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APPLICATION OF DYNAMIC MEMBRANES IN WASTEWATER TREATMENT

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MUBBSHIR SALEEM

I dedicate this endeavour to my mother Momina Saleem, late father M. Saleem Ullah, and to my family. This effort is a part of my struggle to ameliorate the sufferings of millions around the globe affected by contaminated water

There is nothing for man except what he strives for'

Quran (53;39)

TABLE OF CONTENTS

S N	LIST OF TABLES	Page No

S N	LIST OF FIGURES	Page No

CHAPTER 1

INTRODUCTION

1 Introduction

1.1 Wastewater generation, estimates and impacts: The UN report 2017

The 2017 United Nation World Water Development Report emphasized on improved wastewater management while considering it inevitable for achieving sustainable water management. The report delineates the severity of the situation arising from indiscriminate discharge of municipal and industrial effluents in the natural environment. Some of the important points are summarized below, demonstrating the importance of this research in a much border humanitarian context.

The report presented the estimate that approximately two thirds of world's population is facing water scarcity at least one month in a year. On the other, availability of water is inherently connected to its quality which is further associated with the degree of contamination. Although access to safe drinking water is considered as a birth right of every human being however, it is a distant dream for most of world's population, causing millions of deaths annually. Unfortunately 842,000 deaths were recorded in the year 2012 only in the middle to low-income countries because of consuming contaminated water or due to inadequate sanitation and hygiene. An estimated 3,928 km^3 per year of fresh water is drawn globally. A major share of this water, around 44% (1,716 km^3 per year) is consumed only by agriculture while the remaining 56% (2,212 km^3 per year) is discharged into the environment as wastewater consisting of mainly industrial and municipal effluents.

Wastewater roughly contains only 1% of contaminants including, suspended, colloidal and dissolved solids however, 99% is still water. The treatment and removal of fewer 1% contaminations from a wastewater stream is generally considered expensive and capital intensive especially for centralized treatment systems. This is the reason why the ratio between wastewater generated and treated by a country is highly dependent on country's economy. As a matter of fact, high-income countries treat almost 70% of the municipal and industrial wastewater they produce followed by 38% in upper middle-income countries, 28% in lower middle-income countries and only 8% undergoes any kind of treatment in low-income countries. Similarly, estimates also indicate that globally over 80% of all the wastewater generated is discharged into the environment without receiving any treatment. This indiscriminate discharge of wastewater further deteriorates water quality, aggravating water scarcity and increase threat to human health, its socioeconomic development and the environment.

For the same reasons wastewater treatment and disposal, is not only desirable, but also indispensable. Nowadays the paradigm of wastewater treatment demands sustainability, there is a

dire need to develop technologies that can offer affordable, yet efficient wastewater treatment options that are comparable to modern day advance treatment technologies.

1.2 Problem Statement

Wastewater is a multifaceted resource that is full of a wide variety of contaminants, including pathogens (disease organisms), nutrients (nitrogen and phosphorus), solids, chemicals and even hazardous substances. These contaminants should receive a certain amount of physico-chemically and/or biological treatment (using microorganisms) that involves the breakdown of complex organic and inorganic compounds into simpler one that are stable and nuisance-free and poses no danger to human and environment. Talking about providing low cost treatment options, biological treatment is the brightest candidate that utilizes ubiquitous microorganisms present in the waste or augmented from an external source (e.g. inoculum from a similar treatment plant). These microorganisms use an enzyme mediated biological reaction that breaks down and converts complex organic and inorganic substances into nontoxic forms.

The retention of these microorganisms inside the bioreactor is an essential factor for efficient biological treatment. Retention of microscale bacteria or bacteria flocks is challenging because of their very small size due to which they tend to flow with the effluent if suitable solid-liquid separation mechanism is not provided. Moreover, some species of bacteria have a very slow growth rate (e.g. anaerobic, nitrifiers and Anammox etc.) and thus their retention inside the bioreactor becomes even more challenging. Similarly, the retention of metabolic products produced as a result of biological activity is essential because they play a key role in the breakdown of complex substrate and considered as the rate limiting step in determining the overall efficiency of the biological treatment system (Stuckey, 2012). These aspects highlight the importance of the development of reactor technologies allowing long retention times of biomass and solids (sludge retention time - SRT) decoupled by the hydraulic retention time (HRT) of the bioreactors.

In the wake of advancement in biological wastewater treatment technologies the role of membranes in developing robust, efficient and flexible wastewater treatment system is remarkable. Membranes in biological reactors not only obviate the chance of biomass washout through effective solid-liquid separation, producing a superior quality effluent, at the same time they ensure successful retention of biomass, a prerequisite for efficient bioreactor operation, working at very low HRT, without the need of separate clarifier unit (Judd., 2006, 2010). The latter can also be translated in terms of considerable area saving which makes MBR systems an attractive treatment technology for the areas where the cost of the land is very high (Stuckey., 2012: Zhang et al., 2010).

Despite all these advantages that MBR technology has to offer, its widespread application is still limited by membrane fouling which, by decreasing the permeability, increases the capital investment and management cost (Wang et al., 2013). Thus, MBR performance is directly related to membrane fouling and hence, the economic viability of MBR depends mainly on effective fouling control with modest energy input for steady and prolonged MBR operation.

Three major factors determine the rate of membrane fouling in MBR systems including the nature of the feed, membrane characteristics (membrane material, its pore size and nature of its surface, etc.) and the hydrodynamic conditions (crossflow velocity air sparging) inside the reactor. Characteristics of the feed (COD/BOD, C:N:P ratio, fluctuations in flow) are in some way not controllable however, all the other factors can be monitored by adopting efficient design and operational strategies. One such strategy is to operate the system below a certain flux threshold called Critical flux which is defined as the flux below which reversible fouling takes place and above which the relation between TMP and flux becomes nonlinear. Similarly, in order to reduce costs, there is a trade-off between capitals (flux-related) and operating costs (energy and chemicals). Generally lower fluxes are applied, well below the critical flux, so a biomass cake over the membrane surface is avoided, if possible. However, this strategy demands more membrane specific area (m^2 m^{-3}) per unit reactor volume and incurs an additional capital cost. Moreover, membrane fouling is inevitable, even if working below critical flux and due to the same reason transmembrane pressure (TMP) rises over time. Therefore, working below the critical flux only allows a longer operation time for membrane use before the occurrence of severe fouling that ultimately require chemical cleaning or in some cases complete membrane replacement (Meng et al, 2009).

1.3 The idea of dynamic membrane (DM)

A new perspective for a possible evolution of MBR systems is the advent of dynamic membrane (DM) technology. The basic idea behind the filtration effect of a dynamically formed membrane (also known as a secondary membrane) is the formation of cake layer over an underlying support material such as filter clothes and meshes that have been used to develop DM (Alepu et al., 2016; Ersahin et al., 2013; Saleem et al., 2017). The technology is low-cost with comparable solids retention and efficient treatment performance as that of conventional MBR systems (Ersahin et al., 2016, 2012; Hu et al., 2016; Rezvani et al., 2014). Since DM is a purpose-built fouling layer (cake layer) and the rejection of solids is carried out by a regenerative cake layer, its permeability can be controlled by controlling its thickness (Jeison et al., 2008: Zhang et al., 2010). DM layer is made up of mixture of colloidal matter and biomass flocs (Liu et al., 2012; Wang et al., 2011; Zhang et al.,

2014) that is used as a mean of solid-liquid separation medium instead of conventional membranes (Ersahin et al., 2016; Salerno et al., 2017; Xiong et al., 2016).

Mechanism for the formation of DM is governed by several parameters including the concentration of the foulants (EPS and SMP), MLSS content in the bioreactor, filtration flux, TMP and hydrodynamic conditions during the formation stage (Xiong et al. 2016).

As far as the structure of DM is concerned, there is no consensus in the reported literature. Hwang and Hsueh (2003) reported the existence of layer stratification. They pointed the formation of DM begins with the formation of a gel layer, 10-20% of the total DM thickness, yet contributing to about 90% of the total filtration resistance. Zhang et al, (2014) have reported a size-stratified DM structure due to their different interaction with the hydraulic conditions governing DM formation. A different DM structure was also proposed by Liu et al, (2009) based on a three-layer scheme. The stratification from inner to outer consist of the following layers

1. A substrate layer formed by large particles (0.1 mm diameter);
2. A Separation layer (sludge particles arranged with decreasing size from inner to outer) with a pore size comparable to micro-filtration range;
3. A final fouling layer made of large sludge particles, solutes and colloids.

DM technology offers many advantages, including reproducibility, low maintenance, and high flux (J) operation at low transmembrane pressures (TMP) with lower energy consumption as compared to conventional membranes

Since DM are formed over cheaper underlying support material they provide direct cost savings over capital investment, at least one order of magnitude lower than that of conventional MBRs (Zhang et al. 2014). Similarly, their high flux operation (30 – 50 LMH in DM as opposed to an average 10 – 25 LMH in MBRs) at lower TMPs in comparison to conventional MBRs plus chemical free (air or water backwash, air or biogas spargning etc.) less intense cleaning requirements further increases process economy by reducing the plant footprint and operational cost (Zhang et al. 2014) (Ersahin et al. 2012). The concept of dynamic membrane and its application to treat different kind of wastewater is still in its early stages and there are important process parameters and bottlenecks that require complete understanding before its full-scale application for different wastewater streams.

Most of the studies assessed the performance of DM on lab-scale or pilot-scale for the treatment of synthetic wastewater (Alibardi et al., 2016, 2014; Ersahin et al., 2016; Saleem et al., 2016), and municipal wastewater (Hu et al., 2016; Liu et al., 2009; Y. Xiong et al., 2016; Zhang et al., 2010). A

limited number of studies have been performed on high strength, complex wastewater such as landfill leachate (Dong et al., 2007; Xie et al., 2014), textile industry effluents (Sahinkaya et al., 2017) and excess sludge digestion (Liu et al., 2016; Yu et al., 2016).

1.4 Aims and objectives of the study

The thesis was aimed at investigating the formation mechanism and performance of DM in biological wastewater treatment using diverse wastewater streams under aerobic and anaerobic conditions. For this purpose polyamide nylon mesh of porosities 10, 21, 52, 85, 135 and 200 µm were used as underlying support material. The behaviour of DM formed over these meshes was investigated for their solid-liquid separation performance while treating different wastewater streams including

1. Synthetic wastewater under anaerobic conditions for methane production
2. Leachate treatment under aerobic and anoxic conditions for total nitrogen removal
3. Synthetic wastewater under anaerobic conditions for H_2 production
4. Synthetic wastewater under anaerobic conditions for the enrichment of Anammox bacteria

The aforementioned research activities were undertaken due to the lack of data on the application of DM under these conditions. The experiments on leachate treatment were performed under ambient temperature conditions while the rest of the experimentation under anaerobic conditions was performed at mesophilic temperature conditions. Similarly, submerged and side-stream configurations were used under aerobic and anaerobic conditions respectively. Physical and biological performance of DM was evaluated in connection to the changes in feed wastewater characteristics and operating conditions. The aims of this study were met by setting out a clear design strategy and achieving the following objectives:

1. To present a strategy to expedite the formation of DM and reducing the biomass loss during the formation stage by reducing the time of DM formation.
2. To discuss the effect of mesh pore size on DM formation and performance under different operating conditions, type and concentration of feed wastewater, applied filtration mode (constant flux or constant TMP) and bioprocess used (aerobic and anaerobic).
3. To propose and evaluate an effective cleaning mechanism and strategy for cleaning excessively fouled DM layer effectively.
4. To study the effect of change in the type of influent feed wastewater on the filtration behaviour of the sludge and response of DM's solid-liquid separation efficiency
5. To study the role of DM in solid retention (retention of slow growing microorganisms) under aforementioned conditions and its effect on biological performance of the bioreactor.

6. To clearly state the shortcomings of DM technology and identify the areas that requires further investigation for future research.

1.5 Thesis outline

In order to meet aforementioned aims and objectives 35 months of continuous lab-scale experimentation was performed and the results obtained from these experiments are organized and discussed in eight chapters presented in this thesis. The outline of the thesis is structured as follows:

Chapter 2 is focused on assessing the effect of applied filtration flux on the rate of development of DM in an anaerobic dynamic membrane bioreactor (ADMBR). The study also presented a discussion on the bioreactor's performances resulting from different applied flux, HRTs and organic loading rates (OLRs) observed in two separate experimental runs with similar experimental arrangements.

Chapter 3 evaluates the main mechanisms governing DM formation and to discuss the effects of variation of operating parameters including TMP, mesh porosity and MLSS concentration on DM development and performance. Gravity driven filtration tests were conducted under a set of diverse operating conditions in a specifically designed filtration apparatus using anaerobic sludge. Hermia's blocking models were then fitted with the observed data to predict the most probable fouling mechanism occurring in DM filtration.

Chapter 4 evaluates the formation and performance of DMs for treating old landfill leachate characterised by high influent ammonia concentration and low organic content. The study was performed in a pre-anoxic and post-aerobic bioreactor configuration and the effect of the use of different mesh porosities on the development and performance of DM was evaluated. The study also evaluates the behaviour of developed DM in conjunction with the effect of change in feed characteristics and operating conditions.

Chapter 5 also focuses on the application of DM in landfill leachate treatment like the previous study while the main difference was the change in operating strategy to achieve stable bioreactor operation and the size (dimensions) of the bioreactors used. The main focus of the study was to reaffirm the observations of the previous experiment (Chapter 4) regarding the effect of change is DM filtration performance due to the change in the influent feed wastewater plus to achieve a stable biological nitrogen removal performance comparable to conventional MBR systems. Furthermore, this study also provides a comparative performance valuation of the system investigated in this study with the results reported in literature for conventional MBR systems treating LFL.

Chapter 6 focusses on synergizing the benefits of biological H_2 production and DM technology. Filtration behaviour and performance of DM formed over different mesh porosities was evaluated. The study was performed at mesophilic temperature conditions using an untreated mixed bacterial culture under high OLR. The study also highlighted the change in the filtration behaviour of DM and its relation with the influent organics (sucrose) concentration and operating parameters. Furthermore, an innovative manually operated pneumatic internal cleaning mechanism was also tested for the periodic cleaning of excessively fouled dynamic membrane simultaneously in combination with backwashing.

Chapter 7 evaluates the ability of DM coupled anaerobic bioreactor for the enrichment of slow growing Anammox bacteria from a mixed culture (aerobic and anaerobic). The study was performed over a period of 456 days and Anammox bacteria enrichment was assessed by evaluating the progress of total nitrogen removal performance of the system. Furthermore, this study also proposed a pneumatically operated internal cleaning mechanism for cleaning excessively fouled DM while maintaining the anaerobic environment inside the bioreactor.

Chapter 8 provides a thorough discussion on most important results of the performed research activities. This chapter summarizes the overall conclusions to better understand the formation and behaviour of DM formed in successive studies while treating different waste streams. Furthermore, the chapter also highlighted the limitations of DM technology and in return presented few recommendations for delineating future research directions for the successful scaling-up of DM technology.

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CHAPTER 2

EFFECT OF FILTRATION FLUX ON THE DEVELOPMENT AND OPERATION OF A DYNAMIC MEMBRANE FOR ANAEROBIC WASTEWATER TREATMENT

Abstract

Dynamic membrane represents a cost effective alternative to conventional membranes by employing fouling as a means of solid-liquid separation. This study evaluated the effects of initial flux on both development rate of dynamic membrane and bioreactor performance during two consecutive experiments. The dynamic membrane was developed over a 200µm mesh and the reactor was operated under anaerobic conditions. It was found that the effect of an initial higher applied flux on dynamic membrane development was more pronounced than mixed liquor suspended solid concentration inside the bioreactor. The development of the dynamic membrane was therefore positively associated with the applied flux. The rapid development of the dynamic membrane during the second experimental run at high initial fluxes and lower MLSS concentrations also affected the performance of the bioreactor in terms of more efficient COD removal and biogas production. A major shortcoming of applying higher initial applied flux was the formation of a denser and robust dynamic membrane layer that was resistant to applied hydraulic shear to control desired permeability and thus represented an obstacle in maintaining a long-term operation with sustainable flux at lower transmembrane pressure (TMP).

This chapter is based on:

Saleem, M., Alibardi, L., Lavagnolo, M.C., Cossu, R., Spagni, A., 2016. Effect of filtration flux on the development and operation of a dynamic membrane for anaerobic wastewater treatment. Journal of Environmental Management, 180: 469-465.

2 Effect of filtration flu on the development and operation of dynamic membrane for anaerobic wastewater treatment

2.1 Introduction

Membrane bioreactors (MBRs) are nowadays extensively applied for the treatment of municipal and industrial wastewater since they allow for rapid start-up, small footprint, less sludge production and improved effluent quality if compared with conventional activated sludge processes (Pretel *et al.*, 2015; Gabarrón *et al.*, 2014; Ferraris *et al.*, 2009; Judd, 2010). Although MBRs have mostly been applied for aerobic processes, their application in anaerobic treatments represents the ideal combination of membrane filtration and biological process. The very efficient solid-liquid separation of membranes enables in fact a complete decoupling of solids retention time (SRT) from hydraulic retention time (HRT). Anaerobic MBRs could therefore be characterised by short HRTs but long SRTs and high concentrations of bacteria inside bioreactors, the latters being key factors for efficient anaerobic treatments due to the low growth rates and yields of anaerobic microorganisms (Smith *et al.*, 2012). Membrane fouling decreases permeate fluxes and are considered the most significant drawback in the application of MBR technologies for wastewater treatment (Judd, 2010). Different results have been reported in different studies on fouling propensities using aerobic and anaerobic sludge filtration through conventional membranes under different operating conditions in conventional MBRs. For instance, Yurtsever *et al*, (2015) and Spagni *et al*, (2010) have reported severe fouling during anaerobic MBR operation as compared to aerobic MBR operation). On the contrary, Xiong et al, (2016) observed lower fouling propensity in anaerobic MBR than aerobic MBR treating municipal sewage. Similarly, release of biofoulants due to biomass activity under different operating conditions affects fouling in both aerobic and anaerobic MBR systems (Robles et al., 2012). Therefore, different solutions attempting to reduce membrane fouling and improving aerobic and anaerobic sludge filterability have been evaluated (Trzcinski and Stuckey, 2016; Wong *et al.*, 2015; Yang *et al.*, 2012).

In this view, application of dynamic membrane technology in biological treatments can offer benefits over traditional membranes by precluding the need for frequent replacement of costly membrane modules, improving membrane fluxes and reducing the energy consumption (Alibardi *et al.*, 2014; Ersahin *et al.*, 2012). A dynamic membrane (DM) is formed by the deposition of suspended solids, colloids and microbial cell particles over an underlying support material which can be of different nature and characteristics (Loderer *et al.*, 2013; Ersahin *et al.*, 2012; Li *et al.*, 2011). While fouling represents an important drawback for conventional membrane filtration, in the

innovative approach of DM filtration it is purposefully exploited to create a low-cost, regenerative, self-forming filtration surface (Alibardi *et al.*, 2014; Ersahin *et al.*, 2014; Zhang *et al.*, 2014).

Solids rejection of DMs is not comparable to microfiltration (MF) and ultrafiltration (UF) membranes due to the very different cut-off (Alibardi *et al.*, 2014). Nevertheless, DM could represent a compromise between solids removal and plant costs; such a compromise appears even more significant in anaerobic plants since post-treatment is usually considered (e.g. nutrient removal or recovery) prior to final water discharge (Puchongkawarin *et al.*, 2015; Sánchez-Ramírez *et al.*, 2015; Zhang *et al.*, 2014).

DM filtration has initially been studied for aerobic wastewater treatment systems (Wang *et al.*, 2013; Ren *et al.*, 2010; Fan *et al.*, 2002; Kiso *et al.*, 2000). Nevertheless, owing to the benefits offered by anaerobic process, recent studies mainly focused on exploiting DMs under anaerobic conditions (Alibardi *et al.*, 2016; Ersahin *et al.*, 2014; Zhang *et al.*, 2010).

Meshes are indicated as interesting underlying support materials in DM filtration to curtail capital and management costs of MBRs (Alibardi *et al.*, 2014; Loderer *et al.*, 2013; Jeison *et al.*, 2008). Recent studies reported that DM formation evolves from phases characterised by cake layers loosely bounded to the support materials, to phases where thick, stable and robust biofilms are formed (Alibardi *et al.*, 2014; Zhang *et al.*, 2010; Alavi Moghaddam *et al.*, 2002). However, DM formation process is greatly affected by the different materials, pore sizes and structures of meshes (Zhang *et al.*, 2014; Ersahin *et al.*, 2013) and to the best of Authors' knowledge operating conditions specifically affecting DM development have not been studied yet.

This study aimed at assessing the development of the DM in an anaerobic dynamic membrane bioreactor (ADMBR) when different filtration fluxes (*J*) were applied. The study also evaluated the reactor performances resulting from different *J*, HRTs and organic loading rates (OLRs).

2.2 Materials and methods

2.2.1 Experimental setup

The research was conducted on a bench-scale ADMBR, coupled with an external cross-flow filtration module (Fig. 2.1). The external configuration was preferred since it can facilitate membrane maintenance operations while preserving anaerobic conditions in reaction tank.

The reactor had a total volume of 898 mL (WxHxD: 9.5x10.5x9 cm) and a working volume of 684mL. A monofilament woven mesh made of polyamide/nylon (SaatiMil PA 7 XXX, Saatis.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 with a total volume of 48 mL (WxHxD: 20x1.2x2 cm) and a filtration area of 40 cm^2 (LxW: 20x2 cm).

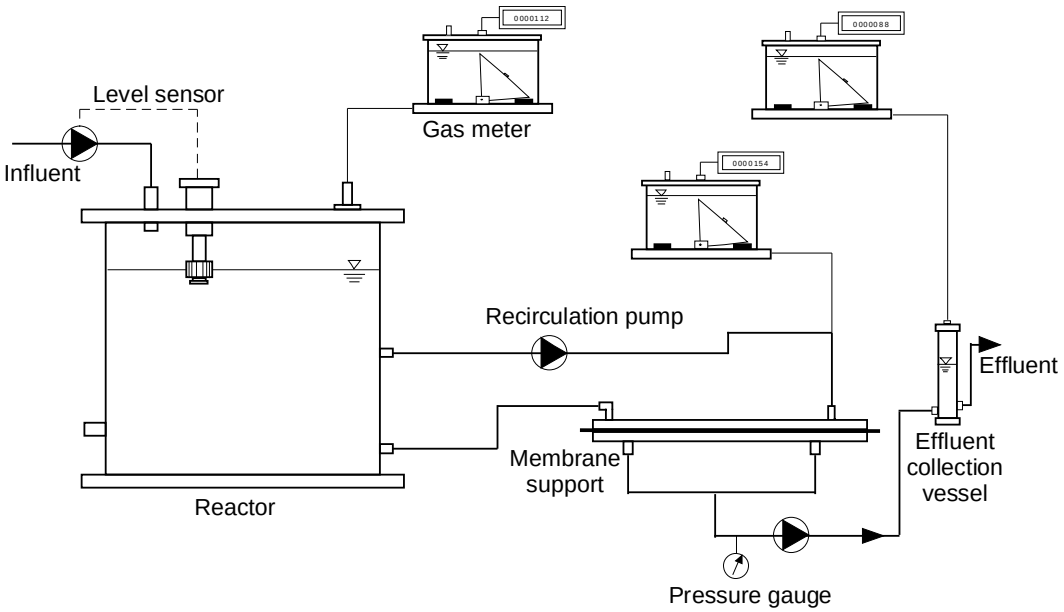

Figure 2.1 Schematic diagram of the experimental setup.

The reactor was fed by using a peristaltic pump (Watson Marlow 401U/D1) controlled by a level sensor in order to maintain a constant working volume in the bioreactor. The cross-flow regime was established in the external module by using a peristaltic pump (Watson Marlow 403U/R1, Falmouth, Cornwall, UK) that continuously circulated mixed liquor along the mesh surface. Permeate extraction was facilitated by means of another peristaltic pump (Watson Marlow 401U/DM3). The permeate passed through a small airtight vessel of approximately 100 mL (Fig. 2.1) in order to account for the presence of oversaturated biogas in the effluent.

TMP was measured by using a U-tube pressure gauge filled with water. Three home-made wet-tip gas meters were used to measure biogas production; they were connected to the experimental system in three different locations, i.e. directly on the anaerobic reactor, on the external cross flow module and on the effluent collection vessel (Fig. 2.1).

Reactor was maintained at mesophilic conditions (35 ± 1°C) by using a thermostatic bath (ISCo. GTR 2000 "11x", Italy). Mixing of sludge in the reactor was carried out by using a magnetic stirrer (Variomag, Thermo Scientific, Italy).

Two consecutive experimental runs were performed during this study, lasting 68 and 27 days, respectively. Details of the start-up for the first experiment are reported elsewhere (Alibardi *et al.*, 2014). The bioreactor was operated in both runs at similar process conditions but different initial fluxes to assess their effects on DM development and on reactor performances. The filtration area

was kept constant during the entire study therefore, any change in flux on the membrane resulted in a change in HRT on the reactor. Furthermore, for both the experiments it was decided to keep the rise in TMP value up to 20 kPa and this value was set as the upper limit of TMP rise.

During the first run the initial J was set to 1.0 L m^{-2} h^{-1} and then increased up to 7.2 L m^{-2} h^{-1} (mean value of 3.3 L m^{-2} h^{-1}) corresponding to HRTs changing from 6.8 to 1.0 d (mean value of 3.1 d). During the second run, initial J was set to 2.9 L m^{-2} h^{-1} and then increased up to 7.0 L m^{-2} h^{-1} (mean value of 5.1 L m^{-2} h^{-1}) corresponding to HRTs changing from 2.4 to 1.0 d (mean value of 1.4 d).

2.2.2 Synthetic wastewater and inoculum

The reactor was fed with a synthetic wastewater composed of sucrose as carbon source at a concentration of 5 gCOD L^{-1}. To ensure alkalinity, macro and micro nutrients the followings compounds were also added: $NaHCO_3$ (2g g COD^{-1}), NH_4Cl (0.04g N g COD^{-1}), KH_2PO_4 (0.01g P g COD^{-1}), $FeCl_3*6H_2O$ (2.1 mg Fe L^{-1}), $CaCl_2*2H_2O$ (8.2 mg Ca L^{-1}), $MgCl_2*6H_2O$ (2.4 mg Mg L^{-1}), $Na_2MoO_4*2H_2O$ (0.22 mg Mo L^{-1}), $ZnSO_4*7H_2O$ (0.23 mg Zn L^{-1}), $CuSO_4*5H_2O$ (0.128 mg Cu L^{-1}), $NiCl_2*6H_2O$ (0.1 mg Ni L^{-1}), H_3BO_4 (0.007 mg B L^{-1}). These chemicals were dissolved in tap water.

The reactor was inoculated with anaerobic sludge (TS of 13.5g L^{-1} and VS of 7.1g L^{-1}) obtained from a full-scale mesophilic sludge digester treating the excess sludge of a municipal wastewater treatment plant located in Padova, Italy.

2.2.3 Analytical Methods

Chemical oxygen demand (COD), total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), were measured according to Standard Methods (APHA, 2005). Biogas production was measured by home-made wet tip gas meters. 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.

2.3 Results

2.3.1 Bioreactor performances

The operative conditions of J, cross flow velocity (CFV), HRT and OLR are reported in Figure 2.2. The average reactor performances during the two experimental runs are reported in Table 1 while biogas production and methane concentrations are reported in Figure 3.

In the first experimental run, HRT was reduced from 6.8 to 1.0 d (Fig. 2.2) resulting in increasing OLR from 0.7 to 5.0 kg COD m^{-3} d^{-1}. In the second experiment, HRT varied from 2.4 to 1.1 d (Fig.

2.2) corresponding to OLR increasing from 2.0 to 4.5 kg COD m^{-3} d^{-1}. The HRT, and thus OLR, variations did not greatly disturb bioreactor performance in terms of COD removal efficiency and biogas quality. During the first run, the average COD removal efficiency was 75% while in the second run it slightly increased to 80 %. The average methane (CH_4) concentration in the biogas was 67% and 63% for first and second experimental run, respectively.

Although similar performances were achieved in the two runs in terms of COD removal and CH_4 concentration, a significant difference can be seen on mixed liquor (ML) solid concentration and biogas production. MLTSS and MLVSS concentrations were much higher in first experiments than in the second one while biogas production almost doubled during the second run (Table 2.1). Therefore, the second experiment with a lower MLSS concentration seemed to show better specific biomass activity since the reactor was able to reach similar COD removals in the two runs.

Dynamic membrane filtration did not allow a complete TSS and VSS removal. Average effluent TSS concentration resulted of 219 and 268 mg L^{-1} during the first and second run, respectively. A VSS/TSS ratio of about 60% was recorded for the mixed liquor in the bioreactor while the VSS/TSS ratio of effluent increased to 89 and 81% for first and second run, respectively. During both experiments, the reactor was operated at infinite SRT since no sludge was extracted to control the SRT of the system, except for the amount of solids wasted in the effluent or used to carry out analysis.

Table 2.1 Average reactor performances during the two experimental runs

Parameter	Unit	Run I	Run II
MLTSS	g L^{-1}	18.0 ± 4.8	7.0 ± 0.9
MLVSS	g L^{-1}	10.6 ± 2.6	4.3 ± 0.5
ML VSS/TSS	%	59	61
Effluent TSS	mg L^{-1}	219 ± 108	268 ± 35
Effluent VSS	mg L^{-1}	197 ± 103	216 ± 22
Effluent VSS/TSS	%	89	81
TSS removal	%	99	96
Effluent COD	mg L^{-1}	1254 ± 420	990 ± 250
COD removal	%	75 ± 8	80 ± 5
Biogas production	mL L^{-1} d^{-1}	373 ± 252	912 ± 440
CH4 concentration	%	65 ± 13	63 ± 8

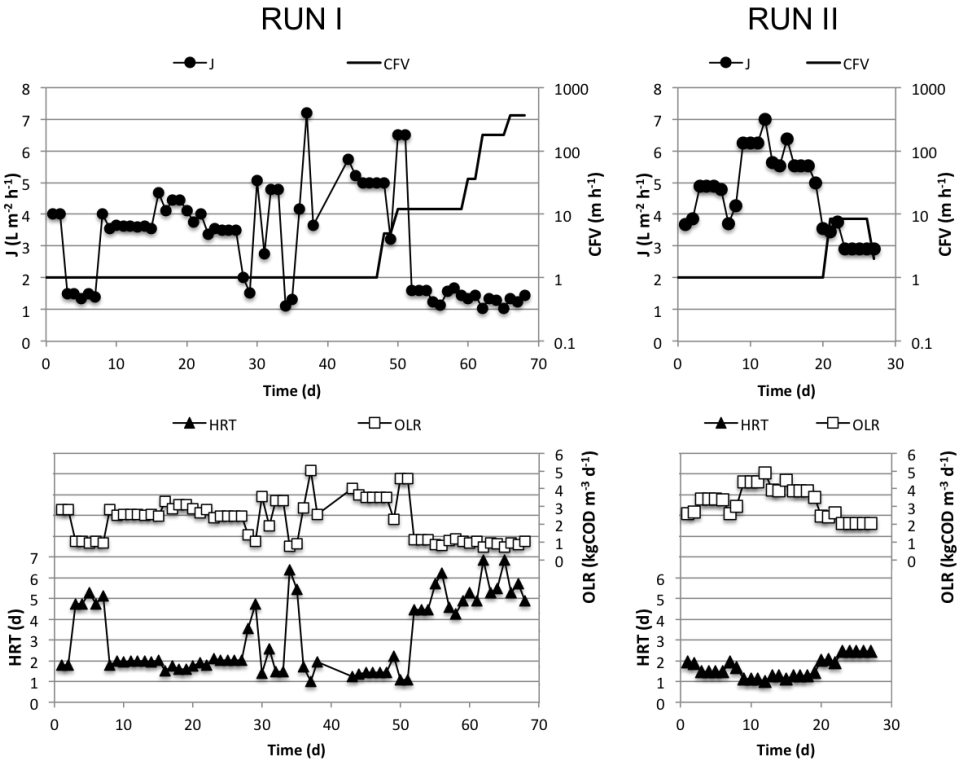

Figure 2.2 Applied membrane flux (J), cross flow velocity (CFV), hydraulic retention time (HRT) and organic loading rate (OLR) during the first (RUN I) and second (RUN II) experiments.

2.3.2 Dynamic membrane development

The operative conditions of *J*, CFV, HRT and OLR applied during the two experimental runs are reported in Figure 2.2. The observed trends of Trans Membrane Pressure (TMP) are presented in Figure 4 while the solid fluxes on the filtration module over time are shown in Figure 2.5.

For both experiments a CFV of 1.0 m h^{-1} was applied in order to allow a gradual build-up of material on the mesh surface and improve DM development maintaining a low hydraulic shear inside the cross-flow module. The value of CVF is lower than those usually applied in conventional MBRs in external configurations (Judd, 2010) and in dynamic MBRs (DMBRs) (Ersahin *et al.*, 2012; Ho and Sung, 2009). This value was chosen also to evaluate an operative condition reducing energy consumption during sludge recirculation for cross flow filtration.

In the first experimental run, with an applied average flux of 3.3 L m^{-2} h^{-1} (Fig. 2.2), the TMP always remained below 1.0 kPa during the first 15 d of operation suggesting a very slow development of the cake layer forming the DM. No considerable rise in the TMP was observed until day 33 of continuous operation; thereafter, TMP gradually increased demonstrating the development of the DM. Although with an up and down trend, TMP reached approximately 20 kPa

in two weeks and then continued at this value with fewer variations for the rest of the run. TMP was not affected by the increased of CFV (up to 360 m h^{-1}) and *J* reduction in the final phase of the run (from day 45 to 68) suggesting the formation of a strong cake layer over the mesh.

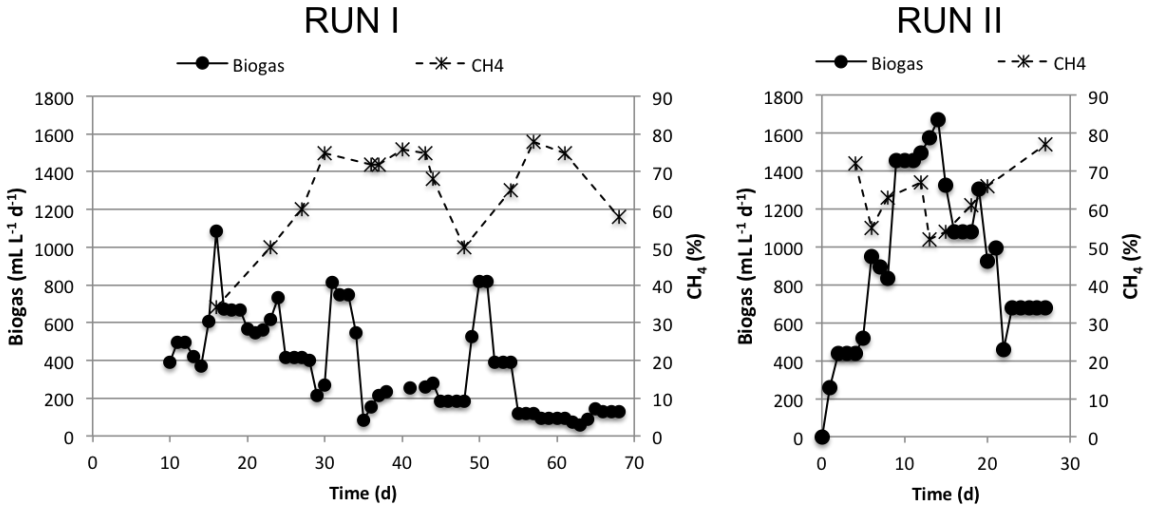

Figure 2.3 Biogas production and methane concentration for first (RUN I) and second (RUN II) experiments.

In the second run, an average *J* of 5.1 L m^{-2} h^{-1}, approximately 50% higher than the first run average value was applied. TMP showed a gradual increase up to 4 kPa in the first 10 d. After this initial phase, TMP increased rapidly reaching 20 kPa in 5 d and then remained stable at this value until the end of the second run even if CFV was increased to 8.5 m h^{-1} and *J* reduced to 3 L m^{-2} h^{-1} suggesting again the formation of a strong cake layer not favourable for sludge filtration.

2.4 Discussion

The similar COD removal achieved in the two runs despite having different biomass concentrations in the reactors could be attributed to the rapid formation of DM at higher applied fluxes which could have aided the formation of a uniform and dens cake layer that helped to maintain a steady bioreactor operation. Recent studies (Alibardi *et al.*, 2016; Smith *et al.*, 2015) have demonstrated that the biofilm composing the cake layer on the membrane is very active and greatly concur in organic removal in MBRs. This biofilm activity, thus, could have supported the similar COD removal observed in this study in spite of different biomass concentration inside the reactor. Similarly, biological performance is greatly affected by the variations in reactor's operating conditions (e.g. OLR and HRT etc.) (Leitão *et al.*, 2006; Torkian *et al.*, 2003) and therefore, lesser variations in the HRT and OLR observed during the second experiment (Fig 2.2) helped to improved biological performance of the system regardless of lower MLSS concentration as compared to first experiment.

Application of DMs does not ensure complete removal of SS in the effluent like conventional membranes (Ersahin *et al.*, 2012). However, the results obtained in this research study indicate that solids removal up to 99 % can be obtained in DMBRs as also other studies have reported (Alibardi *et al.*, 2014; Zhang *et al.*, 2014). The slightly higher TSS concentration during the second experiment, if compared to the first run, could be attributed to the higher applied fluxes. Even under these conditions of high flux, the DM was able to retain more than 96% of SS inside the system under both experimental conditions.

Biogas production in both experiments was quite variable (Fig. 2.3) and fairly dependent on the COD removal efficiency and applied HRT of the bioreactors as observed in the previous study (Alibardi *et al.*, 2014). During the start-up of first experiment, there was no biogas production in the initial phase due to issues with biomass acclimation and therefore, it was decided to increase the HRT from 2 d to 5 d (Fig. 2.2) until biogas production was observed i.e. on day 10. The individual performances of bioreactors in these experiments were quite satisfactory and are comparable to the results reported by other Authors in their studies on ADMBR (Ersahin *et al.*, 2014; Ma *et al.*, 2013; Zhang *et al.*, 2010).

The results obtained from both experiments indicate that DM development is a phased process in which initial TMP behaviour was characterised by a slow and almost linear increase followed by a second phase of rapid increase. This particular behaviour in the initial phase of TMP profiles (Fig. 2.4) ~~behaviour~~ is similar to that proposed by the theory of the local flux for conventional MF and UF MBRs (Cho and Fane, 2002). According to this theory, the first slow TMP increase is caused by a gradual and slow deposition of fouling materials (likely extracellular polymeric substances). The following rapid TMP increase occurs when the membrane permeability does not sustain the applied flux, since it exceeds the critical flux in some regions of the membrane (Cho and Fane, 2002). In fact, the second phase of rapid increase in TMP is the formation phase of DM and after this phase consolidation of already formed DM took place. The TMP evolution observed in this study also confirms the three-stage DM development proposed by (Zhang *et al.*, 2010). During the initial formation stage, particles deposit on the mesh surface with a thickness that is not sufficient to significantly affect the TMP values. Thereafter, a sharp increase in TMP occurs due a rapid deposition of particles and biofilm growth over the already deposited cake layer, causing complete clogging of the pores of the mesh filter. In the final consolidation stage, the deposited cake layer is likely to become thicker and denser (Zhang *et al.*, 2010). However, contrary to previous studies and to the two theories proposed for TMP behaviour in conventional MF/UF MBRs (Cho and Fane, 2002) and in DMBR (Zhang *et al.*, 2010), this study showed a rather stable TMP values at

approximately 20 kPa when the DM seemed well developed (i.e. after 50 and 15 d for the first and second experiment, respectively). It is of note that these pressures were developed and maintained without any cleaning procedures (e.g. chemical cleaning, backwash) typically applied for MF/UF MBRs and also for membrane maintenance in DMBRs (Ma *et al.*, 2013).

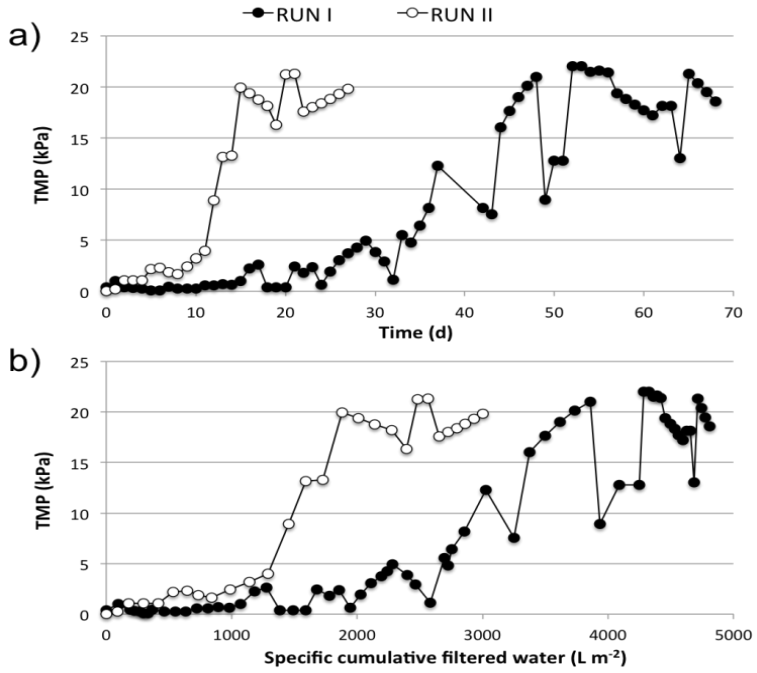

Figure 2.4 Transmembrane pressure (TMP) profiles for the two experimental runs as function of time (a) and of the specific cumulative filtered water (b).

Even though the general shape of both TMP trends resulted similar, their development over time was different with a much faster increase of TMP during the second experiment performed at higher J (Fig. 2.4a). The more rapid development of the DM is also confirmed observing the ~~the~~ evolution of TMP vs the cumulative specific filtered volume (Fig. 2.4b). During the first run a cumulative volume of about 4000 L m^{-2} was filtered before reaching a TMP of 20 kPa while during the second run only 2000 L m^{-2} were filtered before reaching the same TMP value.

Since solids deposition on to the filtration surface is affected by their transport over that surface (in this case a nylon mesh) (Wang and Song, 1999), it could be speculated that the higher flux produced a higher solid load (i.e. J * TSS concentrations) and thus a faster DM development and increase of TMP was observed. On the contrary, the lower TSS concentration in the reactor measured during the second experimental run (Table 2.1) produced lower specific solid loads if compare to those measured during the first experimental run (Fig. 2.5). Therefore, despite the lower TSS concentration, the higher *J* applied during the second run significantly increased the solids flux over the mesh surface during the initial phase and thus produced a faster development of DM that

was thicker and more resistant to filtration (Fig. 2.5). This effect was facilitated by the low CFV applied during the two runs. A low CFV can in fact facilitate the deposition of solids over the filtration mesh (Liu et al., 2009). During the first run at lower fluxes, the equilibrium between particles deposited on the membrane surface and those removed due to shear generated by the CFV produced a cake layer that sustained a prolonged filtration condition at low resistance. On the contrary, during the second run with higher applied fluxes (greater hydrodynamic forces on the particle), this equilibrium shifted more towards particle deposition as compared to removal that stimulated a faster formation of a thick fouling layer characterized by a rapid increase in TMP (Chang *et al.*, 2007).

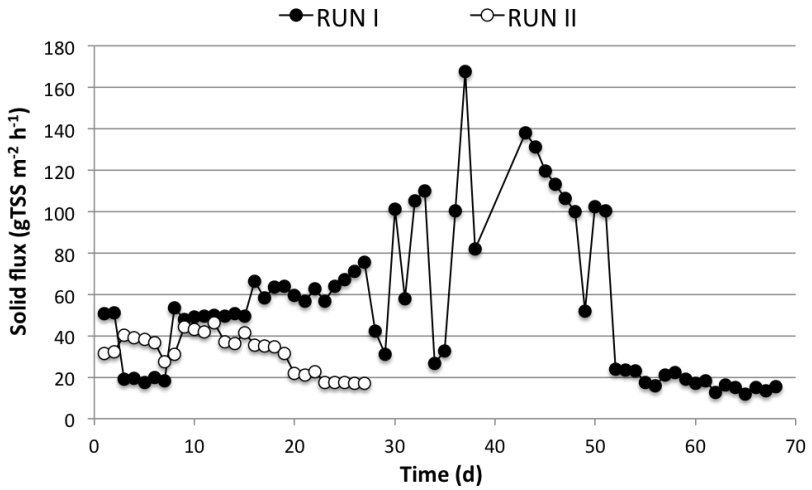

Figure 2.5 Solid flux for the two experimental runs as function of time.

The results therefore indicate that for the development and management of a DM in cross flow configuration, filtration fluxes and CFVs need to be balanced in order to produce equilibrium between the driving forces stimulating the compaction of the cake layer (filtration flux) and the forces producing suspension and removal (CFV).

The results of this study also suggest that the applied fluxes play a more significant role as compared to the MLSS concentration in the development of the DM layer. Even though, high MLSS concentration might have increased the number of particles depositing on the mesh surface in the first experiment, DM developed faster in the second run when higher *J* was applied. Similar results were reported by Chang *et al.* (2007), while operating an MBR coupled with nonwoven fabric. They reported that the effect of high MLSS concentration was reflected only above a certain specific flux and it was insignificant below that value. However, it cannot be concluded that formation of the DM is independent of MLSS concentration in the bioreactor and only depends upon the flux passing through the mesh surface on the basis of limited data obtained in this study

and hence, the effect of these parameters require further experimental investigations. Studies have shown that fouling behaviour is directly linked with sludge characteristics in MBR systems applying conventional membranes (Lousada-Ferreira *et al.*, 2015; Sabia *et al.*, 2013; Meng *et al.*, 2009). Similarly, long term operation of MBR systems applying DMs at higher MLSS concentration has indicated the reduction of the filtration time and more severe fouling at higher filtration fluxes (Zhang *et al.*, 2014; Chang *et al.*, 2007). Therefore, the role of MLSS concentration is significant in achieving a long term sustainable flux and cannot be evaluated independently without considering other associated operational parameters like applied fluxes.

Once the DM membrane reached a consolidated fouling condition, the increase of the CFV affected very slightly the TMP trend. For example, the CFV increase from 1 m h^{-1} to 5 m h^{-1} performed on day 48 caused a slight improvement of the TMP. However, TMP returned to values observed before the CFV increase in four days, supporting the scarce effect of the CFV on the TMP trend (at least under the applied operating conditions of this study). Also further increase of the CFV up to 360 m h^{-1} caused very small fluctuations of the TMP confirming the limited effect of the CFV.

Although higher CFVs than those tested in this study could be applied to control the TMP, it should be stressed that the high CFVs applied in conventional MF/UF MBRs (e.g. 1 m s^{-1}; Judd, 2010) significantly increase the energy consumption making non-competitive the use of DM if compared to conventional membranes.

2.5 Conclusions

The results of this study demonstrate that high permeate fluxes are positively linked with the rate of development of DMs, at least at the applied operating conditions. Effect of higher initial applied flux was found to be more pronounced as compared to MLSS concentrations inside the bioreactor on the development of DM that ultimately resulted in a robust fouling layer, which was more resistant against applied hydraulic shear. Moreover, the rapid development of the DM ensured steadier bioreactor performances for which COD and solids removal efficiencies were comparable to conventional high rate anaerobic treatment systems.

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CHAPTER 3

ANALYSIS OF FOULING DEVELOPMENT UNDER DYNAMIC MEMBRANE FILTRATION OPERATION

Abstract

This research is a contribution towards evaluating the appropriate fouling mechanism responsible for the flux decline under dynamic membrane (DM) filtration and its formation mechanism by using gravity-driven filtration in a specifically design experimental setup. Series of extended short term filtration experiments were performed at varying operating conditions of mixed liquor suspended solids (MLSS) concentrations, trans-membrane pressures (TMP) and mesh pore sizes. Blocking models were applied to identify the fouling mechanisms occurring in DM development. The results demonstrated that cake filtration model can adequately describe fouling mechanisms during DM filtration. According to the Analysis of variance, DM development, as described by flux (J) trends during filtration, was significantly affected by TSS MLSS concentration only while effluent turbidity was significantly affected by MLSS concentration and TMP. On the contrary, J and effluent turbidity trends during filtration were not significantly influenced by mesh pore size, at least in the range used in this study (10-200 μm).

This chapter is based on:

Saleem, M., Alibardi, L., Cossu, R., Lavagnolo, M.C., Spagni, A., 2017. Analysis of fouling development under dynamic membrane filtration operation. Chemical Engineering Journal 312, 136–143.

3 Analysis of fouling development under dynamic membrane filtration operation

3.1 Introduction

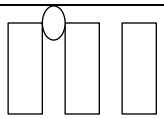
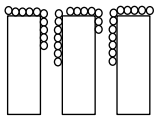
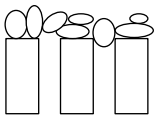
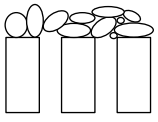
The use of membranes in wastewater treatment is finding growing application due to their complete solid retention, flexibility in operation and small footprints. However, high capital and operating cost and inevitable fouling phenomenon hinders their extensive application (Le-Clech, 2010). In this regard, dynamic membranes (DMs) represent an attractive alternative to the use of conventional microfiltration and ultrafiltration (MF/UF) membranes by positively employing the fouling cake as a mean for solid liquid separation (Alibardi et al., 2014; Zhang et al., 2014; Ersahin et al. 2012). DM is a fouling surface that is formed by the deposition of suspended solids, colloids or microbial cell particles over an underlying support material (meshes, filter cloth etc.) (Ersahin et al. 2012; Li et al., 2011; Liu et al., 2009). Meshes of different porosity ranging from 10 to 500 µm have been reported in literature as a suitable support material for developing DMs (Alibardi et al., 2016; Loderer et al., 2013; Jeison et al., 2008., Kiso et al., 2000). The filtration mechanism of DMs is quite different to conventional MF/UF membranes in a way that, after the formation of DM layer, the filtration resistance is exclusively caused by the cake layer (Liu et al., 2009). However, an excessive growth of a thick and dense fouling layer hinders a long term filtration operation due to excessive loss of permeability (Alibardi et al., 2014; Alavi Moghaddam et al., 2002). Therefore, the identification of operating parameters (e.g. nature and characteristics of the constituting particles, underlying support, suspended solids concentration, mesh pore sizes and hydrodynamic conditions) affecting the DM development remains crucial for practical large scale applications (Zhang et al., 2014; Ersahin et al., 2013; 2012; Chang et al., 2007) but the management of these parameters to ensure performance reproducibility and control of transmembrane pressure (TMP) still represents a challenging task for DM implementation.

Mathematical modelling is a useful tool that has been widely applied to analyse fouling in conventional membranes. Four models have been proposed to assess the fouling evolution over time in the form of complete blocking, intermediate blocking, standard blocking and cake filtration models. Complete pore blocking mechanism takes the assumption that the particles reaching the membrane surface block membrane pores without superposing other particles whereas, in intermediate blocking mechanism particles have an equal probability to deposit on other particles that ultimately cause pore blocking. Standard blocking, assumes that the particles deposit on the pore inner surface that gradually leads to pore constriction and ultimately to pore blocking. Cake formation mechanism is based on the assumption that particles reaching the membrane surface are larger than the membrane pore size and hence, they do not block them, rather form a layer on the membrane surface.

Hermia (1982) derived mathematical equations describing flux evolution over time under constant pressure filtration for these four blocking mechanisms (Table 3.1). Some authors have stated that the fouling process could also be governed by a combination of these mechanisms occurring simultaneously or at different stages during a filtrations operation depending upon the characteristics of the membrane, feed and operating parameters like filtration flux (*J*) and TMP (Sabia et al., 2016; Iritani., 2013; Kim et al., 2013; Bolton et al., 2005; Ye at al., 2005).

Although fouling processes play a decisive role in DM development, very few authors have tried to specifically understand how these phenomena occur in DMs by model-based analyses (Li et al., 2011; Liu et al., 2009). Moreover, the use of mathematical modelling to elucidate the formation mechanism of DMs in conjunction with the effect of changes in operating conditions on model response and its interpretation is still limited.

Table 3.1 Summary of characteristic equations for constant pressure filtration laws proposed by Hermia (1982).

Fouling Mechanism	Model	Blocking constant	Physical Description	Schematic representation
Complete Blocking	$J = J_o e^{-K_b t}$	K_b	Pore blocking	
Standard Blocking	$J = \frac{J_o}{\left(1 + \frac{K_s J_o^{\frac{1}{2}} t}{2}\right)^2}$	K_s	Pore constriction	
Intermediate Blocking	$J = \frac{J_o}{(1 + K_i J_o t)}$	K_i	Pore blocking + surface deposition	
Cake Filtration	$J = \frac{J_o}{\left(1 + 2K_c {J_o}^2 t\right)^{\frac{1}{2}}}$	K_c	Surface deposition	

To date, studies have mainly been focused on evaluating the effect of different operating conditions and process variables on the development and performance of DM (Saleem et al., 2016; Alibardi et al., 2016; 2014; Zhang et al., 2014; Liang et al., 2013; Loderer et al., 2013; Ersahin et al., 2013; 2012; Alavi Moghaddam et al., 2002; Jeison et al., 2008.; Kiso et al., 2000), while very few studies have discussed the mechanism governing the formation of DMs (Li et al., 2011; Liu et al., 2009).

To the best knowledge of the authors, there is a lack of information about the effect of changing parameters on the mechanisms involved in DM formation.

The aim of this study is to understand the main mechanisms governing DM formation and to evaluate the possible effects of variation of operating parameters on DM development and performance. Filtration tests were carried out with a set of diverse operating conditions in a specifically designed experimental set-up. The results obtained from these experiments were analysed by blocking models proposed by Hermia (1982) to predict the most likely fouling mechanism occurring in DM filtration.

3.2 Materials and methods

3.2.1 Experimental setup

The study was conducted at laboratory-scale in a specifically designed apparatus (Fig. 3.1). The experimental setup consisted of a 10 L stirring tank connected to an external filtration vessel by means of a peristaltic pump (Watson-Marlow 604U, Italy). The contents of the stirring tank were kept completely mixed by using an overhead stirrer (LS F201A0151, VELP Scientifica, Italy) operating at 200 rpm. The filtration vessel was made from a 1 mm thick Plexiglas tube having an internal diameter of 42 mm and a length of 180 mm. The filtration module contains a nylon mesh wound over a cylindrical support made of plastic with an external diameter of 35 mm and a length of 68 mm. The openings (6 mm x 5 mm) of the supporting cylinder were uniformly distributed with an effective filtration area of 58.3 cm^2. The cylindrical support was placed concentric to the filtration vessel in order to maintain a uniform hydraulic regime around the mesh surface. The stirring tank was filled with anaerobic sludge (initial TS and VS of 12.3g L^{-1} and 7.13g L^{-1}, respectively) collected from a full-scale mesophilic sludge digester treating the excess sludge of the municipal wastewater treatment plant of Padova, Italy. The required MLSS concentration was then attained by concentrating the sludge through settling or diluting by adding the supernatant of the same sludge.

3.2.2 Filtration experiments

A series of short term filtration experiments were performed at different operating conditions of mesh pore sizes, mixed liquor (total) suspended solids (MLSS) and TMP. Five different mesh pore sizes (10, 52, 85, 135, 200μm) were tested at three different MLSS concentrations (4, 8, 15 g/L) and three different TMPs (5, 10, 18 kPa). Details of the used meshes are reported in Table 3.2. Combination of these parameters was organised in a set of 45 experiments (Table 3.3), each lasting for 5h. Constant pressure gravity-driven filtration mode was employed. Permeate and concentrate

were recirculated to the stirring tank in order to maintain (almost) constant the MLSS (with the exception of the very small samples collected for analysis and of the mass of solids forming the dynamic layer); the volume of the stirring tank (10 L) was chosen large enough in order to make negligible the effect of solids loss during sampling and DM layer formation on the MLSS concentration.

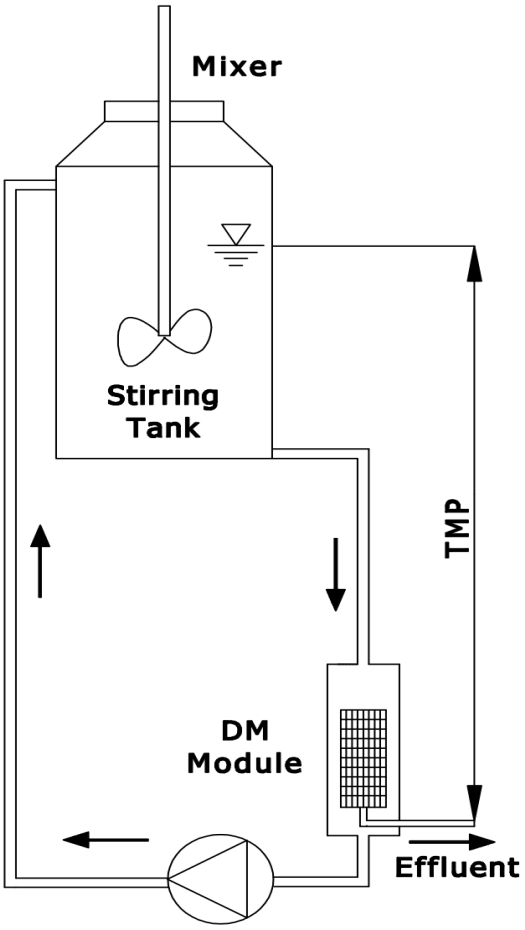


Figure 3.1 Schematic diagram of the experimental setup.

A constant cross flow velocity (CFV) of 30 m h^{-1} was maintained by the MLSS recirculation in all the experimental runs. The application of cross-flow was necessary to avoid possible sedimentation of the sludge inside the external module that could affect the MLSS concentration close to the mesh support (Loderer et al., 2013). The applied CFV in this study was anyway two-three orders of magnitude lower than the values usually applied in conventional membrane filtration (Ersahin et al., 2012) and it was maintained as low as possible to reduce the effect of hydraulic shear on the developing DM layer and to maintain the hydraulic regime as close as possible to dead-end filtration mode.

Table 3.2 Properties of the meshes used in this study. Data reported by the manufacturer.

Product information	Mesh material	Mesh opening (µm)	Open area (%)	Mesh count (/cm)	Thread diameter (µm)
SaatiMil PA 7 XXX	Polyamide Nylon	200	39	31	120
SaatiMil PA 10 XXX	Polyamide Nylon	135	39	46	80
SaatiMil PA 15 XXX	Polyamide Nylon	85	49	81	37
Saatifil PA 52/32	Polyamide Nylon	52	32	110	38
Saatifil PA 10/4	Polyamide Nylon	10	4	200 x 220	30 x 38

Table 3.3 Operational conditions applied during filtration experiments.

Experiment Number	MLSS (g/L)	PORE SIZE (µm)	TMP (kPa)
1, 2, 3	4	10	5, 10, 18
4, 5, 6	4	52	5, 10, 18
7, 8, 9	4	85	5, 10, 18
10, 11, 12	4	135	5, 10, 18
13, 14, 15	4	200	5, 10, 18
16, 17, 18	8	10	5, 10, 18
19, 20, 21	8	52	5, 10, 18
22, 23, 24	8	85	5, 10, 18
25, 26, 27	8	135	5, 10, 18
28, 29, 30	8	200	5, 10, 18
31,32, 33	15	10	5, 10, 18
34, 35, 36	15	52	5, 10, 18
37, 38, 39	15	85	5, 10, 18
40, 41, 42	15	135	5, 10, 18
43, 44, 45	15	200	5, 10, 18

Filtration flux was calculated by measuring the time to collect a known volume of permeate by using a graduated cylinder. Effluent samples were collected at regular intervals and analysed for total suspended solids (TSS) concentration and turbidity. TSS in the stirring tank was measured before and after every experimental run to ensure constant concentration. TSS was measured according to Standard Methods (APHA, 2005). Turbidity measurements were performed by using a turbidimeter (2100P, HACH).

3.2.3 Data analysis

Curve fitting of the experimental observations was performed with classical constant-pressure dead-end filtration equations proposed by Hermia (1982) and reported in Table 3.1. More precisely, filtration flux (J) versus filtration time data was plotted and fitted in a Microsoft Excel worksheet. Sum of squared errors (SSE) between numerical predictions and experimental observation was calculated and minimised to optimise the parameters K_b, K_s, K_i, K_c for every experimental run using the Solver add-in of Microsoft Excel. To evaluate accurate model prediction for the observed data, two statistical indices, root-mean-square error (RMSE) and adjusted coefficient of determination (R^2_{adj}), were used. The former statistical index shows the spread of errors between modelled and observed data while the latter is used as a measure of the strength of linear dependence between modelled and experimental observations. RMSE was preferred over other estimation of residuals, such as the sum of square and the mean square error, since it returns results in the same units of the models.

In order to assess the effect of the applied operating condition and their interactions on the filtration behaviour, factorial analysis of variance (ANOVA) considering two-way interaction between parameters was performed by using IBM SPSS Statistics software on measured J and effluent turbidity values after specific time intervals of continuous filtration. Moreover, ANOVA was also applied on the optimised values of blocking constants (K) for the model that best fits the experimental data.

Due to the short duration of the experiment in this study (i.e. 5 h), the four basic fouling models (Table 3.1) were independently applied as the aim of this study was to assess the effect of operating conditions on fouling mechanism and DM filtration performance. This approach is different to the other studies where combinations of fouling mechanisms were also applied (Sabia et al., 2016; Li et al., 2011; Liu et al., 2009) but it has been reported that combined models, although can improve data fit, could result in inaccurate estimation of model parameters (Sabia et al., 2016). Regression analysis of J values measured at the end of the experiments *vs* different mesh porosities used in this study was also carried out using IBM SPSS Statistics software.

3.3 Results and Discussion

3.3.1 Flux trends

Flux profiles of dynamically formed membrane in all experiments demonstrated a similar decreasing trend, irrespective of the variations in operating conditions (Fig. 3.2). These trends were similar to the flux decline trend observed for conventional MF/UF membranes during dead-end filtration at constant TMP with a sharp decline in first few minutes, followed by a much gradual decline in the later stages of the experiment. However, a significant difference was observed in the magnitude of initial filtration flux and its rate of decline which were much higher as compared to conventional membranes (Mendret et al., 2009). This behaviour was due to the difference in pore size between conventional membranes and the nylon meshes used in this study. In fact, the results showed very high initial filtration fluxes, ranging from 329 to 9880 $Lm^{-2}h^{-1}$ depending upon the applied operating conditions. Nevertheless, the following rapid increase of the filtration resistance (i.e. decrease of J) demonstrated the rapid development of the DM (within few minutes of filtration). As a matter of fact, fluxes reduced to less than 20% of their initial values (J_o) after only 4 minutes of filtration, and later on it further reduced to around 0.2 to 2% of J_o at the end (5 h) of each experimental run (Fig. 3.2).

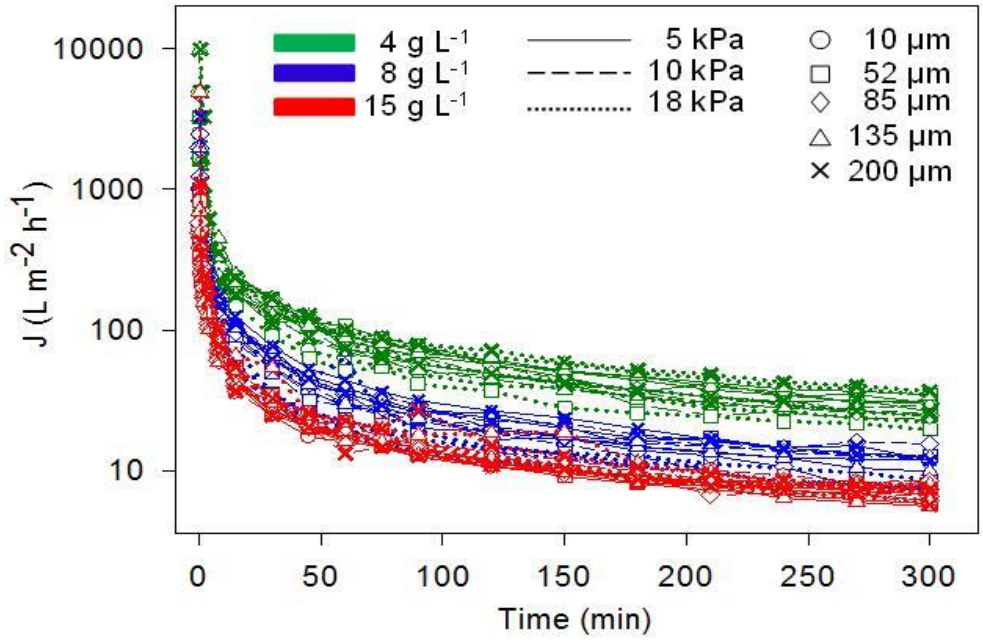


Figure 3.2 Filtration flux profiles under different operating conditions. Colour for MLSS concentration; Line type for applied pressure; Symbol for mesh pore size.

Figure 3.2 also illustrates three distinct levels of flux profiles grouped together with respect to three different MLSS concentrations used in this study. Therefore, it can be observed that higher MLSS concentration tend to increase the rate of fouling, reducing filtration fluxes right from the beginning

of the filtration test. Thus, the highest MLSS concentration showed the lowest values of J_0 (i.e. measured over the first min of filtration). The influence of MLSS concentration on fouling development is also clearly evident by observing the trend in flux decline. In fact, as a general behaviour, the higher the MLSS concentration, the lower the filtration flux (Fig. 3.2). On the contrary, the effect of TMP and pore size cannot be clearly identified by looking at the flux profiles (Fig. 3.2).

To support this argument, the final values of the flux measured at the end of the 45 experiments were compared (Fig. 3.3). Fouling development could be considered as an evolutionary process and thus final values of filtration fluxes carry the history of the fouling process and represent the effect of operating parameters on the filtration characteristics of DMs (Fig. 3.3).Small variations on final fluxes can be observed for experiments at same MLSS but different TMPs and mesh porosities, with the exception for the trends measured using low MLSS concentration (i.e. 4g/L) at high TMP value (i.e. 18kPa) where with increasing mesh pore size seems to favour higher final (after 5 h) filtration flux values (Fig. 3.3c). Linear regression analysis of final fluxes *vs* pore size confirms that data obtained by TMP 18 kPa and 4 g/L of MLSS returns a slope (s=0.0953 L m^{-2} h^{-1} μm^{-1}= 95,344 L m^{-3} h^{-1}) significantly different to zero (p=0.026) while all other regressions have slopes that are not significantly different to zero (data not showed). Therefore results suggest that pore size seems not to affect the final (i.e. after h) filtration fluxes with the exception of sludge with low suspended solids concentration at high pressure.

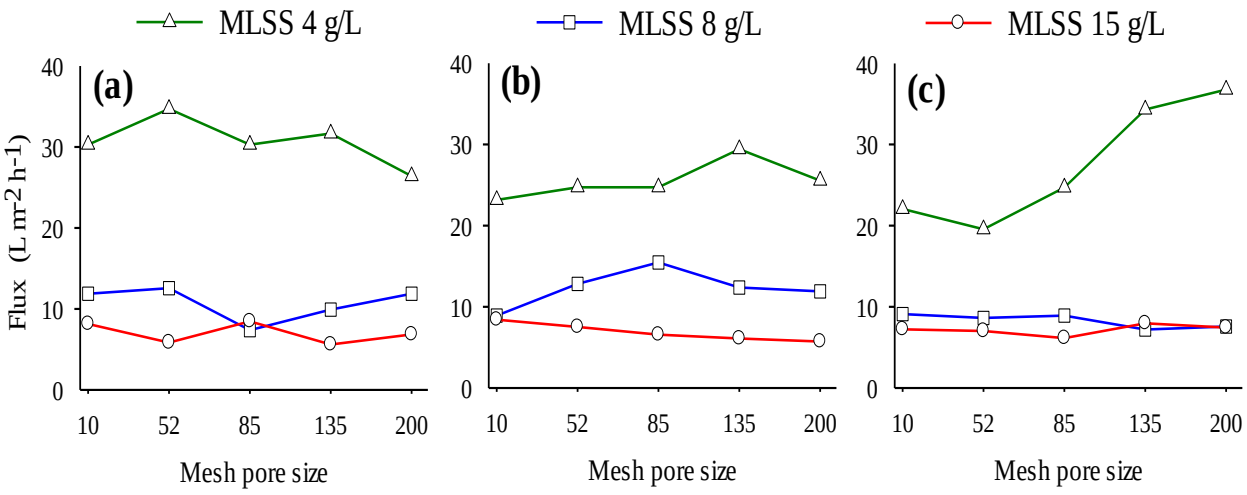


Figure 3.3 Effect of mesh pore size and MLSS concentration on final flux values at (a) 5kPa, (b) 10kpa and (c) 18kPa

The observation that low MLSS concentration at high TMP, is favourable for high filtration fluxes is in agreement with the observation made by Li et al. (2012), who, however, applied TMPs up to 0.62 kPa (63 mm-H_2O), which is much smaller as compared to the values applied in this study.

3.3.2 Curve fitting estimation of fouling models

Curve fitting analyses of fluxes obtained from filtration experiments suggest that cake filtration model best describes the fouling behaviour in DMs while imperfect agreements of intermediate and standard blocking models with experimental data were observed. An example is reported in Figure 3.4 for results obtained for the experiment at 4 g/L MLSS, 5 kPa TMP and with 10 µm mesh pore size. In most of the experiments the observed data seem to have reasonable agreement with modelled results shown by the measured R^2_{adj} values which, excluding the complete blocking model, were always above 0.94 (Fig. 3.4). These results are also confirmed by the RMSE values, which are the highest for pore blocking models and the lowest for cake filtration model (Fig. 3.5). However, the efficiency of the four models in describing the experimental varied at changing operating conditions.

3.3.3 Complete blocking analysis

The result of curve fitting with complete blocking model usually showed poor data fits between predicted and experimental values (Fig. 3.6) as depicted by high RMSE values (Fig. 3.5). It implies that fouling mechanism in DM filtration was not a result of complete pore blocking by the particles reaching the mesh surface. Although this model failed to depict fouling development in DMs, RMSE values changed with varying operating conditions and the lowest values were observed for experiments with smaller pore size meshes (10 µm) and high MLSS concentration (15 g L-1) (Fig. 3.5). Although complete blocking model showed the lowest efficiency in fitting the experimental data, it is of note that R^2_{adj} often achieved values higher than 0.90 (Fig. 3.4), demonstrating that even this model can reasonable describe the fouling occurrence in DMs.

3.3.4 Standard blocking analysis

Curve fitting of the experimental data obtained during this study by using the standard blocking model was not completely satisfactory (Fig. 3.6). Li et al. (2011) assumed standard blocking fouling to be the major blocking mechanism at the initial stage of DM filtration by reasoning that due to large difference between mesh pore and particle size, all other blocking mechanisms were improbable and thus fouling would be initiated by particle adhesion. However, model response observed in this study do not support this hypothesis particularly at lower MLSS, higher TMP and high mesh porosity as represented by high RMSE values (Fig. 3.5).

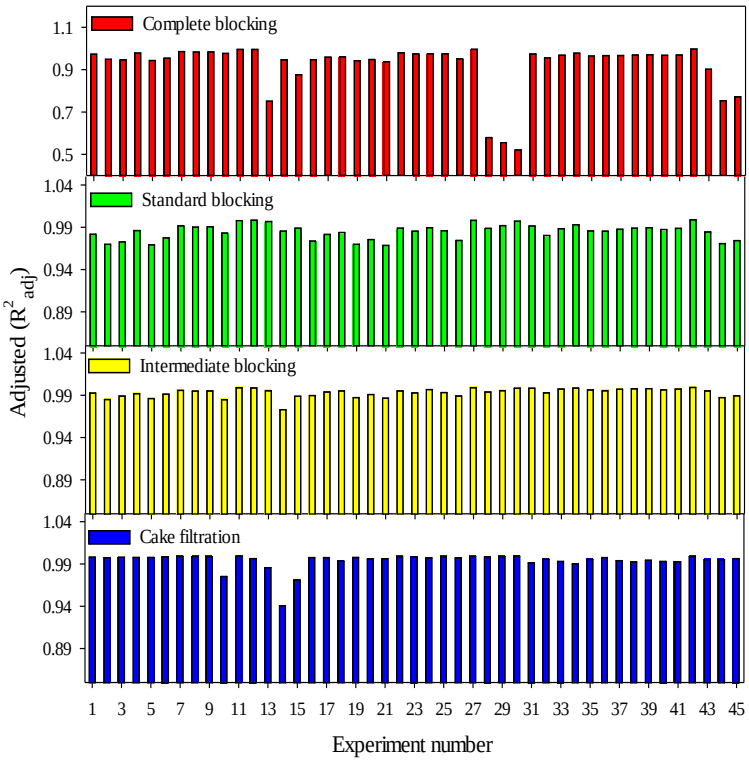


Figure 3.4. Adjusted R2 (R^2_{adj}) values for curve fitting analysis of Hermia's models.

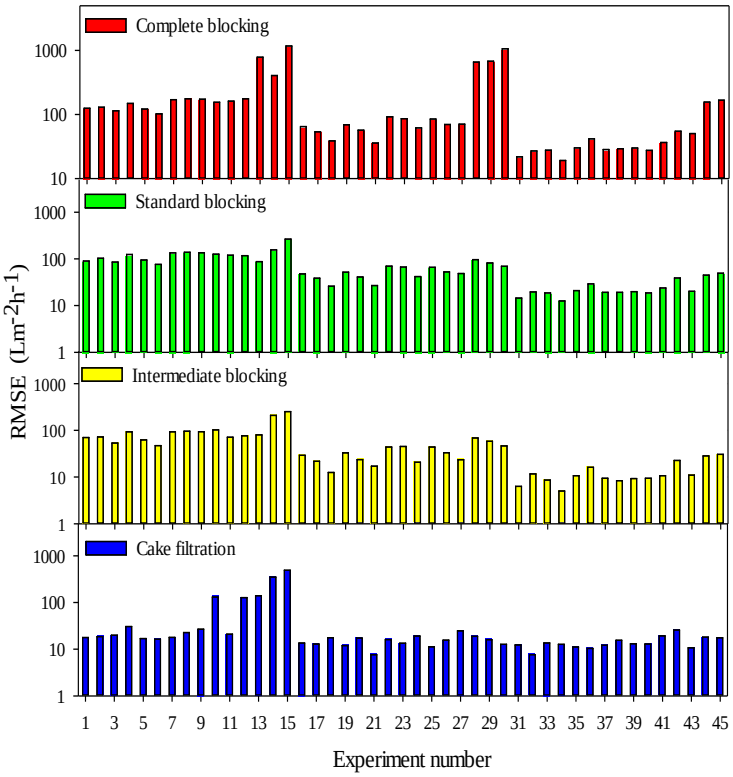


Figure 3.5. RMSE values for curve fitting analysis of Hermia's models.

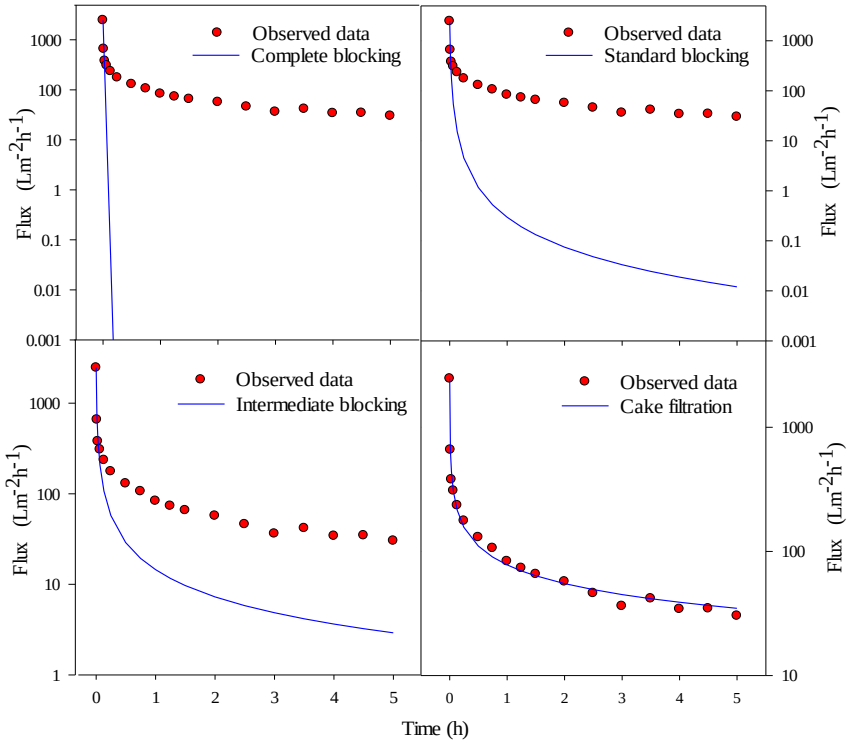


Figure 3.6 Curve fittings for the observed flux profiles with Hermia's models at MLSS 4 g/L, TMP 5 kPa and mesh with 10 µm pore size with (lines: predicted data; symbols: experimental results).

It was observed that the effect of MLSS concentration on model response seemed more prominent as compared to TMP and mesh porosity. This is highlighted by comparing average RMSE values at 4, 8 and 15 g/L of MLSS concentration which were 122, 55 and 24 $Lm^{-2}h^{-1}$, respectively (Fig. 3.5) suggesting that the standard blocking model improves the description of the experimental data increasing suspended solids concentration.

3.3.5 Intermediate blocking analysis

Model predictions made by intermediate blocking model were in much better agreement with the experimental data as compared to complete pore blocking and standard blocking models for the experimental conditions tested during this study (Fig. 3.6). As for standard blocking models, the value of RMSE obtained by using intermediate-blocking fouling model tends to reduce with the increase in MLSS concentration and decrease in mesh pore size (Fig. 3.5).

3.2.6 Cake filtration analysis

Cake filtration model shows the best fit of the experimental data during DM filtration (Fig. 3.6) as confirmed by the highest R^2_{adj} (usually > 0.99) and the lowest RMSE values (Figures S1 and S2, supplementary materials). Therefore, the model analysis of the main fouling models confirms that

the assumption of cake filtration model (i.e. a cake layer that can act as a solid-liquid separation medium) is actually valid in describing the behaviour of the flux trends in DM development and filtration.

The response of cake filtration model on varying operating conditions showed (as for the other fouling model) a similar trend of decreasing RMSE with an increase in MLSS concentration. It follows, therefore, that the model slightly deviated from ideal cake filtration behaviour at low MLSS concentration. This effect is confirmed by studies on conventional MF/UF membranes indicating that membrane fouling and cake formation is enhanced at high MLSS concentration (Lousada-Ferreira et al., 2015).

The effect of the applied operating conditions (i.e. TMP, pore size and MLSS concentration) was evaluated by ANOVA. The statistical analysis was performed on the optimised values of cake blocking constants (*Kc*) since the results of curve fitting supported cake filtration model to be the governing fouling mechanism in DM filtration. ANOVA analysis demonstrated that MLSS concentration proved to have the most significant influence on the value of K_c ($p < 10E\text{-}11$) in F-test among other parameters and their respective interactions (Table 3.4). Surprisingly, neither the pore size nor the applied pressure demonstrated a statistically significant ($p>0.05$) effect on the values of the measured cake fouling constants (at least for the applied conditions in this study). The reason for such a significant influence of MLSS on fouling processes in DMs can be explained by analysing the primary assumptions of cake filtration model (Hermia, 1982), where K_c is defined by the following expression:

$$K_c = \frac{\alpha \gamma s \mu}{A^2 P(1 - ms)} \qquad (3.1)$$

Where, α is the specific cake resistance, γ is the filtrate density, s is the mass fraction of solids in the filtrate, μ is filtrate viscosity, A is the surface area, P is the TMP and m is the mass ratio wet/dry of the cake. The MLSS concentration or mass fraction of solids in the filtrate has a dual effect on the value of K_c: a direct proportional effect expressed by the parameter *s*, and an indirect one by influencing other factors involved in the expression such as α, γ and m.

As far as the TMP is concerned, no significant effect ($p > 0.05$) was found on the value of K_c. However, the interaction of MLSS concentration and TMP was significant ($p = 0.003$ for MLSS*TMP), demonstrating that the relationship between MLSS and K_c also depends on the applied TMP (Table 3.4). Pore size, therefore, does not significantly affect *J* trends during cake filtration (at least using K_c values). This clearly demonstrated that the filtration by DMs is exclusively carried out by the cake layer while the mesh only acts as supporting material.

ANOVA performed on final *J* (i.e. measured after 5 h filtration) confirms, once again, that MLSS is the only statistically significant parameter characterising DM development, at least under the applied operating conditions of this study (Table 3.5). In addition, it is of note that the final *J* values were not statistically affected by the combined MLSS*TMP parameter, suggesting that the cake fouling constant (K_c) better reassumes the fouling phenomenon than a specific *J* values.

Table 3.4 Factorial ANOVA for evaluating the effect of MLSS, TMP and mesh pore size on the value of blocking constant for cake filtration model.

Parameters	Sum of square	Mean square	F	P
MLSS	1.510E-06	7.552E-07	185.961	< 10E-11
TMP	6.427E-09	3.213E-09	0.791	0.470
Pore size	4.132E-08	1.033E-08	2.544	0.080
MLSS x TMP	1.056E-07	2.641E-08	6.504	0.003
MLSS x Pore size	2.153E-08	2.691E-09	0.663	0.716
TMP x Pore size	6.545E-08	8.181E-09	2.015	0.111

Liu et al (2009) proposed a four stage formation mechanism of DMs over silk mesh, since they observed the progressive development of DM which was characterised by the four classic filtration models occurring sequentially. Li et al (2011), on the contrary, proposed a two-stage model to describe the cake formation and flux decline in DM operation by using different mesh filters and under varying operating conditions. They proposed standard blocking as the governing mechanism during the initial stage of filtration whereas the complete blocking and cake filtration dominated in the final stage. This study, on the contrary, demonstrates that cake filtration can adequately describe the *J* behaviour during DM formation and filtration at least during short-term experiments and under the applied operating conditions.

3.4 Effluent quality and suspended solids rejection

Dynamic membrane development was very rapid under all experimental conditions during this study (See Section 3.3.1) thus resulting in an effluent quality that improved quickly both in terms of turbidity and TSS concentration (Fig. 3.7 and 3.8). Moreover, during filtration, the effluent turbidity decreased more rapidly than did the permeate flux (Fig. 3.2, 3.7). In fact, while *J* decreased to 2% of the initial values (J_0) in 5 h (see Section 3.3.1), solids rejection was usually higher than 99% after only 8 minutes of filtration, corresponding to values typically less than 100 NTU and 20 mg/L for

turbidity (Fig. 3.7) and TSS (Fig. 3.8). Furthermore, whereas *J* continuously decreased during the filtration phase, NTU values seemed to show almost stable values after the first few minutes.

The effect of MLSS on effluent turbidity can be observed from Figure 3.7. The results demonstrate that the lowest effluent turbidity values were obtained at lower MLSS concentrations while no clear effects on turbidity can be observed for mesh sizes and TMPs. Turbidity values after 60 minutes of filtration at 4 g/L MLSS were well below 20 NTU for the majority of the experiments (Fig. 3.9). The negative impact of high MLSS concentration was also evident from the effluent SS profile (Fig. 3.8).

Table 3.5 Factorial ANOVA for evaluating the effect of MLSS, TMP and mesh pore size on final flux values.

Parameters	Sum of square	Mean square	F	P
MLSS	3771.906	1885.953	180.387	< 10E-10
TMP	26.827	13.414	1.283	0.304
Pore size	17.336	4.334	0.415	0.796
MLSS x TMP	85.800	21.450	2.052	0.135
MLSS x Pore size	79.254	9.907	0.948	0.507
TMP x Pore size	80.453	10.057	0.962	0.497

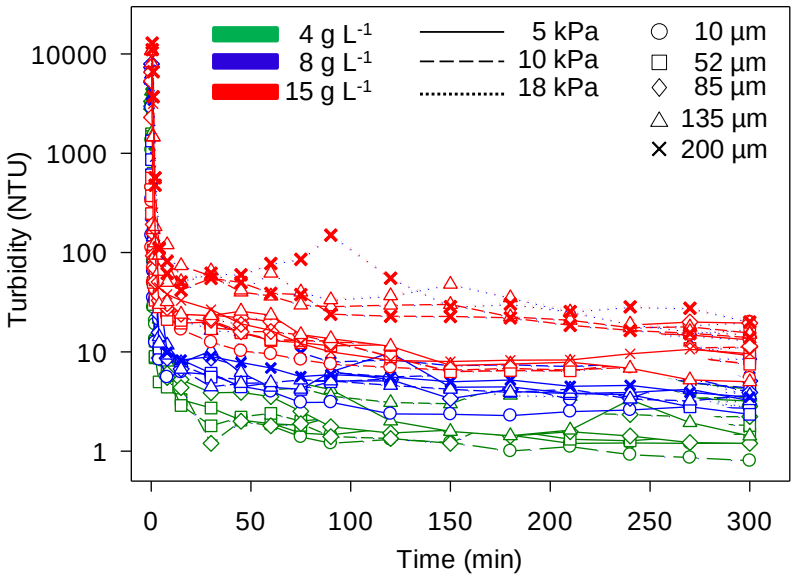


Figure 3.7. Effluent turbidity profiles under different operating conditions. Colour for MLSS concentration; Line type for applied pressure; Symbol for mesh pore size.

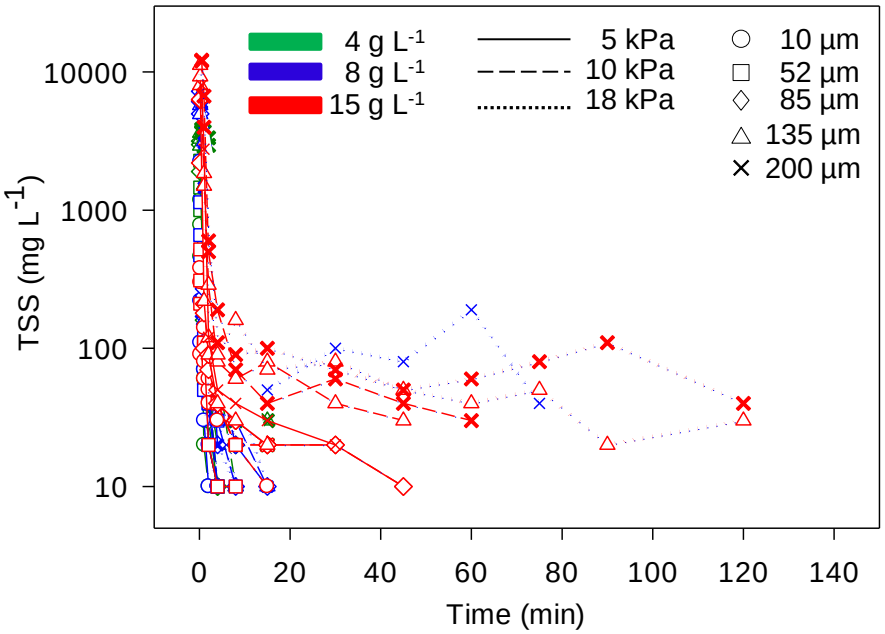


Figure 3.8 Effluent TSS values under different operating conditions applied. Colour for MLSS concentration; Line type for applied pressure; Symbol for mesh pore size.

ANOVA performed on effluent turbidity values measured after 60 minutes of continuous filtration with respect to varying operating conditions of the experiments confirms that MLSS concentration plays a statistically significant role (Table 3.6). Moreover, contrary to the cake filtration constant (K_c), turbidity was also significantly (p=0.08) affected by TMP (Table 3.6). Similar results were obtained by ANOVA on the turbidity values measured at the end of the filtration (i.e. after 5 h of filtration; Table 3.7).

As a general trend, an increase in filtration resistance is followed by a concomitant reduction in effluent fluxes (Fig. 3.2) and improvement in effluent quality (Fig. 3.9) in terms of solids rejection (Alibardi et al., 2014; Xiong et al., 2014; Liu et al., 2009). A slight deviation from this trend was observed in this study at high MLSS concentration for which filtration resistance tends to follow the expected increasing trend. However, effluent quality was slightly compromised as compared to the quality of the effluent at low MLSS concentration (Fig. 3.9). A plausible reason of lower effluent quality at high MLSS concentration could be the presence of higher number of smaller sludge particles per unit volume at high MLSS concentrations and thus, chances of these particles passing through the fouling layer acting as a DM are higher at high MLSS concentrations.

Table 3.6 Factorial ANOVA for evaluating the effect of MLSS, TMP and mesh pore size on effluent turbidity values measured after 60 minutes of continuous filtration.

Parameters	Sum of square	Mean square	F	P
MLSS	5835.006	2917.503	7.787	0.004
TMP	4923.811	2461.905	6.571	0.008
Pore size	3631.665	907.916	2.423	0.091
MLSS x TMP	2694.338	673.585	1.798	0.179
MLSS x Pore size	2977.771	372.221	0.993	0.477
TMP x Pore size	3369.520	421.190	1.124	0.399

Table 3.7 Factorial ANOVA for evaluating the effect of MLSS, TMP and mesh pore size on effluent turbidity values measured after 300 minutes of continuous filtration (i.e. at the end of experiment).

Parameters	Sum of square	Mean square	F	P
MLSS	5748.361	2874.181	5.292	0.017
TMP	6011.720	3005.860	5.535	0.015
Pore size	4839.088	1209.772	2.228	0.112
MLSS x TMP	2940.163	735.041	1.353	0.294
MLSS x Pore size	4309.949	538.744	0.992	0.478
TMP x Pore size	4834.278	604.285	1.113	0.405

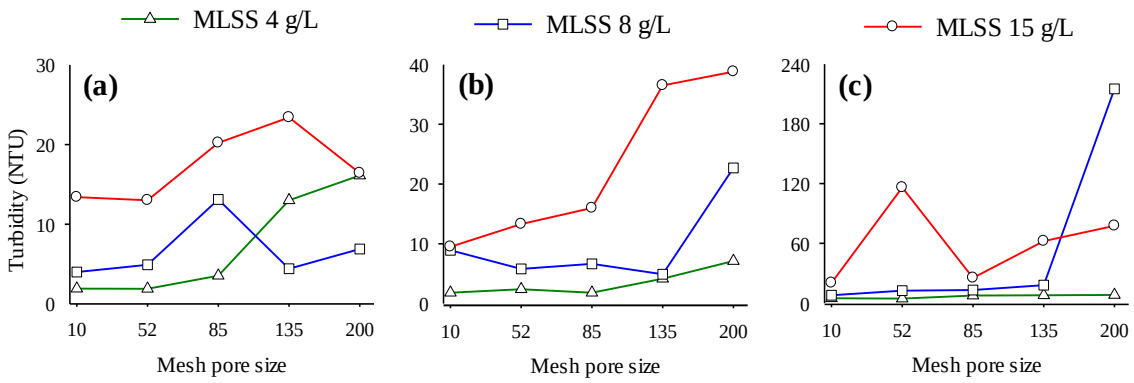


Figure 3.9 Effect of mesh pore size and MLSS concentration on effluent turbidity values after 60 minute of filtration at (a) 5kPa, (b) 10kpa and (c) 18kPa.

3.5 Conclusions

The results of short-term filtration experiments performed in this study using anaerobic sludge at varying operating conditions of MLSS, TMP and mesh pore size can be summarised under the following conclusions:

- DM development under constant pressure was very rapid (less than 5 minutes) and was mainly attributed to very high filtration fluxes observed at the beginning of every experiment.
- Initial filtration fluxes ranged from 329 to 9880 $Lm^{-2}h^{-1}$ and were followed by much lower fluxes after the formation of the DM.
- MLSS concentration was found to be the major factor affecting the filtration performance of DMs. Fluxes increased at decreasing MLSS. The relationship between MLSS and *Kc* also depends on the applied TMP. On the contrary, pore size (in the range used in this study) did not significantly affect DM development and *J*.
- The cake filtration mechanism can be effectively used to model DM formation and fluxes behaviour.
- Turbidity reduction was very rapid and concomitant to DM development as a result of which, suspended solid removal was usually higher than 99 % within 10 minutes of filtration. Effluent quality in terms of turbidity was negatively related to MLSS concentration and TMP, while was not affected by mesh pore size.

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CHAPTER 4

ASSESSMENT OF DYNAMIC MEMBRANE FILTRATION FOR BIOLOGICAL TREATMENT OF OLD LANDFILL LEACHATE

Abstract

This study investigates the behaviour of dynamic membranes (DMs) while treating stabilised landfill leachate. For this purpose, a bench-scale submerged pre-anoxic and post-aerobic dynamic membrane bioreactor (DMBR) was operated under ambient temperature. Four meshes with different openings (10, 52, 85 and 200 µm) were tested to support the development of DM. Marked differences among the meshes were observed in supporting the development of the cake layer constituting the DM. Moreover, the operation of the experimental reactor with landfill leachate affected the sludge characteristics which, in turn, deteriorated the filtration behaviour of the DM. The deteriorated filtrate characteristics caused the rise in the effluent turbidity which was often higher than 100 NTU for larger mesh pore size (85 and 200 µm). Low effluent turbidity was achieved with meshes with 10 and 52 µm (13±2 and 26±4 NTU, respectively), although at low membrane fluxes (lower than 10 L m^{-2} h^{-1}). The bioreactor exhibited moderate organics removal of 50-60% and fair ammonia oxidation (80-90 %). However, due to increased concentrations of free ammonia and free nitrous acid, incomplete nitrification was observed and nitrite accumulation resulted in effluent concentration up to 1062 mgNO_2^--N L^{-1}. Due to the refractory nature of a large fraction of the organic matter of landfill leachate, denitrification was also limited resulting in a total nitrogen removal of 19±2.6%.

This chapter is based on:

Saleem, M., Spagni, A., Alibardi, L., Bertucco, A., Lavagnolo, M.C., 2017. Assessment of dynamic membrane filtration for biological treatment of old landfill leachate. Journal of Environmental Management. Under review

4 Assessment of dynamic membrane filtration for biological treatment of old landfill leachate

4.1 Introduction

Solid waste production associated with population growth is one of the major issues faced by modern day municipalities. Despite being placed at the bottom of waste management hierarchy, sanitary landfills have been acknowledged as the most economically viable ultimate disposal option for municipal solid waste in most parts of the world (Fudala-Ksiazek et al., 2016; Wang et al., 2016). A major concern arising during landfill operation is the production of leachate resulted from the decomposition of waste (Wang et al., 2016; Kurniawan et al., 2006). If not properly managed, leachate could severely contaminate aquifers, raising concerns regarding the protection of natural environment and public health (Kurniawan et al., 2006).

Landfill leachate (LFL) treatment holds a great challenge because of the potentially high level of contaminants including organics, ammonia, inorganic substances, heavy metals and toxic hydrocarbons (aromatic and phenolic compounds) and of the variability in its quantity and quality in both space and time (Moravia et al., 2013; Renou et al., 2008; Kulikowska and Klimiuk, 2008). Moreover, the worldwide application of recent environmental legislation is changing the waste management chain for reducing the disposal to landfills and, as a result, changing the leachate production and composition (Fudala-Ksiazek et al., 2016).

Leachate recirculation back into the landfill, accelerating biodegradation and stabilisation of waste, is a cost-effective onsite treatment option (Reinhart and Al-Yousfi, 1996). Onsite treatment also includes various physicochemical and biological treatment options where physicochemical treatment is mostly applied as a pre/post treatment options targeting a particular contaminant (Renou et al. 2008; Tatsi et al., 2003). Biological processes has been proved to be effective in treating young leachates (Renou et al., 2008) whereas their efficacy reduces with the increase of leachate age due to shortage of biodegradable matter and increase of refractory organics (Ahmed and Lan, 2012; Renou et al., 2008; Spagni et al., 2007). In these conditions, an integration of biological treatment with physical, chemical or physicochemical processes has been documented to effectively exploit the advantage of each single process involved in the treatment trail (Ahmed and Lan, 2012; Hashisho and El-Fadel, 2016; Lin and Chang, 2000; Wang et al., 2008; Mariam and Nghiem, 2010)

Membrane bioreactor (MBR), which consists in the integration of microfiltration or ultrafiltration (MF/UF) membranes with biological reactors, has gained much appreciation over the last decade and has been perceived as an advanced treatment process considering its excellent effluent quality and flexible operation (Judd, 2016). Studies on MBR treating leachate have demonstrated to be very

effective under a wide range of loading conditions as compared to conventional biological treatment systems, particularly in treating stabilised LFL (Alvarez-Vazquez et al., 2004; Hashisho et al., 2016; Sadri et al., 2016 Hashisho and El-Fadel, (2016). However, the application of high loading conditions, long hydraulic retention time (HRT) and solids retention time (SRT), high amount of contaminants can increase membrane fouling (Ahmed and Lan, 2012). In addition, excessive amount of humic and fulvic acids usually present in LFL have shown to speed up membrane fouling (Sutzkover-Gutman et al., 2010).

In a recent review on MBR application treating LFL, Hashisho and El-Fadel (2016) reported consistent high removal efficiencies of biochemical oxygen demand (BOD) and ammonium nitrogen. However, COD removal varied from 23% to 98% depending upon the maturity of leachate. The same authors (Hashisho and El-Fadel, 2016) further concluded that membrane fouling was the main bottleneck in the widespread application of MBR in leachate treatment due to its high fouling potential especially while treating stabilised LFL.

In this regard, dynamic membranes (DMs) could represent an out of the box approach for fouling by purposefully exploiting it as a mean for solid liquid separation (Alibardi et al., 2016, 2014; Saleem et al., 2016; J. Xiong et al., 2016; Zhang et al., 2014). DM is defined as a self-forming and regenerative fouling surface that is formed by the deposition of suspended solids, colloids and microbial cell particles over a coarse underlying support material (Ersahin et al. 2012; Li et al., 2011; Liu et al., 2009).

Most studies on DM have been carried out on synthetic or real wastewater under aerobic or anaerobic conditions (Alibardi et al., 2016, 2014; Saleem et al., 2016; Ersahin et al., 2016; Jeison et al., 2008; Li et al., 2011; kiso et al., 2000; Hu et al., 2016; Xiong et al., 2014; Zhang et al., 2010; Liu et al., 2009) and for anaerobic sludge digestion (Liu et al., 2016; Yu et al., 2016). Xie et al. (2014) studied the performance of an anaerobic dynamic MBR for the treatment of leachate having mixed characteristics of young and old LFL (high ammonium nitrogen and COD content) by using a 40 µm mesh as a support material. Although those authors achieved solids retentions of the DM not comparable to conventional membranes, they reported a better effluent quality than conventional anaerobic treatment systems. To the best knowledge of the authors, there are no studies on the evaluation of LFL treatment by using DMs for the optimisation of organic matter and nitrogen removal. In addition, the effect of the use of meshes with different pore sizes as support material on the filtration performances of DMs treating LFL is also lacking.

This study aims to evaluate the application of DMs in anoxic-aerobic process for the treatment of stabilised LFL. In particular, the effect of the use of different mesh sizes on the development of the

DM was evaluated. Moreover, the behaviour of developed DM was studied in conjunction with the effect of change in feed characteristics and operating conditions.

4.2 Materials and methods

4.2.1 Experimental setup

The study was conducted using a laboratory-scale, continuously mixed, anoxic-aerobic system (Fig. 4.1a and 4.1c). The experimental setup consisted of a pre-anoxic tank with a working volume of 2.8 L connected to an aerobic tank with a working volume of 7.5 L. The tanks were made up of 5 mm thick Plexiglas cylinders having an internal diameter of 24 cm and a depth of 30 cm for the aerobic tank while an internal diameter of 18 cm and a depth of 30 cm for the anoxic one.

DM was developed over nylon meshes wound over cylindrical frames, which were inserted in the aerobic vessel (Fig. 4.1d and 4.1e). The frames were made up of plastic having an external diameter of 15 mm and a length of 70 mm with uniformly distributed openings of 5mm X 3mm. The total surface area of the filtration module that was 33 cm^2 of which approximately 61 % was the effective filtration area of each mesh. Three different nylon meshes were simultaneously immersed in the aerobic vessel resulting in a total effective filtration area of 60 cm^2. Filtration fluxes were controlled through a three line peristaltic pump (Watson Marlow SCI 400) which was connected to the three modules.

Four different meshes with pore sizes of 10, 52, 85 and 200 µm were tested in this study (details are summarized in Table 4.1). Meshes with porosities of 10, 85 and 200 µm were initially evaluated; however, due to changes in filtration behaviour of the sludge of the bioreactor, after 105 days of continuous operation the mesh with openings of 200 µm was replaced with a new one of 52 µm pore size.

The study was performed at ambient (laboratory) temperature (21±1 °C). Aeration of the aerobic tank was provided by aquarium blower and diffusers. The air flow was controlled by using an air flowmeter (ColeParmer 1-800-323-4340). Leachate was fed to the anoxic tank through another peristaltic pump (Watson Marlow SCI 400) connected to a level sensor. Sludge recirculation was approximately of four to five times the influent flow and was provided by means of a peristaltic pump (Watson Marlow SCI 400). The two bioreactors were kept completely mixed by using two overhead stirrers (LS F201A0151, VELP Scientifica).

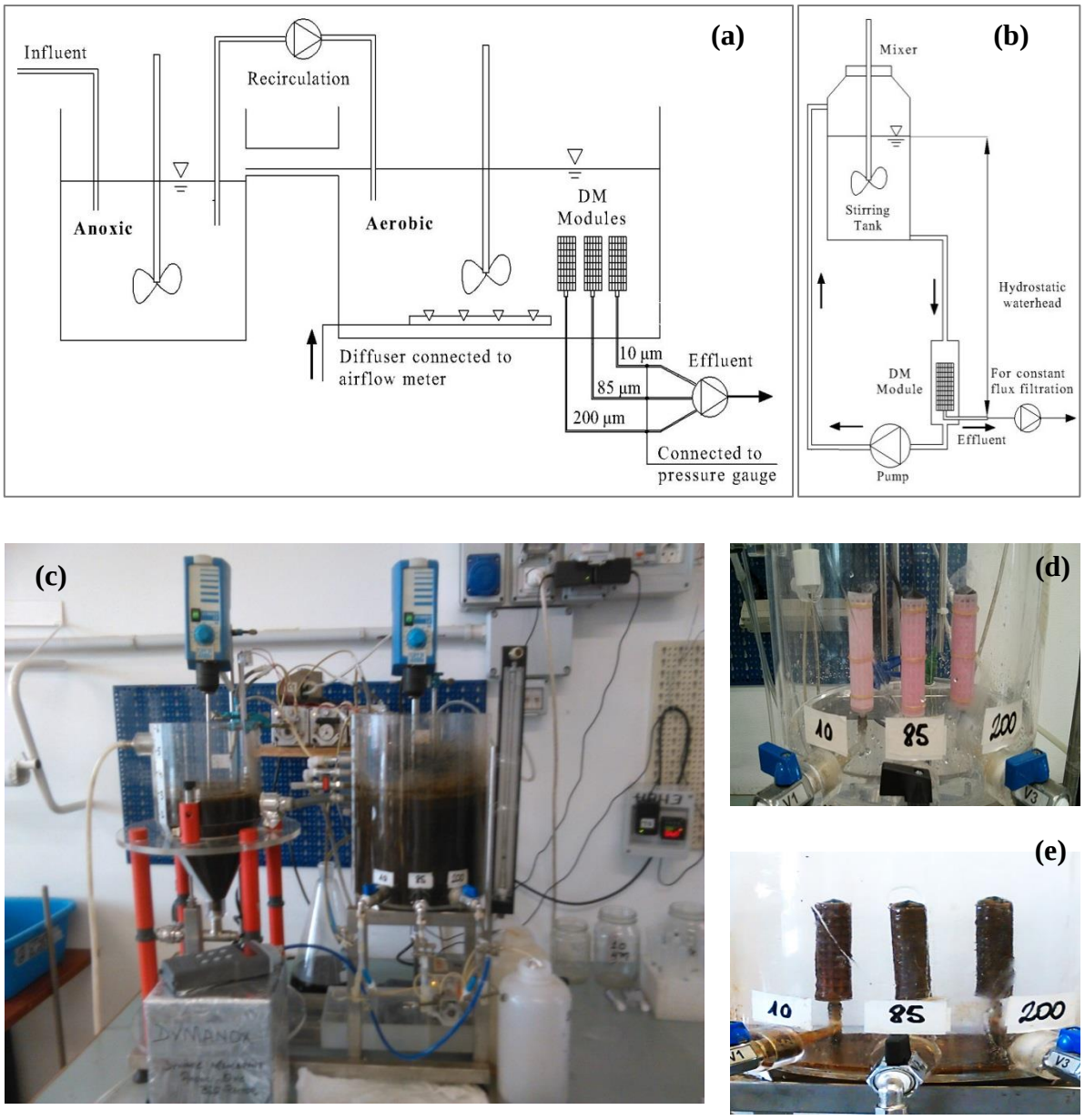

Figure 4.1 (a) Experimental setup schematic diagrams (b) short-term filtration test set-up (c) Experimental arrangement (d) clean meshes € fouled meshes

4.2.2 Inoculum and Feed

Sludge collected from a full-scale municipal wastewater treatment plant (Padova, Italy) was used as inoculum. The sludge had a total suspended solids (TSS) concentration of 8.7 g L^{-1} and volatile suspended solids (VSS) of 5.4 g L^{-1}. No sludge was voluntarily withdrawn from the experimental reactor except for a very small amount for analysis throughout the entire study. Similarly, the solids lost through the effluent were returned to the bioreactor through gravity settling after collecting the sample for solids analysis.

Table 4.1 Properties of the meshes used in this study.

Product information	Mesh opening (µm)	Open area (%)	Mesh count (/cm)	Thread diameter (µm)	Resistance (Clean mesh) (1/m-1) [2]	Tap water permeability (L/m2.h-1.kPa-1) [3]
SaatiMil PA[1] 7	200	39	31	120	5.46×10^9	1572.3
SaatiMil PA 15	85	49	81	37	5.42×10^9	1583.2
Saatifil PA 52/32	52	32	110	38	5.61×10^9	1528.6
Saatifil PA 10/4	10	4	200 x 220	30 x 38	6.46×10^9	1328.4

(1) PA is an acronym for polyamide
(2) Resistance of the mesh measured at TMP of 5 kPa
(3) 20 ^{o}C normalised permeability measured at TMP of 5 kPa

The feed to the reactor consisted of raw LFL, characterised by high ammonium concentration: the main LFL characteristics are reported in Table 4.2. The LFL samples can be considered as stabilised and typical of old landfills still in operation (Kjeldsen et al., 2002; Stegmann et al., 2005). The leachate was collected approximately every month and stored at 4 °C before use. To ensure the availability of essential micronutrients to support biomass activity following micronutrients were added in the feed wastewater: $Na_2MoO_4*2H_2O$ (0.22 mg Mo L^{-1}), $ZnSO_4*7H_2O$ (0.23 mg Zn L^{-1}), $CuSO_4*5H_2O$ (0.128 mg Cu L^{-1}), $NiCl_2*6H_2O$ (0.1 mg Ni L^{-1}), H_3BO_4 (0.007 mg B L^{-1}), Ne_2SeO_3 (0.06 mg Se L^{-1}), $MnCl_2*4H_2O$ (0.56 mg Mn L^{-1}) and $CoCl_2*6H_2O$ (0.124 mg Co L^{-1}).

Since the leachate used in this study has low BOD_5/N ratio (Table 4.2), as typical of old landfills (Kjeldsen et al., 2002; Stegmann et al., 2005), after approximately two months of operation, sodium acetate was also added with the leachate to the anoxic vessel in order to support the denitrification process. The amount of sodium acetate was provided according to the residual nitrate and nitrite concentration measured in the effluent.

4.2.3 Short-term filtration experiments

Short-term filtration experiment is a simple way to evaluate the performance of the coarse meshes used to develop DM (Li et al., 2012). These experiments were performed in a separate filtration system (so to reduce interference in bioreactor operation) according to the procedure previously described in Saleem et al. (2017). Briefly, filtration was performed under a constant transmembrane

pressure (TMP) of 3.43 kPa provided by the hydrostatic water head maintained above the filtration module connected to a 5 L stirring tank by a peristaltic pump (Fig. 4.1b). Filtration fluxes were estimated by measuring the time required to collect a known volume of permeate. Six short-term gravity driven filtration experiments were carried out with 200, 85 and 10 μm meshes. The experiments were carried out for the initial inoculum and for the sludge sampled from the aeration tank after 67 days of continuous bioreactor operation. Since the experiments were performed to assess potential change in the filtration behaviour of the sludge, new meshes were used. TSS and VSS concentration inside the bioreactor on 67th day of the continuous bioreactor operation was 7.4 and 4.3 g L^{-1} respectively.

Table 4.2 Characteristics of the leachate samples.

PARAMETERS		VALUE	UNIT
BOD		479 / 325 / 425	mg L^{-1}
TOC		1110 / 1154 / 1590	mg L^{-1}
NH_4^+		1380 / 1426 / 2272	mg L^{-1}
Average NO_3^-N		none	mg L^{-1}
Average NO_2^--N		7.65	mg L^{-1}
Average total phosphorus		9.63	mg L^{-1}
Average pH		8.56	-
Average alkalinity		14583	mg CaCO3/L
	Cd	< 10	μg/L
M	**Cr**	753	μg/L
E	**Cu**	51,5	μg/L
T	**Fe**	3860	μg/L
A	**Mn**	172	μg/L
L	**Ni**	148	μg/L
S	**Pb**	< 10	μg/L
	Zn	112	μg/L

In addition, for the second experiment only, when the fluxes reduced to less than 5 % of the initial values, the filtration fluxes were increased to approximately 100 L m^{-2} h^{-1} by means of a peristaltic pump (Watson Marlow 505U).

4.2.4 Dynamic membrane operation and cleaning

Periodical cleaning of the excessively fouled DM layer was performed at the same time for all meshes whenever the TMP value was higher than 20 kPa for 200 μm or whenever the fluxes were lower than 2 L m^{-2} h^{-1} for any of the mesh under investigation (set as the lower limit for this study) in order to maintain the designed HRT of the system. The meshes were cleaned in situ (i.e. inside the aerobic bioreactor) with the help of a brush.

Since the formation of DM layer after every cleaning operation could greatly compromised the effluent quality in terms of suspended solids removal (Alibardi et al., 2014; 2015), after every cleaning operation, in order to expedite the process of DM formation, very high initial filtration fluxes of 5,000 to 10,000 L m^{-2} h^{-1} were applied (Saleem et al., 2016; 2017) under gravity driven filtration mode obtained by constant hydrostatic water head of 1.7 kPa. After the development of DM layer, characterised by the production of a "clear" permeate (visual inspection), constant flux filtration operation (to maintain the design HRT) was resumed. Permeate collected during this interval was returned to the bioreactor. Ersahin et al. (2012), Alavi Moghaddam et al. (2002) and Fan and Huang (2002) also proposed a similar recirculation strategy for the start-up of DMs in full scale systems.

4.2.5 Analytical Method and Measurements

Total suspended solids (TSS), volatile suspended solids (VSS), Ammonium, nitrates, nitrites nitrogen, total phosphorous, 5-day biochemical oxygen demand (BOD_5), were measured according to Standard Methods (APHA, 2005). Organics matter and alkalinity were estimated measuring the total carbon (TC) and total organic carbon (TOC) by using Shimadzu TOC-V_{CSN} analyser. The concentrations of free ammonia (FA) and free nitrous acid (FNA) were estimated according to Anthonisen et al., (1976). Transmembrane pressure (TMP) was measured separately for each DM by using an electronic pressure gauge (COMARK C9505/IS, Pressure Meter, 0–30 PSI). Darcy's equation was used to estimate total DM resistance as follows (Li et al., 2012):

$$\boldsymbol{R} = \frac{\boldsymbol{\Delta P}}{\boldsymbol{\mu \cdot J}} \qquad (1)$$

Where J is the permeate flux, ΔP is TMP across the membrane, μ is the viscosity of permeate (assumed of clean water), and R is total membrane resistance.

Dissolved oxygen (DO) concentration inside the bioreactors was monitored by using a DO meter (HANNA HI 9147). Effluent turbidity and pH were measured using a turbidimeter (HACH 2100 P ISO TURBIDIMETER) and a pH-meter (Crison GLP 22), respectively. Average daily fluxes from the three DM modules were estimated by dividing the volume of the filtrate collected from each filtration module by the filtration area of each module.

4.3 Results and Discussion

4.3.1 Dynamic membrane behaviour

Filtration was started by applying very high fluxes in order to speed up the formation of the cake layer on the mesh supports (Saleem et al., 2016). Effluent quality improved very rapidly within 10 minutes of filtration and effluent turbidity values reduced to less than 10 NTU, indicating a very rapid formation of DM for all meshes. The rapid formation of the DM also confirms previous results obtained under batch and continuous conditions (Saleem et al., 2017).

The TMP profile of different meshes throughout bioreactor operation showed similar trends, characterised by gradual increase of the measured TMP during filtration (Fig. 4.2). However, over the entire duration of the study (approximately of four months), these TMP trends showed a significant change in the behaviour (Fig. 4.2). First 25-30 d of the experiment showed a slow and gradual rise in TMP, according to the local flux theory proposed for conventional membranes (Cho and Fane, 2002). Very low and stable TMP of approximately 1-2 kPa was observed during the first week of bioreactor operation irrespective of the considerable difference in the porosities of the meshes used. TMP values, then, gradually and almost steadily increased up to approximately 60 kPa in about 20 days. Owing to the high TMP, the mesh supports were cleaned following the procedures described in Section 4.2.4. Thereafter, contrariwise to the first month of operation, the TMP trends were characterised by sharp and rapid increase in their values after every procedure of mesh cleaning. Moreover, while during the first month of the study TMP behaved similarly for the three mesh pore-size tested, thereafter, TMP showed clearly different trends.

The result obtained over the first month of operation confirms a previous study (Saleem et al., 2017) which demonstrated that the mesh pore size does not significantly affect the filtration flux (Fig. 4.2). However, after the first month of operation (which roughly corresponded to the first filtration period ending with mesh cleaning), the different meshes showed decreasing TMP values (Fig. 4.2) with increasing mesh pore-size suggesting lower resistance (Fig. 4.3) with cake layer developed on larger pore size. It is of note that the maximum TMP achieved during filtration cycles (where a filtration cycle can be identified between two cleaning procedures) decreased during the entire duration of the study (Fig. 4.2). This is particularly evident for the mesh with large pore size (i.e.

200 and 85 µm) where maximum TMP achieved during continuous filtration (between different cleaning operations) decreased from approximately 60 kPa to less than 20 kPa (Fig. 4.2a and 4.2b).

The TMP variation during the filtration affected the achieved fluxes (Fig. 4.2). The observed flux (for every continuous filtration between two successive cleaning procedures) varied according to the mesh porosity and was higher for larger mesh pore size. Due to the very variable J, HRT of the system showed a fluctuating profile (Fig. 4.4).

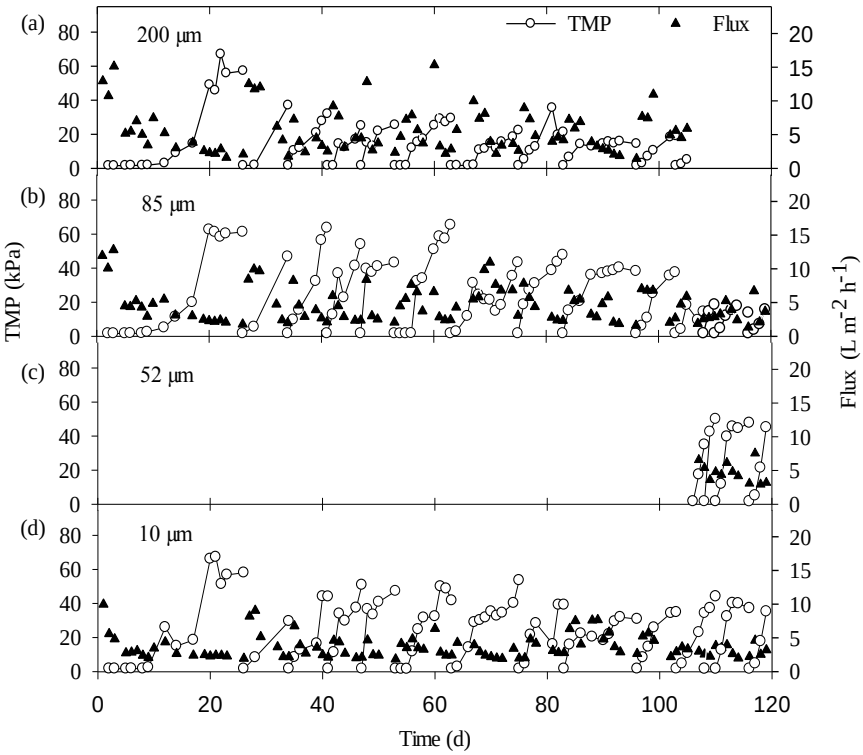

Figure 4.2 Observed filtration flux and TMP profiles for (a) 200 µm, (b) 85 µm, (c) 52 µm and (d) 10 µm

The variation of the behaviour of the developed DM was also evident on the behaviour of the resistance, as a result of the applied J and the achieved TMP (according to equation 1). Over the first filtration cycle (approximately first month of operation), in fact, DM resistance gradually increased from approximately 1.0×10^{12} to 1.0×10^{14} m^{-1} for all the meshes under investigation (Fig. 4.3) and, once more, without large difference among the three meshes. It is important to mention that DM resistance was the main contributor of the total resistance of the filtration module, since the intrinsic resistance of all the meshes measured using tap water was of the order of 10^9 m^{-1} (Table 4.1).

After the first month of operation, the DM resistance always showed rapid build-up demonstrating once more the different behaviour of the formed DM over the entire duration of the study. It is of

note that the initial resistance measured after every mesh cleaning was always much higher than the values measured at the beginning of the experiment indicating that the initial filtration characteristics were not achieved. Moreover, the initial resistance increased with decreasing mesh pore size. This confirms that the formation of the cake layer over the mesh showed different behaviour in sludge filtration during the experiment.

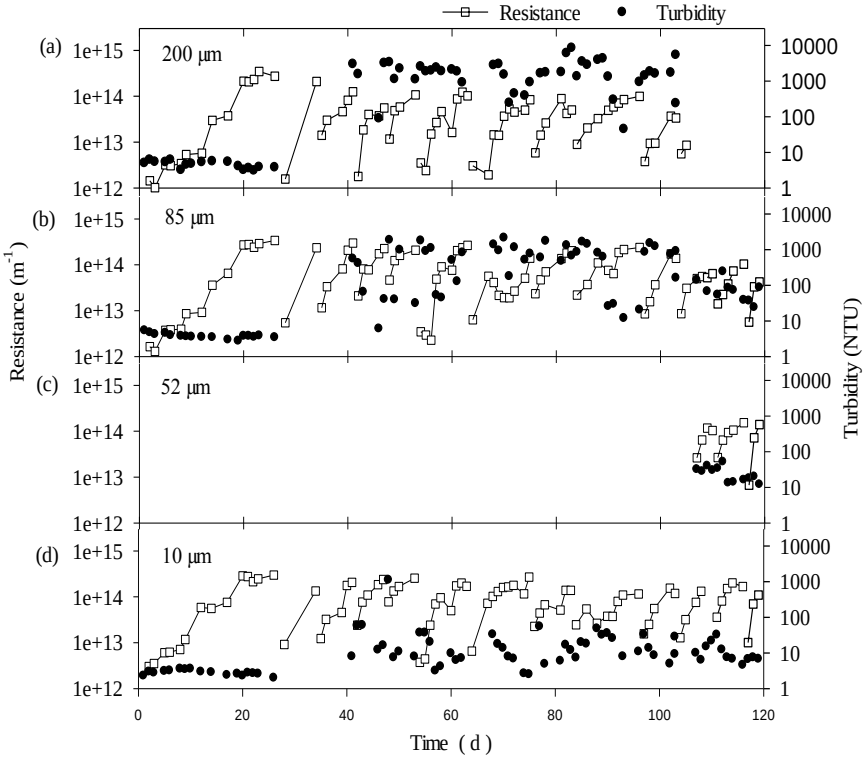

Figure 4.3 Dynamic membrane resistance profiles along with effluent turbidity values (a) 200 µm, (b) 85 µm, (c) 52 µm and (d) 10 µm

The change of the characteristics of the sludge and the DM formed on the mesh is also evident on the measured effluent turbidity (Fig. 4.3). It is noteworthy that the DMs developed on different meshes showed very low effluent turbidity values over the first month of operation (with values less than 5 NTU for all meshes), demonstrating an excellent solid retention of DM during that time period regardless of the difference in mesh openings. Moreover, while TMP greatly increased during filtration (Fig. 4.2), effluent turbidity values remained almost stable (Fig.4.3) confirming the formation of a stable cake layer over the mesh independently, once more, of the different mesh pore size (at least during the first 20-30 days of operation). However, just after the first procedure of cleaning, the effluent turbidity greatly increased and never reached the low values measured during the first two-three weeks (Fig. 4.3). Moreover, the larger the mesh opening the higher the effluent turbidity (Fig. 4.3); the related mean turbidity values for 200, 85 and 10 µm meshes were

2126±253, 615±81 and 37±20 NTU, respectively. These observations demonstrate, once more, that the applied operating conditions greatly affect the development of the DM. As a result of the turbidity, the different DM showed different suspended solid retention. In fact, the DM developed over the mesh of 10 μm exhibited very high suspended solids removal which was always above 95%, while the meshes with openings of 85 and 200 μm achieved mean solid retention of approximately 85 and 55 %, respectively (data not shown).

Since the solids lost through these meshes during the operation was always returned to the bioreactor (except the small amount collected as a sample for analysis) the mixed liquor suspended solids concertation (MLSS) inside the aerobic and anoxic tank remained almost constant around 6.4±0.15 and 4.1±0.24 g L^{-1} respectively.

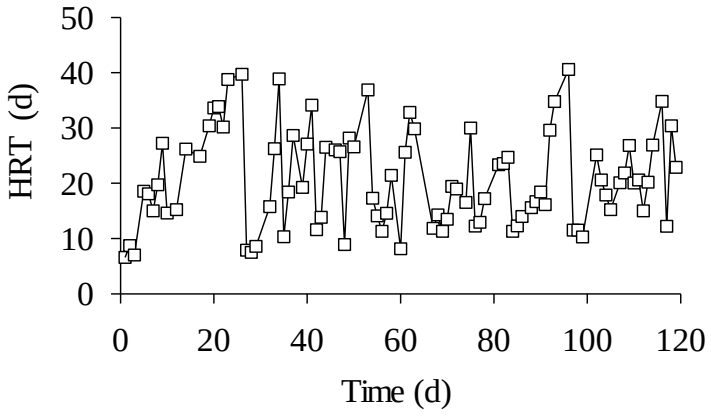

Figure 4.4 Hydraulic retention time (HRT) of the bioreactor

The difference in behaviour of the DMs developed over the different meshes between the first experimental period (of approximately one month before the first cleaning) and the following three months of operation could be due to two reasons. Firstly, the applied procedures, adopting water-flushing supported by intense brushing, was not enough to completely remove the fouling material as identified by Li et al. (2016) or, secondly, the applied operating conditions changed the sludge characteristic greatly increasing its fouling propensity. Li et al. (2016) have recently observed by scanning electron microscopy that a significant amount of fouling material remained deeply entrapped inside the mesh of an anaerobic bioreactor even after intense water flushing and scraping. However, it is also well documented in literature that filtration sludge in membrane- and dynamic membrane- assisted bioreactor are affected by operating conditions (e.g. Sabia et al., 2013; Ersahin et al., 2017). In addition, changes in the sludge filtration characteristics could also be triggered by the treatment of landfill leachate which has a high fouling propensity, at least in conventional membrane bioreactor (Ahmed and Lan, 2012).

The results obtained in this study disagree with a previous study on the evaluation of DM development in short term filtration experiments, where the authors reported that mesh pore size does not affect DM development (Saleem et al., 2017). However, other authors (Wu et al., 2003) reported that large mesh pore size favours high filtration fluxes under similar conditions of applied TMP. This study, clearly demonstrated that operating condition and/or the feed and sludge characteristics influence the development of the cake layer composing the DM and, thus, its filtration characteristics. As a consequence, the use of meshes with different opening could affect the filtration behaviour of the developed DM depending on the characteristics of the sludge. Moreover, excessive amount of organic foulants present in stabilized LFL (humic and fulvic substances) would have contributed towards much faster DM fouling in the later stage of bioreactor operation (Ahmed and Lan, 2012).

In order to find a trade-off between high filtration fluxes observed for 200 and 85 μm meshes and high effluent quality of 10 μm mesh it was decided to evaluate the performance of 52 μm mesh instead of 200 μm mesh on day 104 of the continuous bioreactor operation. The results showed that the solids retention performance of 52 μm mesh was comparable to that of 10 μm in terms of effluent turbidity (Fig. 4.3c and 4.3d). Furthermore, the average filtration fluxes of the DM developed on the mesh with openings of 52 μm was higher than those obtained for 10 μm mesh and was rather comparable to 200 and 85 μm meshes (Fig. 4.2).

4.3.2 Short term filtration experiments

Under gravity driven filtration, the inoculum showed flux reduction to less than 10 % of the initial values in 30 minutes (Fig. 4.5a). On the contrary, when the sludge collected from the aerobic tank after more than two months of leachate treatment was used, fluxes reduced to less than 5 % of their initial values within 10 to 15 min demonstrating a much higher fouling propensity of the biomass of the bioreactor than the inoculum (Fig. 4.5b). As a result, the fluxes measured filtering the inoculum were of approximately 10 time higher than those obtained by using the sludge from the reactor (compared after approximately the same filtration time). This result clearly demonstrated the mush higher fouling propensity of the bulk sludge if compared with the inoculum, which can also be evinced in the filtration resistance profiles (Fig. 4.6).

Since the fluxes reduced very quickly to values well below 5% of the initial flux when using the bulk sludge, the fluxes were increased (using a peristaltic pump) to constant values of approximately 100 L m^{-2} h^{-1} in order to assess the behaviour of the DM filtration under constant flux as operated in the membrane bioreactor. The constant flux filtration caused a slight decrease of

filtration resistance for 200 and 85 meshes which was, however, quickly followed by its increase (Fig. 4.6b).

The initial decrease of the filtration resistance for 200 and 85 μm meshes suggested a weak DM structure that was not resistant to the increased flux and the resulting TMP (Fig. 4.5b). Alibardi et al, (2014) have reported a similar observation, during flux-step experiment performed to assess the strength of DM formed under anaerobic conditions treating synthetic wastewater. These results suggest that larger mesh pore size formed unstable DM which can be easily destabilised with sudden increase of flux. However, the high flux caused a further deposition of material on the meshes which caused the following increase of the membrane resistance (Fig. 4.6). When the mesh of 10 μm openings was used, the increased flux operated by using the peristaltic pump caused a sudden increase of the resistance (Fig. 4.6b) which determined a drop of the flux (Fig. 4.5b).

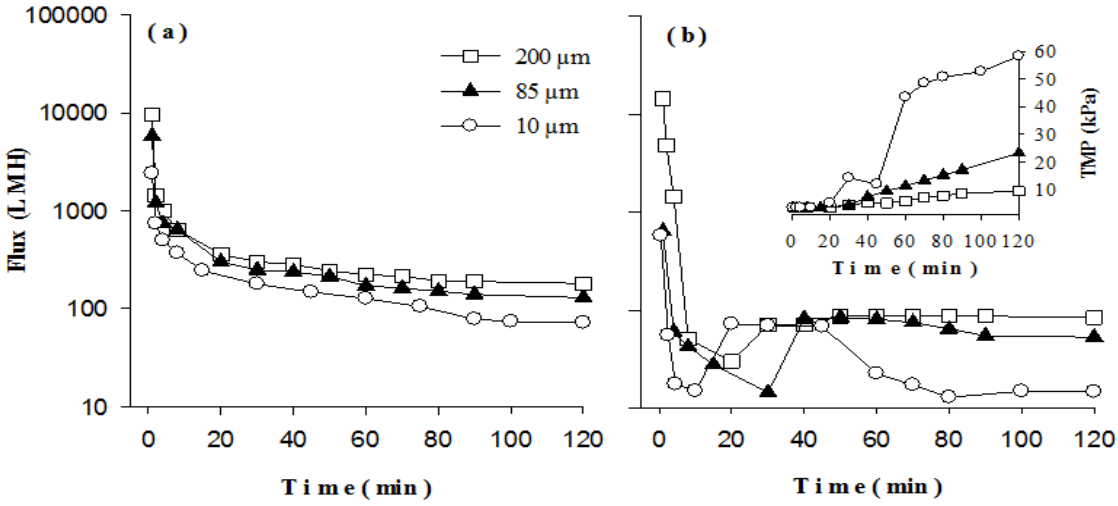

Figure 4.5 Flux profiles for the short-term filtration tests (a) initial inoculum under gravity driven filtration mode at a constant TMP of 2 kPa (b) MLSS from the aerobic tank under gravity driven filtration followed by constant flux filtration mode

The different behaviour of the bulk sludge as compared with inoculum was also well evident in the turbidity measured in the short term experiments. Effluent turbidity reduced in 5-10 min to values less than 10 NTU when the inoculum was filtered with all meshes under investigation (Fig. 4.6a). Moreover, no particular differences were observed in the turbidity among the three different meshes. When the bulk sludge was used, on the contrary, turbidity remained above 400 NTU with much lower flux and higher resistance of the sludge than the inoculum. These results demonstrated that the cake layer formed on the mesh is instable and cannot effectively retain solids when the bulk sludge in filtered contrary to the inoculum. Therefore, the results confirm that the operation of a

DM treating landfill leachate cause a significant deterioration of biofilm on the mesh, at least for its filtration characteristics.

When the peristaltic pump was switched on to increase the membrane flux (for the experiment using the bulk sludge only), the three meshes behaved differently. Turbidity values increased markedly for 85 and 200 μm mesh (Fig. 4.6) and effluent quality deteriorated due to the loss of loosely bounded particles in the effluent at high TMP (Fig. 4.5). In contrast, the continuous deposition of materials on the 10 μm mesh formed a DM which was more resistant to much higher TMP values averaging around 50 kPa (Fig. 4.5). As a result, contrary to the other two meshes, effluent quality improved after the start of constant flux filtration operation and final effluent turbidity progressively reduced to 22 NTU at the end of the experiment (Fig. 4.6d). The use of large mesh openings seems beneficial on the basis of higher filtration flux and lower operating TMP values for 200 and 85 μm meshes than those measured for 10 μm mesh (Fig. 4.6); however, such advantages were associated with highly deteriorated effluent quality due to loss of biomass in the effluent. These results are not in agreement with the previous study (Saleem et al. 2017) where turbidity and flux were not significantly affected by using 5 different meshes with pore sizes ranging from 10 to 200 μm. Therefore, these results demonstrated that the behaviour of the DM is affected by the characteristics of the filtered sludge and by the operating conditions applied (i.e. flux and TMP).

The short term experiments demonstrated that landfill leachate has very high fouling propensity for DM as also previously observed for conventional membrane bioreactors (Hashisho and El-Fadel., 2016). In addition, the operation of the reactor under DM filtration may have most likely affected the sludge characteristics. For example, it is well known that filtration and specific reactor operation can cause changes of the particle size in conventional membrane (Lousada-Ferreira et al., 2015) as in DM bioreactors (Ersahin et al., 2014).

4.3.3 Landfill leachate treatment

Dissolved oxygen concentration of the aerobic bioreactor was always maintained above 1.0 mg L^{-1} (data not shown) during the entire bioreactor operation to assure nitrification conditions. The pH value of aerobic bioreactor varied between 6.4 and 8.9 (Fig. 4.7).

The bioreactor exhibited moderate organics removal performance since recalcitrant organics constituted a significant fraction of the available organic matter present in the leachate (Table 4.2). The average TOC removal recoded after 20 days of continuous bioreactor operation and before the addition of supplemental organics was 58±1.4% (Fig. 4.8). Moderate organic matter removal from leachate collected in old landfill is also usually observed applying conventional biological processes

(e.g. Spagni et al., 2007). In addition, Ahmd and Lan, (2012) reported in their review that conventional MBRs treating stabilized LFL achieved similar organics removal performances in terms of COD removal efficiencies ranging from 54-78%. NH_4^+-N concentration in the effluent showed a fluctuating trend throughout the study, due to which the observed NH_4^+-N oxidation was 70 to 99%; nevertheless, considering the high influent NH_4^+-N concentration (1073-1767 mgN L^{-1}), the average NH_4^+-N oxidation was of 84±1.4% (Fig. 4.9b). Although the system exhibited high NH_4^+-N oxidation, the biological nitrification process was incomplete and NO_2^--N was the main product of ammonia oxidation (Fig. 4.9b). Along with the increase in influent NH_4^+-N concentration a progressive increase in effluent NO_2^--N concentration was observed, reaching NO_2^--N values as high as 1062 mg L^{-1} (Fig. 4.9a).

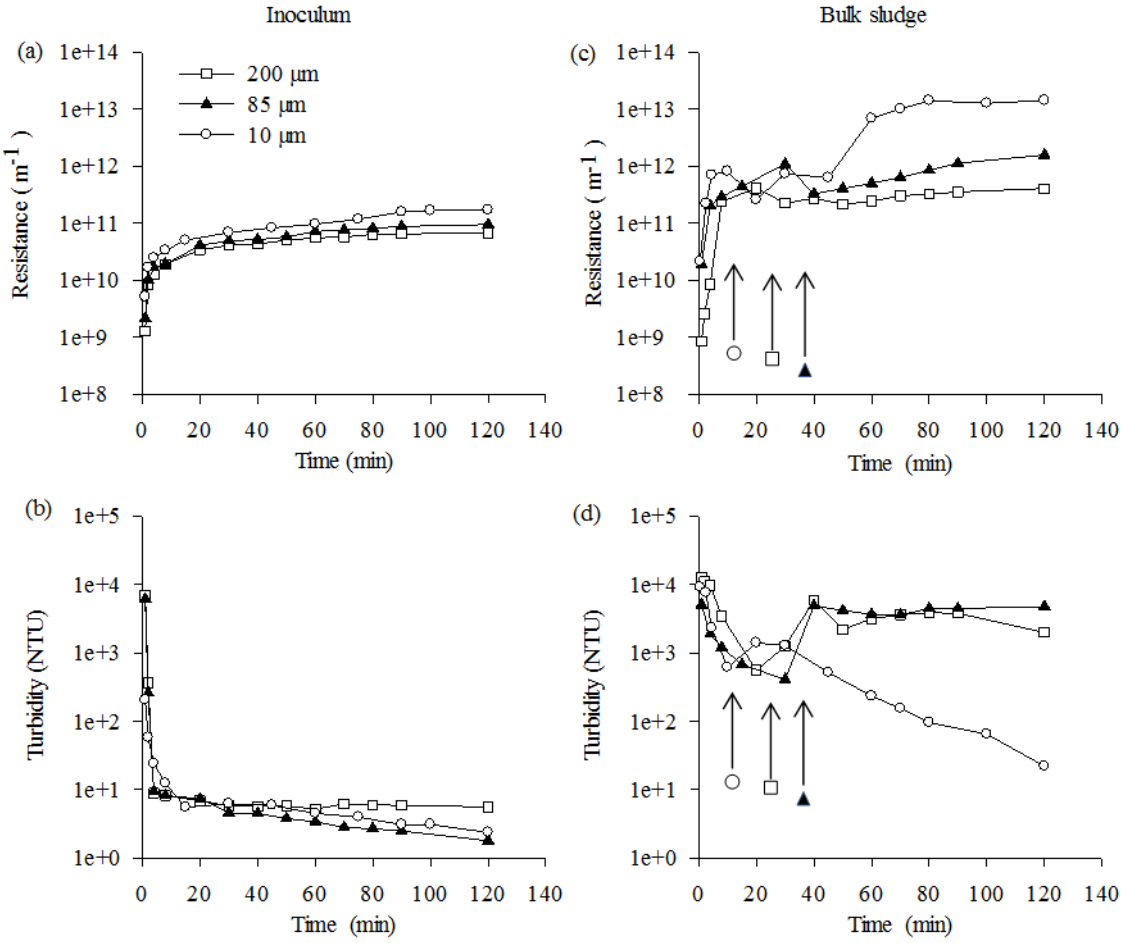

Figure 4.6 Results of short-term gravity driven filtration tests: resistance (a, c) and turbidity (b, d) profiles for initial inoculum and for bulk sludge, respectively. Arrows indicate when the peristaltic pump was switched on (second experiment only) to increase membrane flux; circle, square and triangle below the arrows stay for mesh of 10, 200 and 85 µm, respectively.

As a consequence of incomplete nitrification, the effluent NO_3^--N concentration always remained below 160 mgN L^{-1} (Fig. 4.9) with average concentration of 86±6 mgN L^{-1}, showing a limited activity of nitrite oxidising bacteria (NOB). The severe inhibition of NOB activity can be well explained by considering the free ammonia (FA) and free nitrous acid (FNA) concentrations inside the bioreactor as their presence above a minimum threshold was proved to be toxic for ammonia oxidising bacteria (AOB) as well as NOB (Anthonisen et al., 1976). These authors reported the inhibitory concentrations for FA and FNA for NOB bacteria ranged from 0.1 to 150 mgN L^{-1} and 0.2 to 2.8 mgN L^{-1}, respectively. Similar inhibitory concentrations have also been confirmed by other authors (Kim et al., 2006; Zhou et al., 2011). Fig. 4.10 shows that inhibition of NOB was triggered by FA concentrations since this compound was mostly above the highest toxicity limit (according to Anthonisen et al., 1976) while FNA was only occasionally higher than the toxicity concentration.

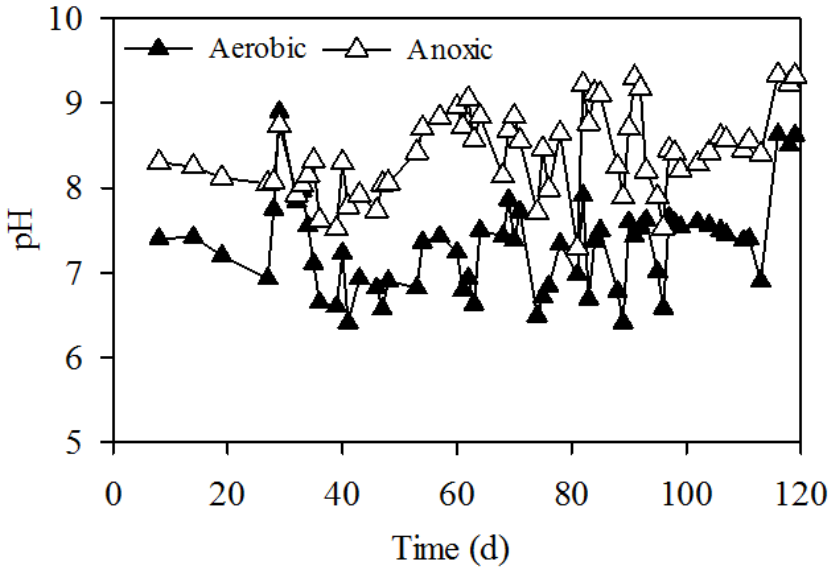

Figure 4.7 Observed pH profile inside the aerobic and anoxic tank

Even though denitrification via the nitrite rout could have offered considerable cost savings in terms of organics and aeration requirements (Peng et al., 2008; Spagni and Marsili-Libelli, 2010), the denitrification performance and, thus, total nitrogen removal was rather poor (Fig. 4.7b). Average total nitrogen removal after 20 days of continuous bioreactor operation and before the addition of supplemental organics was only 25±3.0 %. Moreover, the gradual addition of external organics did not bring significant improvement in the denitrification performance (Fig. 4.7b). Furthermore, it can also be inferred that the contribution of heterotrophic denitrification in total TOC removal was very limited and a large fraction of TOC was removed in the oxic tank instead of anoxic one (data not shown).

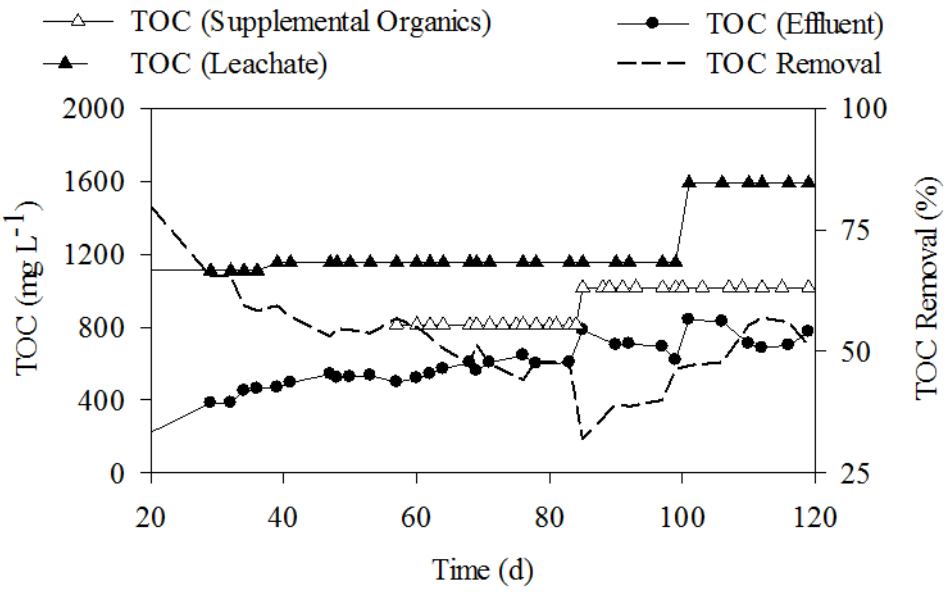

Figure 4.8 Influent and effluent TOC profiles and TOC removal performance.

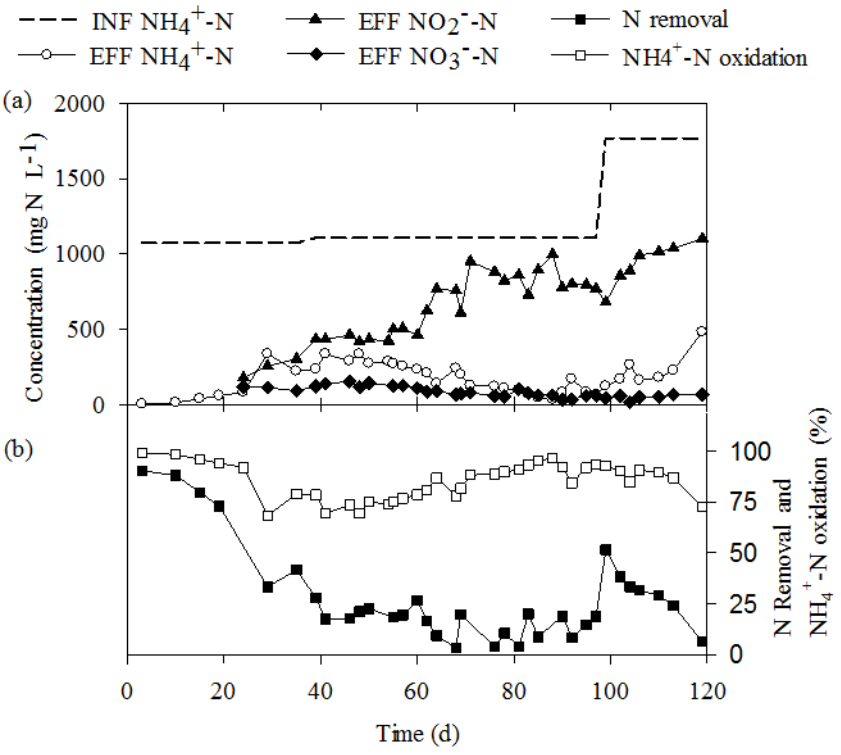

Figure 4.9 (a) influent (INF) and effluent (EFF) ammonia and effluent nitrite and nitrate concentration; (b) ammonia oxidation and nitrogen removal performance

Zhou et al, (2011) summarised the results of several studies done on determining the toxicity threshold of FNA concentration on denitrification activity. Depending upon the microbial community structure and operating conditions (pH and temperature etc.), FNA concentration as low as 0.01-0.025 mgHNO_2-N L^{-1} can initiate inhibition (up to 40%) while concentration up to 0.2

mgHNO_2-N L^{-1} was proved to be extremely toxic on denitrification activity. In this study, the observed FNA concentration in the anoxic tank ranged from 0.001 to 0.079 mgHNO_2-N L^{-1} and averaging around 0.011 mgHNO_2-N L^{-1} that might have contributed to the poor denitrification activity of the system (Fig. 4.7b). Galleguillos et al. (2011) evaluated the performance of a pilot MBR with a microfiltration membrane in treating stabilised LFL. The system exhibited high BOD and ammonia removal of 94% and 98% respectively; however, COD removal was rather low (approx. 40%) due to the high concentration of recalcitrant organics, confirming the results obtained in this study using a DM bioreactor.

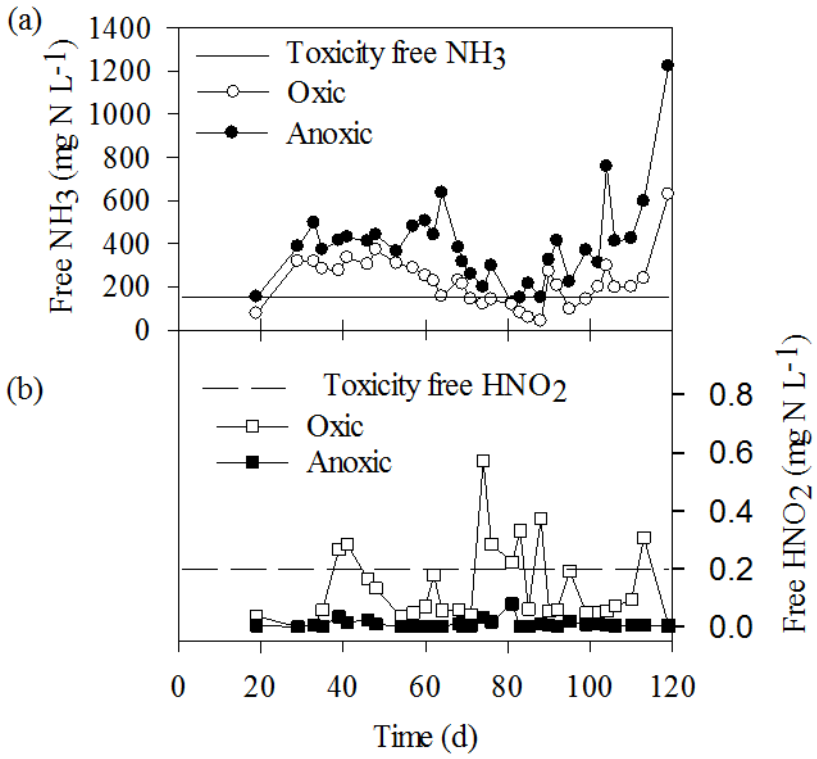

Figure 4.10 Free ammonia (a) and free nitrous acid (b) concentration and values of toxicity for nitrifying microorganism according to Anthonisen et al. (1976).

4.4 Conclusion

This study demonstrates the possibility of using DM developed over nylon meshes (10, 52, 85 and 200 µm) in a two stage anoxic/aerobic bioreactor for the treatment of stabilised LFL. The results show the change of the filterability characteristics of the bulk sludge due to the applied operating conditions and to the use of stabilised LFL. As a consequence, severe DM fouling was observed, which was characterised by very sharp increase in TMP. Moreover, DM solids retention was also deteriorated and the effect of mesh porosity on solid-liquid separation was heightened. Effective solids removal was achieved with the mesh with the smallest openings tested in this study of 10 µm, though at low permeate fluxes (approximately of 5 L m^{-2} h^{-1}). In this regard, among the four meshes

tested in this study, 52μm mesh showed to be a reasonable compromise in terms effluent turbidity and achievable operating fluxes.

The bioreactor achieved organics removal similar to values reported in literature for conventional MBR systems. Even though bioreactor exhibited high ammonia oxidation, the increased concentrations of free ammonia (FA) and free nitrous acid (FNA) inside the system severely affected the nitrification and denitrification performance that resulted in high nitrite accumulation.

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CHAPTER 5

A LAB-SCALE STUDY ON DYNAMIC MEMBRANE BIOREACTOR (DMBR); PERFORMANCE EVALUATION AND COMPARATIVE ASSESSMENT WITH CONVENTIONAL MBR FOR THE TREATMENT OF OLD LANDFILL LEACHATE

Abstract

This study was undertaken to evaluate the performance of a lab-scale submerged dynamic membrane bioreactor coupled with a pre-anoxic and post-aerobic bioreactor treating stabilized landfill leachate (influent NH_4^+-N concentration 1770 to 1730 mgL^{-1}) under ambient temperature conditions. The bioreactor was fed with gradually increasing concentration of landfill leachate mixed with tap water starting from 20% leachate to 100% leachate during 220 days of continuous bioreactor operation. The development of dynamic membrane was expedited by applying high fluxes under gravity driven filtration mode followed by constant flux filtration to avoid biomass loss during the formation stage of dynamic membrane. The results suggested that the filtration behavior of the sludge was significantly affected by the change in the characteristics of the feed wastewater (i.e. from municipal wastewater to stabilized landfill leachate) and increase in LFL concertation ($p < 10E-7$), resulting in higher fouling rate, deteriorated effluent quality and frequent DM cleaning. Under these conditions solid-liquid separation performance of a 52 µm mesh out performed 200 and 85 µm meshes, achieving greater than 99% solids retention corresponding to turbidity values less than 10 NTU. The system exhibited an unvarying nitrification performance with an average NH_4^+-N conversion efficiency of 98.97±0.2% regardless of gradually increasing LFL concentration in the feed due to the successful enrichment of slow growing nitrifying species by dynamic membrane. The steady state total nitrogen removal was increased up to 98 % corresponding to a nitrogen loading rate of 0.36 $kg\text{-}Nm^{-3}d^{-1}$. Episodes of poor denitrification were

found to be related with high NH_4^+-N concentration inside the anoxic tank (above 200 mg L^{-1}) which was effectively controlled by increasing recirculation flux from 4 to 6 times of the effluent flow for facilitating further NH_4^+-N oxidation in the aerobic tank. Finally, in comparison to conventional membrane bioreactor technology, dynamic membrane bioreactor can also accommodate large variations in operating parameters including influent feed composition and loading rates and thus it can guarantee long term stable bioreactor operation with high effluent quality

Manuscript to be submitted to a scientific journal.

5 A lab-scale study on dynamic membrane bioreactor (DMBR); Performance evaluation and comparative assessment with conventional MBR for the treatment of stabilized landfill leachate

5.1 Introduction

Sanitary landfilling still accounts for managing almost 60% of the total solid waste generated all over the global (Hoornweg and Bhada-Tata 2012; Fudala-Ksiazek et al., 2016). At the European level, despite being placed at the bottom of the waste management hierarchy (2009/98/EC), landfill disposal of waste represents the ultimate destination for nearly 25.3% of the total non-hazardous Municipal Solid Waste (MSW) produced within the 27-member states of the European union (Eurostat., 2017). An inevitable consequence of MSW landfilling is the production of landfill leachate (LFL) and its management represents a critical factor for the landfill environmental footprint in a life-cycle perspective (Manfredi., 2009). LFL is the wastewater resulting from rainwater percolation through a landfill body combined with the inherent moisture of the waste mass. Enriched with substances coming from the degradation of the waste material (including biodegradable and refractory organics, ammonia and organic nitrogen, heavy metals and xenobiotic organic micropullutants) (Kjeldsen et al., 2002; Kurniawan et al., 2006; Wang et al., 2016), LFL poses an imminent threat to the immediate environment and human health if not managed or treated properly (Fatta et al., 1999; Hashisho and El-Fadel., 2016; Kurniawan et al., 2006). Arising from the nature of these risks, LFL treatment and management is subjected to increasingly stringent treatment standards (Robinson., 2017). Another challenging aspect of leachate treatment is to cope with the variability in its quality and quantity in both space and time arising from waste quality and landfill age, the climatic and hydrological conditions and landfill design (Heyer and Stegmann 1998; Renou et al., 2008).

In this regard biological treatment methods are by far the most widely applied, owing to their relatively low cost and environmental impact (Stegmann et al., 2005). Biological methods exploit the capability of selected microbial species to remove specific targeted contaminants, such as biodegradable organics and ammonia (Wiszniowski et al., 2006). However, due to their sensitivity to rapidly changing environment or sudden shock loading conditions, their effectiveness decreases in treating older LFL containing higher refractory organics content, such as humic and fulvic compounds (Heyer and Stegmann 1998; Ahmed and Lan, 2012) . It is therefore, recommended that LFL treatment strategy should evolve with its age in order to be consistent in meeting effluent quality requirements at varying LFL characteristics (Alvarez-Vazquez et al., 2004). For this reason, most modern LFL treatment strategies uses a hybrid treatment scheme including biological

treatment (either in aerated lagoons, activated sludge systems, anaerobic digesters or rotating biological contactors) and physico-chemical treatments (such as flocculation/precipitation, chemical oxidation, adsorption or reverse osmosis) (Ahmed and Lan, 2012; Hashisho and El-Fadel, 2016; Heyer and Stegmann 1998,; Renou et al., 2008).

During the past few decades membrane bioreactors (MBRs) (a combination of biological treatment and membrane filtration) came up as an advanced treatment technology for treating numerous wastewater streams (Judd, 2016), including highly concentrated industrial wastewaters and especially challenging, the LFL (Lesjean and Huisjes, 2008; Ahmed and Lan, 2012; Judd, 2016). Besides providing an exceptional effluent quality and flexibility in operation, MBR offers several other advantages including smaller footprints, enrichment of specialized, slowly-growing microorganisms and low -to virtually no - excess sludge production (Ahmed and Lan 2012).

MBRs have been observed to address more effectively the biological removal of organics and nitrogen even for stabilized LFL while successfully accommodating varying loading conditions (Alvarez-Vazquez et al., 2004; Hashisho et al., 2016; Sadri et al., 2016 Hashisho and El-Fadel., 2016). Explanations suggested that the capability of the membranes (ultrafiltration and microfiltration) in retaining high amount of biomass, extracellular enzymes and biological oxidants helps to create a biological environment capable of degrading even the slowly degradable organics (Ahmed and Lan, 2012). However, few studies have reported limited organics removal because of the presence of refractory organics in elevated concentrations in stabilized LFL (Galleguillos et al., 2011). Similarly, MBR performance in ammonia removal via separate or simultaneous nitrification-denitrification route have shown promising results, achieving very high total nitrogen removal up to 95% in both the pilot and lab-sale setups (Litas et al., 2012; Nuansawan et al., 2016).

However, fouling has been perceived as a major obstacle while using membranes in wastewater treatment (Judd, 2016; Meng et al., 2009) which becomes even more challenging when dealing with LFL (Ahmed and Lan, 2012; Ahmed et al., 2007; Gkotsis et al., 2014; Kaewmanee et al., 2016). Moreover, excessive amount of humic and fulvic acids present as a main component in LFL, demonstrated to aggravate membrane fouling (Sutzkover-Gutman et al., 2010).

In this perspective dynamic membrane (DM) offers a cost effective alternative to conventional MBR. The idea exploits a purpose-built and regenerative fouling layer as a mean of solid-liquid separation medium instead of MF or UF membranes (Ersahin et al., 2016). For this purpose, cheaper materials such as filter clothes, non-woven or woven meshes (Alepu et al., 2016; Saleem et al., 2017) have been used as an underlying support materials to develop DM. The DM layer formed inside the biological systems is usually composed of a gel layer, made of a mixture of colloidal

matter featuring Extracellular Polymeric Substances (EPS) and Soluble Microbial Products (SMP) as major foulants, and a cake layer made of larger sludge flocs (Wang et al., 2011; Zhang et al., 2014). Among the advantages offered by DMs, its reproducibility and low maintenance, high flux (Ersahin et al., 2012) operation at low transmembrane pressures (TMP) and lower energy consumption as compared to conventional membranes (Alibardi et al., 2014; Zhang et al., 2014) are the most addressed. Majority of the studies on DM bioreactors are bench-scale applications, the most utilized substrate was either synthetic wastewater (Alibardi et al., 2016, 2014a; Ersahin et al., 2016a; Saleem et al., 2016) or municipal wastewater (Hu et al., 2016; Liu et al., 2009; Y. Xiong et al., 2016; Zhang et al., 2010) and very few studies have been focused on its application in LFL treatment.

Dong et al, (2007) used a single-chamber, tubular DM bioreactor to treat LFL. They obtained very low effluent turbidity (below 1 NTU) and low effluent NH_4^+-N concentration (with about 98% NH_4^+-N conversion efficiency). Xie et al, (2014) treated a medium aged landfill leachate mixed with synthetic wastewater using an anaerobic dynamic MBR equipped with a 40 µm mesh. Besides observing a moderate COD removal of around 62%, the authors have reported a very limited ammonia nitrogen removal. Similarly, they concluded that even though the solids retention of DM was not comparable to conventional membranes, it was better than conventional anaerobic treatment systems.

Studies on the application of DM in treating stabilized LFL are far being very limited due to which the knowledge of their performance while treating LFL is scarce. Therefore, this study was focused on evaluating the formation and performance of DM while integrating it with a pre-anoxic and post-aerobic treatment scheme for the treatment of stabilized LFL. The behaviour of DM was thoroughly evaluated over the period of bioreactor operation in conjunction with the effect of change in feed characteristics and operating parameters. Furthermore, this study also provides a comparative performance assessment of the system investigated in this study with the results reported in literature for conventional MBR systems treating LFL.

5.2 Materials and methods

5.2.1 Experimental setup

DM filtration was performed in a laboratory scale, continuously mixed, dual chamber bioreactor made of 9 mm thick Plexiglas. The experimental arrangement consisting of a pre-anoxic tank with a working volume of 0.9 L connected to a post-aerobic tank with a working volume of 2.5 L. The total working volume of the system was 3.4 L (Fig.5.1).

DM module was constructed by weaving polyamide nylon meshes over a perforated cylindrical plastic frame with an external diameter of 15 mm and a height of 70 mm. The openings (5mm X 3mm) of the supporting cylinder were uniformly distributed. The effective filtration area of the DM support was approximately 61% of the total surface area of the cylindrical frame measuring around 0.0019 m^2.

Two different commercial polyamide nylon meshes (200 and 52 µm) (Table 5.1) were mainly tested during the experiment in order of their decreasing porosity, details of which are reported in table 1. During the first 24 days a single 200 µm nylon mesh was used, however, due to the changes in the filtration behaviour of the sludge inside the bioreactor as compared to the initial inoculum, it was decided to use a 52 µm nylon mesh instead of the 200 µm mesh on day 26 the continuous bioreactor operation. In between, filtration performance of an 85 µm mesh was also tested for only one day that was also found to be similar to the 200 µm mesh and thus a 52 µm mesh was used almost immediately. Finally, due to the concern of rapid DM fouling two identical 52 µm mesh membrane modules were used in a parallel in the later stages of the experiment to reduce filtration fluxes and thus DM fouling (Fig. 5.2). Filtration modules were submerged inside the aerobic tank under a constant hydrostatic water head of 8 cm. Permeate extraction and thus the required filtration fluxes were controlled by means of a peristaltic pump connected with the filtration modules (Watson Marlow SCI 400) (Fig. 5.1a).

Bioreactor's hydraulic was controlled by peristaltic pumps (Watson Marlow SCI 400) connected at different location within the system. For the purpose of maintaining a constant active working volume inside the system, the peristaltic pump feeding the leachate to the anoxic tank was connected to a level sensor placed within the anoxic tank. Similarly, recirculation from the anoxic to the aerobic tank was provided by another peristaltic pump and the recirculation flow was maintained up to 4 to 6 times of the effluent flow. The two bioreactors were kept completely mixed by placing them over magnetic stirrers (Komet Variomag Maxi), rotating at a constant rate of 300 rpm for the anoxic tank and 400 rpm for the aerobic tank, in order to facilitate oxygen transfer in the aerobic tank and to avoid the same in the anoxic tank.

5.2.2 Inoculum and feed

The bioreactor was fed with aerobic sludge as initial inoculum taken from the aerobic tank of a conventional activated sludge process of a full-scale municipal wastewater treatment plant located in Padova (Italy). The initial inoculum had a total suspended solids (TSS) concentration of 5.50 g L^{-1} and volatile suspended solids (VSS) concentration of 3.76 g L^{-1}. Excess sludge withdrawal was initiated after 45th day of bioreactor operation in order to see the effect of MLSS concentration and

SRT on DM fouling propensities (Saleem et al., 2017). Similarly, a small amount of sludge was also collected periodically for analytical procedures.

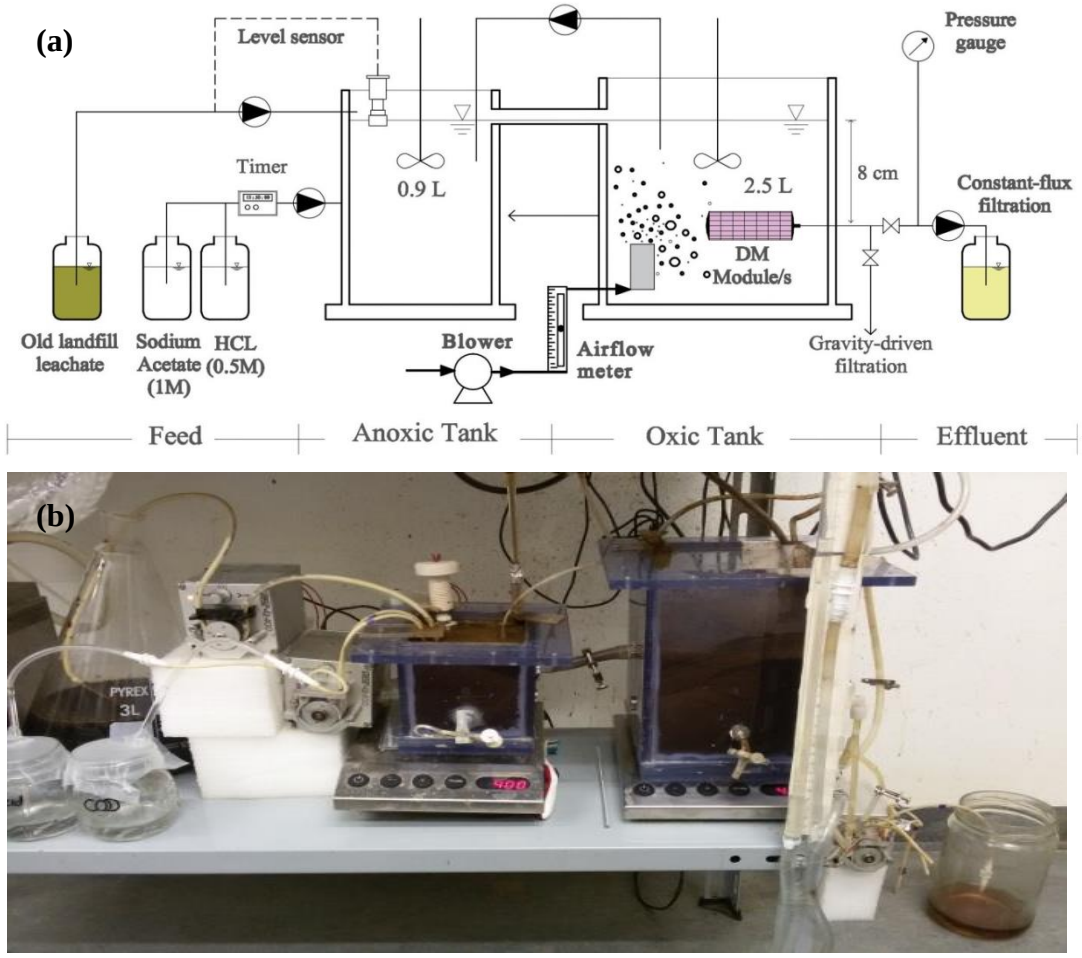
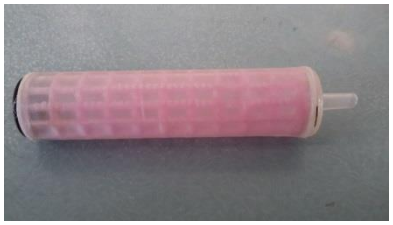


Figure 5.1 Schematic diagrams of the experimental setup

Table 5.1 Properties of the meshes used in this study

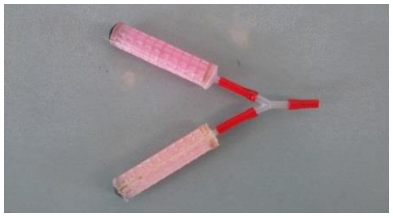
Product information	Mesh opening (µm)	Open area (%)	Mesh count (/cm)	Thread diameter (µm)	Resistance (Clean mesh) (1/m-1) (2)	Tap water permeability (L/m2.h-1.kPa-1) (3)
SaatiMil PA(1) 7	200	39	31	120	5.46×10^9	1572.3
SaatiMil PA 15	85	49	81	37	5.42×10^9	1583.2
Saatifil PA 52/32	52	32	110	38	5.61×10^9	1528.6

(1) PA is an acronym for polyamide
(2) Resistance of the mesh measured at TMP of 5 kPa
(3) 20 ^{o}C normalised permeability measured at TMP of 5 kPa



Day 1 – 24
1 membrane module
200 μm mesh
Filtration area: 0.0019 m^2

Day 26 – 74
1 membrane module
52 μm mesh
Filtration area: 0.0019 m^2



Day 75 – 220
2 membrane modules
52 μm mesh
Filtration area: 0.0038 m^2

Figure 5.2 Description of DM modules used during the experimental period; mesh porosity, effective filtration area and time interval of operation

The feed for the bioreactor was composed of raw leachate mixed with tap water in gradually increasing dilutions starting from 20% to 100% over the period of bioreactor operation. The strategy was adopted to provide sufficient time for biomass acclimatization and to observe the change on the filtration behaviour of the formed DM due to the change in the feed characteristics. Raw leachate was collected from a non-hazardous, municipal solid waste (MSW) landfill situated in northern Italy (Sant'Urbano, Padova), from one of the first sectors being filled on the site (waste disposal began in 1990). Based on the the age of the disposed waste, it can be regarded as around 25-year-old. Leachate was sampled twice: one collected in spring (L1) was fed to the reactor from day 1 to 137 and the one collected in autumn (L2) was used from day 137 onwards and to avoid further degradation samples were stored at 4 °C before use. The chemical characteristics of leachates L1 and L2 are summarized in table 5.2. The basic pH, low BOD_5/COD ratio, high content in reduced nitrogen forms (NH_4^+-N) and low content of oxidized nitrogen species (NO_2^- N and NO_3^- -N), are all coherent with the properties of stabilized LFL (Kjeldsen et al., 2002; Stegmann et al., 2005). Given the lack of bio-available organics in the raw leachate, supplemental readily biodegradable COD was supplied as carbon source to the anoxic tank to support heterotrophic denitrification in the form of tri-hydrated sodium acetate ($CH_3COONa \cdot 3H_2O$). No external nutrient solution was added to support biomass growth. 1 M Supplemental COD solution and 0.5 M HCL

solution for pH adjustment were added separately and simultaneously in the anoxic tank by using a 3-line peristaltic pump operating intermittently and controlled by an electronic timer. pH adjustment was necessary in order to avoid the build-up of toxic unionized ammonia (Anthonisen et al., 1976) because the alkalinity consumption in the aerobic tank (due to nitrification) was not sufficient enough to balance the combined alkalinity, produced in anoxic tank (due to denitrification) and alkalinity inflow from the leachate feed.

Table 5.2 Characteristics of the leachate samples

Parameter	Unit	Leachate 1	Leachate 2
TKN	mg $_{N}$ / L	1 780	1 760
NH_4^+-N	mg $_{N}$ / L	1 770	1 730
NO_3^--N	mg $_{N}$ / L	5	4
NO_2^--N	mg $_{N}$ / L	8	< 0.02
COD	mg $_{O2}$ / L	1430	1930
BOD_5	mg $_{O2}$ / L	190	230
BOD_5/COD	-	0.13	0.12
VFA	mg $_{CH3COOH}$ / L	336	332
P tot	mg $_{P}$ / L	9.6	10.3
TSS	mg / L	410	90
VSS	mg / L	360	76
pH	-	8.56	7.66
EC	mS /cm	15 .6	20.1
Alkalinity	mg $_{CaCO3}$ / L	14 583	9 944
Cd	µg / L	< 10	< 10
Cr	µg / L	753	235
Cu	µg / L	51.5	520
Fe	µg / L	3 860	4900
Mn	µg / L	172	214
Ni	µg / L	148	265
Pb	µg / L	< 10	< 10
Zn	µg / L	112	316

5.2.3 DM; Operation control and cleaning

DM cleaning was performed when the fouling become excessive and TMP values exceeded 15 kPa (set as the upper limit for TMP) or when the permeate flow (and thus membrane filtration fluxes) was less than 5 L m^{-2} h^{-1} (LMH) to maintain the desired HRT of the system below 10 d (chosen as an arbitrary upper limit for HRT). The cake layer on the top of the nylon mesh was manually removed with the help of a brush and solids were always returned to the aerobic tank. The reformation stage of DM layer after every cleaning operation could greatly compromise the effluent quality in terms of suspended solids removal (Alibardi et al., 2016, 2014). In order to avoid huge biomass loss during this time interval, DM formation was expedite by applying very high filtration fluxes (initially around 500 to 2000 LMH) under gravity-driven filtration mode. During this time a constant TMP of 0.78 kPa was provided by the hydrostatic water head maintained above the filtration modules. The quantity of the effluent collected during this time interval (usually less than 20 ml) was always returned to the aerobic tank. After the development of DM layer (within 2 minutes), characterised by the production of a "clear" permeate (visual inspection), constant flux filtration operation was resumed. The effectiveness of high filtration fluxes in rapidly developing the DM layer is well documented in lab-scale applications (Alibardi et al., 2014; Saleem et al., 2016), and the permeate recirculation has also been proposed as a strategy for full scale systems (Ersahin et al., 2012).

The feed rate of sodium acetate was adjusted based on the COD consumption by heterotrophic denitrification (both from nitrate and nitrite reduction and biomass yield on carbon source). During the experiment, the feed rate was regulated based on the analysis of residual TOC in the anoxic tank and in the effluent (data not shown) and any discrepancy between the measured values was always adjusted by increasing or decreasing the rate of sodium acetate addition to the anoxic tank. TOC was kept 10-20% higher than that required in the anoxic tank in order to avoid poor denitrification performance due to the lack of carbon source. It was also accepted that some additional carbon was also consumed inside the aerobic tank by aerobic heterotrophs, as long as it has little or no effect on the oxygen availability for ammonia oxidation.

5.2.4 Analytical methods and equipment

The bioreactor performance was assessed by analysing both physical and chemical parameters of the system. All the analytical procedures were based on Standard Methods (APHA, 2005). Ammonia nitrogen, nitrates and nitrites were periodically measured on the filtered samples (0.45 μm Polytetrafluoroethylene membranes) taken from the two compartments and from the effluent. All dissolved nitrogen species were analysed with UV-visible spectrophotometer (Shimatzu UV-

1601). Mixed liquor suspended solids and volatile suspended solids concentrations (MLSS and MLVSS) were periodically measured to assess biomass growth inside the system. Dissolved organic content in the influent, effluent and inside the bioreactors were measured as TC and DOC by a Shimadzu TOC-VCSN analyser.

The study was performed at ambient temperature, measured by using an electronic thermometer (Hanna Checktemp °C). Transmembrane pressure (TMP) was measured by means of a U-shaped manometer having water as a manometric fluid. Trans-membrane pressure (TMP) was then measured as the level difference between the two limbs of the U shaped manometer pipe, plus the constant hydrostatic head of 8 cm maintained over the filtration modules throughout the experiment.

Daily pH measurements were performed for both the bioreactors by using an electronic pH meter (Crison GLP 22). Effluent turbidity, as a measure of DM solids retention performance, was also measured with the help of (Hach 2100p iso Turbidimeter). Average daily fluxes from DM modules were estimated by dividing the volume of the filtrate collected from the filtration modules by the total filtration area of the modules.

5.3 Results and Discussion

5.3.1 Dynamic membrane formation, filtration behaviour, performance and Cleaning

The bioreactor operation was initiated with the formation of DM by applying high filtration fluxes in order to expedite the formation of the cake layer over the 200 µm mesh (as discussed in Section 5.2.3). The rapid formation of the DM layer (within 10 minutes) was characterised by the absence of suspended solids in the effluent (visual observation), resulting in an improved effluent quality of turbidity less than 10 NTU (data not reported). Saleem et al, (2017, 2016) have also reported similar results obtained under batch and continuous modes using anaerobic sludge. The TMP profile for the complete bioreactor operation showed a shift in the trend of rise in TMP (Fig. 5.2). A short-term initial start-up phase (lasting for 12 days) characterised by slow and gradual rise in TMP and low effluent turbidity values (Fig 5.2a) followed by a second, much longer phase with very sharp increase in TMP and lower filtration fluxes (Fig. 5.2b and 5.2d).

The initial filtration behaviour observed up to 12 days could be well defined by the local flux theory proposed for conventional membranes (Cho and Fane, 2002). This behaviour is attributed to slow and gradual rise in TMP followed by a gradual reduction in the filtration flux (Fig. 5.2a and 5.2c). Recent studies on DM filtration performance have also confirmed this observation under aerobic and anaerobic conditions (Li et al., 2011; Hu et al., 2016; Xiong et al., 2016). During the first 12 days the rise in TMP was very slow and TMP did not rise above 0.784 kPa up to first few days until

it was increased above 4.0 and 21.0 kPa before the first and second cleaning cycle respectively (Fig. 5.3). After first 12 days the filtration behaviour of the MLSS inside the aerobic tank was changed and DM fouling was intensified followed by a very sharp rise in TMP, subsequent reduction in filtration fluxes (Fig. 5.2a and 5.2c), demanding more frequent membrane cleaning (Fig. 5.3). In the meanwhile the solids retention performance of the DM layer formed over 200 µm was also deteriorated causing high effluent turbidity in the effluent up to 1000 NTU (Fig. 5.2a).

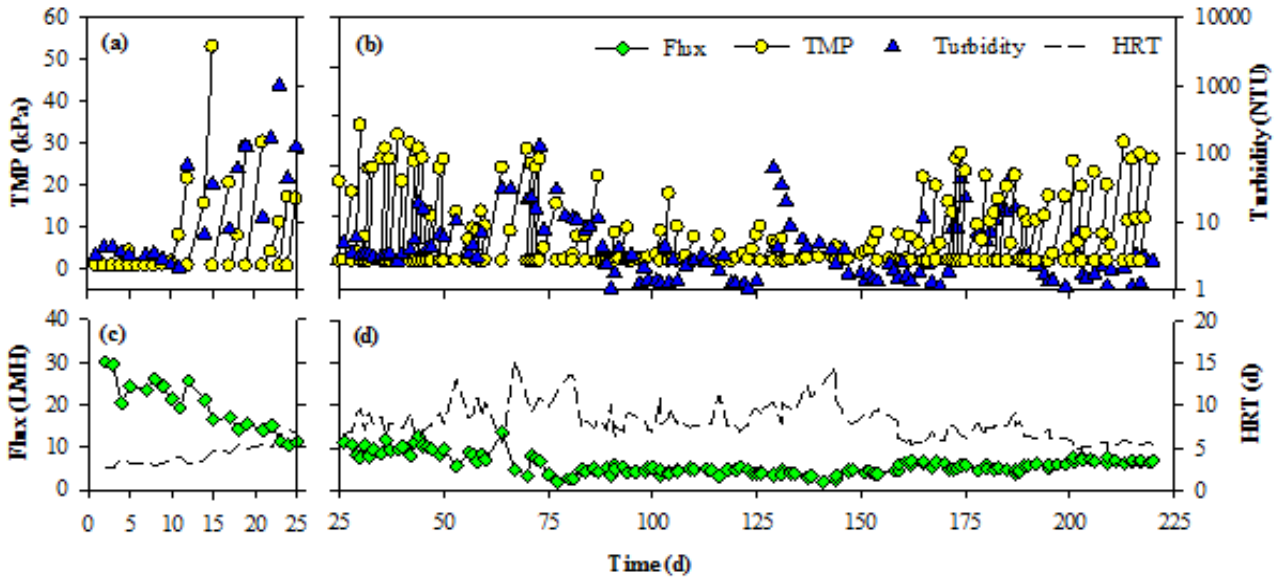


Figure 5.3 Observed TMP and turbidity profiles for (a) 200 µm mesh, (b) 52 µm, (c) Observed HRT and filtration flux profiles for 200 µm mesh and (d) 52 µm mesh

Although DM is a fouling layer and rapid fouling should have helped to improved its solid-liquid separation performance however, the rapid rise in TMP in a short time interval would have exerted pressure high enough to rupture newly formed DM and consequently deteriorating the effluent quality (Alibardi et al., 2014; Salerno et al., 2017). Henceforth, DM formation and its solids removal performance for 200 µm mesh could not be revived to its previously observed state during the first 12 days of the system start-up. In fact, the results reported here are peculiar because the change in the filtration behaviour of DM observed in this study as the experiment progressed was inconsistent with the invariable behaviour of DM previously reported in scientific literature despite having different operating conditions (Ersahin et al., 2012; Rezvani et al., 2014; Salerno et al., 2017; Zhang et al., 2014).

In order to understand the shift in the filtration behaviour of the MLSS inside the bioreactor, an inference can be drawn from the studies on conventional membranes that have evaluated the role of different operating conditions on membrane fouling. In this regard, type of MLSS (aerobic or anaerobic) reported to have contradictory, yet significant effect on fouling propensities (Spagni et

al., 2010; Y. Xiong et al., 2016; Yurtsever et al., 2015). Besides that, Robles et al, (2012) reported that the biofoulants produced as a result of biomass activity was the main reason of fouling, irrespective of the operating conditions applied in both aerobic and anaerobic MBRs. Similarly, it has also been reported that excessive fouling in DMs is significantly affected by the amount of biofoulants produced during the biological process involved (aerobic or anaerobic) (Hu et al., 2016; Liang et al., 2013; Yu et al., 2015). Furthermore, LFL is also rich in organic foulants (humic and fulvic substances) that must have contributed to further aggravate fouling (section 5.1). Based on this discussion the change in the filtration behaviour of the sludge can be attributed to the change in the feed characteristics (i.e. from municipal wastewater to stabilized LFL), as other operating conditions were unchanged during this time.

In order to avoid further loss of biomass through the effluent it was decided to use lower mesh pore sizes. From day 24 to 26, an 85 µm mesh was tested instead of 200 µm mesh however, due to no considerable improvement in the effluent quality (Fig. 5.2a) it was replaced by a 52 µm mesh on day 26 of the continuous bioreactor operation (Fig. 5.2b). The use of lower mesh porosity (i.e. 52 µm) greatly helped to improve effluent quality in terms of turbidity values averaging less than 10 NTU except for few occasions (Fig. 5.2b). The solids retention corresponding to turbidity values less than 10 NTU was greater than 99% (data not reported) and it was recorded for more than 80% of the observations. The excellent solids retention of the formed DM was comparable to the values found in the effluent of conventional membrane bioreactor (Hosseinzadeh et al., 2013; Naghizadeh et al., 2011)

However, due to lower particle cut-off of 52 µm mesh DM fouling was aggravated that resulted in more rapid rise in TMP (Fig. 2b) and thus entailed more frequent DM cleaning between day 30 to to 75 (Fig. 5.3). Another important reason for performing frequent DM cleaning was to lower the HRT of the system that was increased from 3.2±0.1 d to 10±1.7 d (Fig. 5.2c and 5.2d) during first 75 days of bioreactor operation. To overcome this issue the effective filtration area of the mesh was doubled on day 75 due to which the average HRT of the system was reduced to 8.9±0.3 d (Fig. 5.2d) and DM cleaning frequency reduced to almost half (or even lower) up to day 150 of the experiment (Fig. 5.3). Later on in the experiment the HRT of the system was further reduced up to 6.3± 0.14 d by increasing the fluxes to almost 28% (from 4.7 to 6.0 LMH) (Fig. 5.2d). During this time period, DM cleaning frequency first increased due to the increase in the filtration fluxes. However, after 195 days of continuous bioreactor operation the limit of the maximum allowable TMP observed before every cleaning operation (arbitrary value) was increased from 20 to 30 kPa due to which DM cleaning frequency started to reduce (Fig. 5.3). These upper limits for TMP were

only set to avoid biomass loss at high TMPs due to the expected rupture of the formed DM under excessive pressure (Alibardi et al., 2014). Nevertheless, contrary to the speculation the formed DM not only sustained the high TMP but also this strategy allowed more time between two successive DM cleaning intervals without any deterioration in the effluent quality (Fig. 5.2b). From the TMP and flux profiles shown in figure 5.2b and 5.2d after day 150, it can also be observed that unlike conventional MBR, underlying support of 52 µm did not encounter any irreversible fouling after physical cleaning because the flux values could almost completely be recovered for the similar rise in TMP. In fact, unlike conventional membranes (Table 5.3), no chemical cleaning was ever performed during 220 days of continuous bioreactor operation.

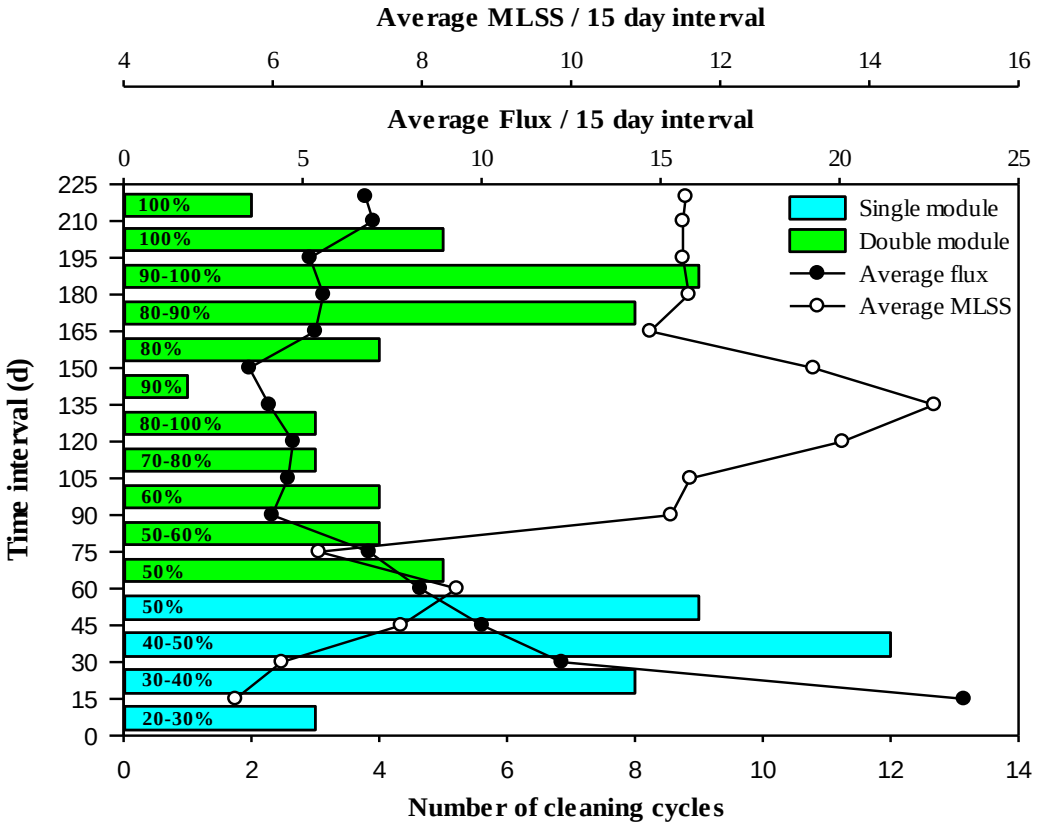


Figure 5.4 Evolution of the mesh cleaning frequency along the experimental period of 220 days (single and double module). Evolution of average MLSS concentration inside the aerobic tank and resulting average filtration flux observed over 15 day time interval

The formed DM in this study exhibited an excellent solid retention performance except for a limited time interval while using 200 µm mesh between 15 to 25 days. During the first 60 days no excess sludge was drawn from the system except for the small amount loss in the effluent or collected for sampling due to which the SRT of the system was theoretically infinite. However, excess sludge was withdrawn from the bioreactor to adjust SRT around 10 days. Thereafter, no sludge was withdrawn from the system up to 135 day to allow enrichment of slow growing nitrifying species to

coup-up with gradually increasing concentration of the LFL in the feed (Fig. 5.3). During this time the MLSS content increased to more than 15 g L^{-1} in the aerobic bioreactor without any effect on the excellent solid-liquid separation performance of DM, that was in fact, comparable to conventional MBRs (Judd, 2016). Finally in order to reduce excessive DM fouling associated with high MLSS concentration (Saleem et al., 2017) once again the SRT was gradually reduced and kept up to 10 d till the end of bioreactor operation (Fig. 5.3). Statistical analysis showed that MLSS concentration has a significant effect on DM cleaning and it has an inverse and moderate correlation (r_p = -0.623, p = 0.0016 < 0.05) with DM cleaning similarly, filtration fluxes also found to have a moderate positive effect (r_p = 0.666) on the cleaning frequency. Besides these operating parameters (MLSS, Flux and TMP), gradually increasing concentration of influent leachate in the feed found to have a significant effect (p < 10E-7) (Fig. 5.3) on fouling rate and thus on DM cleaning frequency in this study. Although the concentration of foulants (humic and fulvic acids) was not measured during this study however, their presence in stabilized LFL in high concentrations (Sutzkover-Gutman et al., 2010) and their role in membrane fouling (Mariam and Nghiem,. 2010) has been studied and hence, their effect on fouling rate in DM filtration cannot be overlooked.

5.3.2 Ammonia conversion and total nitrogen removal performance of the system

During the entire bioreactor operation, the average total nitrogen removal was around 78.4±2.6% except for first 20 days due to the technical problems that causes the blockage in the recirculation system (Fig. 5.4). As the proportion of the leachate in the influent was gradually increased from 20% to 100% over 220 days of bioreactor operation, the nitrogen loading rate (NLR) also increased from 0.05 to 0.36 kg-$Nm^{-3}d^{-1}$ (Fig. 5.4). The system showed an excellent nitrification performance with an average NH_4^+-N conversion efficiency of 98.97±0.2% (Fig. 5.4b). The major product of nitrification was always NO_3^--N which shows a fluctuating profile while effluent NH_4^+-N and NO_2^--N concentrations averaged around 11.4±1.9 and 19.4±3.2 mg L^{-1} respectively (Fig. 5.4a). The fluctuations in the effluent NO_3^--N concentration was usually followed by the subsequent increment in the influent LFL concentration in the feed (Fig. 5.4a). In fact, after every increment, the amount of supplemental organics fed to the anoxic tank to cater for the demand of excess NO_3^--N produced was needed to be adjusted according to the consumption in the anoxic tank for denitrification (section 5.2.3).

On day 132 when influent LFL concentration in the feed was increased from 80% to 100%, poor denitrification was observed and soon after that effluent NO_3^--N concentration reached up to 465 mg L^{-1} on day 132 (Figure 4a). It was speculated that the sudden increase in NLR from 0.18 to 0.23 kg-$Nm^{-3}d^{-1}$ followed by a simultaneous increase in the NH_4^+-N concentration in the anoxic tank

above 250 mgL^{-1} (data not shown) could have inhibited the heterotrophic denitrifying bacteria. Anthonisen et al, (1976) have studied the inhibitory effect of free ammonia production caused by the combined effect of high NH_4^+-N concentration and pH inside the bioreactor on the nitrification and denitrification performance. Therefore, in order to increase the transfer of NH_4^+-N from the aerobic to anoxic tank, MLSS recirculation was increased from 4 to 5 times of the effluent flow. Moreover, on day 140 the influent NH_4^+-N concentration in the leachate was also reduced to 75% from 100%, and later on to 80% (Fig. 5.4a). These strategies greatly helped to resumed denitrification performance and total nitrogen removal increased above 90% (Fig. 5.4b).

The effect of increasing NLR on denitrification performance was evaluated by increasing the effluent fluxes on day 159 (Fig. 5.2d). It was found that even at NLR up to 0.25 $kg\text{-}Nm^{-3}d^{-1}$ (Fig. 5.4b), denitrification performance was not deteriorated and total nitrogen removal was maintained above 90% at a recirculation ratio of 5 times of the effluent flow (Fig. 5.4a). Since then the influent LFL concertation was again gradually increased up to 90% and the corresponding total nitrogen removal was still above 90% (Fig. 5.4a). However, when the reactor was fed with 100% LFL, the denitrification performance was again affected by the increase in the NH_4^+-N concentration in the anoxic tank from less than 100 mg L^{-1} to above 200 mg L^{-1}(data not shown). Once again the fast transfer of NH_4^+-N from anoxic tank to aerobic tank was facilitated by increasing the recirculation ratio up to 6 times of the influent flow. The increase in the recirculation ratio was followed by a gradual recovery of denitrification performance and effluent NO_3^--N concentration reduced to less than 15 mg L^{-1} from 482.2 mg L^{-1} within last 10 days of continuous bioreactor operation (Fig. 5.4a). The corresponding total nitrogen removal was increased up to 98% by the end of bioreactor operation on day 220 while the NLR also increased up to 0.36 $kg\text{-}Nm^{-3}d^{-1}$ (Fig. 5.4b). Interestingly, during this time interval the system showed almost invariably ammonia removal down to below 20 mg L^{-1} regardless of increase in influent NH_4^+-N concentration, provided that no inhibition in nitrification occurred (Fig. 5.4b) that shows the robustness and efficacy of DM in retaining high concentration of slow growing microorganisms, such as nitrifying bacteria.

5.3.4 Comparative performance assessment of conventional MBRs with DMBR treating LFL.

This section is an attempt to make a comparative performance assessment of recent researches performed on MBRs treating LFL with the results obtained in this study. To examine this, results related to (a) Membrane characteristics (total filtration area etc.) (b) operating parameters (fluxes, HRT, SRT and cleaning method and frequency) and (c) biological performance (total nitrogen removal and NH_4^+-N conversion efficiency) were compared with researches conducted in the last 10 years or so. Since supplemental organics was provided to support denitrification in this study

therefore, the organics removal performances was not evaluated and only total nitrogen and NH_4^+-N removal were compared and selected as biological performance indicators. Similarly, few studies have also applied pre-treatments and/or post-treatments methods however, the removal efficiencies of MBR was only compared by considering the feed characteristics entering into MBR only.

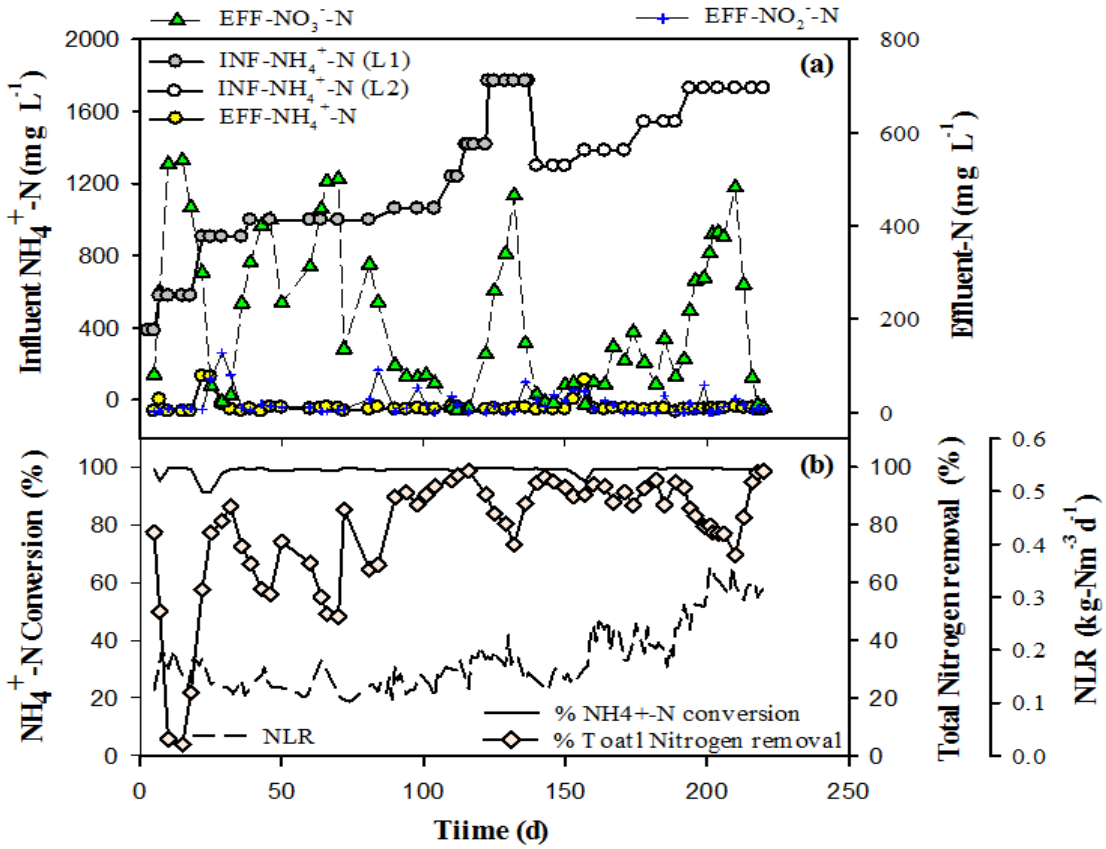


Figure 5.5 Total nitrogen removal and NH_4^+-N conversion performance of the bioreactor (a) influent (sample L1 and L2) and effluent NH_4^+-N and effluent NO_2^--N and NO_3^--N concentration profiles (b) % NH_4^+-N conversion, % total nitrogen removal profile and NLR profile along the bioreactor operation

While treating wastewater that aggravates fouling like LFLs, requirement for specific membrane area (m^2/m^3) becomes more prominent in order to curtail capital and management cost of the treatment plant and to maintain long term sustainable flux. The specific membrane area used in this study (0.56-1.12 m^2/m^3) is comparable to the ones reported for MBR (Table 5.3) with an exception of Tsilogeorgis et al, (2008), who however, have applied very low filtration fluxes in their study (Table 5.3).

Although the fluxes reduced greatly over the entire bioreactor operation in comparison to the fluxes obtained during the start-up of the bioreactor however, an average flux of around 6.4-7.7 LMH maintained during the last 20 days of bioreactor operation (100% influent LFL concentration in the feed). The average flux recorded was comparable with the average fluxes reported for conventional

MBR treating municipal wastewater (18.5 ± 4.8 LMH) and close to the range reported for MBR treating LFL (9–14 LMH) (Judd, 2016). Similarly, a review of the data presented in table 5.3 also shows the that the average filtration flux observed in this study is in very close proximity with the data reported in the literature for conventional MBRs (Table 5.3) with and exception of Kaewmanee et al., (2016) mainly due to the difference in leachate characteristics and origin (leachate from MSW collection trucks).

HRT and SRT are important parameters while deciding the biological performance and size of the biological system. Considering the high NH_4^+-N concentration in the feed leachate and low biodegradable organic content, the applied HRT in the last 20 days of this study (4.8-6.0 d) falls inside the wide range of HRT reported in table 5.3. It is important to mention that the recorded HRT in this study was not the lowest achievable HRT of the system under applied operating conditions and that, the system's HRT could also be reduced by increasing the effective filtration area of the DM. Ahmed and Lan, (2012) in their review, have also reported HRT between 3.6 h to 12.9 d for MBR treating LFL regardless of its age. Similar to conventional MBRs (table 5.3), the exceptional solid retention performance of DM observed in this study (discussed in section 5.3.1) allowed operating the system at virtually infinite SRT occasionally, giving it a marked distinction from conventional treatment systems (Ahmed and Lan, 2012; Judd, 2016).

The efficacy of conventional MBRs in treating LFL with high NH_4^+-N concentration is well established (Ahmed and Lan., 2012; Alvarez-Vazquez et al., 2004). In comparison, the NH_4^+-N removal performance of the DM assisted bioreactors as observed in this study is similar to majority of the studies reviewed in table 5.3, where over 90% NH_4^+-N removal efficiency were reported irrespective of the age of LFL. Furthermore, in this study the nitrification performance did not experience any inhibitory effect of high NH_4^+-N concentration, which is otherwise, a major concern while treating stabilized LFL (Ahmed and Lan., 2012). Usually in such conditions of high concentration of influent NH_4^+-N, MBR treatment is followed by a pre-treatment stage (like ammonia stripping) to reduce the NH_4^+-N concentration in the feed to MBR (Hasar et al. 2009; Wichitsathian et al). However, in this study, NH_4^+-N removal remained relatively constant over different feed concentrations, HRTs, and NLRs (section 5.3.2). Furthermore, in this study, DMBR NH_4^+-N removal efficiencies were relatively constant and high variations in effluent NH_4^+-N concentrations were also not observed, indicating the robustness in its ability to cope with variations in the feed and varying loading conditions. Besides, the steady state total nitrogen removal performance of the system has exhibited high removal efficiency (Table 5.3) despite high influent NH_4^+-N concentration, In fact the observed total nitrogen removal performance was even better

than what is reported by Nuansawan et al. (2016) (table 5.3) at almost similar NH_4^+-N concentration while treating even young LFL. Finally, an economically attractive aspect of using DM as an alternative of conventional membrane is the ease of cleaning they offer without the use of chemical treatment (Ersahin., 2014). Physical means, like water or air backwash, or simply brushing as performed in this study are usually employed. Although the cost of chemical cleaning used in conventional MBR is only 0.5-2% of total operating cost (Brepols., 2011) however, fouling in MBR is inevitable, which in many cases, require complete replacement of the membrane its self (Meng et al, 2009).

5.4 Conclusion

The results obtained in this study can be summarised under following main conclusions:

- The strategy of gradually increasing the concentration of LFL in the influent feed proved to be effective in maintaining steady performance of the system in order to avoid possible inhibition due to excess NH_4^+-N concentration. Similarly, high recirculation ratio also proved to be effective in order to overcome these conditions.
- Based on this discussion the change in the filtration behaviour of the sludge can be attributed to the change in the feed characteristics (i.e. from municipal wastewater to stabilized LFL), as other operating conditions were unchanged during this time.
- Due to which the effect of mesh porosity on solid liquid separation performance became prominent and solid-liquid separation performance of a 52 µm mesh out performed 200 and 85 µm meshes achieving greater than 99% solids retention corresponding to turbidity values less than 10 NTU
- Similar to conventional membranes, DM formed in this study achieved very high solids retention performance and showed a possibility of enriching slow growing bacteria like nitrifying bacteria.

The system achieved a total nitrogen removal up to 98% at the end of bioreactor operation similarly; it has also exhibited an unvarying NH_4^+-N conversion efficiency of 98.97±0.2% regardless of gradually increasing LFL concentration in the feed up to NLR of 0.36 kg-$Nm^{-3}d^{-1}$.

Table 5.3 Performance of MBR treating landfill leachate and details of operating parameters

Leachate Type (Landfill Location)	Reactor Type /Bioprocess	Feed Leachate Characteristics		MBR configuration			Operating and management parameters					Removal Performance		Reference
		COD (mgL^{-1})	NH_4^+ - N (mgL^{-1})	Volume (L)	Membrane configuration and type	Specific Filtration Area (m^2/m^3)	NLR ($kg\ m^{-3}\ d^{-1}$)	HRT (d)	SRT (d)	Flux (LMH)	Cleaning (frequency/mode/method)	NH_4^+-N removal (%)	TN removal (%)	
Young (MSW transfer station, Thailand)	Lab scale, pre- anoxic post aerobic	86460	2170 - 2690	30	Submerged, Hollow fibre	12.83	0,464 - 1,128	2,5 - 5	-	4,17 - 8,33	N.A.	77,4 - 87,3	78,3 - 86,4	Nuansawan et al. 2016
Young (MSW collection trucks, Thailand)	Lab scale, pre- anoxic post aerobic	8908 - 20657	82 - 201	6	Submerged, Hollow fibre	2000	0,094 - 0,255	1,00 - 1,67	-	13 - 21	Two modes of chemical cleaning: with chemical enhanced back flushing	62 - 96	69 - 95	Kaewmane e et al. 2016
Young, (Thailand)	Lab scale, pre- anoxic post aerobic	9389	105 - 174	-	Submerged, Hollow fibre	-	0,105 - 0,174	1	virtually infinite	-	-	83 - 91	-	Boonyaroj et al 2012
Old, (Germany)	Lab scale, Aerobic, anoxic tank	2200	1200	50	External, Tubular (UF)	-	0,169 - 0,411	2,9 - 7,1	100	-	-	90 - 99	-	Svojitka et al 2009
Old, (Greece)	Lab scale, SBR (anoxic, aerobic sequences)	1391 - 3977	310 - 509	5	Submerged, Hollow fibre (UF)	0.0094	0,031 - 0,051	10	virtually infinite	0.443	-	almost 100%	up to 88%	Tsilogeorgis et al 2008
Old mixed with synthetic WW (Greece)	Pilot scale, Intermittently aerated CSTR	1459 - 3898	194 - 347	300	Submerged, Plate and frame (MF)	-	0,022 - 0,039	9	virtually infinite	-	-	up to 99%	76 - 95	Litas et al. 2012

Young (Turkey)	Lab scale, Intermittent aeration CSTR	6300 - 7300	200 - 800	1.5	Submerged, Thin-film hydrophobic membrane	0.026	-	0,15 - 0,625	10 - 50	2,56 - 10,7	-	87 - 91	85 - 92	Hasar et al 2009
Young (transfer station, Thailand)	Lab scale, Aerobic MBR	4778 ± 1187	68 ± 26	22	Submerged, Hollow fibre	45	0,111 - 0,465	0,146 - 0,608	30	1,2 - 5,1	Water backwash automatically activated at the set point of 40 kPa TMP	55 - 88	56 - 85	Thanh et al. 2013
old and young, (from a landfill and a transfer station, Thailand)	Lab scale, aerobic MBR	1200	1000 - 1700	6	Submerged, Ceramic membrane module	6.67	1,0 - 1,7	1	virtually infinite	6.25	Pressurized water spraying, followed by chemical cleaning	60 - 75	60 - 75	Visvanathan et al. 2007
MSW with ~70% as organic food waste Beirut, Lebanon	Lab scale, pre- anoxic post aerobic	9000–11,000	1800–4000	-	Submerged, Hollow fibre (UF)	-	-	-	-	-	Air scouring and backwash for fouling control / chemical cleaning	96	95	El-Fadel and Hashisho, 2017
MSW landfill Manitoba, Canada	Lab scale, Aerobic stirred tank reactor	2737 - 4079	617 - 671	7.5	Submerged, Hollow fibre		1.3-8	1-3.5	30-60	-	Air scouring and backwash for fouling control	> 99	-	(Sadri et al., 2016)
Stabilized LFL, MSW landfill site (Italy)	Lab scale, pre- anoxic post aerobic	585-1154 (as TOC)	2225-2276	3.4	Submerged, Cylindrical dynamic membrane support (52 µm mesh)	0.56-1.12	0.05-0.36	4.8-6.0	10 days - virtually infinite	6.3 - 7.7	Manual cleaning with a brush 1 -12 times per 15 day time interval	up to 99	up to 98% at steady state	**This study**

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CHAPTER 6

BIOLOGICAL HYDROGEN PRODUCTION VIA DARK FERMENTATION PROCESS BY USING A SIDE-STREAM DYNAMIC MEMBRANE BIOREACTOR: STATE-OF-THE-ART AND PERFORMANCE EVALUATION

Abstract

The study manifested the possibility of using low cost dynamic membranes as a solid-liquid separation medium for fermentative H_2 production in three successive lab-scale experimental runs under mesophilic temperature conditions. For this purpose two commercially available polyamide-nylon meshes (52 and 21 µm) were used to develop dynamic membranes. The fouling behaviour of dynamic membrane was studied in response to the effect of change in the influent COD concentration, operating parameters and associated biological performance of the system. It was found that change in influent feed characteristics affected the filtration behaviour of the sludge due to which 52 µm mesh was replaced with a 21 µm mesh during the study for a better solid retention performance. However, the effect of influent COD concentration on solid-liquid separation performance was more prominent and it was found to be strongly and negatively correlated (ρ = -0.95) with dynamic membrane solids retention performance. High influent COD concentration (>30 g L^{-1}) and associated high organic loading rates tend to aggravate fouling in dyanimc membrane and favoured the accumulation of metabolites (volatile fatty acids and solvents), leading to the inhibition of biological activity. On the other hand, low influent COD (10-30 g L^{-1}) concentration allowed operating the system at lower HRT (<1 d), facilitating the removal of metabolites and keeping high biomass concentration inside the system up to 13 g L^{-1}. Under these conditions the bioreactor showed consistently improving biological performance with an unvarying carbohydrates conversion efficiency of more than 99% and a H_2 yield of 8 L H_2/mole of sucrose in the third experimental run. Furthermore, pneumatic internal cleaning mechanism supplemented with

backwashing precluded the need of chemical cleaning and was found to be very effective in restoring the permeability of excessively fouled dynamic membrane layer.

Manuscript to be submitted to a scientific journal.

6 Biological hydrogen production via dark fermentation process by using a side-stream Dynamic Membrane Bioreactor: State-of-the-art and performance evaluation

6.1 Introduction

The compulsive demand for alternative and more sustainable energy resources, arising from rapidly depleting fossil fuel reserves (Das and Veziroğlu, 2001) and the escalating environmental misdeeds caused by their intensive use has made hydrogen (H_2) as the most appealing fuel for future (Nikolaidis and Poullikkas, 2017). Furthermore, Its exceptional energy content (122 kJ/g) among other known fuels and negligible pollution content makes it the most desirable candidate among its compere (Pereira et al., 2017). At present, about 0.1 GT of H_2 is produced worldwide annually and according to the trends, its consumption is increasing by 6% per year (Nikolaidis and Poullikkas, 2017). However, according to Das and Veziroğlu, (2001) a major portion (nearly 90%) is still produced through processes that involves the consumption of fossil fuels or hydrocarbons (Decourt et al., 2014; Das and Veziroğlu, 2001) that have severe environmental consequences (Lee et al., 2009). In this perspective, biological H_2 production through dark fermentation process has gained much attention due to its potential as a sustainable alternative to the conventional methods for H2 production (Das and Veziroğlu, 2001). Unlike the chemical or electrochemical ones, the biological processes are catalysed by the enzymes produced by ubiquitous microorganisms at ambient temperature and atmospheric pressure and consequently, making the process even cheaper. In particular, bio-hydrogen ($bioH_2$) production through dark fermentation from wastes rich in biodegradable organics could attract various benefits such as waste minimization, waste utilization and also simultaneous energy generation (Hafez et al., 2009). The process is mediated by a wide range of microorganisms that have different preferences over type of substrate, pH and temperature (Wang and Wan, 2009). Due to the same reason and from a practical stand point mixed cultures of H_2 producing microorganisms are preferred over pure cultures especially in full-scale fermenters due to their robustness against the variations in environmental conditions and process parameters (pH, temperature, feed type and concentration etc.) (de Sá et al., 2013; Wang and Wan, 2008; Wong et al., 2014). However, mixed cultures also contain H_2 consuming microorganisms (hydrogenotrphic methanogens and sulphur-utilizing microorganisms) and thus, inoculum pre-treatment techniques using various physical (aeration, heat treatment, ultraviolet irradiation, ultrasonic and freezing/thawing) or chemical (acid base treatment or organic compounds) methods are

applied to eliminate these microorganisms and maximize H_2 yield (Bellucci et al., 2016; de Sá et al., 2013; Y.-Y. Wang et al., 2011).

Moreover, the process, in general is affected by several operating parameters, including influent substrate concentration, hydraulic retention time (HRT), solids retention time (SRT) and their effective decoupling to retain high concentration of microorganisms inside the system (Lee et al., 2009). As a matter of fact, high concentration of biomass has a direct impact on substrate conversion efficiency and thus on volumetric H_2 production at low HRTs (Hafez et al., 2009; Lee et al., 2007, 2006).

The advent of membrane assisted biological treatment has gain much attention in the past decades due to their peerless solid-liquid separation performance if compared with conventional continuous stirred tank reactors (CSTRs) (Judd, 2016). Application of MBR obviate the potential risk for biomass washout, producing effluent of very high quality, allowing considerable reduction in plant's footprint and provides flexibility in operation (Bakonyi et al., 2014). Similarly, the use of MBR in dark fermentation process has brought significant improvement in H_2 production efficiency over the CSTRs (Lee et al., 2009, 2007). Due to their high solids retention, HRT as low as 1 h and SRT as high as 450 days has been successfully applied without biomass washout and stable bioreactor performance (Bakonyi et al., 2014; Lee et al., 2008; Lee et al., 2007). However, despite the several benefits that MBR offers, fouling phenomena in MBR is the main barrier in its prevalence (Judd, 2016) – especially under anaerobic conditions (Ozgun et al., 2015; Spagni et al., 2010). Despite of extensive research dedicated on fouling control techniques including, physical (backwashing, membrane relaxing and gas sparging), chemical (NaClO, NaOH EDTA or ozone), and more recently evaluated, enzymatic treatment, fouling control still holds a challenging task in MBR application for biological H_2 production (Bakonyi et al., 2014).

Recently, the use of dynamic membrane (DM) has been proposed as an out-of-the-box and cost effective alternative to the use of conventional membranes. The idea of DM exploits fouling itself as a mean of solid-liquid separation medium acting as a secondary membrane instead of conventional membranes (Ersahin et al., 2017; Saleem et al., 2017, 2016). For this purpose, cheap underlying supports, such as filter clothes and meshes (Ersahin et al., 2012) have been used to develop DM layers. The main structural component of DM layer formed in bioreactors includes a preliminary gel layer, mainly composed of Extracellular Polymeric Substances (EPS) and Soluble Microbial Products (SMP), and a superposed cake layer made

up of larger sludge particles (Liu et al., 2012; Wang et al., 2011; Zhang et al., 2014). The most striking aspect of DM technology includes its reproducibility and operation under high filtration flux (Ersahin et al., 2012), low transmembrane pressures (TMP) and comparatively lower maintenance and energy consumption than conventional membranes (Alibardi et al., 2014; Zhang et al., 2014).

Recent studies on DMs were mainly focused on evaluating their performance using synthetic wastewater (Alibardi et al., 2016, 2014a; Ersahin et al., 2016; Saleem et al., 2016), municipal wastewater (Hu et al., 2016; Liu et al., 2009; Xiong et al., 2016; Zhang et al., 2010) and excess sludge digestion (Liu et al., 2016; Yu et al., 2016). Very few studies have been focused on its application in high strength, complex wastewaters such as landfill leachate (Dong et al., 2007; Xie et al., 2014) and textile industry effluents (Sahinkaya et al., 2017). Until recently, Park et al, (2017) have successfully enriched H_2 producing consortia in an external side-stream DM bioreactor. They reported a maximum H_2 production rate of 51.38 L $L^{-1}d^{-1}$ and hydrogen yield of 2.98 mol mol^{-1} glucose at an HRT of 3 h. However, the study lacks to provide any discussion regarding the solid retention performance of DM and its effect on biological performance of the system.

Therefore, this study was based on synergizing the benefits of bacteria mediated H_2 production and DM technology. The overarching aim of this study was to evaluate the formation and performance of DM while integrating it with an anaerobic fermentative bioreactor for the production of H_2 using an untreated mixed bacterial culture at mesophilic temperature conditions. During the entire study the behaviour of DM was thoroughly evaluated over the period of bioreactor operation in conjunction with the effect of change in the feed characteristics, operating parameters and biological performance of the system. Furthermore, a new manually operated pneumatic internal cleaning mechanism alongside of backwashing was also tested for the purpose of periodic cleaning of excessively fouled dynamic membrane layer in this study.

6.2 Materials and methods

6.2.1 Bioreactor setup and operation

The study was performed in a lab-scale, continuously mixed, anaerobic dynamic membrane bioreactor (AnDMBR), with a total volume of 1.0 L. The cube-shaped bioreactor (10 cm × 10

cm × 10 cm) was made of 10 mm thick Plexiglas. The effective working volume of the reactor was 0.8 L and 0.2 L free head space for biogas collection (Fig. 6.1).

DM filtration was performed in an external cross-flow configuration made up of a Plexiglas. The cross-flow filtration mode was carried out by using a peristaltic pump (Watson Marlow 403U/R1, Falmouth, Cornwall, UK) which circulated mixed liquor along the mesh surface and provided a sufficient cross flow velocity (CFV) of 30 m h^{-1} was maintained to avoid the possible sedimentation of the mixed liquor inside the external cross-flow module only while the effect of CFV on fouling control was not evaluated in this study.

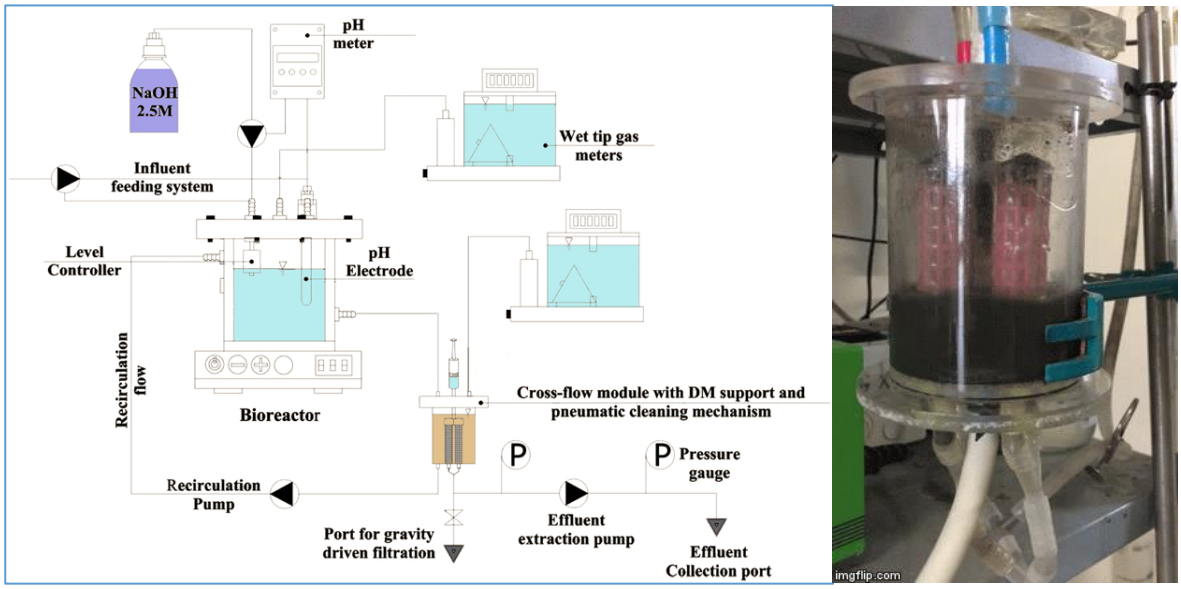

Figure 6.1 Schematic diagram of the experimental setup and external side-stream module coupled with internal cleaning system with 4 dynamic membrans supports

DM was developed over monofilament woven meshes made of polyamide/nylon submerged inside the cross-flow module. Two commercial nylon meshes (Table 6.1) were tested in the three phases of the experiment (three different start-ups) in order of decreasing mesh porosities 52 and 21 µm respectively. In the later phases of the experiment only 21 µm nylon mesh was used due to the concern of avoiding excess biomass loss from the effluent.

The DM modules were built by weaving nylon meshes over a cylindrical plastic frame (15 mm diameter and 60 mm height). Each cylinder had uniformly distributed rectangular openings (3 x 5 mm) and a total surface area of 0.003 m^2. The effective area to carry out filtration was approximately 57 % of the total surface area of the cylindrical frame measuring around 0.00162 m^2.

Table 6.1 Properties of the meshes used in this study

Product information	Mesh opening (µm)	Open area (%)	Mesh count (/cm)	Thread diameter (µm)
Saatifil PA 52/32	52	32	110	38
Saatifil PA 21/17	21	17	200	30

The constant working volume of the bioreactor was maintained by a level sensor directly connected to the influent peristaltic pump (Watson Marlow 401U/D1, Falmouth, Cornwall, UK) (Fig. 1). Similarly, effluent extraction was facilitated by a second peristaltic pump (Watson Marlow 401U/DM3, Falmouth, Cornwall, UK). Biogas production was monitored by using homemade wet-tip gas meters, directly connected on the anaerobic reactor and on the external cross-flow module to account for the biogas production from the different locations inside the system (Fig. 1).

The reactor was operated continuously at mesophilic temperature of 30.9 ± 1°C that was maintained by a thermostatic bath (IS Co. GTR 2000 "11x", Italy). Continuous mixing at 600 rpm was carried out using a magnetic stirrer (Variomag Maxi Direct, Thermo Scientific, Italy). The pH inside the the bioreactor was maintained between 4.9 to 5.1 using am automatic pH controller (Crison 28 carrying a pH probe Crison 53 35) connected to a peristaltic pump (Watson Marlow 401U/D1, Falmouth, Cornwall, UK) for dosing NaOH 2.5 M solution.

Pressure was measured with the help of a U shaped, manometer with water as the manometric fluid. Trans-membrane pressure (TMP), representing the pressure difference across the membrane, was then measured as the level difference between the two limbs of the manometer pipe plus the constant hydrostatic water head of 30 cm provided above the DM modules and maintained constantly throughout the experiment (Fig. 1).

6.2.2 Inoculum and feed

The bioreactor was fed with synthetic feed containing sucrose as carbon source at a concentration between 10 to 100 g COD L^{-1} during the first second and third phases of the

experiment. No inoculum pre-treatment was performed to inactivate the methanogenic microorganisms and to harvest the H_2-producing microorganisms.

To ensure the availability of alkalinity and sufficient amount of macro and micro nutrients, followings compounds were also added in the feed solution (dissolved in tap water) in respective concentration: $NaHCO_3$ (1.2-2 g L^{-1}), NH_4Cl (0.04 g N g COD L^{-1}), KH_2PO_4 (0.01 g P g COD L^{-1}), $FeCl_3*6H_2O$ (2.1 mg Fe L^{-1}), $CaCl_2*2H_2O$ (8.2 mg Ca L^{-1}), $MgCl_2*6H_2O$ (2.4 mg Mg L^{-1}), $Na_2MoO_4*2H_2O$ (0.22 mg Mo L^{-1}), $ZnSO_4*7H_2O$ (0.23 mg Zn L^{-1}), $CuSO_4*5H_2O$ (0.128 mg Cu L^{-1}), $NiCl_2*6H_2O$ (0.1 mg Ni L^{-1}), H_3BO_4 (0.007 mg B L^{-1}), Ne_2SeO_3 (0.06 mg Se L^{-1}), $MnCl_2*4H_2O$ (0.56 mg Mn L^{-1}) and $CoCl_2*6H_2O$ (0.124 mg Co L^{-1}). The bioreactor was inoculated with anaerobic sludge (TS of 14 g L^{-1} and VS of 7.44 g L^{-1}) obtained from a full-scale mesophilic sludge digester treating the excess sludge of a municipal wastewater treatment plant located in Padova, Italy.

During the three experimental phases periodic sludge withdrawal was organized in order to control the desired SRT of the system and to facilitate the removal of metabolic products inhibitory for the H_2 producing bacteria (Khanal et al., 2004). The SRT of the system was a result of biomass concentration in the effluent and in the wasted sludge and was defined as follows:

$$SRT = \frac{V_R X_R}{(Q_E X_E + Q_W X_W)} \qquad (6.1)$$

where SRT is the sludge retention time (d), V_R is the effective working volume of the bioreactor (L), X_R is the MLSS concentration in the bioreactor (g L^{-1}), Q_E and Q_W are the volumetric flowrates for the effluent and waste sludge (L d^{-1}) while, X_E and X_W are the suspended solids concentration in the effluent and in waste sludge respectively.

During the first experiment no excess sludge was withdrawn from the bioreactor and similarly, the solids lost through the effluent were returned to the bioreactor through gravity settling after collecting a small amount of sample for TSS analysis and therefore, the system SRT was theoretically infinite. However, in the second and third experiments an average SRT of 16 d and 12 d was maintained respectively by periodically drawing excess sludge from the bioreactors

6.2.3 DM cleaning and operation

A challenging aspect of using DM under anaerobic environment is to perform cleaning operation of excessively fouled DM layer without disturbing and interfering in the overall bioreactor operation and anaerobic environment. Although external cross-flow configuration facilitates membrane cleaning and management in conventional MBRs (Ho and Sung, 2009) and allows to operate the system at lower filtration resistance and TMP in comparison to the submerged configuration for ADMBR (Ersahin et al., 2017) however, shear generated by CFV to control fouling was found to be less effective in recovering the permeability of excessively fouled DM (Alibardi et al., 2014). Therefore, in this study a new manually operated pneumatic cleaning mechanism was proposed and used to carry out the physical cleaning operation of excessively fouled DM inside the external cross-flow module (Fig. 1). The pneumatic cleaning was further supported by the simultaneous backwashing at a flow rate of 5 L h^{-1}. The whole cleaning procedure was always completed in less than 2 minutes with continuous backwashing and 20 reciprocations of pneumatic cleaning mechanism.

Another strategy for DM operation, inspired by Alibardi et al, (2014) and Saleem et al, (2017, 2016) was adopted to expedite the formation of DM and to avoid excessive biomass loss during the formation stage. High filtration fluxes under gravity-driven filtration mode were applied under the constant hydrostatic head of 30 cm of water constantly maintained above the filtration module. The quantity of the effluent collected during this time interval (usually less than 50 ml) was always returned to the bioreactor. The development of DM (within 2 minutes) was characterised by the absence of suspended solids in the effluent (visual inspection). Soon after that, the constant flux filtration operation was resumed. It is important to mention that gravity driven filtration cannot be performed in a vacuum (under negative pressure). Therefore, it was necessary to avoid the formation of negative pressure in the head space of the bioreactor while performing gravity driven filtration. To serve this purpose the daily produced biogas was collected in a 5 L biogas collection bag and during the gravity driven filtration mode, the possibility of developing negative pressure was avoided by allowing the biogas to enter into the head space of the bioreactor from the biogas collection bag and replacing the drop in the effective volume of the bioreactor (around 20-30 ml) during the gravity driven filtration mode. The collected volume of the filtrate during this time interval was always returned to the bioreactor through the influent feeding system.

6.2.4 Analytical methods and equipment

The analytical procedures were based on Standard Methods (APHA, 2005), otherwise stated elsewhere. The bioreactor performance was periodically measured on filtered samples (0.45 μm PTFE membranes) taken from the effluent. The effluent was characterised for volatile suspended solids (VS), total suspended solids (TSS), volatile fatty acids (VFAs including acetic, propionic, butyric, caproic and valeric acids), measured by a gas chromatograph (Varian 3800) equipped with a flame ionization detector, a 25m×0.53mm×0.70mm CP-Wax 58 (FFAP) CB capillary column (Varian) and using nitrogen as carrier gas. Residual sucrose was analysed using the phenol-sulfuric acid method for reducing sugars (Dubois et al., 1956). Mixed liquor suspended solids and volatile suspended solids concentrations (MLSS and MLVSS) were periodically measured to assess biomass growth inside the system. Biogas composition was measured by a micro-gas chromatograph (Varian 490-GC) equipped with a 10 m MS5A column and 10m PPU column, using argon as carrier gas and a thermal conductivity detector. The continuous monitoring of the temperature was performed by using an electronic thermometer (Hanna Check-temp °C) inserted inside the bioreactor. The effluent flowrate was obtained by simply measuring the effluent volume by means of a graduated cylinder over time interval it was collected. Similarly, the filtration fluxes were calculated by dividing the flowrate by the effective filtration area of the DM membrane modules. DM hydraulic resistance was calculated according to Darcy's equation as follows:

$$R = \frac{\Delta P}{\mu \cdot J} \qquad (6.2)$$

Where J is the permeate flux, ΔP is TMP across the membrane, μ is the viscosity of the permeate, and R is total membrane resistance.

6.3 Results and Discussion

6.3.1 Observed hydraulic retention time, filtration flux, transmembrane pressure and DM resistance profiles

The study was initiated with the idea of maintaining the HRT of the system lower than 1 d for keeping high organic loading rate conditions and to facilitate the inhibition of methanogens through acidification (Akuzawa et al., 2011; Chen et al., 2007; Veeravalli et al., 2017; Zhu and Béland, 2006) while simultaneously avoiding the accumulation of metabolic by products inhibitory to H_2 producing bacteria (Lee et al., 2008). For the three consecutive experimental

runs the filtration performance of the DM was evaluated by measuring physical parameters such as membrane flux (L m^{-2} h^{-1}, LMH), HRT (d), TMP (kPa) and effluent TSS concentration (mg L^{-1}). In figures 6.2, 6.3 and 6.4 it is represented respectively, the behaviour of TMP (kPa) and DM filtration resistance (m^{-1}) profiles over time, the trends of total HRT (d) together with the resulting filtration flux (LMH), effluent TSS (mg L^{-1}) concentration and corresponding DM solids removal performance (%) profiles. First two experiments were terminated due to the inhibition of biological activity (absence of biogas production) and the new experiment was restarted with the addition of fresh inoculum and a new start-up strategy to achieve steady state biological performance.

DM filtration operation was initiated by applying the protocol discussed in section 2.3 to expedite the formation of DM. During the first experimental run a 52 µm mesh was used to develop DM however, due to excess loss of biomass with the effluent it was replaced by a 21 µm mesh instead within 8 days of continuous bioreactor operation (Fig. 6.2a and 6.3a). The DM showed a progressively deteriorated solids removal performance throughout the first experimental run (Figure 3a). Even the reduction in the mesh porosity (from 52 to 21 µm) on day 8, and an increase in the effective filtration area on day 16 (to increase effluent fluxes and to curtail the rise in TMP and DM resistance) did not bring any improvements in the solids removal performance of the formed DM and reduction in the desired HRT to less than 1 day (Fig. 6.2a, 6.3a and 6.4a). It was also observed that the initial solids removal performance and effluent TSS concentration observed during the first few days of every experimental run was better than the rest of the period of experimentation, irrespective of the mesh pore size, MLSS concentration and bioreactor operating parameters (flux, TMP, HRT etc.) (Fig. 6.3). Furthermore, the deteriorated effluent quality was followed along with intensified DM fouling, very sharp increase in TMPs (Fig. 6.2a), subsequent reduction in filtration fluxes and corresponding increase in the HRT of the system (Fig. 6.4a) in the first experiment. Likewise, initial DM resistance showed a rapid shift in its average values, increasing from the order of 10^{12} m^{-1} to more than 10^{13} m^{-1} after only first few days of continuous bioreactor operation in the first and second experiment respectively (Fig. 6.2a, 6.2b).

This sudden change in the filtration behaviour of the formed DM has called attention towards the effect of feed characteristics on the rate of fouling and increase in DM resistance, as other operating condition were unchanged during the start-up phase in all experiments (i.e. lasting for less than 5 days). Since the strategy adopted to inhibit methanogenic activity was through

applying high OLR for subsequent acidification through VFA accumulation, high influent COD concentration (100 g L^{-1} as sucrose) was fed to the bioreactor in the first experiment, However, in conventional MBR systems with fermentative H_2 production high OLR has shown to increase membrane fouling propensities by influencing biomass content, properties of colloidal materials and production of EPS (Shen et al., 2010).

Adding to the discussion, studies have also reported that an excess concentration of biofoulants aggravate fouling in DM (Hu et al., 2016; Liang et al., 2013; Yu et al., 2015), particularly the study made by Yu et al, (2015) under anaerobic conditions. They reported that amount of EPS, (soluble or bound EPS) externally added or produced through biological activity greatly affects DM formation and fouling propensities by influencing properties like particle size of the sludge flocks and compactness of the DM layer. However, the author did not present any result on the effect of intensified fouling on DM solid retention performance as observed in this study.

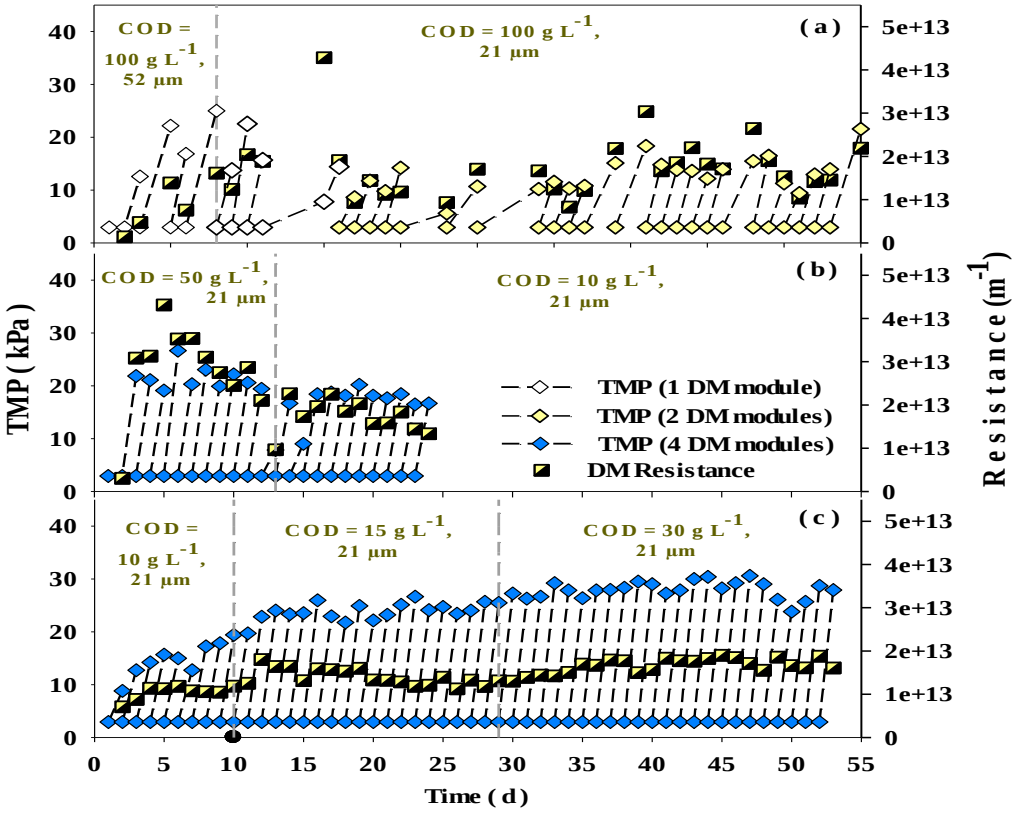

Figure 6.2 Observed TMP and filtration resistance profiles with respective COD concentrations and mesh porosity used for (a) first experimental run, (b) second experimental run and (c) third experimental run.

Although complete EPS analysis (carbohydrates and protein content) was not performed in this study however, based on the discussion it can be inferred that high OLR plus the addition of COD as sucrose; a carbohydrate and a major component of total EPS (Liu et al., 2012; Yu et al., 2015), must have contributed in increasing the amount of biofoulants inside the bioreactor and most possibly responsible for the change observed in the filtration behaviour of the DM and sludge.

On the contrary, an interesting point of argument could be the definition of DM itself as previously introduced as a purpose built fouling layer In section 6.1 and thus, rapid fouling should have improved its solid-liquid separation performance. However, the rapid rise in TMP in a shorter time interval would have exerted high pressure, high enough to break the newly formed DM and deteriorating the effluent quality (Salerno et al., 2017). Alibardi et al, (2014) have reported the same observation with the help of flux-step experiments that DMs can resist TMP up to a certain maximum level depending upon their maturity and strength, and an excessive TMP can break their structure and could lead to a deteriorated effluent quality. For the same reason any effort to reduce the HRT by increasing filtration fluxes (Fig. 6.4a) caused very rapid rise in TMP and subsequent rupture of newly formed DM causing the effluent TSS concentration to reach more than 3.0 g L^{-1} in first and second experiment respectively (Fig. 6.3a, 6.3b).

In order to assess the effect of influent COD concentration on DM performance and to maintain the HRT of the system less than 1 d it was decided to reduce the influent COD concentration to 50 g L^{-1} and to further increase the effective mesh filtration area to 0.00648 m^2 (4 modules) in the second experiment. The increase in the effective filtration area helped to reduce the HRT of the system up to 2.0 d nevertheless, DM solid-liquid separation performance showed a similar trend of progressively deteriorated effluent quality corresponding to the TSS removal as low as 60% (Fig. 3b). No improvement was observed in the solid-liquid separation performance of DM until the influent COD concentration was reduced to 10 g COD L^{-1} from 50 g COD L^{-1} on day 13. Soon after that the effluent quality started to gradually improve with average TSS removal reaching 94% by the end of second experimental run (Fig. 6.3b). The analysis of the data during this interval showed that influent COD concentration has a strong negative correlation (ρ = -0.86) with DM TSS removal efficiency and strong positive correlation (ρ = 0.87) with the DM filtration resistance. In addition the strategy helped to gradually lower DM filtration resistance to more than 42%

(Fig. 6.2b) and thus enabling the system to operate at an HRT of around 1.2 d with an ensuant increase of 33% in the filtration flux during the last 5 days of second experiment (Fig. 6.4b).

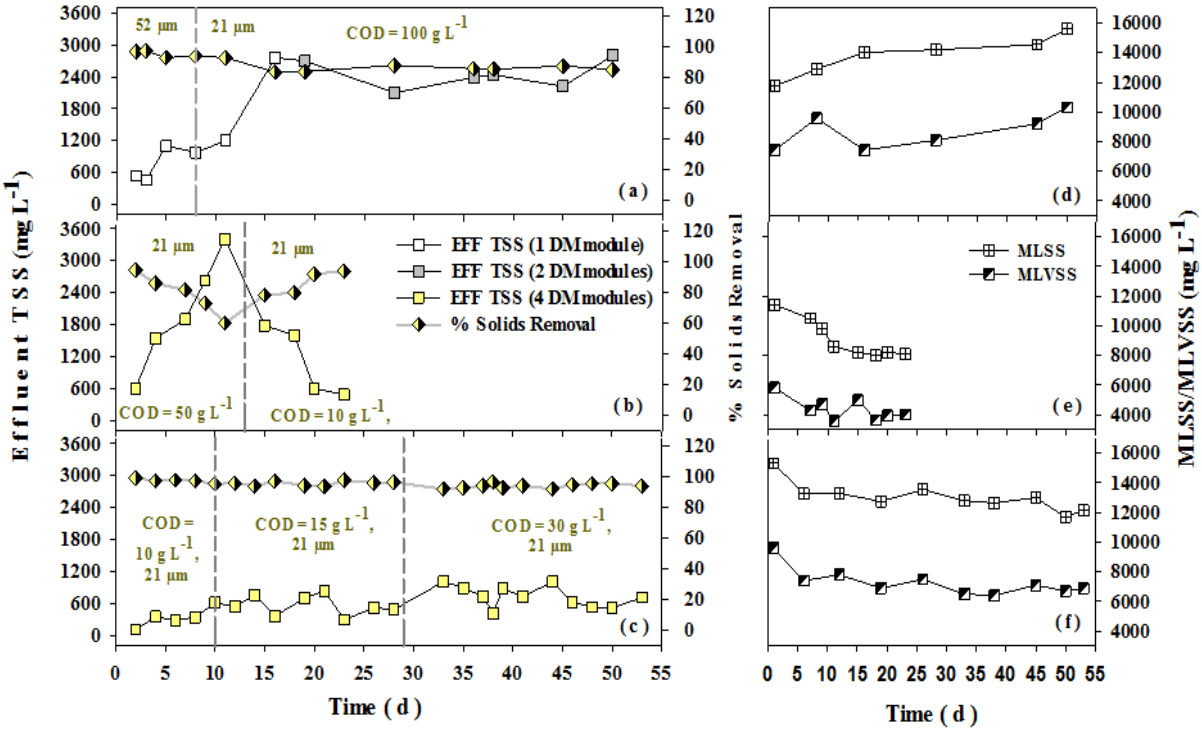

Figure 6.3 Observed effluent TSS concentration and % solids removal performance for (a) first experimental run, (b) second experimental run and (c) third experimental run, respective MLSS and MLVSS evolution profile inside the reactor for (d) first experimental run, (e) second experimental run and (f) third experimental run.

Starting with the experience gained from the previous 2 experimental runs it was inferred that the low influent COD concentration reduced the fouling propensities and allowed operating the system at lower HRT. With these observations in mind, third experimental run was initiated by feeding the bioreactor with an initial concentration of 10 g COD L^{-1} and gradually increasing up to 30 g COD L^{-1} by the end of the third experiment (Fig. 6.2c). Initially the average HRT in the first 10 days was 1 d and the corresponding effluent flux was 5.0 LMH. During this time DM showed excellent solids removal efficiency of around 97.5% (Figure 3c). Since then than the average HRT of the system was gradually reduced to 0.82 d by increasing the effluent fluxes from 5.0 LMH to 6.2 LMH that was comparable to the fluxes applied in conventional membranes for biological H_2 production (Lee et al., 2008).

After every cleaning operation, performed almost every day during the third experimental run, it was expected that an increase in the effluent fluxes to reduce system HRT could

possibly cause rupture of the newly formed DM layer due to rapid rise in TMP as observed in the past two experiments. However, the effect of increase in the effluent flux to around 6.2 LMH and the associated rise in the TMP averaging to 28.5 kPa did not affect the solid-liquid separation performance of the formed DM (Figure 3c). In fact, solids removal efficiency of DM during the whole experimental run was higher than the previous experimental runs, averaging around 95% of TSS removal with average effluent TSS concentration up to 0.6±0.03 g L^{-1} with fever fluctuations in its profile as compared to what was observed for last two experimental runs (Fig. 6.3). The effect of influent COD concentration on the DM resistance and solids retention was more prominent in the third experiment (Fig. 6.2c) showing a much stronger negative correlation (ρ = -0.95) with DM TSS removal efficiency and a stronger positive correlation (ρ = 0.9) with the DM filtration resistance as compared to the second experimental run.

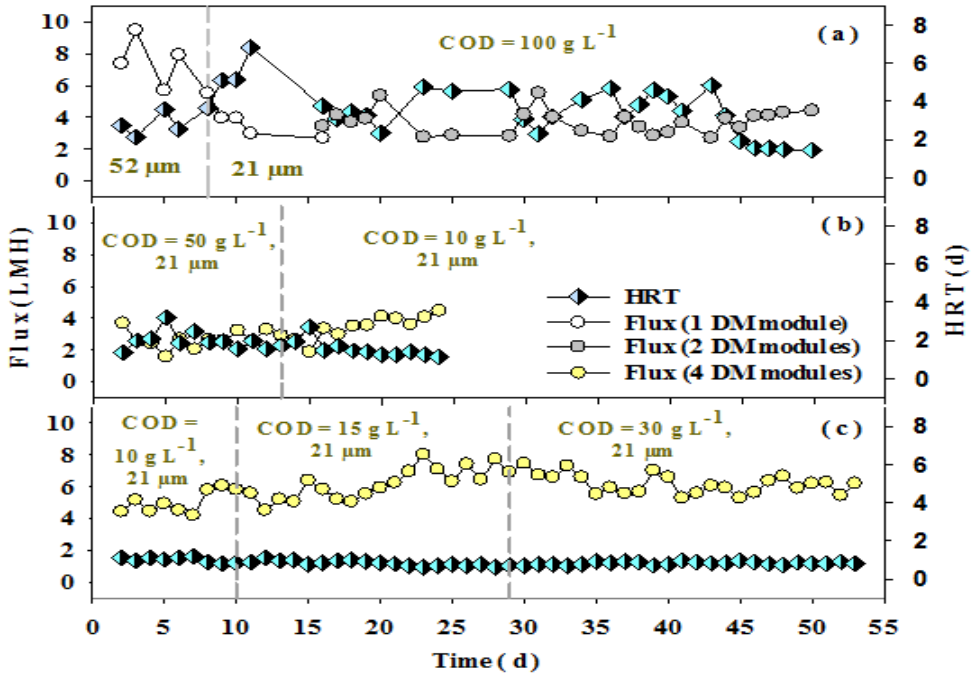

Figure 6.4 Effluent flux and resulting HRT profile for (a) first experimental run, (b) second experimental run and (c) third experimental run

The results related to the influence of feed characteristics and influent COD concentration on DM characteristics and filtration performance are peculiar in comparison to the data previously reported in scientific literature for these parameters despite having different operating conditions as applied in this study (Ersahin et al., 2012; Rezvani et al., 2014; Salerno et al., 2017; Zhang et al., 2014), or even working with bioH_2 production (Park et al.,

2017). Previous studies did not mention any change in the filtration behaviour of the formed DM and the suspended sludge inside the bioreactor with the progress of the experiment as observed during this study in each of the three successive experiments performed.

In relation to DM fouling behaviour and filtration performance, the role of MLSS concentration has been well studied (Li et al., 2012; Saleem et al., 2017). However, in this study the solids removal performance observed during the third experiment (Fig. 6.3c) was far better than previous two experiments, despite having higher average MLSS concentration of around 13 gL^{-1} as compared to second experiment with an average MLSS concentration of 9 gL^{-1} (Fig. 6.3e and 6.3f). Statistical analysis of the data obtained in the third experiment showed that MLSS concentration has a weak negative correlation with DM filtration resistance (ρ = -0.39) and TSS removal performance (ρ = - 0.2) of the formed DM. These results again confirm the previous observation that influent COD concentration has more profound effect on DM filtration resistance and its solid-liquid separation performance, at least under the conditions applied in this study.

6.3.2 Biological Performance

Production of H_2 through dark fermentation involves production of various intermediates (VFAs and alcohol etc.) and their respective concentration dictates process stability and thus H_2 production rates (Khanal et al., 2004). Trends in biogas production together with H_2 production (ml L^{-1}), OLR (g L^{-1} d^{-1}) and H_2 percentage in biogas (%) are reported in figure 6.5, while the respective VFA concentrations profiles (mg L^{-1}) along with carbohydrates conversion percentage (%) are reported in figure 6. The biogas production started almost after 1 to 2 days of bioreactor start-up and mostly followed the trends in OLR in every experimental run.

During the first experiment biogas production and its H_2 content was variable and often related to carbohydrates conversion efficiency and concentration of VFAs inside the bioreactor (Fig. 6.5a and 5.6a). In the first 16 days acetic acid was in higher concentrations followed by butyric acid however, soon after that butyric acid production was increased while propionic, caproic and valeric acids were never produced in high concentrations throughout the first and second experiments (Fig. 6.6). On day 18 H_2 content in the biogas was 50 % producing around 455 mL H_2 L^{-1} and the corresponding total VFA concentration was also highest recorded for the whole experiment around 79 mM and 96 mM for acetic and butyric

acid respectively, indicating a very good biological activity of the biomass inside the system (Fig. 6.6a). Nevertheless, due to the accumulation of high concentration of VFAs (acetic and butyric acids) > 60 mM (Ginkel and Logan., 2005) and possible production of solvents (alcohols etc.) due to solventogenesis at higher OLR (Ginkel and Logan., 2005; van Niel et al., 2003; Veeravalli et al., 2017), the biogas production and carbohydrates conversion were greatly reduced (Fig. 6.5a and 6.6a). One major factor affecting biological performance and H_2 production during this study could also be the range of bioreactor's pH (4.9-5.1) maintained during this study. Lower bioreactor pH (around 4.5) and high OLR have been found to favour the production of solvents and undissociated forms of acetic or butyric acid that is reported to be inhibitory for the H_2 producing bacteria (Ginkel and Logan., 2005; De Amorim et al., 2012). Ginkel and Logan, (2005) have conclusively reported that acetic or butyric acid concentrations (> 25mM) and higher influent substrate concentration (> 40 g glucose L^{-1}) can reduce H_2 yield and initiate solventogenesis.

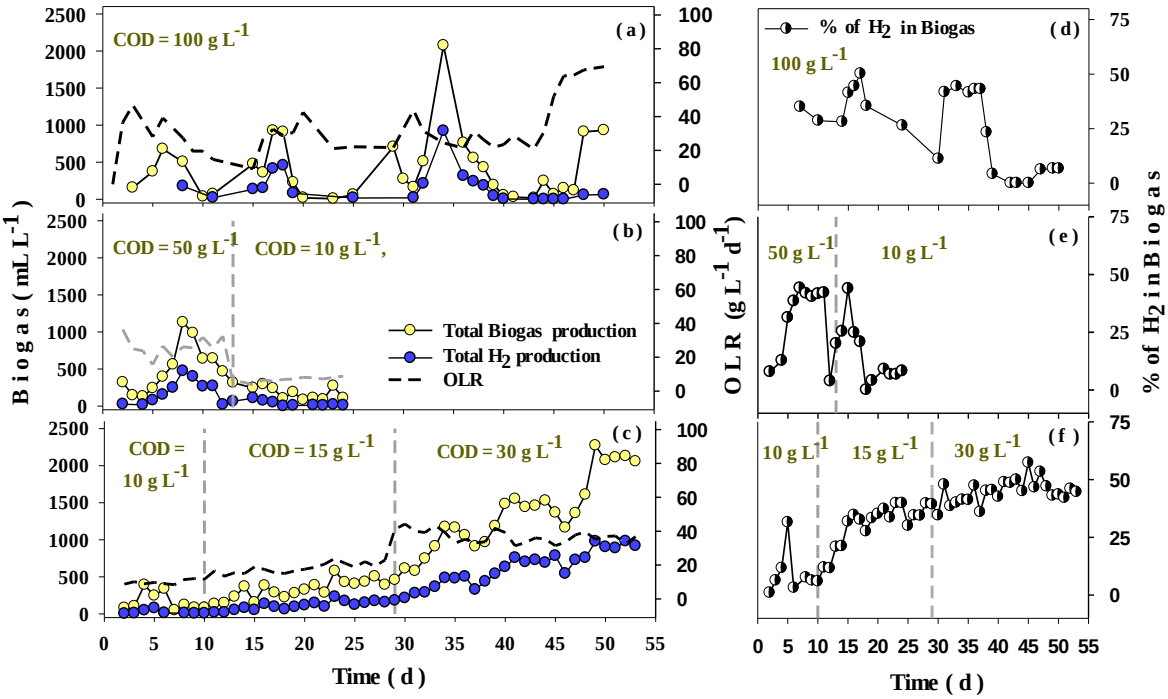

Figure 6.5 Total biogas and hydrogen production along with organic loading rate (OLR) profiles for (a) first experimental run, (b) second experimental run and (c) third experimental run, recorded percentage of H_2 in the biogas for (d) first experimental run, (e) second experimental run and (f) third experimental run.

Since, the bioreactor performance was totally deteriorated, it was decided to flush the accumulated VFAs from the system on day 32 without any addition of COD in the feed and

keeping the same nutrient concentration and alkalinity in the first experiment. The flushing lasts for two days and soon after that the normal feeding operation was resumed. Due to flushing biological performance was temporarily resurrected and soon after that, on day 34 H_2 production showed a peak in its profile measuring around 920 mL H_2 L^{-1} (Fig. 6.6a).

The corresponding increase in the VFA concentration (mainly butyric acid) (Fig. 6.6a) again lead to the deterioration in the biological activity and since then, H_2 production was never revived till the end of the experiment. Even the reduction in the HRT of the system to less than 2 days during the last week of the first experiment (Fig. 6.4a) did not bring any improvements in the biological performance (Fig. 6.5a) and it was decided to shut down the system. The severe inhibition of the biological activity during first experiment can be well associated with high influent COD concentration (100 g L^{-1}), lower operating pH range (4.9-5.1) and the accumulation of excess VFA (> 60 mM) inside the system that might have favoured the production of solvents and undissociated form of VFAs as discussed above (Ginkel and Logan., 2005; De Amorim et al., 2012; Van Niel et al., 2003).

The second experiment was started with an influent COD concentration of 50 g L^{-1} that was further reduced to 10 g L^{-1} on day 13 after observing the gradual reduction in the trends of biogas production. Similar to the previous experiment, a fluctuating profile was observed for biogas production, reaching its peak value of 902 mL L^{-1} with 44% of H_2 content on day 8 of continuous bioreactor operation (Fig. 6.5b). While butyric acid again dominated the total VFA production followed by acetic and propionic acid (Fig. 5.6b). Since then, the biogas production showed a decreasing trend with some fluctuations until the end of the experiment reaching the lowest H_2 content of only 4% in the biogas (Fig. 6.6e). The gradual inhibition of biological activity was again initiated at high acetic and butyric acid concentrations (Fig. 6.6b) while propionic acid was also noticed in concentration greater than > 1.5 mM at the end of the second experimental run. A temporary recovery in H_2 concentration a up to 42 % in the biogas was observed after the reduction in the influent COD concentration and HRT of the system (< 2d) on day 13 however, total biogas production did not show any considerable improvements.

The third experiment was initiated with an initial low COD concentration of 10 g COD L^{-1} that was gradually increased up to 30 g COD L^{-1} in 3 phases during the entire length of the experiment (53 days). The biogas and H_2 production started almost immediately and a gradually increasing trend was observed, following the trends in OLR profile (Fig. 6.5c and

6.5f). The biogas and H_2 production increased more than 2000 and 950 mL L^{-1} respectively (Fig. 6.5c) while the corresponding H_2 yield of of the system reached up to 8 L H_2 $mole^{-1}$ of sucrose (data not reported). Most of the time the system operated at an HRT of < 1 d, SRT of around 12.1±0.6 d and the respective increase in OLR was a result of mainly an increase in the influent COD concentration (Fig. 6.5c). Under these conditions the bioreactor exhibited an unvarying carbohydrate conversion efficiency of more than 99% (Figure 6c) irrespective of the gradual increased in OLR (Fig. 6.5c). Similar performance for carbohydrates conversion efficiency has been reported for conventional MBR systems working at an HRT of less than 1d, provided a sufficient SRT (Bakonyi et al., 2014; Lee et al., 2009a, 2009b; Park et al., 2017; Wong et al., 2014).

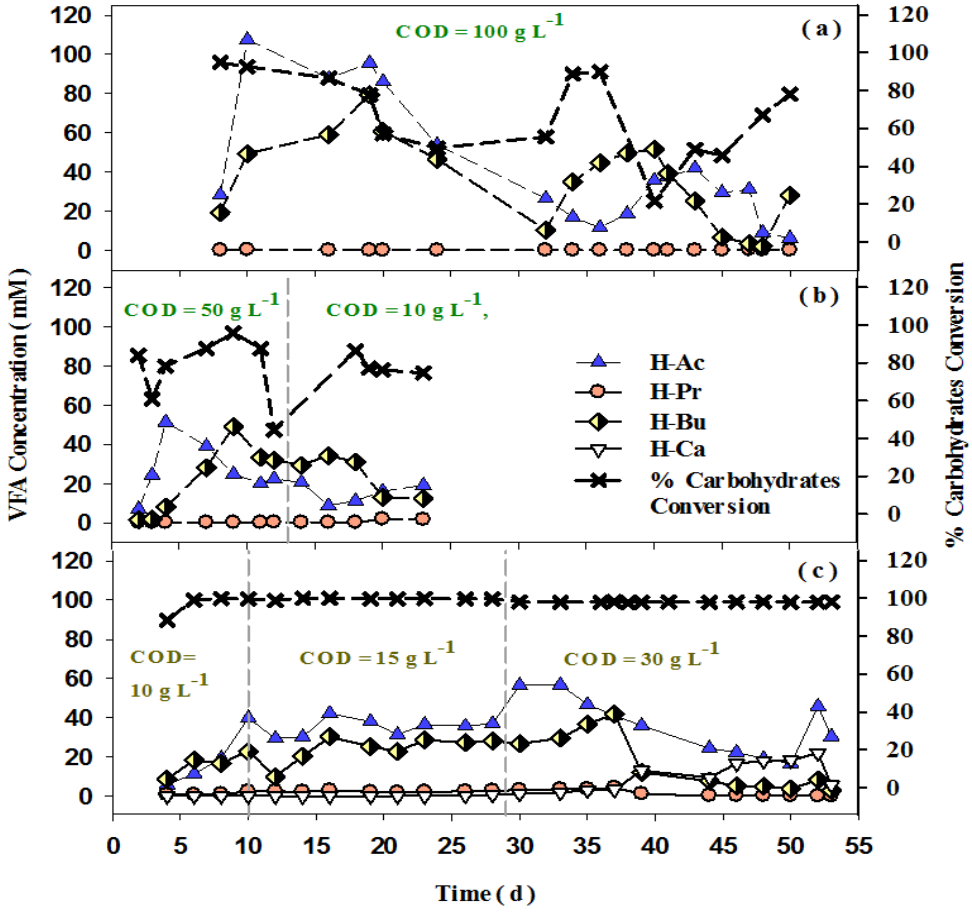

Figure 6.6 Volatile fatty acids (VFAs) concentrations measured in the effluent. HAc = acetic acid; HPr = propionic acid; HBu = butyric acid and HCa = caproic acid along with carbohydrates conversion efficiency for (a) first experimental run, (b) second experimental run and (c) third experimental run.

The distribution of VFA during the third experiment was also similar to the previous experiments with respect to acetic and butyric acids as the main product of carbohydrates conversion with an exception of significant production of caproic acid after 40 days of continuous bioreactor operation (no caproic acid observed in the first 2 runs). The production of caproic acid to more than 20 mM and consequent reduction in the concentration of butyric acid to less than 8 mM from an average of 24±2.1 mM was a result of secondary fermentation of of ethanol butyrate pathway during dark fermentation (Ding et al., 2010). (Ding et al, (2010) showed conclusively from their study that caproate formation is a H_2 producing secondary fermentation step during dark fermentation instead of H_2 consuming, however, this pathway of H_2 production resulted in overall less H_2 production if compared with the respective theoretical H_2 yields for a given substrate. The authors attributed possible solventogenesis for lower H_2 yields, because solvents like ethanol shown to have bactericidal effects (Wong et al., 2014) furthermore, for secondary fermentation to occur, alcohol is one of the main reactants that serve as reducing equivalents not been liberated as hydrogen gas (Ding et al., 2010). Similarly, propionic acid, was also recorded in higher concentrations (i.e. up to 3.0 mM) as compared to the previous experiments (Fig. 6.6c). Although alcohols were not measured during this study however, increase in caproic acid concentration and a simultaneous decrease in butyric acid concentration indicated the occurrence of solventogenesis during the third experiment.

In contrast to the previous experiments, presence of high concentration of of VFAs inside the system did not deteriorate the biological performance of the system and stable percentage of H_2 around 47±1.0% was maintained during the last 15 days of third experiment (Fig. 6.5f). The improved biological performance can be attributed to the lower HRT (< 1 d) maintained during the experiment that avoided the accumulation of metabolic by-products despite reaching concentrations (> 25 mM) reported to retard the biological activity of H_2 producing bacteria (Ginkel and Logan., 2005).

One significant feature of foregoing results is that no, or negligible percentage of CH_4 in the biogas was detected, indicating that the methanogenic bacteria had been successfully inhibited or eliminated even though anaerobic mixed culture was used and retained at high SRT, at least during the first experimental run. Previous researches have reported the revival of methanogenic activity under high SRT conditions over the period of bioreactor operation

(Kim et al., 2004; Mizuno et al., 2000) therefore, there washout from the system is a key to successfully inhibit their activity considering the long-term bioreactor operation.

The successful inhibition and the possible washout of methanogenic bacteria as observed in this study could be associated to the synergistic effect of applying high OLR conditions and maintaining acidic environment inside the bioreactor followed by - not so good solid-liquid separation performance of the formed DM as compared to conventional membranes. The solid-liquid separation efficiency of DMs is not as excellent as conventional membranes (Alibardi et al., 2014; Saleem et al., 2016) that would have allowed the gradual washout of methanogenic bacteria from the system over the entire bioreactor operation, especially in the first experiment when the SRT was theoretically infinite. Interestingly, this aspect of DM technology can be seen as advantageous for successful inhibition of methanogenic activity using mixed anaerobic cultures for continuous H_2 production in situations where very high effluent quality is not required for reuse application.

6.4 Conclusion

The study was an effort towards evaluating the performance of DM while integrating it with dark fermentation process for the production of H_2 gas. Based on the results and main observations of this study are summarized in the form of following conclusions.

1. DM filtration can be successfully applied as a mean of solid-liquid separation in dark fermentation process for the production of H_2 gas under the conditions reported in this study. However, due to the change in the feed characteristics (from municipal sewage to sucrose rich feed) a drastic change in the filtration behaviour of the sludge was observed.
2. Due to the same fact the effect of mesh porosity on solid liquid separation performance became prominent and solid-liquid separation performance of a 21 µm mesh out performed 52 µm mesh achieving greater than 90% solids retention in the third experiment.
3. The strategy of gradually increasing the influent COD concentration as sucrose proved to be effective in maintaining steady performance of the bioreactor and to avoid possible inhibition due to excess VFA accumulation and high OLR in the third experimental run.

4. It was also found that low influent COD concentration and OLR reduces fouling propensities in DM filtration and improves suspended solids removal efficiency. Influent COD concentration was found to have a strong negative correlation (ρ = -0.95) with DM TSS removal efficiency and a strong positive correlation (ρ = 0.9) with DM filtration resistance.
5. Due to low fouling resistance and better solids retention, it was possible to operate the system at lower HRT (<1 d) and keeping high biomass concentration inside the system up to 13 g L^{-1} at an SRT of 12.1±0.6 d. Due to the same fact the bioreactor exhibited stable biological performance with an unvarying carbohydrates conversion efficiency of more than 99% and a H_2 yield of 8 L H_2 mol^{-1} of sucrose. Moreover, the biogas production was found to be a function of applied OLR.

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CHAPTER 7

APPLICATION OF ANAEROBIC DYNAMIC MEMBRANE BIOREACTOR (AnDMBR) FOR THE SUCCESSFUL ENRICHMENT OF ANAMMOX BACTERIA USING MIXED ANAEROBIC AND AEROBIC SEED SLUDGE

Abstract

Numerous studies have used technologies to enrich anaerobic ammonium oxidation bacteria in order to offset limitations related to its slow growth rate. This study investigated a novel lab-scale bioreactor configuration coupled with a side-stream dynamic membrane module for the enrichment of Anammox bacteria. The side-stream module was also equipped with an internal cleaning mechanism for permeability recovery of excessively fouled DM layer. The enrichment was materialized under mesophilic conditions over a synthetic feed and by using a mix of anaerobic and aerobic sludge as inoculum. Dynamic membrane development and performance was analysed over two polyamide-nylon meshes (200 and 52 µm). The excellent solid-liquid separation of 52 µm mesh outperformed 200 µm with an average effluent turbidity of 2.4±0.1 NTU, ensuring gradual enrichment and successful retention of Anammox bacteria. The gradual enrichment enables the system to operate at a maximum nitrogen loading rate of 696 mg $L^{-1}d^{-1}$ and a maximum nitrogen removal rate of 611.6 mg $L^{-1}d^{-1}$ with an average total nitrogen removal efficiency of 87.5±0.56 %. A stable filtration flux of around 10 LMH was applied while the HRT of the system was maintained between 2-3 d. Initially, episodes of poor biological activity were recorded due to the inhibition caused by high nitrite concertation in the effluent (up to 33.7 mg-NL^{-1}) however; a slight reduction in the nitrogen loading rate was helpful in Anammox activity recovery. Additionally, the internal cleaning mechanism was effective in permeability recovery without the use of

chemical cleaning for a period of 456 days. Digital realtime PCR Sequence analysis showed that Planctomycetales belonging to ascertained Anammox-specific genera progressively increased their presence in the reactor consistently with its nitrogen abatement performance. The study demonstrated the effectiveness of this novel bioreactor configuration and formed DM for the enrichment of Anammox bacteria and to provide long term stable bioreactor performance with high effluent quality.

Manuscript to be submitted to a scientific journal.

7 Application of anaerobic dynamic membrane bioreactor (AnDMBR) for the successful enrichment of Anammox bacteria using mixed anaerobic and aerobic seed sludge.

7.1 Introduction

Over the last few decades, stricter legislations for the treatment and disposal of nitrogen rich waste streams have been incorporated in national and international discharge guidelines due to the concerns of eutrophication (Liu and Wang, 2017; Val del Rio et al., 2017). In this regard, conventional biological (nitrification and denitrification), physico-chemical, and until recently evaluated, bioelectrochemical systems have been extensively studied and successfully applied to treat ammonia rich waste streams (Kelly and He, 2014; Liu and Wang, 2017; Nancharaiah et al., 2016). Although conventional biological wastewater treatment technologies are by far the cheapest available options for nitrogen removal however, for wastewater having C/N ratio of less than 2.86 (landfill leachate etc.), addition of supplemental organics for the support of heterotrophic denitrification activity and the consequent increase in excess sludge production has a considerable impact on operational and management cost (Liu and Wang, 2017; Suneethi and Joseph, 2011). The discovery of anaerobic ammonium oxidation (Anammox) in mid-1990s (Mulder et al., 1995) has started a new era for more cost-effective and sustainable way to treat ammonium rich waste streams (Hu et al., 2010; Kuenen, 2008). This bacteria-mediated biological process converts nitrite and ammonium into nitrogen gas under anaerobic conditions (Eq (1)) (Strous et al., 1998). The most attractive aspects of the Anammox process in comparison with conventional nitrogen removal processes are its significant reduction in (1) aeration requirements (64%), (2) sludge production (80–90%), (3) demand for supplemental organics to support heterotrophic growth (100%) and (4) greenhouse gas emission (60%) (Hu et al., 2010; H. Li et al., 2012). As a whole, the Anammox process reduces the energy requirements up to 1 kWh kg-1N compared to 2.8 kWh kg-1N required for conventional nitrogen removal system and an overall 90% savings in operational costs (Wang et al., 2009).

$$1NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (7.1)$$

Despite many advantages offered by the Anammox process, its higher doubling time, usually reported around 11 d (Strous et al., 1997) and for treating sewage, Ma et al., (2016) reported between 15–30 d, possess a great challenge for keeping sufficient Anammox biomass inside the system for efficient treatment performance. Therefore, technologies including attached

growth systems (Lackner et al., 2014; Meng et al., 2014; Tang et al., 2010; Tsushima et al., 2007), sequencing batch reactors (Strous et al., 1998; Tao et al., 2012; Wang et al., 2012), granular sludge bed reactors (Li et al., 2016; Ni et al., 2012; Tang et al., 2009) and membrane bioreactors (MBR) (Li et al., 2016, 2015; Lotti et al., 2014; Suneethi and Joseph, 2011; Tao et al., 2012; Trigo et al., 2006; Van Der Star et al., 2008) have shown to be promising in assuring effective solid-liquid separation and retaining high amount of biomass inside the system. In this perspective nitrogen loading rate (NLR) as high as 26.0 g-NL-1d-1 has been reported while applying high-rate anaerobic Anammox biofilm reactors (Tsushima et al., 2007). It has also been reported that even a slightest loss of biomass with the effluent could delay the complete enrichment required to treat the desired nitrogen loading rate (NLR) for a biological system. Besides, it is inevitable to avoid complete biomass wash out with the effluent in all these technologies except for MBR systems (Li et al., 2015; Trigo et al., 2006).

Application of MBR has demonstrated success in treating municipal and complex industrial wastewater streams including landfill leachate while, membrane capital cost and intrinsic fouling still remains a challenge for its wide acceptance as a low cost wastewater treatment solutions (Judd., 2016). In perspective of cultivating slow growing bacteria like the Anammox biomass, MBRs have shown great success in biomass enrichment and associated total nitrogen removal performance (Li et al., 2015; Trigo et al., 2006; Van Der Star et al., 2008). Since the solid-liquid separation in MBR systems is independent of settling, any disruption in biomass settling ability (e.g. degranulation, in case of granular sludge) due to substrate limitations or inhibitory effect does not raise concerns for potential biomass loss from the bioreactor (Van Der Star et al., 2008).

Although sufficient Anammox biomass is available for seeding from the full-scale Anammox bioreactor in Rotterdam (The Netherlands) for faster start-up, majority of the studies in the recent past were focused in enriching Anammox biomass from sludge samples obtained from aerobic, anoxic, anaerobic or a mix of these sludge samples (Date et al., 2009; Ding et al., 2017; Wang et al., 2012). Recently, (Lotti et al, (2014) have successfully cultivated an almost 100% pure Anammox culture using anaerobic MBR with highest ever reported specific maximum growth rate of 0.21 d−1. Similarly, Van Der Star et al, (2008) have also achieved an unprecedented purity of Anammox enrichment of 97.6% by using a submerged MBR. The successful enrichment potential of MBR allowed to operate the system at NLR up to 5 gNL-1d-1and hydraulic retention time (HRT) in the range of 1–3 days.(Suneethi and Joseph,

2011). Besides this, more severe fouling has been observed in Anammox MBR system as compared to partial nitrification MBR operated under similar conditions due to the hydrophobic nature of Anammox consortia (Niu et al., 2016). Van Der Star et al, (2008) also highlighted fouling as the main problems for their system, requiring membrane module replacement in every 10 days while maintaining anaerobic environment inside the system. Strategies like use of carrier media for biomass immobilization (Zhang et al., 2016) and use of hydraulic shear generated by membrane rotation (Jiang et al., 2013) have shown to be useful in mitigating membrane fouling in Anammox MBRs. However, these strategies are merely for retarding the fouling phenomena and could not prevent it altogether and in fact, complete permeability recovery was also not reported in these studies.

Although perceived as a bottleneck in the wide spread application of MBR technology, fouling itself has an ingenious application as a low-cost solid-liquid separation medium formed by the the deposition of a mixture of colloidal matter and biomass flocs and termed as dynamic membrane (DM) (Ersahin et al., 2016; Liu et al., 2012; Saleem et al., 2016; Salerno et al., 2017; Xiong et al., 2016; Zhang et al., 2014). DM technology offers many benefits of conventional membranes including comparable solid-liquid separation and treatment performance etc. (Ersahin et al., 2016, 2012; Hu et al., 2016). Yet the most attractive feature of this technology is its reproducibility, low capital and operational cost due to the use of cheap underlying support materials (meshes and filter cloths etc.) and high flux operation at low transmembrane pressures (TMP) with lower energy consumption as compared to conventional membranes (Alibardi et al., 2014; Saleem et al., 2017; Zhang et al., 2014).

Still the application of DM and evaluation of its performance is mostly limited to synthetic (Alibardi et al., 2016; Ersahin et al., 2016; Saleem et al., 2016) and municipal wastewater (Hu et al., 2016; Liu et al., 2009; Y. Xiong et al., 2016; Zhang et al., 2010) under both aerobic and anaerobic conditions and very few studies were targeted to evaluate its potential for enriching slow growing bacteria like Anammox biomass. In this regard, Meng et al, (2014) and Ni et al, (2009) have reported excellent enrichment of more than 97% of Anammox biomass with bioreactor performance comparable to conventional MBR. However, they used non-woven membranes with porosity 0.1 µm instead of coarse underlying support materials like nylon meshes mostly used to develop DM. Similarly, these studies fall short in explaining the behaviour of DM with respect to parameters including rise in TMP and DM resistance, filtration flux, cleaning frequency and DM reproducibility.

To the best knowledge of the authors this is first time DM formed over a coarse underlying support material has been used for the enrichment of Anammox biomass. Therefore, the primary aim of this study was to propose a cost effective and innovative bioreactor configuration coupled with a side-stream DM module capable of enriching Anammox bacteria from a mixed culture (aerobic and anaerobic) for practical application. A pneumatically operated internal cleaning mechanism for ensuring complete anaerobic environment inside the system was also tested in this study. Our group has previously shown the application of innovative approaches in detecting the presence and metabolic efficiency of Anammox microorganisms through metagenomics (Rosselli et al., 2016). In the present analysis biomass enrichment was assessed by evaluating the progress of total nitrogen removal performance and quantified by using a real-time PCR assay targeting the 16S rRNA genes of Anammox bacteria in gradually enriching culture.

7.2 Materials and methods

7.2.1 Bioreactor setup

The study was carried out in a 2 L lab-scale, continuously mixed, anaerobic dynamic membrane bioreactor (AnDMBR) made of 10 mm thick Plexiglas sheet. The cube-shaped bioreactor (12 cm × 12 cm × 15 cm) had an effective working volume of 1.6 L while the remaining 0.56 L free head space for biogas collection (Fig. 7.1).

External Cross-flow configuration was selected to carryout DM filtration due to the ease for the management and operation of DM under anaerobic conditions (Ersahin et al., 2017). During the study period two cross-flow modules, made up of Plexiglas were used. Initially a cylindrical module with an internal diameter of 42 mm and a length of 120 mm carrying a single, concentrically placed DM support was used. However, due to the concern of fouling and to maintain constant flux, DM effective filtration area was doubled on day 262 of the continuous bioreactor operation and a rectangular configuration (5.5 cm × 3.5 cm × 10 cm) holding two symmetrically placed DM supports was utilized.

Two commercially available polyamide/nylon meshes were used to develop DM (Table 7.1). Initially a 200 μm mesh was utilized however, due to the concerns of excessive biomass loss; it was replaced by a 52 μm mesh. To support nylon mesh and thus the formed DM the mesh was sewed over a cylindrical plastic frame (15 mm diameter and 60 mm height) provided with uniformly distributed openings (5mm X 3mm). The effective filtration area of a single

plastic support was 0.00162 m^2 i.e. approximately 57% of the total surface area (0.003 m^2) of the supporting frame.

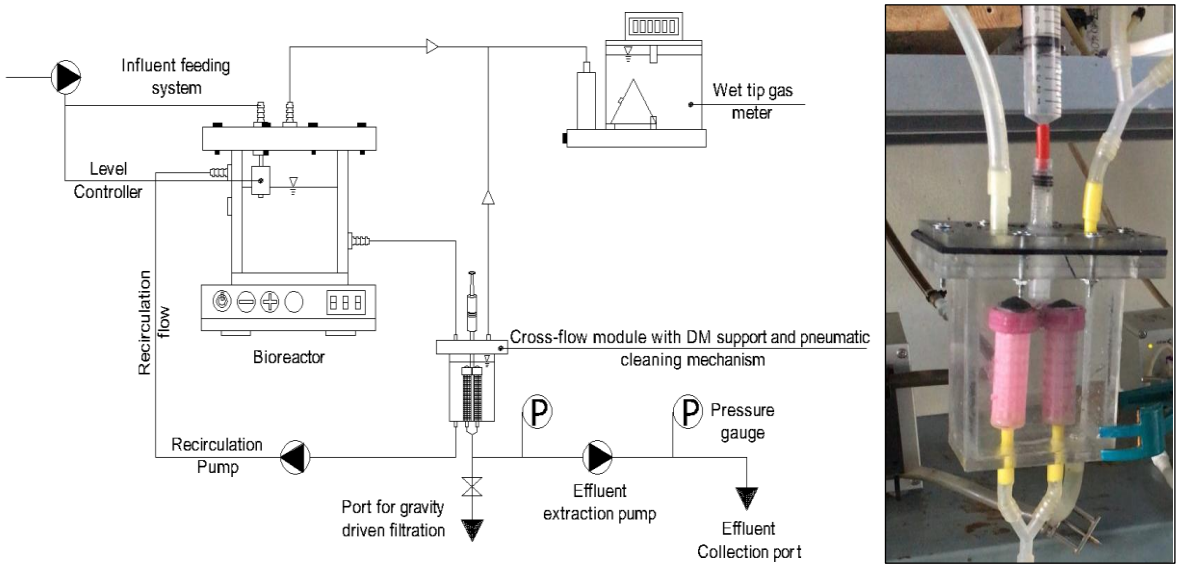

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Figure 7.1 Schematic diagram of the experimental setup and representation of internal cleaning system with 2 dynamic membrane modules

The cross-flow filtration mode was employed with the help of a peristaltic pump (Watson Marlow 403U/R1, Falmouth, Cornwall, UK) connecting the bioreactor with the external crossflow modules. The concentric and symmetrical placement of DM supports helped to maintain the hydraulic regime along the surface of the DM membrane support as uniform as possible. A constant cross flow velocity (CFV) of 50 m h^{-1} was maintained by the circulation of the mixed liquor along the mesh surface in both the external crossflow configurations. It is important to mention here that the magnitude of the CFV was evaluated, and set to 50 m h^{-1}, only to avoid possible sedimentation of biomass inside the cross-flow modules and not for fouling control. A level sensor was suspended inside the bioreactor to keep a constant working volume of 1.6 L. The sensor was then connected with the influent peristaltic pump (Watson Marlow 401U/D1, Falmouth, Cornwall, UK) (Fig. 7.1) to replace the filtered effluent volume with an equivalent volume of the influent feed. Effluent extraction was enabled by a second peristaltic pump (Watson Marlow 401U/DM3, Falmouth, Cornwall, UK).

7.2.2 Inoculum and feed

The bioreactor was dosed with synthetic wastewater containing required amounts of NH_4Cl and $NaNO_2$. The ratio NO_2^-/NH_4^+ was adjusted according to their respective concentration in the effluent and by measuring biological total nitrogen removal activity of the system. To sustain biological activity, alkalinity and sufficient amount of macro and micro nutrients were added in the form of following compounds (dissolved in tap water) in respective concentrations: $NaHCO_3$ (0.4-1.0 g L^{-1}), KH_2PO_4 (0.01 g P g COD L^{-1}), $FeCl_3*6H_2O$ (2.1 mg Fe L^{-1}), $CaCl_2*2H_2O$ (8.2 mg Ca L^{-1}), $MgCl_2*6H_2O$ (2.4 mg Mg L^{-1}), $Na_2MoO_4*2H_2O$ (0.22 mg Mo L^{-1}), $ZnSO_4*7H_2O$ (0.23 mg Zn L^{-1}), $CuSO_4*5H_2O$ (0.128 mg Cu L^{-1}), $NiCl_2*6H_2O$ (0.1 mg Ni L^{-1}), H_3BO_4 (0.007 mg B L^{-1}), Ne_2SeO_3 (0.06 mg Se L^{-1}), $MnCl_2*4H_2O$ (0.56 mg Mn L^{-1}) and $CoCl_2*6H_2O$ (0.124 mg Co L^{-1}). The amount of $NaHCO_3$ was enough to sufficiently buffer the pH of the system around 7.2±0.1 and to serve as a source of inorganic carbon for the autotrophic bacteria.

Table 7.1 Properties of the meshes used in this study

Product information	Mesh opening (µm)	Open area (%)	Mesh count (cm^{-1})	Thread diameter (µm)	Resistance (Clean mesh) (m^{-1}) (2)	Tap water permeability ($Lm^2.h^{-1}.kPa^{-1}$) (3)
SaatiMil PA(1) 7	200	39	31	120	5.46×10^9	1572.3
Saatifil PA 52/32	52	32	110	38	5.61×10^9	1528.6

(4) PA is an acronym for polyamide
(5) Resistance of the mesh measured at TMP of 5 kPa
(6) 20 ^oC normalised permeability measured at TMP of 5 kPa

The bioreactor was inoculated with a mixture of anaerobic (TS of 27 g L^{-1} and VS of 17.6 g L^{-1}) and aerobic (TS of 4.56 g L^{-1} and VS of 2.8 g L^{-1}) sludge mixed in equal proportion by mass (on the basis of total suspended solids). The initial solids concentration of the inoculum was 7.6 g L^{-1} of total suspended solids (TSS) and 5.01 g L^{-1} of volatile suspended solids (VSS). Aerobic sludge was obtained from the aeration tank of a municipal wastewater treatment plant located in Padova, Italy while, the anaerobic sludge was taken from the mesophilic excess sludge digester of the same treatment plant. Due to very slow growth rate of Anammox bacteria no excess sludge was withdrawn from the bioreactor except for the very little amount for suspended solids analysis, and thus the system was operated at an infinite sludge retention time (SRT) theoretically.

7.2.3 Strategy for bioreactor operation and DM formation, cleaning and regeneration

Bioreactor was operated under constant flux operation and the hydraulic retention time (HRT) of the system was maintained between 2-3 d. The concentration of NH_4^+ and NO_2^- were both initially set to around 50 mg L^{-1} and the nitrogen loading rate (NLR) was gradually increased by increasing the concentration of NH_4^+ and NO_2^- in the feed and maintaining NO_2^- -N/ NH_4^+ -N molar ratio to less than 1.0 (lower than the theoretical value Eq. (7.1) in order to avoid toxicity cause by high nitrite (Jin et al., 2012). NH_4^+ removal was monitored and NO_2^- concentration was increased whenever it reduced to less than 5-10 mg L^{-1} to cater for complete NH_4^+ oxidation.

The formation of DM was bought about by applying high filtration flux under gravity driven filtration mode (i.e. under the constant hydrostatic head of 35 cm). Alibardi et al, (2014) and Saleem et al, (2017, 2016) have reported about the efficacy of using such strategy in greatly reducing DM formation time and biomass loss during the formation stage. Effluent collected during this time interval (usually less than 50 ml in this study) was returned to the bioreactor through influent feeding system. The formation of DM that can offer effective solid-liquid separation was identified by measuring effluent turbidity values to less than 5 NTU (i.e. the absence of suspended solids in the effluent). Soon after the formation stage (approximately less than 5 minutes), the constant flux filtration operation was resumed for normal bioreactor operation.

An important technical challenge was to avoid the formation of vacuum in the head space in a closed bioreactor configuration during the gravity driven filtration mode (Fig. 7.1). For this purpose, daily produced biogas collected in a 3 L biogas collection bag was allowed to replace the effective volume of the bioreactor during the gravity driven filtration mode and thus completely avoiding the possibility of developing negative pressure in the head space of the bioreactor. The strategy also helped to maintain the anaerobic environment inside the bioreactor without any intrusion of ambient air.

Similarly, cleaning the excessively fouled DM under anaerobic conditions without disturbing the anaerobic environment was also challenging. Conventional techniques like backwashing and shear induced DM cleaning and fouling control have proven to be less effective in recovering the desired permeability and effluent quality of the excessively fouled DM layer (Alibardi et al., 2014; Ersahin et al., 2017; Hu et al., 2016; Salerno et al., 2017) Furthermore, opening the cross-flow module to perform cleaning at regular intervals (usually after 2-3

days) was also not not feasible, keeping in view the practical application of the same configuration (full-scale plant). Therefore, in this study a manually operated pneumatic cleaning mechanism was used to cleaning excessively fouled DM physically inside the cross-flow module (Fig. 7.1). The mechanism consisted of a 20 mm wide brush encircling the DM modules and connected with a pneumatically driven reciprocation mechanism. The reciprocations were provided manually by using a syringe filled with tap water as hydraulic fluid (Fig. 7.1). The pneumatic cleaning accompanied by simultaneous backwashing at a flow rate of 5 L h^{-1} was found to be very effective in recovering the permeability of DM and removing the fouling layer. The cleaning procedure was performed whenever the TMP started to rise above 10 kPa or when the filtration flux reduced to less than 10 LMH (predefined values to control the HRT of the system between 2-3 days). The total time for running this protocol was always completed in less than 2 minutes with continuous backwashing and 20 reciprocations of pneumatic cleaning mechanism.

The formation stage of DM was also analysed during the study period by using 5 short-term gravity driven filtration experiments for 200 and 52 µm meshes on day 4, 27, 39, 229 and 352 respectively. Experiments were conducted using the same bioreactor set-up under the constant hydrostatic water head of 35 cm (3.43 kPa). Filtration fluxes and effluent turbidity values were measured at regular intervals for 30 minutes in each experiment (Fig. 7.2).

7.2.4 Analytical methods and equipment

The performance of the bioreactor was assessed by measuring NH_4^+-N, NO_2^--N and NO_3^-N concentrations in the effluent. For this purpose periodic sampling was performed on filtered samples (0.45 µm PTFE membranes) taken from the effluent and analysed with UV-visible spectrophotometer (Shimatzu UV-1601) using Standard Methods (APHA, 2005). To assess the biomass growth inside the bioreactor, mixed liquor suspended solids and volatile suspended solids concentrations (MLSS and MLVSS) were periodically measured using the procedure outlined in Standard Methods (APHA, 2005). DM solids-liquid separation efficiency was measured by measuring effluent turbidity values with the help of (Hach 2100p iso Turbidimeter). Bioreactor's pH measurements were performed by using an electronic pH meter (Crison GLP 22). Transmembrane pressure (TMP) across DM was measured by using a U-shaped manometer filled with water as a manomatric fluid. The TMP was the sum of the hydrostatic head (35 cm of water) maintained above the DM module and the level difference between the two limbs of the U-shaped manometer pipe. Daily collected effluent volume was

measured by using a graduated cylinder and the associated average filtration flux was calculated by dividing effluent volume by the effective filtration area of the module/s. Biogas composition was measured by a micro-gas chromatograph (Varian 490-GC) equipped with a 10 m MS5A column and 10m PPU column, using argon as carrier gas and a thermal conductivity detector. The temperature of the bioreactor was maintained under mesophilic temperature of 30±1 ^{o}C, controlled by using a thermostatic bath (IS Co. GTR 2000 ‘‘11×’’, Italy) and measured by using an electronic thermometer (Hanna Check-temp °C). Biogas (Nitrogen) production was monitored by using homemade wet-tip gas meters, directly connected to the Anammox reactor and to the external cross-flow module (Fig. 7.1). To ensure continuous mixing, a magnetic stirrer (Variomag Maxi Direct, Thermo Scientific, Italy) was operated at 400 rpm. DM hydraulic resistance was calculated according to Darcy’s equation as follows:

$$R = \frac{\Delta P}{\mu \cdot J} \qquad (7.2)$$

Where J is the permeate flux, ΔP is TMP across the membrane, μ is the viscosity of permeate, and R is total membrane resistance. Similarly, free ammonia (FA) and free nitrous acid (FNA) concentrations were calculated as described in Anthonisen et al. (1976).

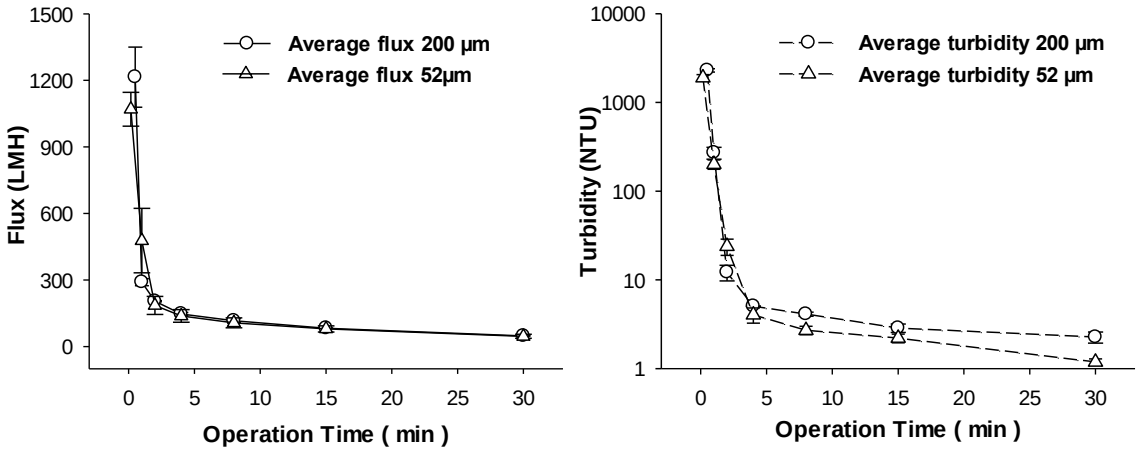

Figure 7.2 Flux and turbidity profiles under gravity driven filtration mode for DM formation at 35 cm (3.43 kPa) of hydrostatic water head for 52 and 200 µm meshes. MLSS for 200 µm was 7.23 g L^{-1} while for 52 µm was 5.34 g L^{-1} at the time of experimentation.

7.2.5 Anammox-specific bacteria detection

Sludge suspension samples were withdrawn from the reactor at three time points (day 30th, 375th and 451st). The initial inoculum used to seed the reactor was also assayed. 300 microliters from the decanted suspensions were extracted using an automatic BioSprint 96 workstation (Qiagen) as described (Stevanato et al., 2013). The final elution was carried out in a 150 µl volume and 1.5 µl were assayed. In order to ascertain the compliance of the observed nitrogen transformation to the Anammox type microbial physiology and to quantify the specific microbiota a Realtime PCR approach was adopted. Primers targeting the taxonomic group of known Anammox-proficient genera within the Plactomycetes Phylum were designed upon aligning the available 16S rRNA gene sequences drawn from the NCBI GenBank repository. The following primers were chosen: F818Scal. 5' ATGGGCACTMRGTAGAGGGRATT (forward) and R1064n 5' CCCAACGTCTCACGACACGAGCTGAC (reverse). The detection involved a Taqman FAM-labelled probe with the following sequence 5'GTGCCCCGCCTGGGGAGTACGGCCGC. To enhance detection sensitivity a QuantStudio^{TM} 3D Digital PCR genotyping System (Thermo Fisher Scientific, USA) was used at the conditions previously described (Stevanato and Biscarini, 2016).

7.3 Results and Discussion

7.3.1 Dynamic membrane formation, rise in TMP, filtration resistance and solid-liquid separation performance

Fig. 7.3, 7.4 and 7.5 illustrates profiles of DM operational parameters including rise in TMP observed for each operational cycle (Fig. 7.4a), effluent turbidity as a measure of DM solid-liquid separation efficiency and maximum DM filtration resistance observed before every cleaning operation (Fig. 7.3b), observed filtration flux and bioreactor's HRT (Fig. 7.4), DM cleaning frequency and fouling rate expressed per 15 d time interval along with solids (MLSS/MLVSS) evolution profile for the entire study (Fig. 7.4). One operational cycle corresponds to the start of filtration until the time when physical cleaning was applied by using the pneumatic cleaning mechanism. The whole operational cycle was divided into three stages including DM formation (less than 5 minutes), constant flux filtration (operational period between 1 to 7 days depending upon applied filtration flux and rate of fouling) and DM cleaning for permeability recovery (less than 2 minutes).

The bioreactor operation was started by using a single DM module with a 200 µm mesh and the filtration cycle began by following the procedure discussed in section 7.2.3 using gravity driven filtration mode. Although the start of constant flux filtration after every DM reformation was marked by observing effluent of turbidity less than 5 NTU (Section 7.2.3) however, the average turbidity observed for the constant flux filtration mode was 44.3±4.2 NTU during the first 32 days of continuous bioreactor operation for 200 µm mesh (Fig. 7.3b). This showed poor solid-liquid separation performance of DM formed over 200 µm mesh under constant flux filtration mode corresponding to the loss of more than 60 mgL^{-1} of suspended solids through the effluent (data not reported). During this time TMP was maintained well below 20 kPa (Fig. 7.3b) and DM resistance increased up to the order of 10^{12} m^{-1}, three orders of magnitude greater than mesh intrinsic resistance measured with tap water (Table 7.1). Filtration flux averaged around 15.3±0.8 LMH (Fig. 7.4) while system's HRT was maintained around 2.8±0.13 d, well between the range decided for bioreactor operation (section 7.2.3). Due to the biomass loss through the effluent the MLSS inside the bioreactor reduced more than 20 %, from 7.23±0.16 gL^{-1} (initial 32 days average) to 5.49 ±0.23 gL^{-1} (rest of the experimental period) (Fig. 7.5a). In order to curtail biomass loss through the effluent it was decided to use 52 µm mesh instead of 200 µm mesh on day 33 of the experiment.

The change in the mesh porosity bought considerable improvement in the effluent quality in terms of turbidity that gradually reduced to less than 10 NTU after 50 days of continuous experimentation. Studies have reported contradictory results regarding the effect of mesh porosity on DM solid-liquid separation efficiency. On one hand it has been reported that large mesh porosity resulted in longer formation time of DM and poor effluent quality due to the loss of biomass particles smaller than the mesh pore size (Ersahin et al., 2012; Hu et al., 2016; Wu et al., 2003). On the other hand, while performing short term gravity driven filtration experiments on different mesh porosities using anaerobic sludge, Saleem et al, (2017) have reported conclusively that DM solids rejection is independent of mesh porosity and significantly affected by MLSS concentration of the filtering suspension. However, Saleem et al, (2017) and Hu et al., (2016) applied the gravity driven filtration mode for effluent collection (i.e. constant TMP operation) and in this study a peristaltic pump was utilised instead for permeate extraction (i.e constant flux operation). The excess hydrodynamic pressure produced due to forced extraction through a pump must have an

effect on the stability of DM layer depending upon the rate of rise in TMP, and its strength and maturity (Alibardi et al., 2014). As a matter of fact, the behaviour of DM formed over 200 and 52 µm meshes was similar during the formation stage under gravity driven filtration mode with similar formation time (i.e less than 5 min) and effluent quality in terms of turbidity values (less than 5 NTU) (Fig. 7.2). Similar results have been reported by Hu et al., (2016) for the formation stage of DM while working with DM bioreactor under aerobic conditions. The deterioration of effluent quality with progressively increasing suction pressure and consequent disruption of DM layer has also been reported by Salerno et al, (2017).

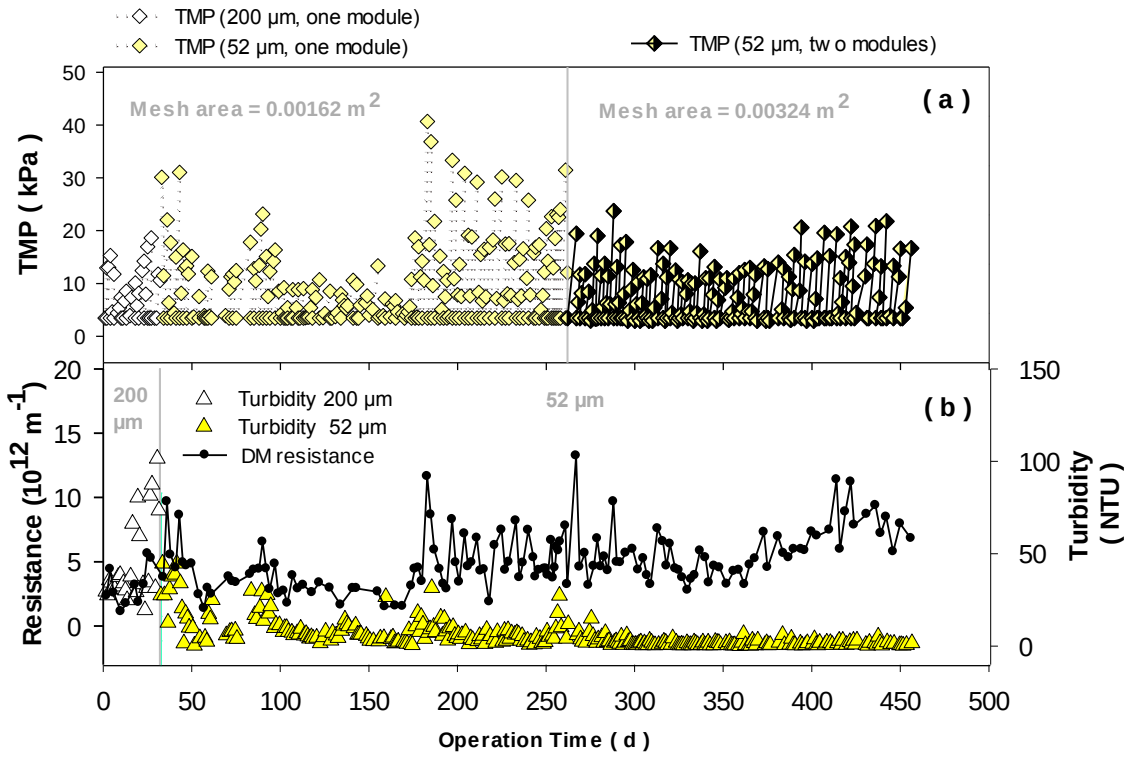

Figure 7.3 (a) Variation of TMP profile along the experimental period and (b) observed turbidity profile and maximum filtration resistance profile for 200 µm and 52 µm meshes

The reduction in the mesh porosity decreased the filtration fluxes by 12% and thus the average HRT of the system was increased to 3.12±0.04 d (Fig 7.4). In order to reduce the HRT (between 2-3 d), effluent fluxes were increased to 15 LMH however; the increase in filtration flux was followed by a rapid increase in the TMP values above 20 kPa between day 180 and 262. The issue was resolved by doubling the effective filtration area of DM to 0.00324 m^2 due to which the average HRT reduced to 2.3±0.04 d (Fig. 7.4) and rise in TMP

was also curtailed to less than 20 kPa again (Fig. 7.3a). Similarly, DM filtration resistance averaged around 5.9×10^{12} m^{-1} (Fig 7.3b). Furthermore, the observed effluent quality from day 262 onwards also improved with an average effluent turbidity of 2.4±0.2 NTU for which the suspended solids in the effluent were mostly undetectable (data not reported). Solid-liquid separation performance of the formed DM in this study was comparable to the values reported by Hosseinzadeh et al, (2013) observed for conventional membranes and also confirmed by Salerno et al, (2017) for DM. The excellent solid liquid separation performance of DM observed in this study demonstrated the robustness of this technology in successfully enriching very slow growing microorganisms, such as Anammox bacteria.

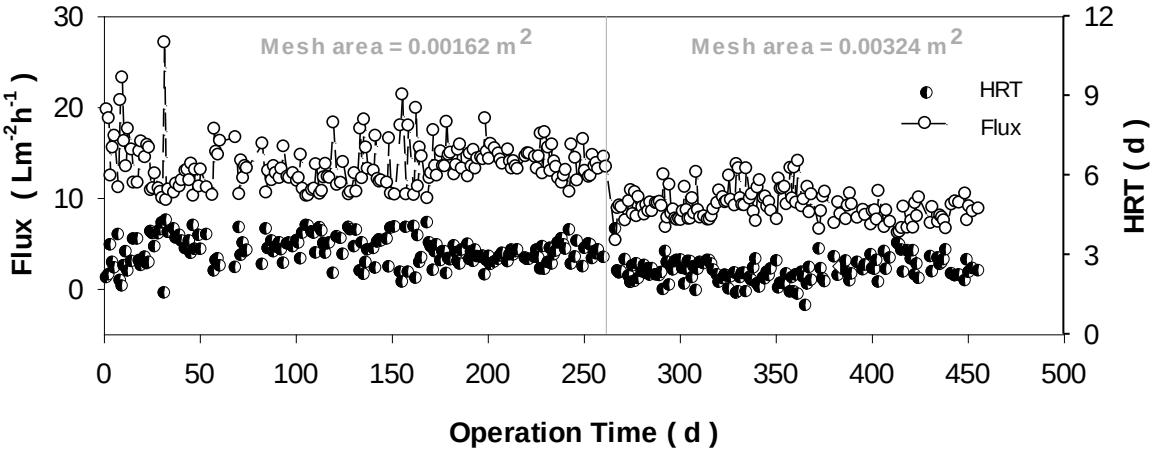

Figure 7.4 Observed HRT and filtration flux profile for the experiment

7.3.2 DM fouling rate and associated cleaning frequency

Start of cleaning operation was identified by the rise in TMP above 20 kPa or when the filtration flux reduced to less than 5 LMH and permeability recovery of the excessively fouled DM was required to maintain the HRT of the system between 2 to 3 d. Initially dynamic membrane cleaning was performed after almost every 3 days of the continuous constant flux operation till day 120. During that time the average fouling rate was variable averaging around 0.23±0.04 kPa h^{-1}. However, between days 130 to 180 the fouling rate suddenly decreased to less than 0.09 kPa h^{-1}, 2.5 times lesser than the previously observed value. The sudden drop in fouling rate was attributed to the reduction in MLSS concentration inside the reactor. MLSS concentration inside the bioreactor continues to decrease between 130 to 180 d of experiment despite excellent solid liquid separation performance of DM (Fig. 7.6a). An investigation inside the bioreactor performed on day 180 revealed that some part of the biomass was attached to the walls of the bioreactor and therefore, the actual MLSS

concentration was not appearing in the solids analysis. After the resuspension of the attached biomass in the bulk MLSS, the solids concentration again increased up to the previously measured concentration of around 5 g L^{-1} (Fig. 7.6a). DM formation and fouling rate have been reported to be significantly affected by the MLSS concentration of the filtering suspension in several studies (Li et al., 2011; W. W. Li et al., 2012; Saleem et al., 2017). The increase of MLSS concentration above 5 g L^{-1} also increased the fouling tendency of DM in this study due to which fouling rate again increased to more than 0.6 kPa h^{-1} and cleaning frequency was also increased from 6 cycles per 15 day to 9 cycles per 15 day time interval. Moreover, the fouling rate measured between 255 to 262 day (for 7 days) was the highest recorded for the entire bioreactor operation with 5 cleaning cycles during this time (Fig. 5b, single vertical bar highlighted in red colour). The increase in mesh filtration area on day 262 reduced the average filtration flux to 9.1±0.16 LMH (observed between day 263 and 456) from 13.3±0.2 LMH (observed between day 33 and 262). Since DM formation and fouling rate is proportional to solid flux (Filtration flux × MLSS) over the mesh surface (Saleem et al., 2016) therefore, DM fouling rate was also reduced, averaging around 0.15± 0.01 kPa h^{-1} however, DM cleaning frequency only slightly reduced to 4.8± 0.3 per 15 day. It is of note that the specific filtration area used in this study (1.0-2.0 m^2m^{-3}) was far less than what was reported for MBR using conventional membranes (6-41 m^2m^{-3}) (Huang et al., 2016; Li et al., 2015; Niu et al., 2016; Wang et al., 2012; Zhang et al., 2016). Therefore, filtration flux, rate of DM fouling and associated DM cleaning frequency could further be reduced by increasing the effective filtration area of DM.

The use of pneumatic internal cleaning mechanism with simultaneous backwashing found to be very effective in recovering the desired permeability of DM without disturbing the anaerobic environment inside the system. In fact, the external cross-flow module was never opened for cleaning or maintenance purposes, except for the addition of another DM module to increase the effective filtration area on day 262. Furthermore, no chemical cleaning was applied during the entire study period of 456 days. The fact that the proposed cleaning mechanism was effective in permeability recovery is also highlighted by observing the flux and turbidity profiles for short-term gravity driven filtration tests performed during the formation stage of DM. Despite being performed on different days (considerably spaced in time (section 7.2.3), and different MLSS concentrations, the trends in flux and turbidity profiles were similar with very little standard error in measurements for 200 and 52 µm

meshes respectively (Fig. 7.2). Rapid reduction in filtration flux and turbidity values is a typical behaviour of DM formation under gravity driven filtration mode frequently observed and reported by other studies (Chu and Li, 2006; Fan and Huang, 2002; Hu et al., 2017; Saleem et al., 2017). Surface brushing has already been suggested as very effective in cleaning excessively fouled DM and removing the cake layer (Ersahin et al., 2012). Study performed by Hu et al, (2016) has shown that surface brushing, was unavoidable in order to completely remove irreversible fouling and to recover underlying support permeability in addition to air backwashing. Biogas sparging to control DM layer permeability was found to be effective in improving filtration flux however, the technique has to be evaluated for additional cost and effect of excess shear on effluent quality (Ersahin et al., 2016). Similarly, until recently Salerno et al., (2017) have reported the effectiveness of continuous air scouring for ensuring long term DM filtration operation however, they found that too intense air scouring could result in deteriorated effluent quality and thus renders unsuitable for complete permeability recovery of the underlying support.

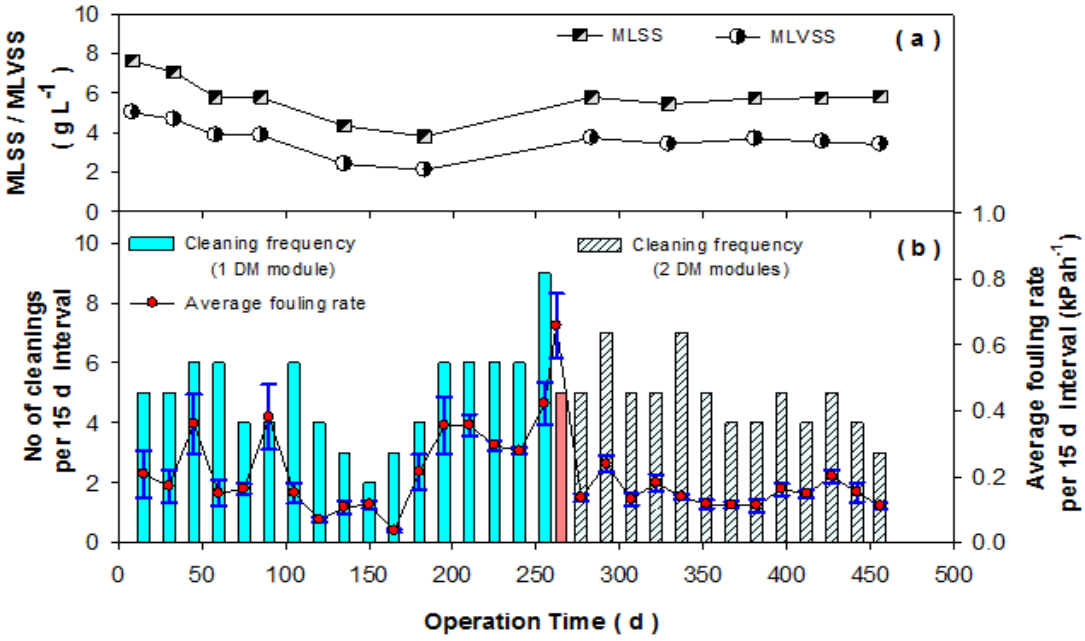

Figure 7.5 (a) Evolution of MLSS/MLVSS inside the bioreactor and (b) mesh cleaning frequency along the experimental period of 456 days (single and double module) with the variation of average fouling rate during the study (red vertical bar represents 7 day time interval instead of 15 just before the increase in mesh effective filtration area

7.3.3 Nitrogen species and total nitrogen removal performance

During the course of 456 days, bioreactor's biological performance was evaluated by observing trends and distribution of nitrogen species (NH_4^+ -N, NO_2^- -N and NO_3^- -N) in the effluent and their respective loading rate (NLR), increasing rates (NIR) and removal rates (NRR) represented in figure 5 and figure 7.7. Based on effluent nitrogen profile (Fig. 7.6), the start of the biological activity was observed after 16 days of the continuous experimentation when total nitrogen removal efficiency slightly increased to more than 20% after showing a negative trend (Fig. 7.7a) in its profile. The negative trend could be due to the presence of higher NH_4^+-N concentration inside the sludge sample (from aerobic tank and anaerobic digester) as compared to the influent NH_4^+-N concentration in the feed (38.9 mg L^{-1}). The decrease in NH_4^+-N and NO_2^--N concentrations and subsequent production of NO_3^--N on day 16 onwards suggested the progressive enrichment of Anammox bacteria (Fig 7.6). During the course of experimentation the influent nitrite and ammonia concentration was increased gradually from 50 to 2400 mg L^{-1} (15.2-730.4 mg-N L^{-1}) and 50 to 1000 mg L^{-1} (38.9-777.8 mg-N L^{-1}) respectively (Fig 7.6), resulting in an increase in the nitrogen loading rate (NLR) from 14.5 to 696 mg$L^{-1}d^{-1}$ (Fig. 7.7a). The colour of the inoculum also changed from black to rusty near the end of bioreactor operation.

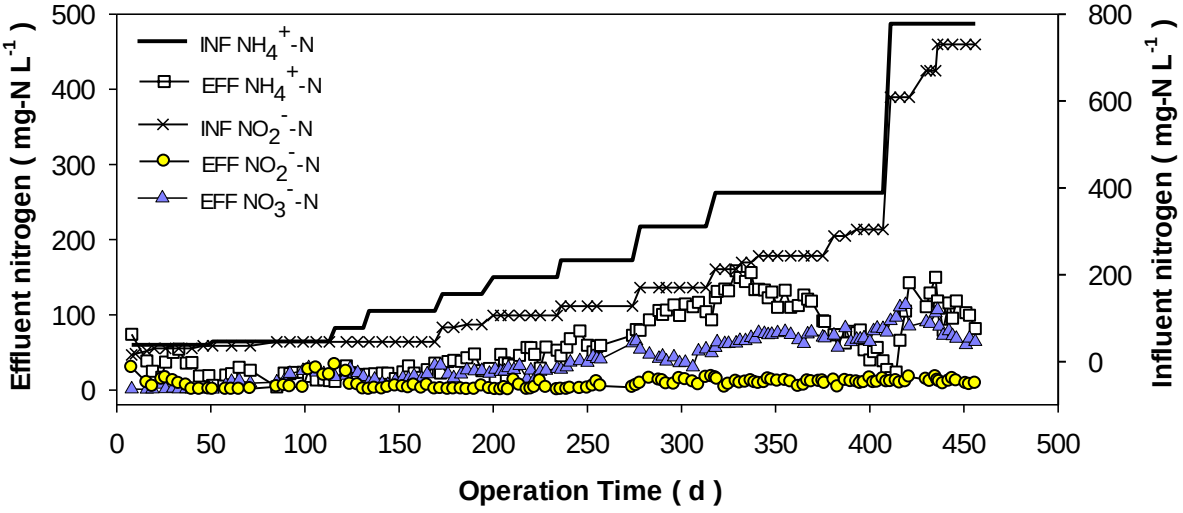

Figure 7.6 Influent NH_4^+-N and NO_2^--N profiles along with the observed effluent NH_4^+-N, NO_2^--N and NO_3^--N profiles for the entire study period

Initially the system exhibited fluctuating nitrogen removal efficiency profile and total nitrogen removal efficiency decreased to less than 20% between day 26 to 33 and 102 to 122

of the experiment (Fig. 7.7a). After analysing the effluent nitrogen species profiles (Fig. 7.6) it was found that during these two instances effluent NO_2^--N concentration showed peaks in its profile measuring up to 16.2 mg-NL^{-1} on day 26 and 33.7 mg-NL^{-1} on day 116. NO_2^--N / NH_4^+-N molar ratio was also the highest (0.98) observed for the entire study between day 102 to 122 (Fig. 7.8). Similarly, the loss of total nitrogen removal efficiency to less than 20 % was also coincided with nitrite accumulation in the bioreactor. Furthermore, NO_2^--N removal rate was also decreased to less than 5 mg L^{-1} d^{-1} during the same time intervals, demonstrating a strong relation between nitrite accumulation and inhibition of Anammox biological activity (Fig. 7.7c). High nitrite concentration has been repeatedly reported to be inhibitory for Anammox bacteria in varying concentrations depending upon the experimental mode and conditions (Bettazzi et al., 2010; Ding et al., 2017.; Kimura et al., 2010; Ni et al., 2009; Strous et al., 1998). Although reported results have contradictions over toxicity threshold values ranging between 5 to 280 mg-N L^{-1} (Jin et al., 2012), the inhibitory values of nitrite observed in this study (i.e. 16.2 and 33.7 mg-N L^{-1}) falls within this range. Neither FA, nor FNA was found to be higher than the inhibitory concentrations for Anammox bacteria reported in literature, which are 37 to 76 mg L^{-1} for FA and 3 to 11 µg L^{-1} for FNA respectively (Li et al., 2016). The average theoretical values of FA and FNA in this study were found to be 1.96±0.2 mg L^{-1} and 0.56 µg L^{-1} respectively (data not reported). Addition of trace concentrations of Anammox intermediates (hydroxylamine or hydrazine) were stated to be conducive in reviving Anammox activity from nitrite inhibition (Strous et al., 1999). However, the loss of Anammox activity in this study was not severe and a slight reduction in the filtration fluxes to decrease the NLR was helpful in restoring the Anammox activity without reducing the influent NH_4^+-N and NO_2^--N concentration (Fig. 7.6 and 7.7a). Similar observation for Anammox activity recovery after nitrite inhibition was also reported by Tao et al, (2012) and Tang et al, (2009).

After day 134 onwards no inhibition was observed and bioreactor exhibited gradual improvement in Anammox activity. Influent NH_4^+ concentration was progressively increased to 500 mg L^{-1} from 100 mg L^{-1} (77.8 to 388.9 mg-N L^{-1}) while NO_2^-concentrations was also increased from 150 to 1000 mg L^{-1} (45.7 to 304.3 mg-N L^{-1}) (Fig. 7.6). During this time interval (day 134-409) the NLR was increased from 39.to 429 mg L^{-1} d^{-1}.with a concurrent increase in NRR from 22 to 304 mg $L^{-1}d^{-1}$(Fig. 7.7a). The total nitrogen removal was 72±0.6 %, and NH_4^+-N and NO_2^--N removal efficiencies were 74.4±0.9% and 95.2±0.34%

respectively (Fig. 7.7). Major portion of the effluent nitrogen comprised of 56.9±1.14% NH_4^+-N while NO_2^--N and NO_3^--N were 6.1±0.42% and 37±1.0% respectively. During the same time interval effluent NH_4^+-N showed more fluctuations in its profile than NO_2^--N and NO_3^--N, ranging from 14.7 to 158.5 mg-N L^{-1} (Fig. 7.6).

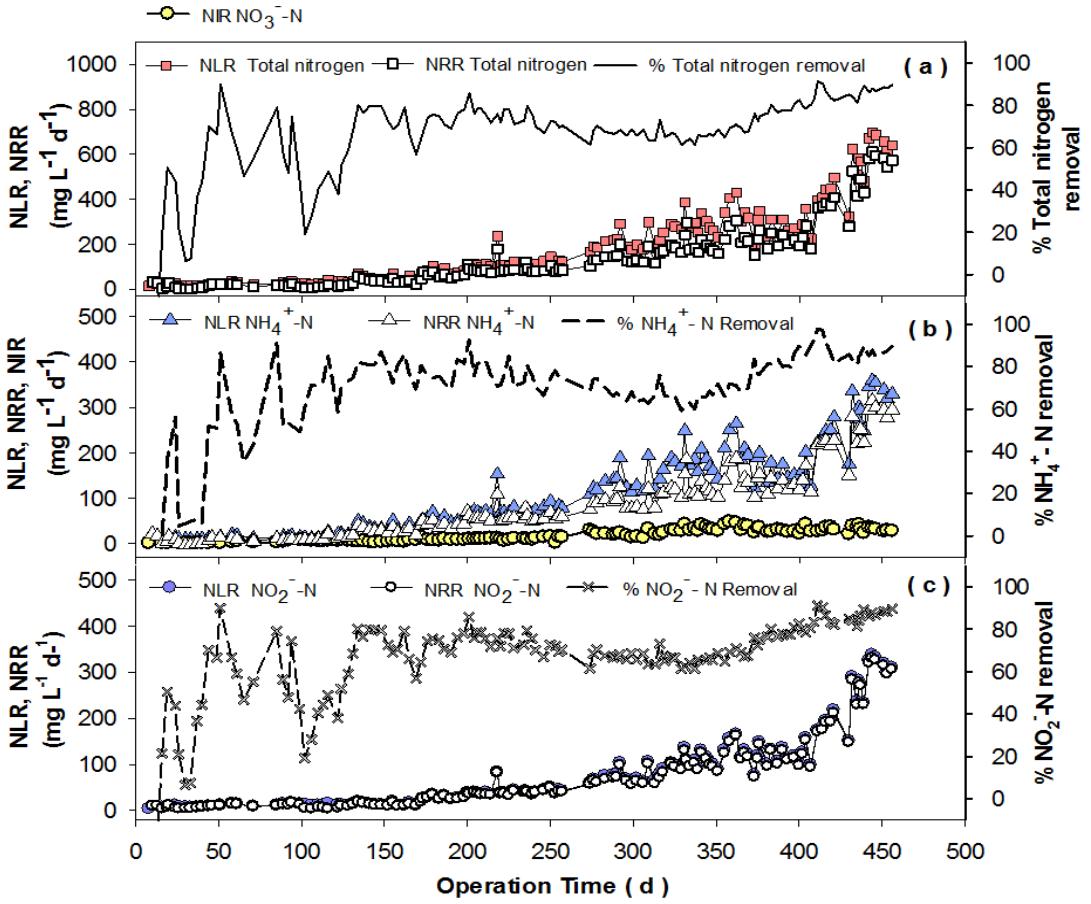

Figure 7.7 NLR, NRR profiles and removal rates for (a) total nitrogen (b) $NH4^+$-N and NIR for NO_3^--N (c) NO_2^--N

The increase in the influent NH_4^+-N concentration from 400 to 500 mg L^{-1} (311.1 to 388.9 mg-N L^{-1}) between day 316 to 409 (Fig. 7.6) was followed by a deterioration in the effluent quality in terms of NH_4^+-N removal efficiency which decreased to less than 60% while NO_2^--N removal efficiency did not show much variation and remained above 85% (Fig. 7.7a and 7.7c). The NRR ($NH4^+$-N) was almost 40% lower than the NLR ($NH4^+$-N) showing limited NH_4^+-N oxidation through Anammox activity (Fig. 7.7b). In order to reduce effluent NH_4^+-N and to improve NH_4^+-N removal efficiency, NO_2^--N / NH_4^+-N molar ratio was increased from 0.55 to 0.78 (Fig. 7.8) by gradually increasing the influent NO_2^- concentration from 700 to 1000 mg L^{-1} (213 to 304.3 mg-N L^{-1}) over a period of 58 days (between day 332-390) (Fig.

7.6). The additional NO_2^--N was consumed for ammonia oxidation and did not show up in the effluent due to which NO_2^--N concentration remained stable averaging around 10.9±0.5 mg-N L^{-1} (Fig. 7.6). As a result, NH_4^+-N removal efficiency showed improvement in its profile and reached more than 90% (Fig. 7.7a) consequently the share of NH_4^+-N in total effluent nitrogen also decreased, measuring less than 30 mg-N L^{-1} on day 409 of the continuous bioreactor operation (Fig.7.6). The discrepancy between NRR ($NH4^+$-N) and NLR (NH_4^+-N) also reduced to almost 10% (Fig. 7.7b). After observing a steady state total nitrogen removal of around 80% from day 397 to 411 (Fig 7.7a), the influent NH_4^+ concentration was increased from 500 to 1000 mg L^{-1} (388.9 to 777.8 mg-N L^{-1}) while NO_2^- concentration was also doubled (i.e. from 1000 to 2000 mg L^{-1}) (Fig. 7.6). Effluent $NH4^+$-N again tend to increase and reached 150 mg L^{-1} on day 135 (Fig. 5) however, NH_4^+-N removal efficiency was still above 80%, while NO_2^--N removal was above 98 % (Fig. 7.7a and 7.7c). Once again an increase in NO_2^--N / NH_4^+-N ratio by increasing the influent NO_2^- concentration from 1000 to 2400 mgL^{-1} (304.3 to 730.4 mg-N L^{-1}) from 0.78 to 0.94 (Fig. 7.8) bought improvement in NH_4^+-N removal efficiency up to 89% (Fig. 7.7b). The bioreactor showed a steady total nitrogen removal of 87.5±0.56% during the last 20 days of bioreactor operation (Fig. 7.7a), while effluent nitrogen consisted of 54±1.2% NH_4^+-N, 6.0±0.46% NO_2^--N and 40±1.2% NO_3^--N respectively. Maximum achieved NRR for total nitrogen, NH_4^+-N and NO_2^--N during this time interval were 611.6, 314.8 and 330.1 mg L^{-1} d^{-1}respectively. Due to the oxidation of NO_2^--N to NO_3^--N for the generation of energy for Anammox bacteria, the increase in influent NO_2^--N concentration was followed by an increase in effluent NO_3^--N concentration from 1.0 to 113 mg-N L^{-1} (Fig. 7.6). The NIR value increased from 0.3 to 47.3 mg L^{-1} d^{-1} during the entire bioreactor operation (Fig. 7.7b).

Throughout the experiment average effluent pH was maintained at 7.46±0.02 with a low standard deviation of only 17%, indicating that influent alkalinity (section 7.2.2) provided the sufficient buffering capacity for the system. The recorded pH falls within the optimum pH range for Anammox bacteria (6.7–8.3) (Strous et al., 1999).

Analysis of biological activity of enriched Anammox biomass was also performed on the basis of molar ratios for removed NH_4^+-N : NO_2^--N : produced NO_3^--N : N_2 gas observed after 134 days of continuous bioreactor operation (Fig. 7.8). This theoretical molar ratio has been reported by Strous et al, (1999) to be 1:1.32:0.26:1.02 for Anammox activity. Neither the removed NO_2^--N : NH_4^+-N ratio, nor the produced NO_3^--N: removed NH_4^+-N ratio

observed under varying influent NO_2^--N : NH_4^+-N ratio conformed with the reported ratio of 1.32 and 0.26 by Strous et al, (1999) in this study. The observed values for removed NO_2^--N : NH_4^+-N and produced NO_3^--N : removed NH_4^+-N ratios in the last 20 days (436-456) were smaller than the proposed values averaging around 1.07±0.007 and 0.11±0.007 respectively (Fig. 7.8). The molar ratio for produced N_2 and consumed NH_4^+-N was highly variable and averaged around 1.05±0.16 which is close to the theoretical value of 1.02. The high variability could be due to the changes in influent $NO2^-$-N : NH_4^+-N ratio throughout the experimental period similar observation was reported by Ni et al, (2009).

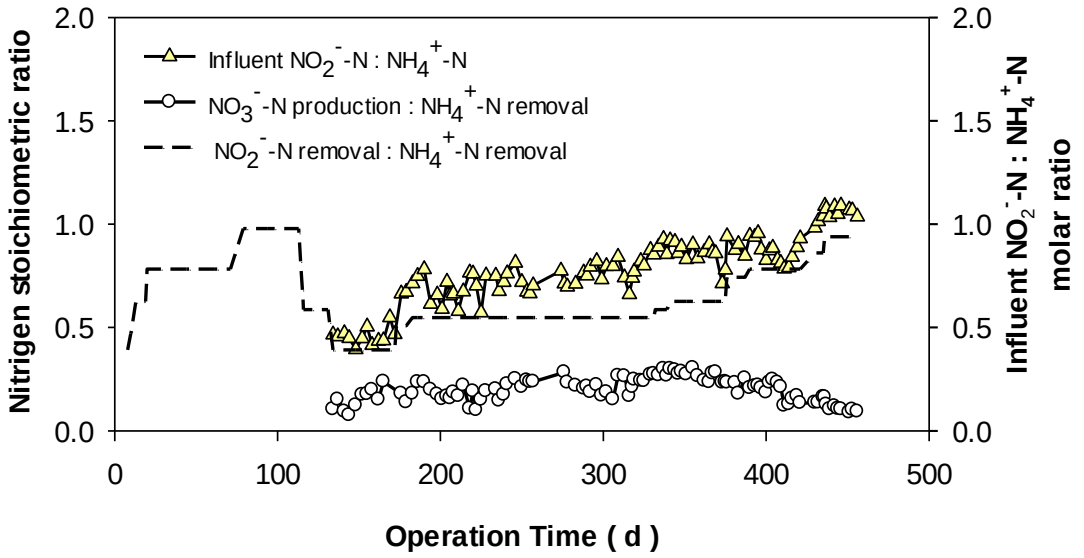

Figure 7.8 Stoichiometric molar ratios obtained between day 134 and 456.

7.3.4 Microbial community evolution and enrichment of Anammox biomass

The analysis was performed by targeting the 16S ribosomal operon DNA of a group of microorganisms that are known to be involved in the Anammox process and that all belong to the Planctomyceyes phylum The primers used were designed to encompass all currently ascertained genera capable of such metabolism, which include *Kuenenia, Brocadia, Scalindua, Jettenia,* and *Anammoxoglobus*. The detection of the resulting 246 bp amplicon was achieved by an internal specific Taqman probe. The corresponding RealTime PCR assay took advantage of the digital PCR extension module, a technique that spreads the reaction over a chip plate enhancing over 100-fold the detection limit in comparison to ordinary RealTime PCR and that has recently been shown to be functional on plant and environmental genomic and metagenomic DNA (Stevanato et al., 2016).

The analysis was performed on samples from three progressive time points withdrawn from the reactor, plus the initial inoculum. As positive control a sample taken from an industrial-scale Anammox plant operative at a local swine farm and continuously fed by the liquid digestate fraction resituating from anaerobic digestion was used. The results, expressed in number of gene copies per microliter of dPCR reaction were the following: Initial inoculum Below detection limit; Time point 1 (day 30): Below detection limit; Time point 2 (day 375): 1307 gene copies/µl;; Time point 3; day 451: 7192 gene copies/µl. The positive control, which at the sampled date had an efficiency of nitrogen abatement of 66%, scored 8048 gene copies/µl.

7.4 Conclusion

Experimental results have shown that DM can effectively retain slow growing Anammox bacteria and ensure stable bioreactor operation. In this regard pore size of underlying support played an important part and solid-liquid separation performance of 52 µm mesh was better than 200 µm mesh with an average turbidity value of 2.4±0.1 NTU. The HRT and TMP of the system were kept within the predefined range of 2-3 d and less than 20 kPa respectively. DM fouling rate and cleaning frequency were found to be affected by MLSS concentration and effective filtration area of DM. The proposed internal cleaning mechanism was effective in permeability recovery of the excessively fouled DM without the application of chemical cleaning during the entire study period of 456 days. However, the proposed cleaning mechanism needs further investigation in similar pilot and full-scale applications. The system showed robust Anammox activity during the gradual enrichment period, reaching NRR for total nitrogen up to 611.6 mg L^{-1} d^{-1} while applying a maximum NLR of 696 mg $L^{-1}d^{-1}$ that corresponds to an average total nitrogen removal of 87.5±0.56%. Similarly, the bioreactor easily coped with the episodes of poor Anammox activity due to high nitrite concentration by just reducing the applied filtration flux and in return, the NLR to the bioreactor. The observed stoichiometric values of Anammox activity process were in agreement with the reported literature. Quantification of the Anammox-specific bacteria by RealTime PCR confirmed the genuine presence of the expected microbiota and its progressive enrichment consistent with the increasing performance of the reactor in abating nitrogen. The results obtained from this study demonstrated that the proposed bioreactor configuration holds a great promise to be considered as a cost-effective alternate for conventional MBR systems for enriching and applying Anammox process.

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CHAPTER 8

OVERALL CONCLUSIONS AND FUTURE RESEARCH DIRESTIONS

8 Overall conclusions and future research direction

8.1 Conclusions

The study was undertaken to evaluate the performance (solid removal) of DM assisted biological treatment of synthetic wastewater and landfill leachate. The experiments using synthetic wastewater were conducted to investigate the performance of DM coupled bioreactors for the production of biogas (CH_4 and H_2) and its potential for the enrichment of slow growing Anammox bacteria. The evaluation of DM formation mechanism and performance was based on selecting the appropriate porosity of underlying support (i.e. nylon meshes of 10, 22, 52, 85, 135 and 200 µm pore size) together with assessing the effect of operational parameters (TMP, filtration flux, effective filtration area and effluent turbidity etc.). A wide variety of experimental conditions including mode of filtration (constant TMP and constant flux), type of biological process (aerobic and anaerobic), cleaning procedure (cross-flow and surface brushing) and bioreactor configuration (external and submerged) were applied and studied. Procedure to expedite DM formation and to reduce biomass loss during its formation stage was outlined and thoroughly investigated. The characteristics of the DM (resistance and fouling rate etc.) and filtration behaviour were also investigated in response to the change in feed wastewater characteristics.

Based on the results and main observations of these experiments following conclusions can be drawn:

- It was possible to form DM and use it as a mean of solid-liquid separation under variety of conditions applied and tested in this study.
- Rate of development of DM was positively associated with the magnitude of applied flux and higher filtration flux tends to increase the rate of formation of DM.
- DM formation under gravity driven filtration and high filtration flux was found to be effective in expediting DM formation (within few minutes) and avoiding biomass loss in the formation stage of DM for both, external cross-flow and submerged configurations. However, it was particularly challenging to apply gravity driven filtration under anaerobic conditions due to the formation of vacuum in the head space. The problem was encountered by allowing the daily collected biogas to replace the drop in the effective volume of the bioreactor in the head space during the formation stage of DM, simulating similar conditions of gravity driven filtration.

- The results obtained under short-term gravity driven filtration tests suggested that cake filtration mechanism can be effectively used to model DM formation and filtration flux behaviour.
- DM resistance was the major contributor of overall resistance of DM module accounting for more than 99% of total resistance.
- Contrasting results regarding the effect of mesh porosity on the development and performance of DM were recorder. During the short-term gravity driven filtration experiments DM formation and its solid-liquid separation performance was found to be be independent of the range of mesh porosities tested in this study instead, MLSS concentration was the major factor affecting the filtration performance of DMs. However, in the experiments using landfill leachate for total nitrogen removal and synthetic wastewater for bioH_2 production lower mesh porosity showed better solids removal performance as compared to higher mesh porosity.
- DM formation and filtration behaviour was found to be significantly affected by the type of influent feed wastewater and its characteristics. DM fouling was aggravated and solid-liquid separation performance was deteriorated during landfill leachate treatment and bioH_2 production when the characteristics (concentration of organics during bioH_2 production) and type (landfill leachate instead of municipal wastewater) of influent feed were changed.
- High total nitrogen removal and NH_4^+-N conversion efficiencies of around 98% and 99% were observed for DM bioreactor treating landfill leachate. The observed biological removal efficiency was comparable with conventional MBR systems.
- The strategy of gradually increasing the leachate concentration in the influent feed allowed the gradual enrichment of slow growing nitrifying bacteria which proved to be effective in maintaining steady performance of the bioreactor while avoiding possible inhibition due to excess free ammonia and free nitrous acid concentration.
- DM was successfully used for bioH_2 production from high strength synthetic wastewater applying high OLR and using untreated suspended anaerobic biomass. However, DM solids removal performance showed a strong negative correlation (ρ=-0.95) with influent organics (in the form of sucrose) concentration due to which lower mesh porosity (21 µm) showed better solids removal performance.

- The strategy of gradually increasing the influent organics concentration as sucrose proved to be effective in maintaining steady performance of the bioreactor at low HRT (around 1 d) to avoid possible inhibition due to excess VFA accumulation and high OLR.
- Due to the same fact the bioreactor exhibited stable biological performance with an unvarying carbohydrates conversion efficiency of more than 99% and a H_2 yield of 8 L H_2/mole of sucrose.
- DM formed over 52 µm can effectively retains slow growing Anammox bacteria producing high quality effluent of average turbidity value of 2.4±0.1 NTU and ensuring stable bioreactor operation.
- The system showed robust Anammox activity during the gradual enrichment period of 456 days, reaching NRR for total nitrogen up to 611.6 mg $L^{-1}d^{-1}$ that corresponds to an average total nitrogen removal of 87.5±0.56%.
- Application of shear generated by applying high cross-flow velocity to control DM layer thickness and its permeability was ineffective. Therefore, surface brushing was applied for permeability recovery of excessively fouled DM layer in both the configurations (external cross-flow and submerged) used in this study. The proposed pneumatic internal cleaning mechanism along with simultaneous backwashing used under anaerobic conditions (bioH_2 production and Anammox enrichment) was effective for in situ cleaning of DM layer and recovery of underlying support permeability without the application of chemical cleaning.
- Although the main focus of this study was to assess DM's physical solid-liquid separation performance under variety of conditions however, the formed DM was also biologically active and accounted for an average 9.2±5.0% of the total nitrogen removal during landfill leachate treatment. Keeping in view the small amount of biomass forming the DM as compared to the amount of biomass inside the bioreactor, DM layer showed a considerable biological activity.
- DM coupled bioreactors studied in this study exhibited comparable physical and biological treatment performances in terms of contaminates removal and applied fluxes, plus its low capital investment, low TMP operation and reproducibility makes it a promising alternative for conventional MBR systems.

Nevertheless, DM is still in its infancy and requires more consolidated research efforts to investigate and standardize this technology. Some of the main areas to commence future research directions are discussed below.

8.2 Future research direction

Based on the contrasting results reported in this thesis for the effect of mesh porosity on DM formation and performance while treating different wastewater streams, more research should be focused to identify the important parameter/s linked to the change in the behaviour of formed DM. This is the first time that the change in the filtration behaviour of DM and worsening of solid removal performance along the experimental run was observed and reported. Type and characteristics of influent feed are reported as a major factor causing the change in the filtration behaviour of the filtering suspension and thus the formed DM layer. However, the parameter that is responsible for this change was not studied and experimentally identified in this thesis. Although studies have identified excessive concentrations of biofoulants including Extracellular Polymeric Substances (EPS) and Soluble Microbial Products (SMP) aggravate fouling in DM (Hu et al., 2016; Liang et al., 2013; Yu et al., 2015) under aerobic and anaerobic conditions. However, the effect of change in feed characteristics in connection to the production of these biofoulants and their respective optimum concentration for the development of an effective DM layer has not been studied due to which application of DM technology cannot be standardized for treating different kinds of wastewater streams.

The application of DM is still limited to municipal and synthetic wastewater treatment mostly performed on lab-scale or pilot scale bioreactors and very few studies were focused on its application in high strength complex wastewater streams like landfill leachate and industrial effluents. In this thesis an effort was made to understand DM formation and behaviour for the treatment of landfill leachate, production of H_2 and for the enrichment of Anammox biomass. Information related to their performance while treating variety of high strength industrial effluents is very limited, which in fact, is a major consumer of conventional MBR technology (Judd, 2016). Therefore, DM technology cannot be considered as a reliable alternative for conventional MBR systems unless its application is extended to treat industrial effluents in full-scale applications.

The strategy to rapidly develop DM under gravity driven filtration mode was found to be effective in avoiding biomass loss during the formation stage of DM in this study however,

the application of the same strategy has to be investigated at pilot and full-scale set-ups. The quantities of effluent collected during the formation stage and its recirculation back into the bioreactor plus the time required for the formation of DM providing effective solid-liquid separation are the most important parameters to be investigated.

The specific filtration area (m^2m^{-3}) used in this study was similar or even less as compared to conventional MBR systems nonetheless, an appropriate design of DM module is also challenging that can provide maximum effective filtration area per unit bioreactor volume occupied.

As discussed in this study that commonly used DM cleaning methods (backwashing and air or biogas sparging etc.) have limited performance for long term steady DM operation while physical cleaning through surface brushing was shown to be effective in permeability recovery. The proposed internal cleaning mechanism must be evaluated for pilot-scale and full-scale applications in order to investigate the engineering challenges arising with the scaling up of the proposed internal cleaning mechanism. Definition of an effective cleaning mechanism and DM formation protocol is mandatory to successfully apply this concept in full-scale treatment plant set-up.

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