removal efficiency usually ranges between 75% and 99% (Skouteris *et al*., 2012; Lin *et al*., 2013). The AnDMBR developed showed a slightly higher efficiency than granulated technologies, which anyway usually work with OLRs higher than 10 kgCOD m^{-3} d^{-1}. In fact, according to Martinez-Sosa *et al*. (2011), the removal efficiency of total COD in UASBs working under temperate ambient temperature

ranges usually between 60% and 75%. Anyway, it should be noticed that the comparison of the findings with literature data may not be reliable, as in most of the works it is not specified if the removal efficiency if calculated on the total or filterable or soluble COD, i.e. a clear distinguish between total and biological removal efficiency is not performed.

Figure 3.6 revealed a similar pattern for total, filterable and soluble COD concentration in the effluent. Consequently, it is likely to assume that biological COD removal efficiency is linked to solids retention capability in the system, i.e. solids concentration in the effluent.

The TSS and VSS effluent concentration throughout the experiment is reported in Figure 3.8. The Mixed Liquor TSS and VSS concentration are reported in Figure 3.9, along with the VSS/TSS ratio.

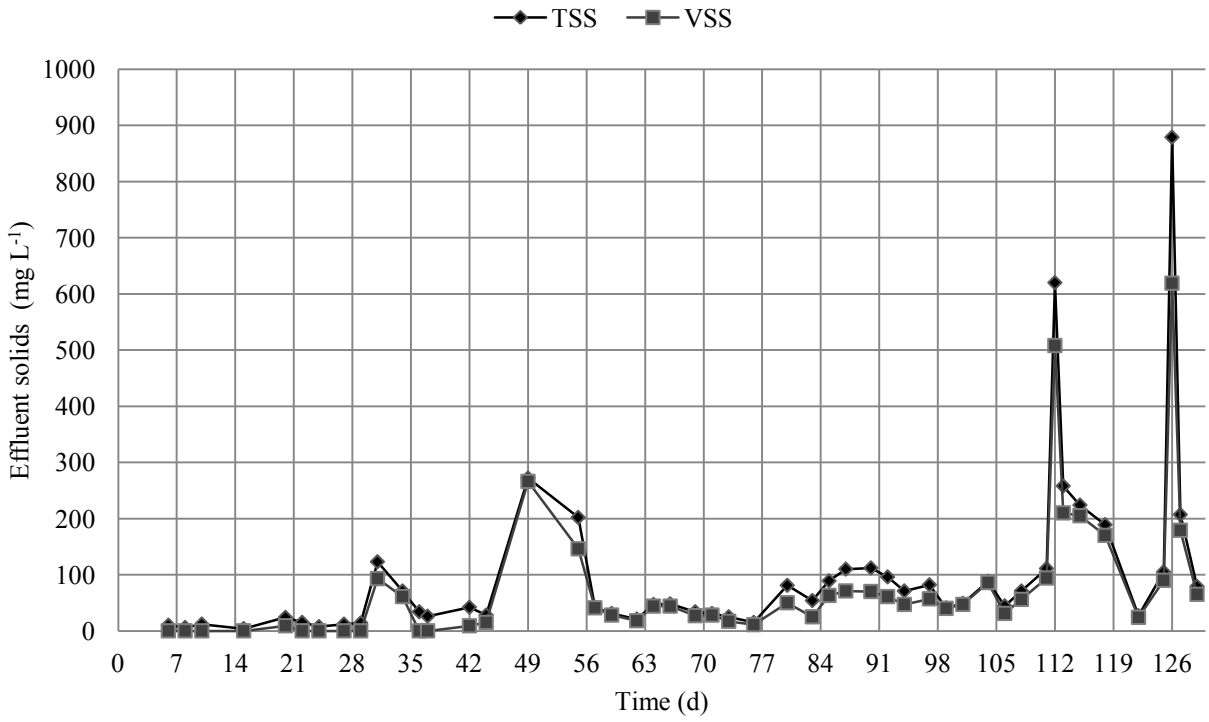

Figure 3.8: Total Suspended Solids and Volatile Suspended Solids during the AnMBR operation.

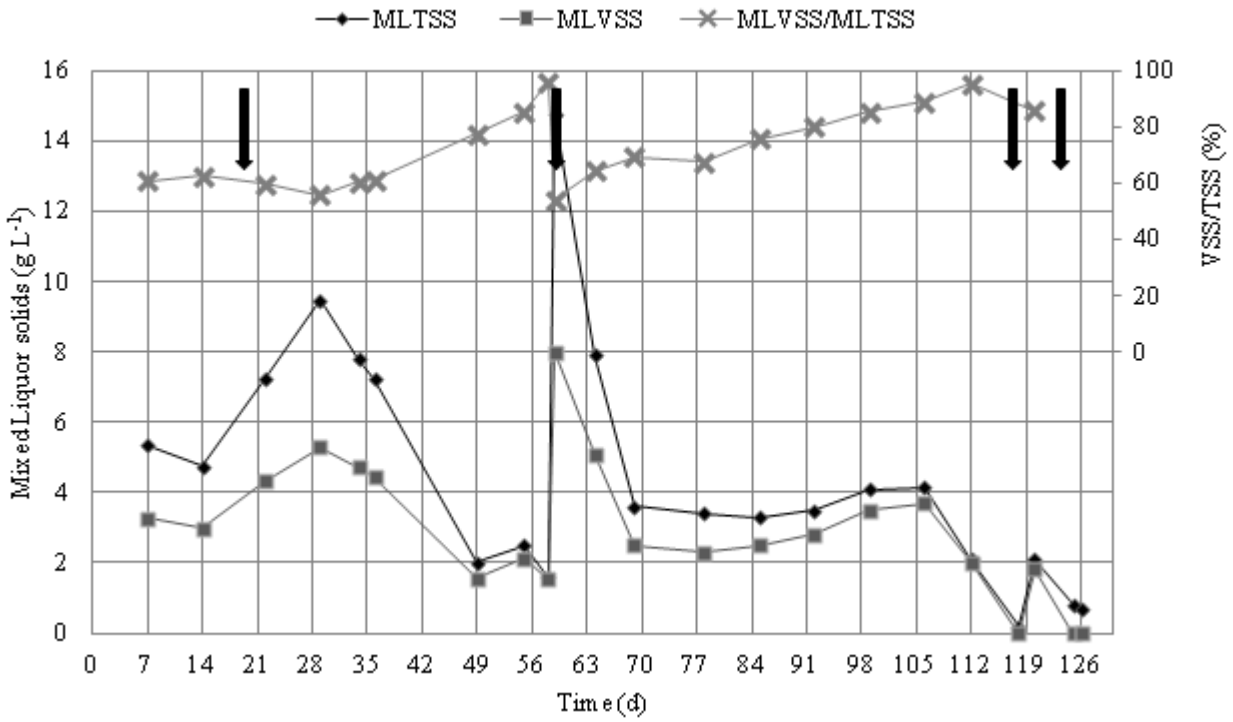

Figure 3.9: Mixed Liquor Total Suspended Solids and Volatile Suspended Solids during the experiment. Black arrows indicate the further seeds performed along the experiments.

During the first month the solids effluent concentration kept lower than 25 mg L^{-1}. Even though data about the first days of experiment were not available, it seemed that the system reached quickly good solids retention. This is in accordance with Ma *et al.* (2013), who stated that the effluent quality reached nearly stable conditions in 1-2 d. Anyway, as stated above, data about effective effluent solids concentration are probably underestimated. The initial MLTSS and MLVSS content measured on day 7 were equal to 5.4 and 3.3 mg L^{-1}, respectively. Volatile solids accounted for about 60% of TSS in the mixed liquor. The MLSS began to rise after the first additional inoculum, which was performed on day 17 after the membrane support had been opened because of clogging problems. On day 29, the MLTSS and MLVSS peaked at 9.5 and 5.3 g L^{-1}, respectively. During the second month of experiment, more fluctuations in effluent solids content were notice. After the peak observed on day 29, solids in the reactor decreased gradually down to values around 2 g L^{-1}. The effluent solids concentration increased up to about 100 mg L^{-1} on day 31, probably as a response to the increasing in mixed liquor concentration observed the previous days. Anyway, TSS and VSS concentration returned quickly close to the low values observed in the first month. On day 25, 60 g of granular sludge were seeded in the reactor to try to enhance biomass acclimation. This is the reason of high solids content in the effluent detached on day 49 and 55. The simultaneous occurrence of negative pattern of mixed liquor solids concentration and low solids content in the effluent leads to the assumption that

solids originally in the reactor were accumulating on the mesh, forming a cake layer. The low COD removal observed in this period shows that the biomass in the system –both in the reactor and on the mesh- had not acclimatized yet. As MLTSS and MLVSS concentration of barely 2 g L^{-1} was detached on day 58, new sludge was inoculated in the reactor the following day. The MLTSS and MLVSS climbed up to 15 and 8 g L^{-1}, respectively. Anyway, in less than two weeks the ML solids concentration decreased of about 75%. Therefore, on day 70 the MLTSS and MLVSS were equal to 4 and 2 g L^{-1}, respectively. Anyway, the effluent solids concentration kept lower than 50 mg L^{-1}. Consequently, solids were still accumulating onto the porous mesh. As previously stated, in these days a significant overall enhance in COD removal efficiency occurred and the filterable and soluble COD concentration in the effluent were under the detachable values. This proved the acclimation of biomass. Moreover, it is likely to assume that biological reactions started to occur on the cake layer on the mesh. From day 70 to day 105, when the system was run at HRT of 2 and 1 d, the MLTSS and MLVSS solids kept around 4 and 2-4 g L^{-1}, respectively. Nevertheless, a light increase in biomass content was observed, leading to an enhancement in COD removal. From day 105 to day 119, the solids in the reactor dropped to nearly zero. A new inoculum was therefore performed, so that MLTSS and MLVSS increased slightly. Solids and total COD concentration in the effluent rose up to 600 and 220 mg L^{-1}, respectively. Anyway, filterable and soluble COD kept below 125 mg L^{-1}. Is therefore likely to assume that the biochemical reactions taking place in the membrane supplied partially to the reduction in microbial activity in the reactor induced by ML solids loss. On day 119 a new additional seed was performed. Anyway, the retention capability of the system had deteriorated, so that biomass was washed out from the system and solids content of Mixed Liquor returned again to values close to zero. Another inoculum was therefore performed on day 125 but also this time the solids were washed out and the effluent quality deteriorated quickly.

On overall, effluent solids concentration kept under 100 mg L^{-1} with few exceptions. Anyway, this value is higher than those reported for conventional MBRs as MF and UF membranes achieved a nearly total retention of solids (Judd, 2006). Under stable conditions the system managed to keep quite low ML solids concentration (about 3-4 g L^{-1} for MLTSS and 2-4 g L^{-1} for MLVSS). These values are much lower than those normally reported in literature for AnMBRs. Skouteris *et al.* (2012) report that the good solids retention property of conventional MF/UF membranes allows working with MLSS up to 40 g L^{-1}. The ML solids concentration achieved in the present studies are lower than those reported by Alibardi *et al*. (2014). In fact, in the previous work the MLVSS concentration kept always above 5 g L^{-1} and showed a less fluctuating pattern. These differences may be explained by the better environmental conditions

provided to biomass in the previous study, as the system was run at optimal mesophilic temperature and the inlet COD concentration was 5-times higher. Operating conditions are therefore proved to affect significantly biomass behavior.

Italian Legislation concerning municipal wastewater treatment (D.Leg 152/2006) fixes a maximum COD concentration of 125 mg L^{-1} and a minimum removal efficiency of 75% for facilities higher than 10000 PE. As shown in Figure 1, when the system was run at 2 and 1 d and reached stable working conditions, the total effluent COD was lower or at least equal to the limit value. The limit was slightly exceeded during the second month of operation because of low acclimation of biomass and clogging problems. The limit was overcome again from day 97 on, in parallel with the deterioration of solids retention capability of the system. The total COD removal efficiency was higher than 75% throughout the experiment, with the only exception of the last period when the system was run at HRT of 0.25 d.

The SS discharge limit for superficial water bodies is set at 35 mg L^{-1} (D.Leg 152/2006). Data reported in Figure 3.8 revealed that this limit was not usually met, even though solids concentration values higher than 100 mg L^{-1} were rarely detached. Solids removal efficiency is slightly lower than those often observed in CAS clarifiers (Chong *et al*., 2012).

3.3.1 Contribution of the Dynamic Membrane to the overall COD removal efficiency

The outstanding biological COD removal observed in the systems even at low MLSS values leads to the hypothesis that the dynamic membrane forming gradually on the support mesh plays an active role in filterable COD removal, besides solids separation. In order to evaluate the contribution of the biochemical reactions taking place within/on the cake layer, the overall biological COD removal efficiency was split in two contributions: the reactor biological removal efficiency and the membrane biological removal efficiency. The first term is the difference between inlet COD and filterable COD measured in the reactor divided by the inlet COD. The second one is calculated as the difference between the COD measured in the reactor and the effluent filterable COD divided by the inlet COD. The pattern of the filterable COD concentration of the mixed liquor and the filterable COD of the effluent are reported in Figure 3.10, together with the biological removal efficiency of the membrane.

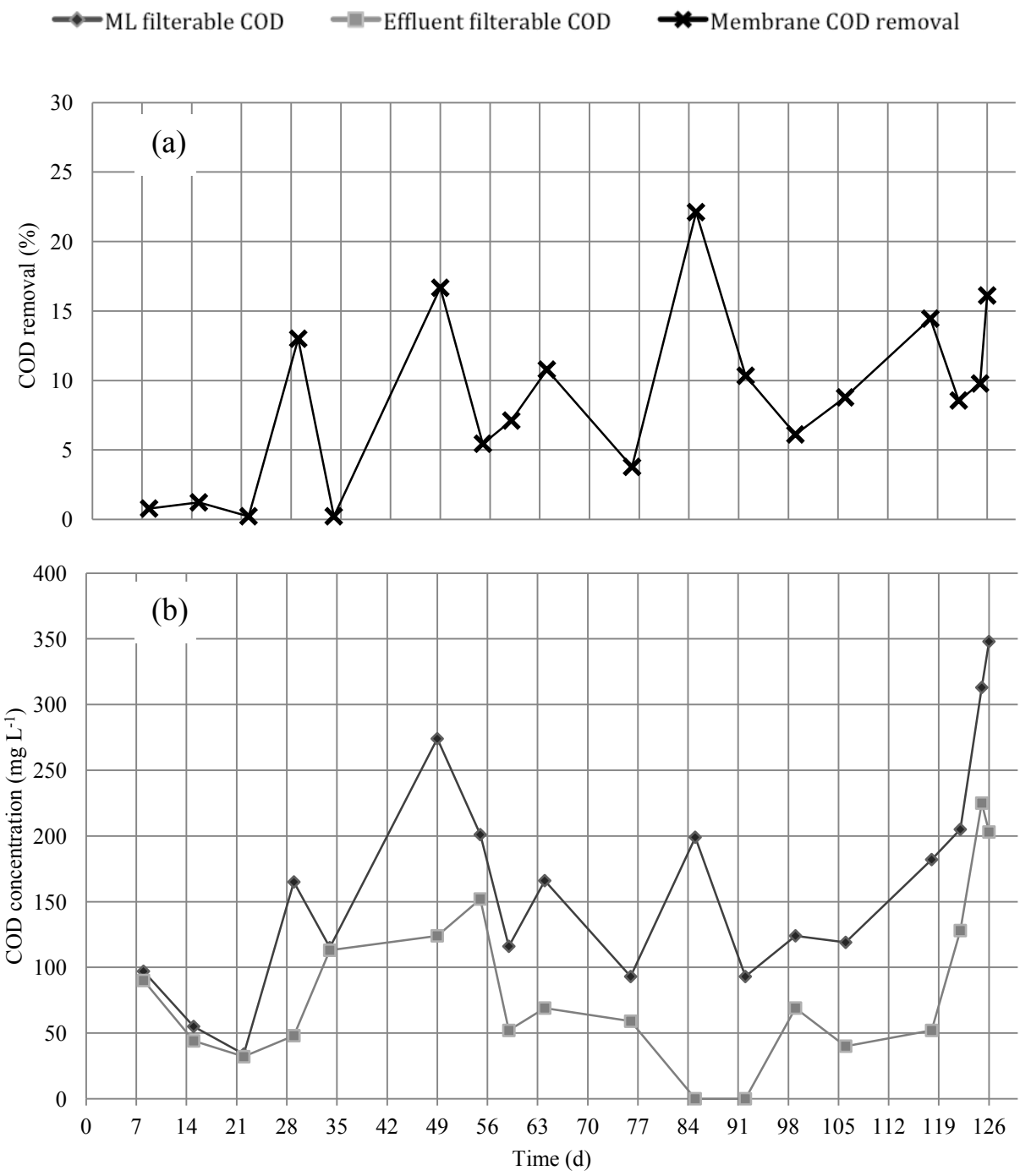

Figure 3.10: (a) Membrane biological removal efficiency and (b) filterable COD in the effluent and in the mixed liquor during the AnDMBR operation.

The filterable COD in the ML showed significantly lower concentration with respect to the inlet. In fact, the concentration did not usually overcome 200 mg L^{-1}. These low values are due to microbial organic matter degradation. Anyway, COD removal is likely to be enhanced by the sludge cross-flow movement within the external membrane support, which induced the recirculation of biomass and oxidized compounds into the reactor. The only higher concentration values are detached in the second month (day 49) when the biomass had not acclimatized yet, and in the last week of operation (day 125 and day 126), when the system was run at low HRT and the solids retention capability had been deteriorated. In these cases, the filterable COD concentration was equal to 275 and nearly 350 mg L^{-1}, respectively. Along the experiment time, the filterable ML COD pattern followed roughly the trend of the effluent filterable COD. Up to day 21, the value of ML COD was coincident with the value in the effluent. The biological removal efficiency operated by the membrane was therefore equal to zero. From the third week on, the membrane biological removal increased slightly, even though the trend was unstable. The peak was reached on day 85, when ML concentration was nearly 200 mg L^{-1} and the effluent concentration was under the detachable limit. Consequently, the membrane biological removal efficiency was 22%. After that, the membrane biological removal attested at slightly lower values. The average value observed throughout the experiment was nearly 10%. Anyway, it has been proved that the cake layer attached onto the support mesh can account for even one fifth of the overall filterable COD removal. This finding is coherent with Smith *et al*. (2010), who managed a conventional MF-AnMBR at psychrophilic conditions for the treatment of domestic wastewater. The Authors noticed significant soluble COD removal across the membrane, which accounted on average for the 21% of the total COD removal. Similar results were obtained by Ho and Sung (2010) working on a MF-AnMBR at 15 and 25°C. Anyway, to the best of our knowledge, the filterable COD removal efficiency performed by a dynamic membrane only had not been quantified yet.

3.4 Biogas production

Total daily biogas production pattern is represented in Figure 3.11, along with HRTs applied. Figure 3.12 reports the biogas fractioning according to the four measuring points (i.e. reactor, membrane, postmembrane, and exiting vessel).

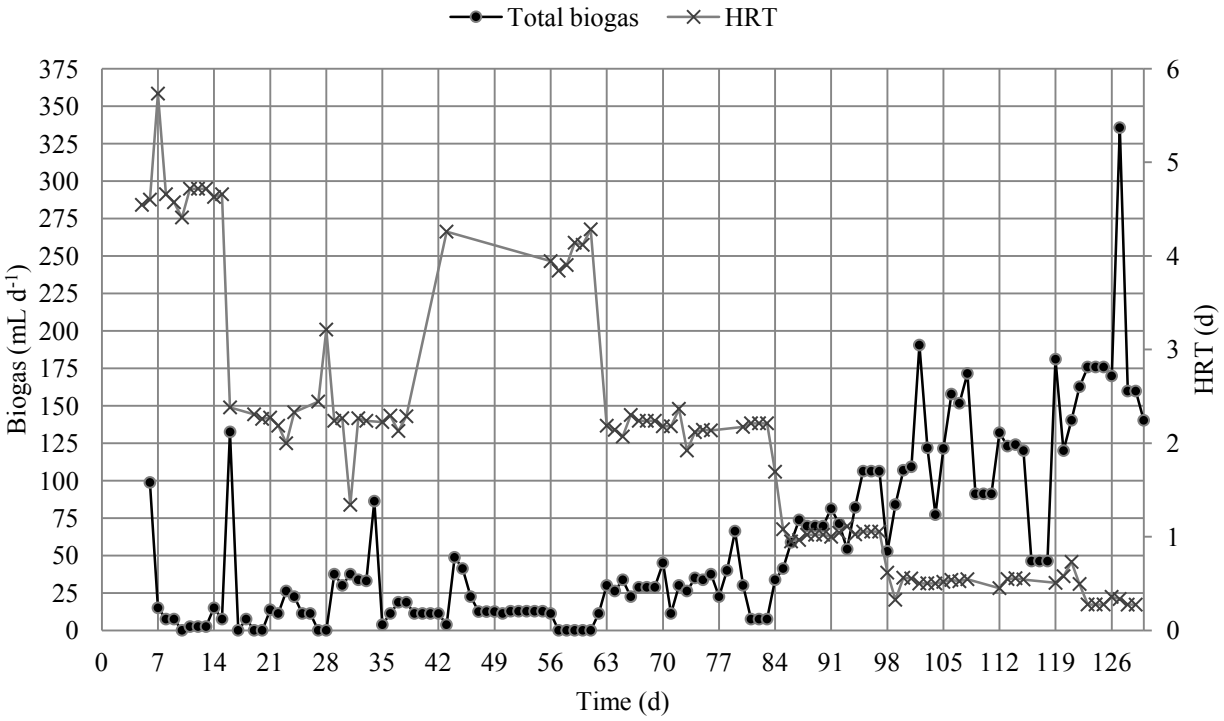

Figure 3.11: Total biogas daily production and applied HRT during the study.

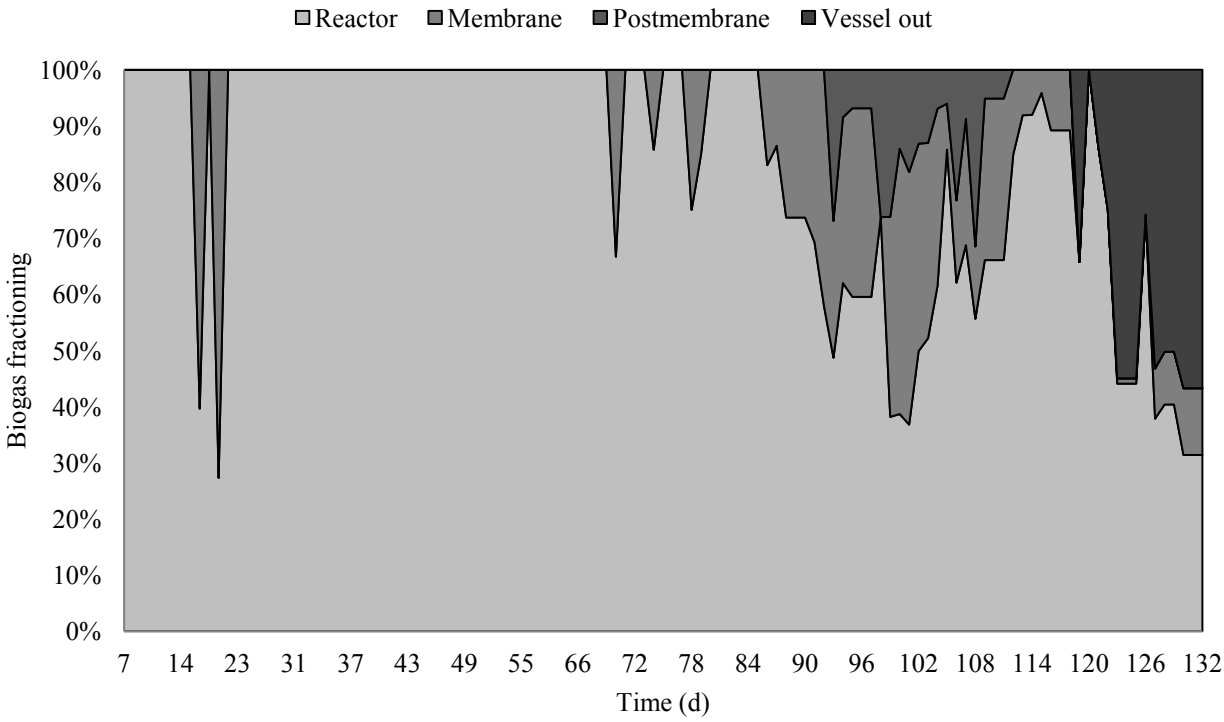

Figure 3.12: Biogas produced by the different four compartments of the system.

Biogas production was variable thorough the experiment. The volume of gas emitted from the system in the first two months was not significant. A peak was observed on day 14. Anyway, the chromatographic analysis performed revealed null methane content. This high value can be therefore caused by air bubbles entrapped in the system, which was characterized in that period by significant clogging problems. Slightly higher biogas emission was observed from day 29 to 34 as the system was feed with solution of acetic acid to enhance methanogens kinetic. Anyway, on day 35, biogas production dropped again to values close to zero. The low biogas production is probably due to the slow kinetic of biomass, which had not acclimatized yet to new conditions. These findings seem validating the hypothesis that the high filterable and soluble COD removal (> 95%) detached in the first month of operation is actually overestimated, as discussed in Paragraph 3.3. Biogas production kept lower than 50 mL d^{-1} even during the second month, when sucrose was used as sole COD source in the feed. The low methanogenic activity in this phase is coherent with the lower filterable and soluble COD removal observed in this phase (85-90%) with respect to the average value (>90%). In the first two months, the vast majority of biogas was released by the reactor, which therefore accounts for nearly 100% of the total volume measured. Biogas emission increased slightly from day 63 on, when the HRT was reduced to 2 d and the system reached more stable conditions. Accordingly, the COD removal efficiency increased up to values higher than 95%. This enhancement in biogas production may be due to both higher OLR applied and acclimation of biomass. Daily production ranged around 25 mL with peaks of nearly 75% until day 80. In this phase, the membrane, too, released biogas even though this volume accounted for only 4% of overall production. This finding proves that a biologically active cake layer was developing onto the support mesh, coherently with the increasing contribution of membrane on overall filterable COD removal (5-10 of the whole removal efficiency), as reported in Paragraph 3.3.1

A drop in biogas production was observed between day 80 and day 83. Values close to zero might be due to lower MLVSS detached with respect to the previous phase. Biogas production rose up again after the HRT was lowered to 1 d on day 83. In fact, the daily volume increased steadily from 35 mL (day 84) to 175 mL (day 108), even though MLVSS kept nearly stable at quite low values (2-4 mL d^{-1}). Biogas production rose steadily up to values of 175 mL d^{-1}, even if significant fluctuations were observed. Lower values were detached between day 116 and day 119 as MLSS had been washed out from the system. Anyway, after the inoculum performed at day 119, biogas production rose up again to the level detached in the previous days. The biogas production kept at quite high levels even in the last phase of the experiment, when the solids retention capability of the system had deteriorated. Consequently, it is likely to assume that the

biomass in the dynamic membrane had compensated for the reduction in MLVSS. The role of membrane in biogas production became important from the beginning of the third month. The volume escaping from the membrane support accounted for even 40% of total production. Moreover, a significant amount of biogas was measured by means of the gas meter placed down-flow the membrane from day 92 to day 111. The average contribution to overall production in these days was 15%, with a peak of 36% on day 108. As this gas is likely to be the product of biomass attached onto the lower face of the mesh support, its contribution can be added to the one reported for the membrane (i.e. second gas meter). By considering together these two contributions, the biomass attached onto the mesh was responsible of even 70% of the overall daily production (day 101). A high amount of biogas was collected from the effluent collection vessel, after the forth gas meter was set in motion on day 119. This gas accounted for about half of the overall daily production. Therefore, a relevant fraction of biogas produced was dissolved in the effluent. As the forth gas-meter was not properly working until day 119, relevant methane losses are likely to had occurred during the experiment. The contribution of the gas measured by the forth gas-meter in the present study is considerably higher than that reported by Alibardi *et al*. (2014), who worked at 35°C. According to the Authors, the dissolved methane released in the vessel accounted for about 6% of the overall production. This comparison stressed the enhancement of dissolution of gases in the effluent when shifting from mesophilic conditions to ambient temperature, coherently with literature data (Gimènez *et al*., 2012a).

The methane content detected in the biogas extracted by the reactor and the membrane support along the experimental time is reported in Figure 3.13.

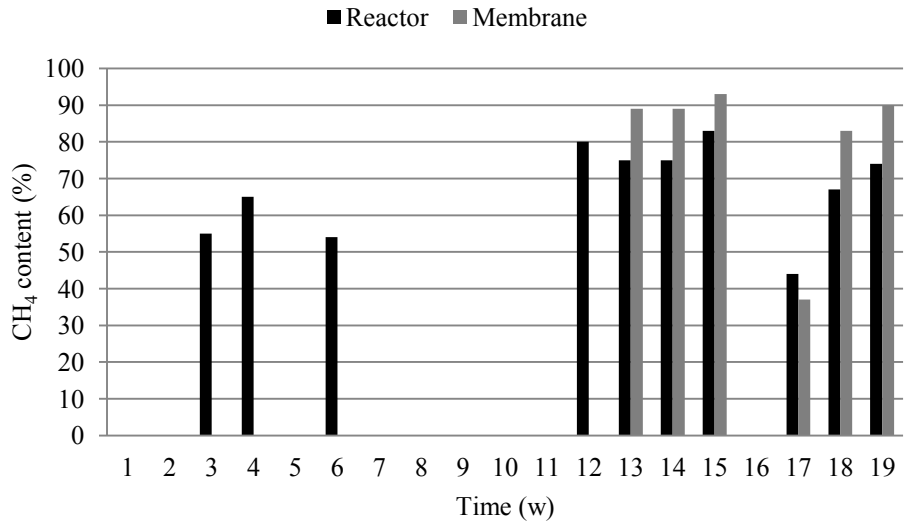

Figure 3.13: Methane concentration in the gas phase measured in the reactor and in the membrane module.

In the first two months, the methane content in the biogas extracted from the reactor was between 55% and 65%. The membrane released no methane. After the start-up, when the system was run at HRT of 1 and 2 d, the percentage of methane from bioreactor rose up to values higher than 75% with peaks at nearly 85%. This increase was probably related to a better acclimation of methanogens and lower HRTs applied. In fact, CO_2 is nearly 25 times more soluble than CH_4, so it is more easily washed out from the system when working at low HRT, especially at low temperatures like those reported throughout the experiment. The different solubility of methane and CO_2 may explain also the higher methane content detached in the biogas escaping from the membrane support. In fact, the membrane support had a lower specific interfacial area with respect to the reactor, so that the liquid-to-mass gas transfer was discouraged. The compounds dissolved in the liquid phase resulted to be easily washed into the reactor again thorough the sludge recirculation system. The tendency of gases of being transported into the reactor is directly dependent on their solubility, so it is higher for CO_2 than for CH_4 especially under ambient temperature.

The lower methane content in the biogas detached on week 17 is probably related to the inoculum performed on day 119 after the MLSS washout. Anyway, in the last weeks the methane content showed a positive trend and values higher than 65% were observed even when the stability of the system had collapsed. The differences in composition between biogas extracted from the reactor and the membrane were slightly higher than those observed in the previous phase. The methanogenic activity of the membrane played therefore an important role until the end of the experiment.

The observed methane content in biogas is slightly higher than that in Alibardi *et al*. (2014), who reported values between 50% and 70%. This difference is likely to be due to the lower HRTs and different ambient conditions, but also to the lower inlet COD applied in the present work. Ho *et al*. (2007) report, in fact, a slight decrease from 92% to 89% in methane content when doubling the feed COD concentration. Such high values in methane content are coherent with literature data. Chen *et al*. (2014) report methane content in biogas of nearly 80% when managing a FO-AnMBR at 25°C. Anyway, few data are available in literature about biogas composition in AnDMBRs and about the differences between biogas extracted from the reactor and from the membrane.

Relatively high solubility of gases at ambient temperature may also explain the consistent biogas production observed in the bioreactor even in the last weeks of experiment, when the MLVSS dropped to values close to zero and the solids retention capability of the system had deteriorated. It is plausible to assume that a relevant percentage of the biogas escaped from the reactor had

been actually produced within the membrane support and then transported dissolved the liquid phase into the reactor, where volatilization of gases is more likely to occur, as the interfacial area is wider. The methanogenic activity of cake layer would be therefore underestimated.

3.5 Nutrients pattern

The influent TKN and the effluent ammonium ion concentration along the experiment are reported in Figure 3.14. The inlet and outlet phosphorous concentration patterns are represented in Figure 3.15.

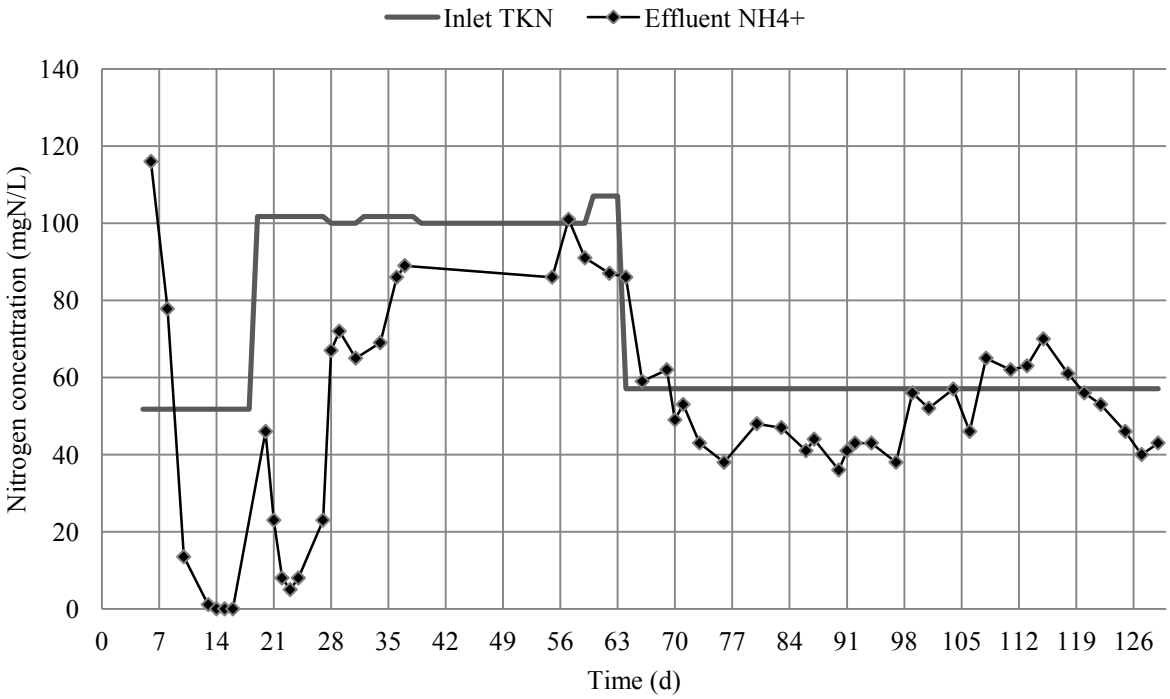

Figure 3.14: Influent TKN and effluent ammonium ion concentration measured during the AnDMBR operation.

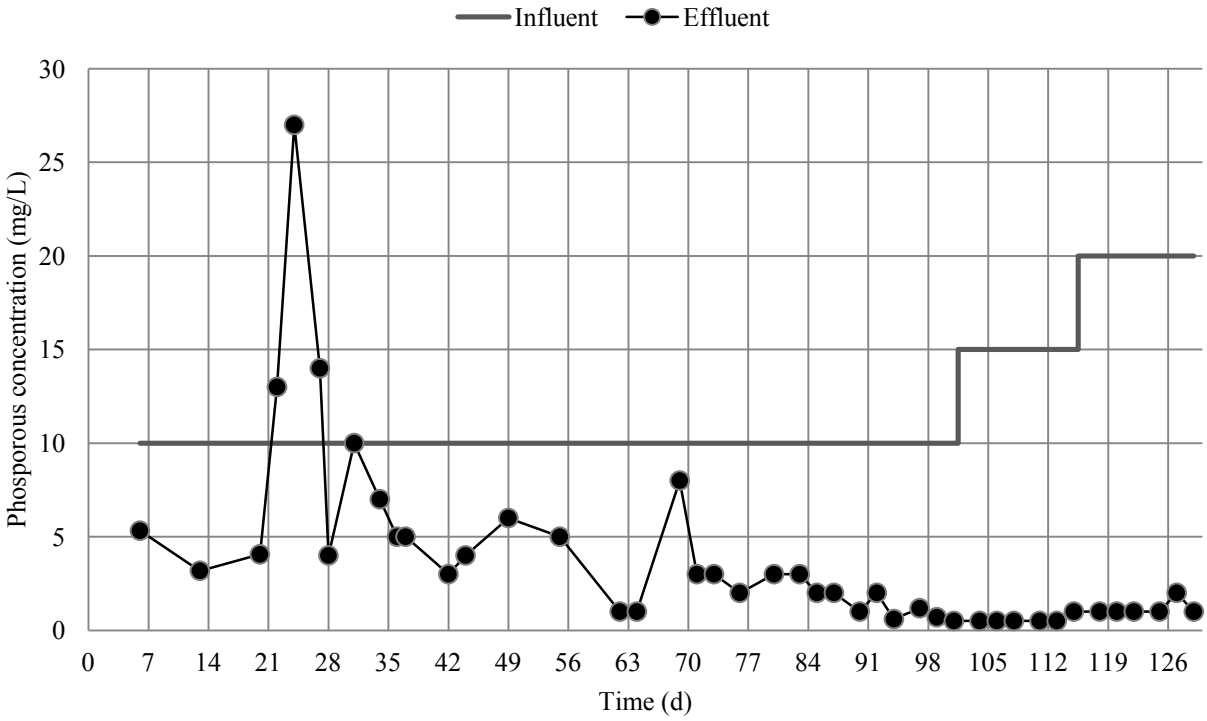

Figure 3.15: Influent and effluent phosphorous concentrations.

The ammonium ion concentration in the effluent showed a fluctuating trend throughout the experimental period. This behavior was particularly remarkable during the first two months, when the system had not reached a stable condition yet. The ammonium ion content observed in the effluent was initially high (nearly 120 mgN L^{-1}). In fact, the sludge taken out from the anaerobic digester and used for the initial biomass inoculum had nitrogen content of 250 mg L^{-1}. Anyway, in one week the ammonium concentration in the outflow plummeted to values close to zero. For this reason, the content of NH_4Cl used as source of readily bioavailable nitrogen was doubled and the total TKN inlet concentration increased from 50 up to 100 mgN L^{-1}. The effluent ammonium concentration rose up to 46 mgN L^{-1} (day 20). Anyway, this increase seems to be due to the biomass inoculum performed on day 17 instead of changes in inlet characteristics. In fact, effluent nitrogen dropped again quickly to values close to zero, similarly to what had been observed in the first week. From day 27, nitrogen effluent concentration increased rapidly to values of about 70 mgN L^{-1}. During the second month of experiment, the ammonium concentration ranged between 30 and 100 mgN L^{-1}. The increase in nitrogen content with respect to the previous phase seems related to the higher HRT applied. On day 64, the inlet NH_4Cl concentration was lowered again down to the initial value of 50 mgN L^{-1}. By considering powder milk feed concentration of 400 mg L^{-1}, the inlet TKN was therefore about 60 mgN L^{-1}. From day 65 to day 106, when the system was run at 2, 1 and 0.5 d, the outlet ammonium

concentration ranged between 36 and 65 mgN L^{-1}. Slightly higher values were observed from day 108 to day 118. This might be caused by the release of nitrogen previously accumulated within the system. Effluent ammonium concentration then decreased steadily down to about 40 mgN L^{-1}. Despite significant fluctuations, the average abatement of nitrogen concentration with respect to the feed is 12%. The nitrogen biomass uptake under anaerobic conditions is 10-13 mgN per 100 mgVSS (Tchobanoglous *et al*., 2003). Anyway, this contribution can be neglected, as the MLVSS in the system kept always lower than 15 g L^{-1}. Other mechanisms should be therefore evaluated to explain nitrogen removal. Among all, the nitrogen abatement might be partially related to the biochemical reactions occurring in the cake layer attached onto the support mesh, which proved to be biologically active. The solids characterization performed on the membrane after the system shut down revealed high VS content. The biomass in the cake layer should be therefore taken into account in the estimation of nitrogen microbial uptake.

Similarly to nitrogen, total phosphorous concentration in the effluent showed an unstable trend. In the first three weeks, the outlet concentration kept between 3 and 5 mgP L^{-1}, with abatement higher than 50%. From day 20 on, the concentration soared up to 27 mgP L^{-1} (day 24). After this peak, the outlet decreased rapidly to values near to 5 mgP L^{-1}, even though fluctuations of nearly 100% were observed until the end of the second month, when the system reached a more stable condition. After the HRT was lowered to 2 d, the effluent concentration started dropping down to values close to zero, even though the inlet KH_2PO_4 content was increase up to 15 mgP L^{-1} (day 101) and then further to 20 mgP L^{-1} (day 115), in order to avoid phosphorous acting as a limiting element in microbial kinetic. The average phosphorous removal during the experiment was higher than 60% and lowered to even 97% after the second month. No clear explanations have been provided for such low phosphorous content in the effluent. The phosphorous biomass uptake under anaerobic conditions is 1-2 mgN per 100 mgVSS (Tchobanoglous *et al*., 2003), i.e. one order of magnitude lower than the one reported previously for nitrogen. Consequently, microbial uptake cannot explain alone the high abatement, even considering the biomass in the cake layer. The variability of N and P concentrations observed in the effluent could be explained by struvite precipitation. However, the formation of struvite was not investigated since it was beyond the scope of the study.

The actual Italian legislation (D. Lgs. 152/2006) fixes the discharge limits in superficial water bodies at 10 mgP L^{-1} for total phosphorous and 15 mgNH_4 L^{-1} for ammonium.

The effluent phosphorous concentration observed in the second part of the experiment does not overcome this limit. On the contrary, post-treatments are necessary to further reduce nitrogen

content before discharge or nutrients-rich water may be used in other applications (e.g. irrigation purposes, Smith *et al*., 2012).

3.6 pH and Alkalinity

The pH pattern in the effluent and in the mixed liquor is reported in Figure 3.16. Figure 3.17 represents the alkalinity and Total Volatile fatty Acid concentration in the effluent along the experiment time.

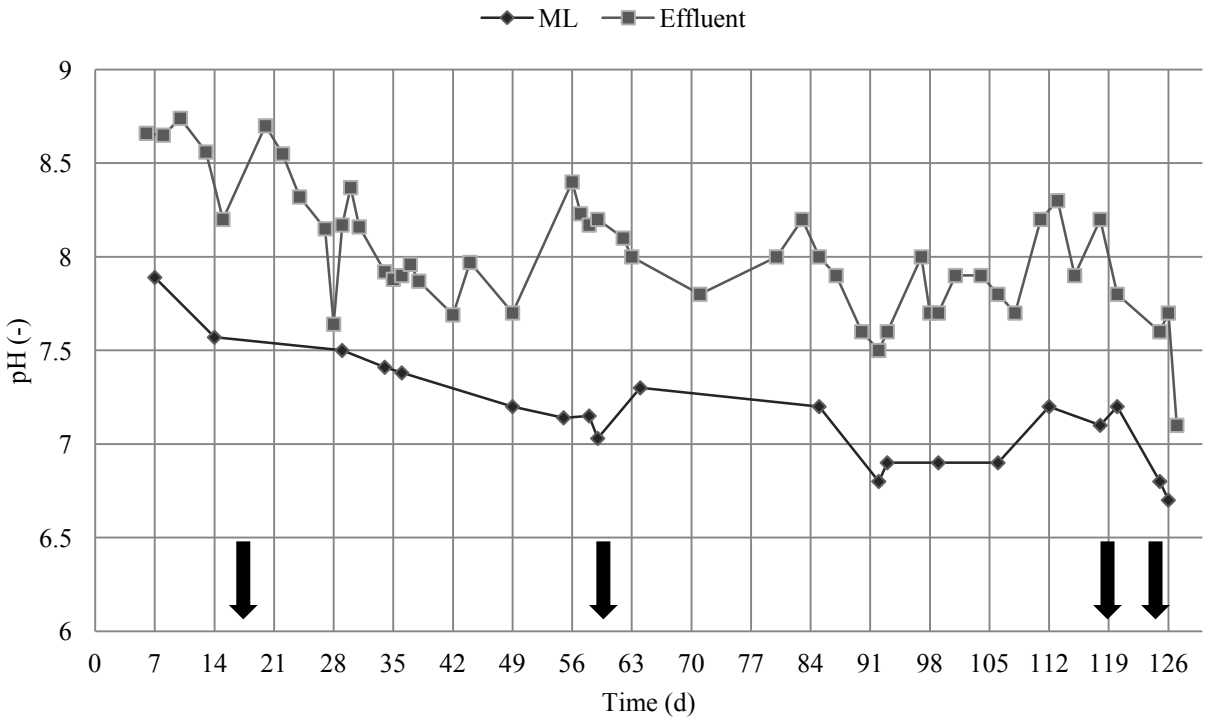

Figure 3.16: pH measured in the Mixed Liquor and in the effluent during the experiment. Black arrows indicate the additional seeds performed along the experiments.

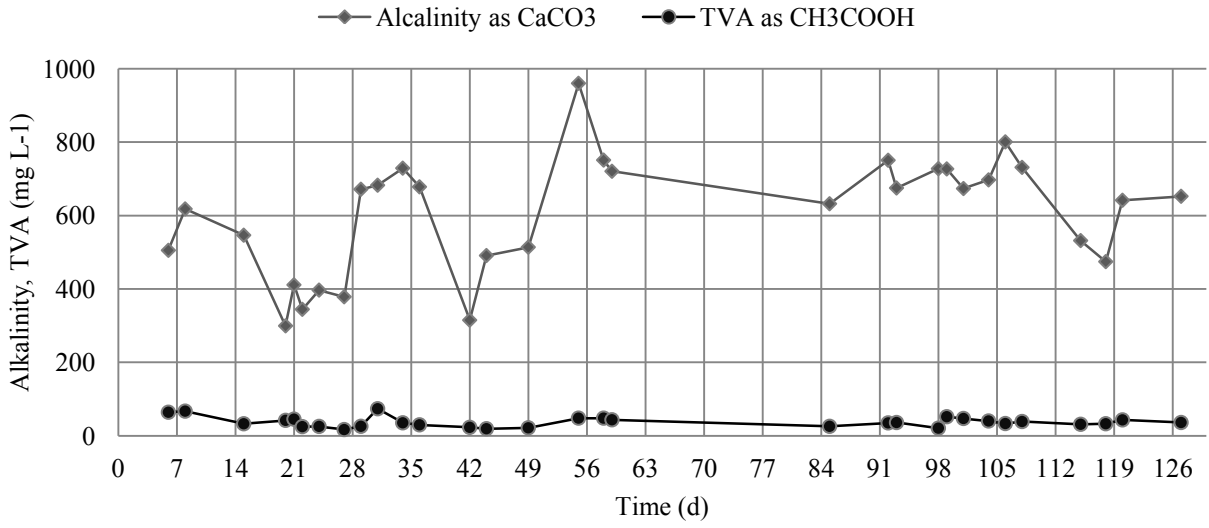

Figure 3.17: Alkalinity as $CaCO_3$ and Total Volatile fatty acids concentration measured in the effluent.

The effluent pH showed a fluctuating pattern, ranging from 8.7 and 7.1. On overall, a slightly decreasing trend was observed, in particular in the last operation week. A similar pattern was observed for mixed liquor pH, even though fluctuations seem less evident. Even though lower pH values were reported from day 90 to day 106, the mixed liquor pH was always above 6.5, which is reported as the lower limit for optimal methanogenic activity (Tchobanoglous *et al*., 2003). The additional seeds performed along the experiment seems not influencing pH pattern significantly. It has to be stressed that effluent showed always a higher pH than mixed liquor, similarly to what observed by Ho and Sung (2010) when running a conventional MF-AnMBR. This behavior may be the consequence of CO_2 dissolved in the liquid phase because, which volatized in the effluent tank. As CO_2 behaves as an acid in water, dissolved CO_2 in the liquid phase might explain the slightly decreasing pH trend along with the enhancement in microbial kinetic, i.e. CO_2 production.

The alkalinity, expressed as $CaCO_3$ concentration, showed a fluctuating pattern along the time, ranging from 300 to 1000 mg L^{-1}. Anyway, by considering the pH trend reported in Figure 3.16, it is clear that the alkalinity provided by the feed solution and the tap water was sufficient to maintain neutral environment optimal for microbial kinetic. The concentration of volatile fatty acids (i.e. intermediate products of organic matter degradation) kept always lower than 100 mg L^{-1} as acetic acid. This is an indicator of balanced conditions within the system and proper environmental conditions for acetophilic methanogens.

3.7 COD Mass Balance

Figure 3.18 represents the COD mass balance performed on the system run at HRT of 2 d, 1 d, and 0.5 d. Quantification of methane dissolved in the liquid phase was performed by considering thermodynamic equilibrium conditions between phases. The results are expressed as percentage on the total COD entering the system through the inlet stream in the whole periods considered. The results of the mass balance performed on the system working at HRT of 0.25 d are not reported. In fact, in the last week of experiment the solids retention capability had deteriorated. Consequently, the COD measured in the effluent turned out to be higher than the inlet COD.

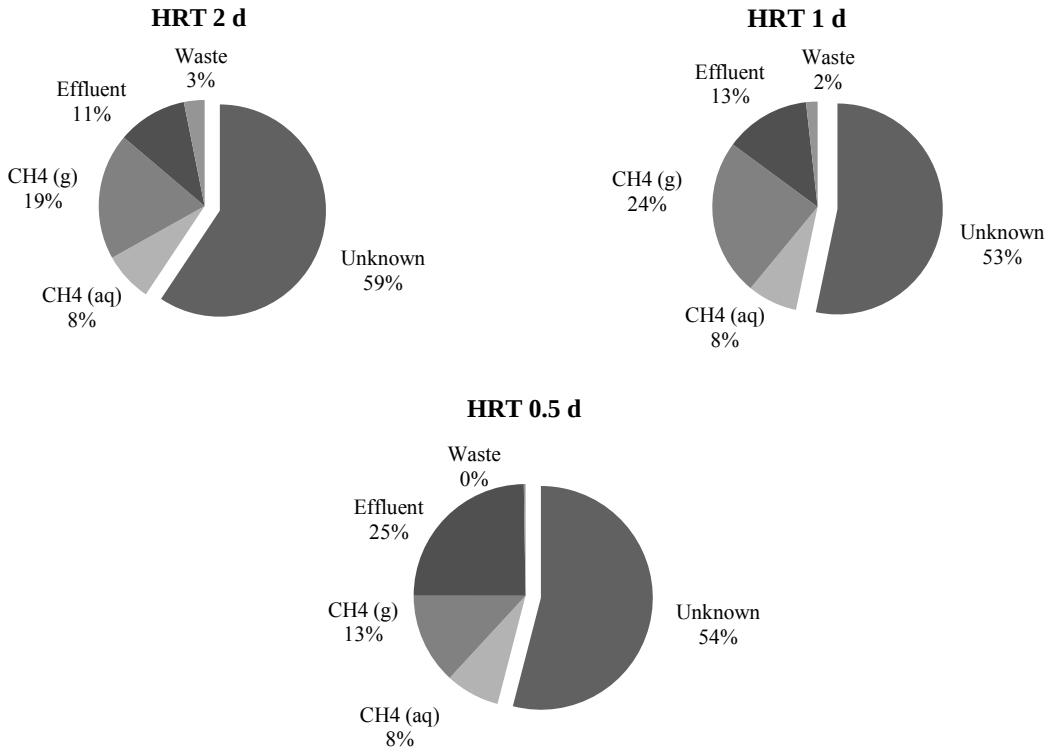

Figure 3.18: Results of mass balance performed on the system run at HRT of 2 d, 1 d, and 0.5 d.

The system was run at HRT of 2 d (OLR of 0.4 kgCOD m^{-3}) for 3 weeks, so that the inlet COD was 6440 mg. The value increased to 9856 mg and 32355 mg for the system managed at HRT of 1 d (OLR of 0.4 kgCOD m^{-3} for 2 weeks) and 0.5 d (OLR of 3.6 kgCOD m^{-3} for 25 days), respectively.

No chromatographic analyses were performed in HRT 2d, so the methane content in biogas was estimated at 80%. On average, methane content in biogas was equal to about 80% and 75% in HRT 1d and HRT 0.5 d, respectively. The methane recovered in the gas phase was equal to 479

mL (i.e. 1244 mgCOD) in HRT 2d, 820 mL (i.e. 2141 mgCOD) in HRT 1 d, and 1621 mL (i.e. 4253 mgCOD) in HRT 0.5 d. In HRT 2d, the methane flowing out the system with biogas stream accounted for about one fifth of the input COD in HRT 2d and one fourth in HRT 1d. The relative contribution in HRT 0.5d is slightly lower (<15%). This drop was in fact due to solids washout, as emissions were anyway higher than in the previous phases. Anyway, gaseous methane is the major defined system electron sink in the three periods.

Dissolved methane concentration in the liquid phase under thermodynamic equilibrium conditions is not dependent on HRT applied, so it did not varied in the three periods analyzed. The only operating parameters influencing equilibrium between phases are working temperature and pressure. Average temperature varied slightly in the three periods (22-25°C), while the working pressure was assumed equal to 1 atm. The dissolved methane concentration under thermodynamic equilibrium was 17 $mgCH_4\ L^{-1}$. This corresponds to a mass of 1244 mgCOD, 2141 mgCOD, and 4253 mgCOD in the three periods (8% of inlet COD). By taking into account both gaseous and dissolved forms, about 30% of total input COD was converted into methane in HRT 2d and HRT 1d. The methane contribution dropped to about 20% in HRT 0.5d because of the increase in effluent COD.

The total COD mass discharged with the effluent was of 685 mg for HRT 2d and 1164 mg for HRT 1d. Consequently, the effluent COD accounted for less than 15% under both conditions. These data are coherent with the high total COD removal (>85%) observed, as reported in Paragraph 2.3. This contribution rose up to 25% in HRT 0.5d because of increase in solids washout. The amount of COD lost during the analysis of the mixed liquor can be neglected independently from HRT.

Considering methane in the gas phase only, the observed yield coefficient $Y_{obs}^{CH_4}$ was 83 $L_{CH_4}kgCOD^{-1}$ for HRT 2d and 101 $L_{CH_4}kgCOD^{-1}$ for HRT 1d. These values rose of 20% and 30%, respectively, if considering dissolved methane, too. In fact, the observed methane yield coefficient $\widehat{Y}_{obs}^{CH_4}$ was 101 $L_{CH_4}kgCOD^{-1}$ for HRT 2d and 133 $L_{CH_4}kgCOD^{-1}$ for HRT 1d. The $Y_{obs}^{CH_4}$ value fell to 5 $L_{CH_4}kgCOD^{-1}$ for HRT 0.5 d because of decrease in overall COD removal. By the way, this drop is less important for $\widehat{Y}_{obs}^{CH_4}$, which was equal to 100 $L_{CH_4}kgCOD^{-1}$. These values are still low if compared to data found in literature about AnMBRs. As an example, Martinez-Sosa *et al*. (2011) report values of 270 and 230 $L_{CH_4}kgCOD^{-1}$ working at 35 and 20°C, respectively. Similarly, in a recent work Chen *et al*. (2014) achieved value of 210 $L_{CH_4}kgCOD^{-1}$ working at 25°C, so very close to the working conditions reported throughout the experiment.

During the work, the observed methane yield coefficient $Y_{obs}^{CH_4}$ was equal to only one third 30% of the theoretical value of 340 $L_{CH_4}kgCOD^{-1}$ (at 25-27°C).

The low methane yield values led to the assumption that the actual dissolved methane concentration in the liquid phase was considerably higher than that calculated under thermodynamic equilibrium conditions. Moreover, as clearly shown in Figure 3.18, COD mass balances performed are not well-closed as the unknown fraction ranged between 50% and 60% in the three cases analyzed. This corresponds to a COD gap of 3822 mg, 4728 mg, and 17848 mg for HRT 2d, HRT 1d, and HRT 0.5d, respectively. The fate of about half of inlet COD was actually unknown. Methane oversaturation (i.e. concentration higher than thermodynamic equilibrium concentration value) in the liquid phase is likely to occur in anaerobic systems working at ambient temperature, as gas solubility and temperature are indirectly proportional and methane is poorly soluble. This behavior has been already reported in literature. Yeo and Lee (2013) found that the actual concentration of methane dissolved in the effluent of an AnMBR run at 23°C and low SRT (20 d) can rise by almost 80% of the equilibrium value, even in case intensive mixing is applied. Similarly, Pauss *et al*. (1990) analyzed gas transfer processes between phases in different anaerobic systems (baffled stirred reactor, sludge-bed reactor, and upflow sludge-bed filter reactor). Even in completely stirred configuration, the liquid phase was 10-12 times more concentrated in dissolved methane than thermodynamic equilibrium conditions. On the contrary, Giménez *et al.* (2012a) reached nearly equilibrium working with a semi-industrial AnMBR even at low temperature (20°C). Part of the produced gas was indeed recirculated into the bottom of the reactor to enhance gas stripping. The global mass transfer coefficient (k_La), which is representative of liquid-to-gas mass transfer rate, changes significantly according to reactor configuration and operation mode (Pauss *et al*., 1990). Consequently, k_La should be determined on site. In the present work, if an oversaturation factor of about 10 were detached, the mass balances performed would be closed and dissolved methane would be the main electron sink in the system.

Moreover, sulfate-reducing bacteria compete with methanogens for COD, leading to a decrease in methane gas production (Tchobanoglous *et al*., 2003). The main source of sulfate in the system is the tap water used to prepare the feed solution. According to the data provided by the supplier, the sulfate concentration in tap water is 13 $mgSO_4^{2-}\ L^{-1}$. The overall extra sulfate contribution given as $CuSO_4$ and $ZnSO_4$ used as macronutrients is negligible. The amount of COD consumed for sulfate reduction ranges between 0.89 and 0.67 gCOD $(g\ SO_4^{2-})^{-1}$ (Tchobanoglous *et al*., 2003). As a consequence, in the system here presented, the organic matter

used for sulfate reduction is 61-81 mgCOD for HRT 2d, 87-116 mgCOD for HRT 1d, and 314-417 mgCOD for HRT 0.5d. Sulfate reduction accounts therefore for only 1% of total input COD.
By means of data of the mass balance, it was also possible to estimate the SRT value for each period. The SRT was calculated as the ratio between the biomass in the reactor and the VSS lost through both the outlet stream and the extracted mixed liquor throughout each of the three periods. SRT was 110 d when the system was run at HRT of 2 d and 40 d in the following period. The SRT value dropped to 5 d when the HRT was decreased to 0.5 d, because of increase in effluent solids losses. These values are significant lower than those reported in literature for conventional AnMBRs, which usually work at SRT higher than 150 d (Skouteris *et al*., 2012) and for anaerobic treatment systems (Tchobanoglous *et al*., 2003).
Anyway, even lower SRT values are reported for MBRs equipped with dynamic membranes. Kiso *et al*. (2000), Ersahin *et al*. (2014), and Ma *et al*. (2013) achieved SRT values not higher than 40 d, working under either anaerobic or anaerobic systems and with mesh porosity down to 10 μm. By the way, the method proposed for the calculation of SRT does not take into account the biomass entrapped in the cake layer on the support mesh, which was proved to play a significant role in COD degradation. Analysis performed on the cake layer after the system shutdown reported an overall VS content of about 750 mg. By considering also the membrane contribution together with MLVSS in the evaluation of system biomass, the SRT rises of about 25% up to 135 d in HRT 2d and 51 d in HRT 1d. Because of the low MLVSS detached, the contribution of membrane biomass in SRT calculation is higher at HRT 0.5d. The SRT value is in fact 40% higher, even though it keeps low (7 d).

3.8 Short-term test on membrane filterable COD removal

A short-term experiment was performed with the setup after the system shutdown (day 129), focusing on the COD removal efficiency performed by the dynamic membrane. Main hydraulic parameters (i.e. HRT and TMP) were monitored, too.
The TMP pattern during the short-term test is represented in Figure 3.19, along with the HRTs applied.

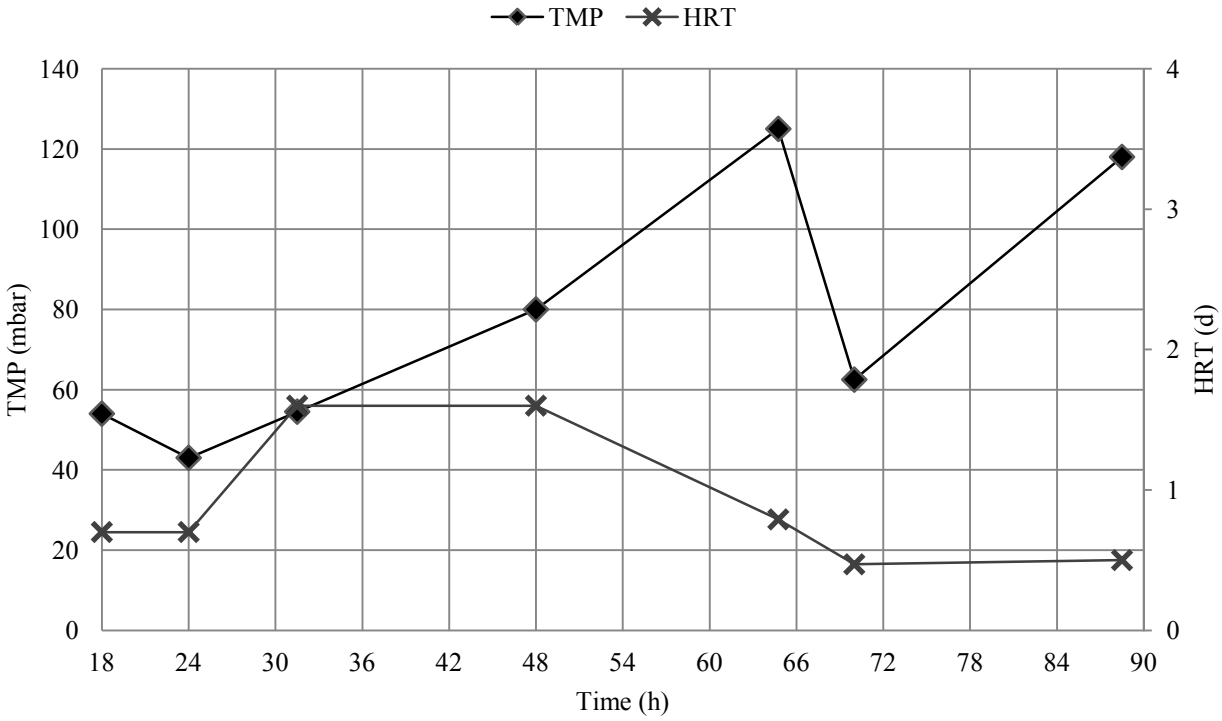

Figure 3.19: TMP e HRT during the short-term test.

The HRT applied in the test ranged between 0.5 d and 1.5 d. In fact, good system stability was observed in the previous experiment under these conditions. Within the first day, TMP stayed at about 50 mbar. As the HRT was increased to about 1.5 d, the TMP started to rise up to 125 mbar. A drop to initial values occurred after the HRT was decreased to 0.5 d. Anyway, TMP rose quickly again to the value of nearly 120 mbar detached at the end of the test. On overall, TMP showed a quite unstable behavior. The starting values were lower than those reported at the end of the main experiment. This datum might be due to the relaxation induced by the temporary stop in operation, which had been performed before the short-test to clean the setup. Even though the peaks of about 200 mbar reported in the main experiment were not reached anymore, in the short-term test TMP was slightly higher than that reported in the main test under the same HRTs. It is likely to assume that the membrane kept memory of the modifications induced by the critical HRT conditions imposed at the end the main experiment. Anyway, the short duration of the test precludes identifying a clear TMP pattern.

The trend of total and biological COD removal during the test is represented in Figure 3.20. Figure 3.21 reports the effluent solids concentration pattern.

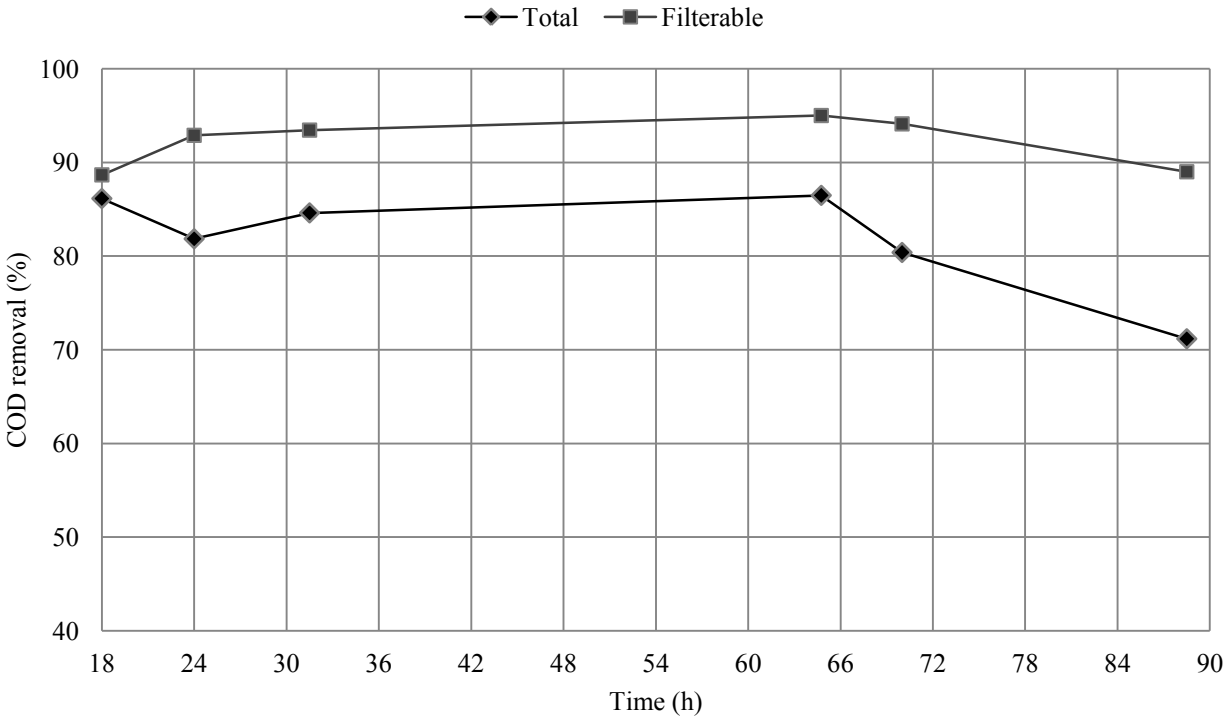

Figure 3.20: Total and filterable COD removal during the short-term test.

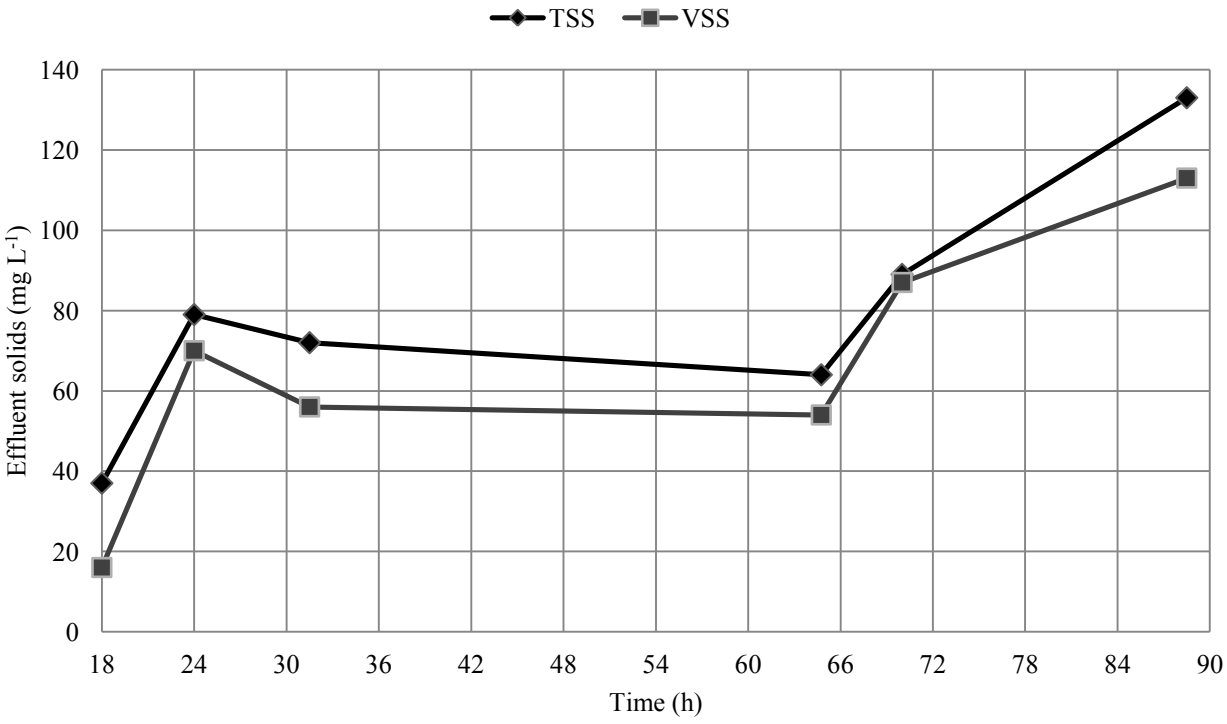

Figure 3.21: Total Suspended Solids and Volatile Suspended Solids in the effluent during the short-term test.

Even though effluent solids concentration doubled within the first day, total COD removal kept between 80% and 90% in the three first days. These findings are coherent with those reported during the previous experiment at the same HRTs. Moreover, no significant fluctuations were

detached. On day 3 (h 66), when the HRT was decreased to 0.5 d, an increase in solids in the effluent occurred. Peaks of 130 mgTSS L^{-1} and 110 mgTSS L^{-1} were observed at the end of the test. Consequently, the total removal efficiency decreased gradually from about 85% to 70% (h 90). The sudden increase in effluent solids concentration at h 66 may be due to the partial damage of the membrane, resulting from increase in cross-fluxes induced by HRT reduction. Anyway, total effluent COD kept lower than 250 mg L^{-1} throughout the whole test.

During the first half of day 1, total and biological COD removal efficiency were nearly coincident, as effluent solids concentration was lower than 40 mg L^{-1}. Biological COD removal increased then slightly until h 66, when a decrease occurred because of biomass losses from the membrane. By the way, during the test, the biological COD removal kept quite constant and ranged marginally around 90%. Values as high as 95% were reached. Good filterable COD removal was also enhanced by the recirculation of organics from the cake layer into the reactor through the flow crossing the external membrane support. Solids also were brought again into the reactor. Figure 3.22 compares in a visual way the feed in the reactor at the beginning of the test and the solution analyzed at the end. Anyway, MLTSS kept lower than 0.5 mg L^{-1} even at the end of the test.

Figure 3.22: Feed in the reactor at the beginning of the short-term test and the solution at the end.

On overall, about 400 mL of biogas were released by the system during the test, having methane content of 82% on average. The higher contribution (62%) was given by the biogas flowing out the effluent collection vessel. This datum stresses the importance of dissolved methane in the liquid phase. Quite surprisingly, the biogas escaping from the membrane support was only 8% of the total production. Moreover, gas emission in the membrane was observed during the first two days, only. About one third of the gas was released from the reactor. As the MLVSS was nearly zero even at the end of the test, it is not likely that this biogas had been actually produced within

the reactor. Likely, this biogas was the result of biochemical organic matter oxidation occurring in or within the cake layer. The configuration of the membrane support did not promote liquid-to-gas mass transfer, so that biogas was transported through the recirculation system into the reactor, where liquid-to-gas transfer was more likely to occur. This consideration can explain why the biogas production of the system did not drop during the main experiment even when MLVVS near zero were detached and proves again that the dynamic membrane was biologically active in organic matter oxidation.

3.9 Dynamic Membrane development

The membrane support was opened on day 17 to evaluate clogging. After that, the support was opened again only after the system shutdown. By comparing the conditions of the cake layer detached on both days, it is possible to assess how the dynamic membrane developed with time. Figure 3.23 reports the pictures of the membrane taken on day 17 and after the shutdown, respectively. Figure 3.24 reports the membrane support after the shutdown.

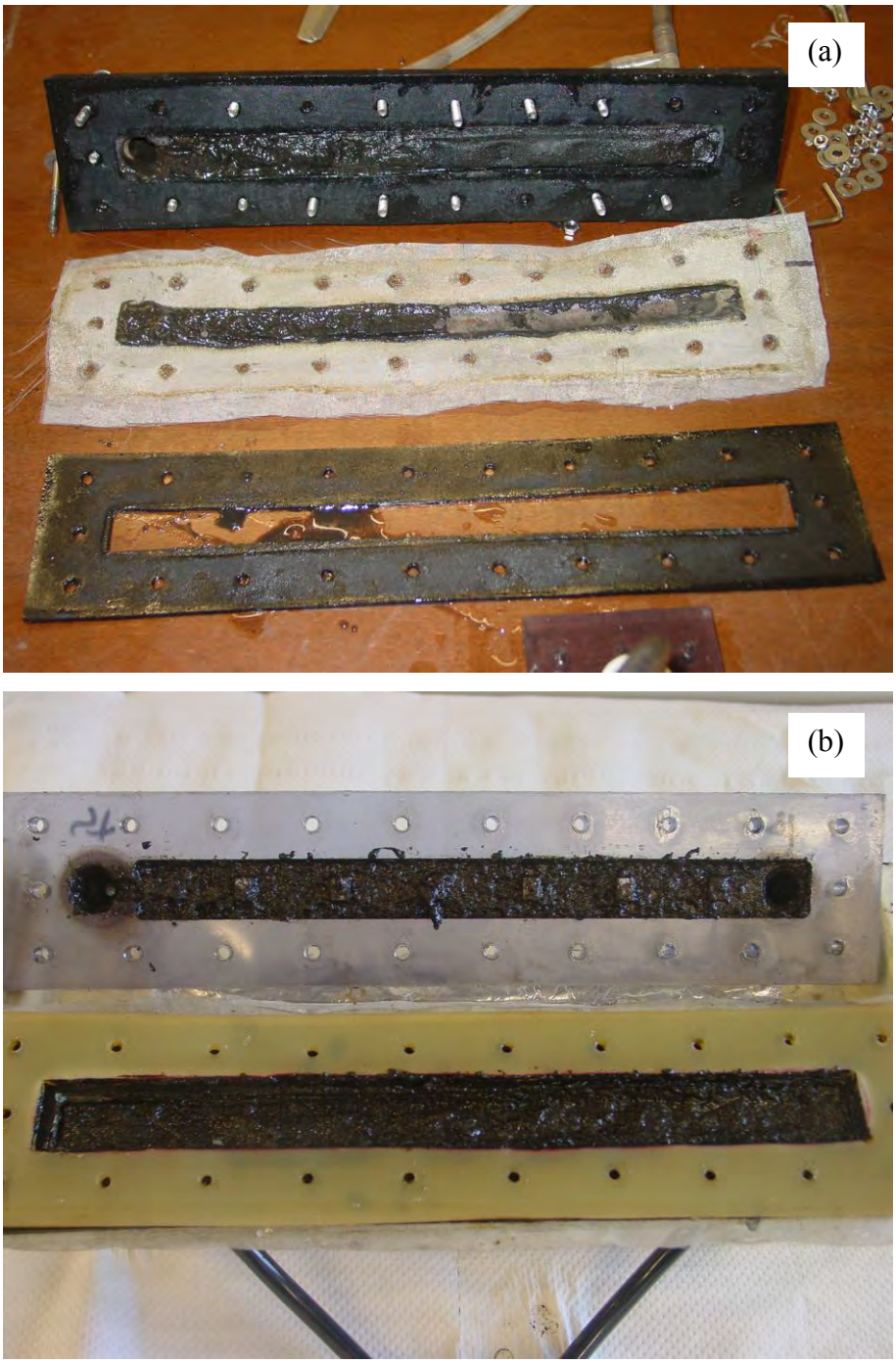

Figure 3.23: Membrane on day 17 (a) and after the shutdown (b).

Figure 3.24: Membran esupport after the shutdown. The mesh and the lower side of the device can be seen properly.

On day 17 the support resulted partially clogged by a dense sludge layer attached onto the upper surface of the support. This layer was removed and sludge characterization was performed. The material had humidity content of 82% and VS/TS of nearly 45%. On the whole, the cake layer was not homogeneous. Because of clogging, only the part of the mesh near the sludge inlet point was actually crossed by liquid flux. The analysis performed after the experiment revealed higher water content (98%). The VS/TS value rose up to 75%. This relevant increase in volatile solids content may be due to growth of biomass on the mesh. Another possibility would be the major propensity of volatile solids to attach onto the mesh with respect to non-volatile solids. Anyway, the VSS/TSS in the mixed liquor showed usually an increasing pattern during the experiment, as reported in Paragraph 3.3. Consequently, the last hypothesis seems less plausible.

The membrane appeared homogeneously subjected to water liquid flux and it was few millimeters thick. Sludge particles were attached on to the lower part of the mesh, so that it may be assumed that also the lower part of the membrane support was biologically active. This result is in accordance with the presence of biogas down-flow the membrane from day 92 to day 111. The sludge attached on the mesh was scoured off for solids characterization. Anyway, some particles stickily attached on the support could not be removed, forming an irreversible biological layer which is likely to enhance the development of a new cake layer after damages to the membrane (i.e. scouring).

4. Conclusions

An AnDMBR system equipped with a coarse filtration mesh (200 μm) was successfully applied for the treatment of synthetic wastewater at ambient temperature. The characteristics of the inlet had been chosen to simulate low-strength municipal wastewater. Particular attention was paid to the COD removal capacity of the dynamic membrane itself.

As mesophilic sludge was used for the inoculum, a long time was needed for biomass to acclimate at lower temperature. In fact, stable conditions were reached in two months. As a result, working parameters (mainly temperature) turned out to greatly influence sludge behavior and, thus,dynamic membrane formation. Anyway, the results demonstrated that homogeneous dynamic membrane build-up can be achieved even on a large-porous mesh under psychrophilic conditions.

HRT turned out to be a fundamental parameter in optimizing system efficiency. Best results in term of COD removal and solids retention were achieved under HRTs of 2 d and 1 d, with OLR ranging from 0.4 and 0.9 $kgCODm^{-3}d^{-1}$. Filterable and soluble COD removal was usually higher than 80 % (with peack almost of 100%). Therefore, at HRT of 2 and 1 d the effluent characteristics met the standards imposed by the current Italian legislation in term of total COD. However, effluent COD concentration increased when the HRT was decreased to 0.5 d. Nevertheless, filterable and soluble COD were kept under the discharge limit at least up to the applied HRT of 6 h when effluent COD concentration build-up was observed. However, it has to the highlighted that the COD removal was still maintained higher than that reported for other anaerobic technologies (e.g. UASB, Chong *et al*., 2012) applied at ambient temperature.

The analysis of fluxes and TMP applied at different HRTs proved the good sludge filterability achievable by means of course-porous support mesh. By applying pressure values lower than 50 mbar (i.e. one order of magnitude lower than those normally reported in conventional MBRs) fluxes of 15 $Lm^{-2}h^{-1}$ were achieved. These fluxes may have therefore practical engineering applications (Ersahin *et al*., 2012). Therefore, the successful build-up of a cake layer onto a course-porous support mesh allowed us to obtain high membrane fluxes applying low pressure.

Analysis on filterable mixed liquor and effluent COD proved that biochemical organic matter oxidation occurred in the cake layer attached on the support mesh. Cake layer contribution accounted for even 20% of total filterable COD removal. Similar results were obtained in the short-term test performed at the end of the experiment, in which removal capability up to 95% was achieved by means of the dynamic membrane, only. These findings are consistent with the observation that biogas production did not drop when MLVSS was nearly zero. Therefore, when

working at psychrophilic conditions, dynamic membrane technology may help in compensating the reduction in COD removal induced by slower microbial kinetic.

HRT also significantly affected both production and methane content. After biomass acclimation, reduction in HRT induced an enhancement in biogas production, even though significant fluctuations were observed. As CH_4 is less soluble than CO_2, methane content in biogas increased at decreasing HRT. Peaks higher than 90% in CH_4 content were achieved. Methane-rich biogas may be obtained in AnDMBRs run under psychrophilic conditions. The short-term experiment proved that methanogenic activity within the cake layer could not be neglected.

Despite the high COD removal, the overall biogas production observed was rather low (30% of the theoretical value at working temperature). The methane dissolved in the liquid phase was therefore likely to be considerably higher than the value calculated under thermodynamic equilibrium conditions. Since dissolved methane in the effluent was not analyzed in the present work, proper investigations should be performed to assess effective liquid-to-gas mass transfer processes.

Even though outstanding filterable COD capability was observed, the effluent characteristics did not meet the limits of nitrogen concentration for discharge into superficial water body. Post-treatments should, therefore, be applied. Different technologies have been already applied to UASBs to improve the overall system efficiency in order to meet local discharge limits (Chong *et al*., 2012) for nutrients.

The investigated treatment system is likely to require less starting and management expenditure with respect to conventional MBRs. Costs reduction results from lower energy consumption, as aeration is not provided and the use of large-porous mesh allows achieving relatively high fluxes at lower pressure. Cheap materials can be used as support for dynamic membrane development.

Moreover, since methane can be recovered, the proposed treatment system can work with much lower energy consumption if compared with conventional wastewater treatment plants.

The use of mesh filters in order to support the development of a biofilm, which acts as a dynamic membrane, could represent a very promising technology for anaerobic wastewater treatment under psychrophilic conditions achieving high efficiency of pollutant removal with low operating costs.

The results of this study also suggest that specific mesh support should be used according to the applied operating conditions.

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