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**DOI**

[10.1016/j.watres.2018.03.015](https://doi.org/10.1016/j.watres.2018.03.015)

**Publication date**

2018

**Document Version**

Accepted author manuscript

**Published in**

Water Research

**Citation (APA)**

Dereli, R. K., Wang, X., van der Zee, F. P., & van Lier, J. B. (2018). Biological performance and sludge filterability of anaerobic membrane bioreactors under nitrogen limited and supplied conditions. *Water Research*, 137, 164-172. <https://doi.org/10.1016/j.watres.2018.03.015>

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## Biological Performance and Sludge Filterability of Anaerobic Membrane Bioreactors Under Nitrogen Limited and Supplied Conditions

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### Abstract

The impact of nitrogen on biological performance and sludge filterability of anaerobic membrane bioreactors was investigated in two lab-scale cross-flow anaerobic membrane bioreactors that were fed with cheese whey at two different COD:TKN ratios (50 and 190). Nitrogen deprivation adversely affected the biological treatment performance and reactor stability, as indicated by volatile fatty acids accumulation. On the other hand, nitrogen (urea) supplementation resulted in a reduced sludge median particle size and decreased sludge filterability. Standard filterability parameters such as capillary suction time and specific resistance to filtration tended to rapidly increase in the nitrogen supplemented reactor. The critical fluxes in the nitrogen limited and supplemented reactors were 20 and 9 L·m<sup>-2</sup>·h<sup>-1</sup>, respectively. The rapid deterioration of sludge filterability under nitrogen supplemented conditions was attributed to abundant growth of dispersed biomass. Thus, the COD:TKN ratio of wastewater affected both bioconversion and filterability performance in the anaerobic membrane bioreactors.

**Keywords:** anaerobic membrane bioreactor; cheese whey; COD:TKN ratio; filterability; nitrogen

### 1 Introduction

Anaerobic membrane bioreactors (AnMBRs) are increasingly being researched in the last decade for the treatment of several wastewater streams due to their many advantages over conventional high rate anaerobic reactors (Dereli et al., 2012). They provide complete sludge retention, very high treatment efficiency, and excellent effluent quality free of suspended solids. The achievable membrane flux in AnMBRs is governed by the cake layer covering the membrane, commonly referred to as membrane fouling, which results from the filtration process (Jeison and van Lier, 2007a; Charfi et al., 2012). In addition to the formation of a dense cake layer on the membrane surface, due to the accumulation of organic and inorganic foulants, clogging of membrane pores may contribute to membrane fouling in AnMBRs, resulting in a subsequent flux decline. Fouling is a very complex phenomenon, and its extent of manifestation

depends on many parameters, such as substrate characteristics, mixed liquor properties, bioreactor design and operating conditions, membrane properties and operation (Meng et al., 2009). Most of the time, it is difficult to identify a single parameter that determines the degree of fouling, because all these parameters are interrelated to each other (van den Broeck et al., 2011).

Among the causes and mechanisms of fouling, the impact of substrate composition is probably the least investigated one. In general, substrate characteristics have an indirect impact on fouling by affecting the bioreactor operating conditions, i.e. applicable organic load, food to mass ratio and hydraulic retention time, microbial species composition, characteristics of extracellular polymeric substances (EPS) and types of inorganic precipitates (Le-Clech et al., 2006). Organic matter and nitrogen content, which are generally referred to as carbon to nitrogen (C:N) or chemical oxygen demand to nitrogen (COD:N) ratio, of wastewaters is considered an important parameter affecting the performance of both aerobic and anaerobic biological treatment systems (Speece, 1996; Rittmann and McCarty, 2001). The COD:N ratio in aerobic membrane bioreactors (MBRs) is commonly investigated with regard to nitrogen removal and denitrification performance (Fan et al., 2014; Babatsouli et al., 2015). Obviously, a high COD:N ratio is in that case desirable since an abundance of readily biodegradable substrate is needed in conventional denitrification systems. There are some contradictory reports about the effect of COD:N ratio on the membrane fouling and sludge filterability characteristics in aerobic MBRs. Sari Erkan et al. (2016) observed a decrease in sludge filterability indicated by lower critical fluxes in an MBR when the COD:N ratio of wastewater was increased. Controversially, several researchers indicated that low COD:N ratios promote fouling rates, deterioration of filterability and higher biomass yields in MBR systems (Feng et al., 2012; Hao and Liao, 2015, Hao et al., 2016). Considering the previous research, it is clear that the COD:N ratio has a substantial impact on mixed liquor characteristics (Ye et al., 2011) which are in close relation to filterability and fouling in aerobic MBRs.

In addition to a biodegradable carbon source, the anaerobic digestion process requires a balanced nutrient cocktail in terms of macro- and micro- nutrients that are required for bacterial and archaeal metabolism. Although the adverse effects of deficiency in micronutrients such as iron, nickel and cobalt have been well documented in literature (Speece, 1996; Demirel and Scherer, 2011; Hendriks et al., 2017), the impact of macronutrient limitation, such as nitrogen (N) and phosphorus (P) in anaerobic treatment is less well documented and macronutrient dosing is generally linked to the COD concentration and composition (van Lier et al., 2008). In general, the nitrogen and phosphorus demand for cell synthesis is low in anaerobic systems, due to very little biomass yield of anaerobic sludge. Speece (1996) reported that substrate COD:N ratio should be 50 and 150 for highly and lightly loaded systems, respectively. Others link this ratio to substrate composition and the expected growth yield, giving a COD:N:P ratio of 1000:5:1 for fully acidified wastewater and 350:5:1 for non-acidified wastewater (Chernicharo, 2007).

Several agro-industrial wastewaters, such as pulp and paper, olive mill, biodiesel production, confectionary and opium alkaloids industry effluents, are known to be nitrogen limited (Astals et al., 2011; Ersahin et al., 2011; Ozgun et al., 2012). In most of these cases, anaerobic processes are considered the most suitable technology available for the treatment of these high strength industrial streams. Owing to a reduced hydraulic selection pressure, anaerobic high-rate reactors are often limited by biomass wash-out when treating concentrated wastewaters. Particularly for these type of wastewaters, AnMBRs are of potential interest (Dereli et al, 2012), because the membrane can act as an absolute barrier against biomass wash-out and produce

solids free effluent with high reuse potential. However, there is very limited information about the effect of nitrogen limitation (high COD:N ratios) on anaerobic treatment systems in particular AnMBRs. Nitrogen limitation in AnMBRs may exert a dual effect impacting the overall performance of the system. On the one hand, nitrogen limitation will restrict sludge production and may thus positively impact the membrane filtration performance. On the other hand, it may negatively affect the overall biochemical conversion process, leading to lower COD removal efficiencies. Nitrogen deficiency will likely induce metabolic changes in microorganisms and limit biomass synthesis. Consequently, sludge production and sludge characteristics will change and product formation and species distribution will be altered. Both will have consequences on treatment efficiency and reactor stability but also on sludge filterability and fouling propensity in AnMBRs.

Sam-Soon et al. (1990) reported that nitrogen deficiency caused poor formation of methanogenic sludge granules in UASB reactors. Controversially, Punal et al. (2000) observed an enhanced biomass adhesion in the start-up phase of anaerobic filters fed with nitrogen limited substrate. The adverse effect of nitrogen deficiency on the biological performance of AnMBRs was first mentioned by Qiao et al. (2013) for the treatment of coffee grounds waste with a C:N ratio of 23.7. In this case, although the feed contained high amounts of organic nitrogen, the retardation of protein degradation by tannins limited the ammonification of organic nitrogen. On the other hand, given the high-enough ammonium nitrogen concentrations, even without nitrogen supplement, in the reactor ( $250\text{-}500\text{ mg}\cdot\text{L}^{-1}$ ) for biomass growth, nitrogen deficiency seems unlikely. Therefore, the most plausible reason for poor reactor stability seems to be micronutrient deficiency as stated by the authors.

The purpose of this study is to investigate the effect of nitrogen limitation on both biological performance and sludge filterability in AnMBRs. According to authors' knowledge this is the first study systematically reporting about its effects in AnMBRs. Two AnMBR systems were operated with nitrogen limited and supplemented cheese whey. The sludge filterability was systematically evaluated under two different COD to total Kjeldahl nitrogen (COD:TKN) ratios with standard parameters in order to achieve an objective comparison.

## **2 Materials and Methods**

### **2.1 Reactor Setup and Operation**

Two lab-scale cross flow AnMBRs with 10 L effective volume were operated under mesophilic conditions. Reactors were equipped with tubular ultrafiltration membranes (Pentair X-Flow) with a pore size of  $0.03\ \mu\text{m}$ . Membrane surface area was  $0.014\ \text{m}^2$ . A cross-flow velocity of  $0.5\ \text{m}\cdot\text{s}^{-1}$  was imposed with a peristaltic pump (Watson Marlow 530U) and the permeate suction and backwash was conducted with a small sized peristaltic pump (Watson Marlow, 120U). A detailed schematic diagram of the reactors was previously presented (Dereli et al. 2014a). Daily biogas production and pH data were recorded online. The pH of the reactors was controlled with a stand-alone controller (Hach Lange SC-1000) and a dosing pump (KNF Stepdos O8 RC) for caustic addition.

The reactors were named R-1 and R-2 and were operated for 158 and 169 days, respectively. R-1 was fed with nitrogen limited substrate for 134 days and at the last stage nitrogen was added to the feed. R-2 operation was started with nitrogen supplemented substrate and nitrogen addition was first sharply and then gradually decreased within 40 days at the final operation phase. Switching of substrate towards the end of operation was applied as a control experiment in order to test and validate the effect of nitrogen limited and supplied conditions on the

bioreactor performance. Both reactors had more than sufficient total phosphorus for biomass growth and COD:TP ratio was in the range of 70-75.

## 2.2 Experimental Methods

### 2.2.1 Analytical Methods

Chemical oxygen demand (COD) and total phosphorus (TP) concentrations were determined with Hach-Lange Kits. Total suspended solids (TSS), volatile suspended solids (VSS), total kjeldahl nitrogen (TKN) and ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) were measured according to Standard Methods (APHA, 1998). Soluble parameters were measured after centrifuging the sludge at 17,500 g for 10 minutes and subsequently filtering the supernatant with 0.45  $\mu\text{m}$  disposable filters. Volatile fatty acids (VFAs) were determined with a gas chromatograph according to Dereli et al. (2015a). Extracellular polymeric compounds (EPS) were extracted and measured according to the methods described in Dereli et al. (2015b). Each sample was measured in duplicate.

### 2.2.2 Substrate Characteristics

Whey permeate obtained from a cheese production plant was used as substrate in the study. As a result of precipitation and removal of proteins during cheese production, the nitrogen content of this whey permeate was quite low. In order to test the effect of nitrogen on sludge filterability, nitrogen was supplemented with the addition of urea to the feed of R-2. The COD:TKN ratio of the substrates fed to R-1 and R-2 was 190 and 50, respectively. The detailed compositions of the substrates fed to each reactor are given in Table 1. The feed was almost a completely soluble and rapidly fermentable substrate. It had high polysaccharide content originating mainly from lactose present in the milk.

**Table 1.** Feed characterization of R-1 and R-2 (mean $\pm$ standard deviation).

Parameters	Unit	R-1	R-2
Total COD	$\text{g}\cdot\text{L}^{-1}$	26.1 $\pm$ 1.3	29.2 $\pm$ 3.3
Soluble COD	$\text{g}\cdot\text{L}^{-1}$	25.4 $\pm$ 0.9	28.9 $\pm$ 3.3
TKN	$\text{mg}\cdot\text{L}^{-1}$	136 $\pm$ 14	600 $\pm$ 135
$\text{NH}_4^+\text{-N}$	$\text{mg}\cdot\text{L}^{-1}$	27 $\pm$ 6	45 $\pm$ 14
TSS	$\text{mg}\cdot\text{L}^{-1}$	730 $\pm$ 315	460 $\pm$ 400
VSS	$\text{mg}\cdot\text{L}^{-1}$	700 $\pm$ 360	340 $\pm$ 215
TP	$\text{mg}\cdot\text{L}^{-1}$	350 $\pm$ 30	415 $\pm$ 45
pH	-	5.1 $\pm$ 0.2	5.3 $\pm$ 0.5
Soluble protein	$\text{g}\cdot\text{L}^{-1}$	1.3 $\pm$ 0.3	1.4 $\pm$ 3.2
Soluble polysaccharide	$\text{g}\cdot\text{L}^{-1}$	11.2 $\pm$ 2.2	14.1 $\pm$ 0.6

### 2.2.3 Sludge Filterability

The critical flux was determined according to flux-step method with 15 minutes of step length and 2  $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  step height. A slope of  $dP/dt \geq 1 \text{ mbar}\cdot\text{min}^{-1}$  was chosen according to weak definition of critical flux (Le-Clech et al., 2003) to confirm that the critical flux was reached. Capillary suction time (CST) and specific resistance to filtration (SRF) were measured following the procedures described in Dereli et al. (2014b). Laser diffraction analysis (Beckman Coulter LS230, USA) was used to determine particle size distribution of the sludge between 0.4 and 2000  $\mu\text{m}$ .

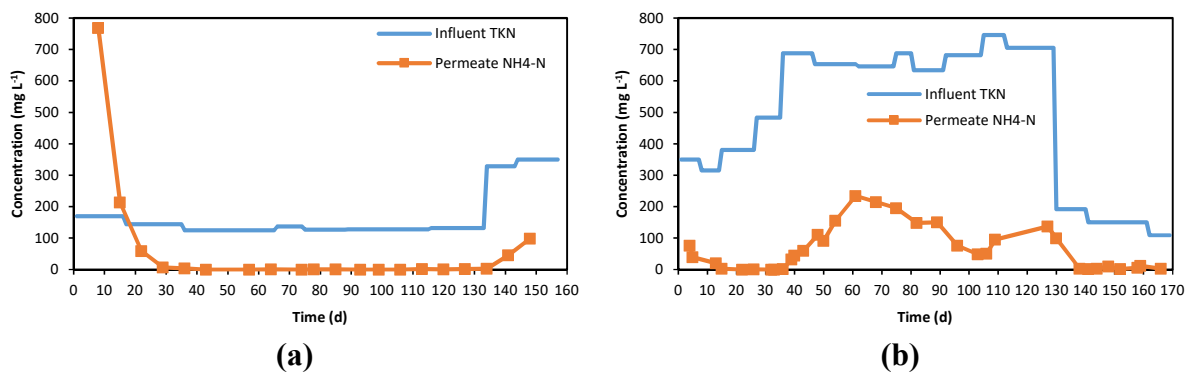
### 2.2.4 Specific Acidogenic Activity Tests

Batch acidification tests were carried out in sealed 250 mL serum bottles for different fractions of the sludge. Sucrose was used as primary substrate and the initial substrate to inoculum ratio was adjusted to 3. The media was buffered with  $5 \text{ g}\cdot\text{L}^{-1}$   $\text{NaHCO}_3$ . The methanogenic activity was inhibited by adding  $10 \text{ g}\cdot\text{L}^{-1}$  2-bromoethanesulfonate (BES). The headspace of the bottles was flushed with 70%  $\text{N}_2$  and 30%  $\text{CO}_2$  gas mixture. The tests were carried out in duplicate. Periodically, samples were collected from each bottle and VFAs were analysed.

### 3 Results and Discussion

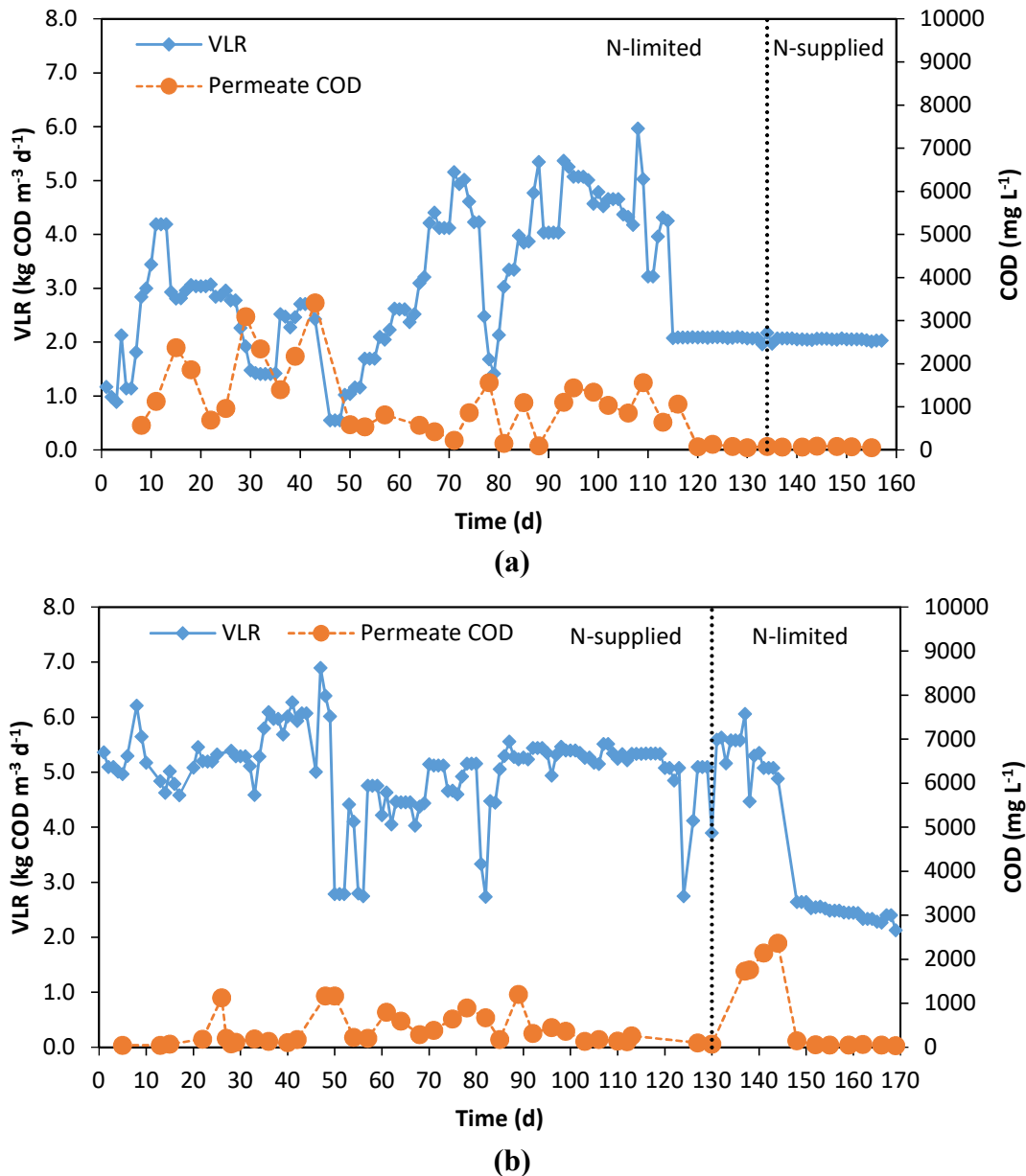
#### 3.1 Biological Performance

The influent TKN and permeate ammonium nitrogen concentrations in both reactors are shown in Figure 1. The lack of ammonium in R-1 permeate confirmed that treatment of cheese whey with high COD:TKN ratio was indeed nitrogen limited. During operation, until day 134 almost no ammonium was detected in the permeate of R-1. When the TKN concentration in the feed was increased to  $330 \text{ mg}\cdot\text{L}^{-1}$  in the last stage of the study, the permeate ammonium concentrations began to gradually increase. Moreover, no nitrogen was detected in R-2 permeate until the feed TKN concentration was increased up to  $700 \text{ mg}\cdot\text{L}^{-1}$ . Then, the permeate nitrogen concentration gradually increased up to  $230 \text{ mg}\cdot\text{L}^{-1}$  and levelled around  $100 \text{ mg}\cdot\text{L}^{-1}$  until day 130, when the feed TKN concentration was first sharply and then gradually reduced. As a result, nitrogen limited conditions were also observed in the last stage of R-2 operation.



**Figure 1.** Feed TKN and permeate ammonium nitrogen concentrations of R-1 (a) and R-2 (b).

Unstable performance was observed in R-1 fed with nitrogen limited substrate (Figure 2). Several attempts were done to increase the volumetric load and maintain it at around  $5 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$  but VFA accumulation restricted the performance (Figure 3). Until day 115, high COD concentrations up to  $4000 \text{ mg}\cdot\text{L}^{-1}$  were observed in the permeate. During this unstable period  $55\pm 8\%$  of permeate COD originated from accumulated VFA. Speece (1996) reported a remarkable decrease in acetate utilization rate when the  $\text{NH}_4\text{-N}$  concentration was below  $70 \text{ mg}\cdot\text{L}^{-1}$ . However, in our present study, propionate was the most dominant VFA building up in R-1 (Figure 3). t-tests were performed in order to test the statistical significance of the results. The means of acetate concentrations measured in R-1 and R-2 were not statistically different ( $p = 0.494 > \alpha = 0.05$ ). Whereas, the means of propionate concentrations in the reactors were significantly different ( $p = 0.004 < \alpha = 0.05$ ).

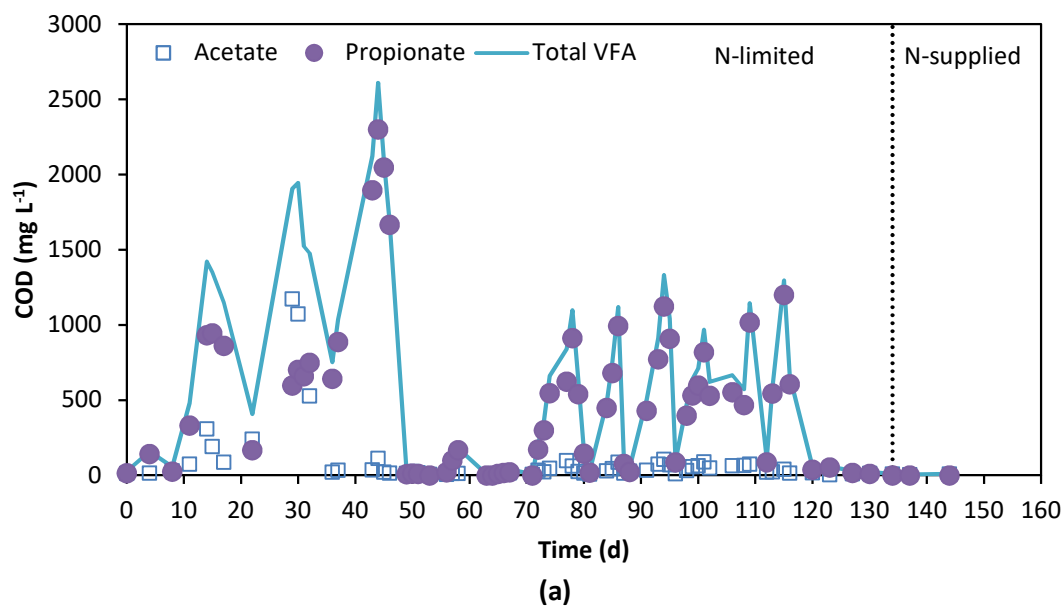


**Figure 2.** Applied load and permeate COD concentrations in R-1 (a) and R-2 (b).

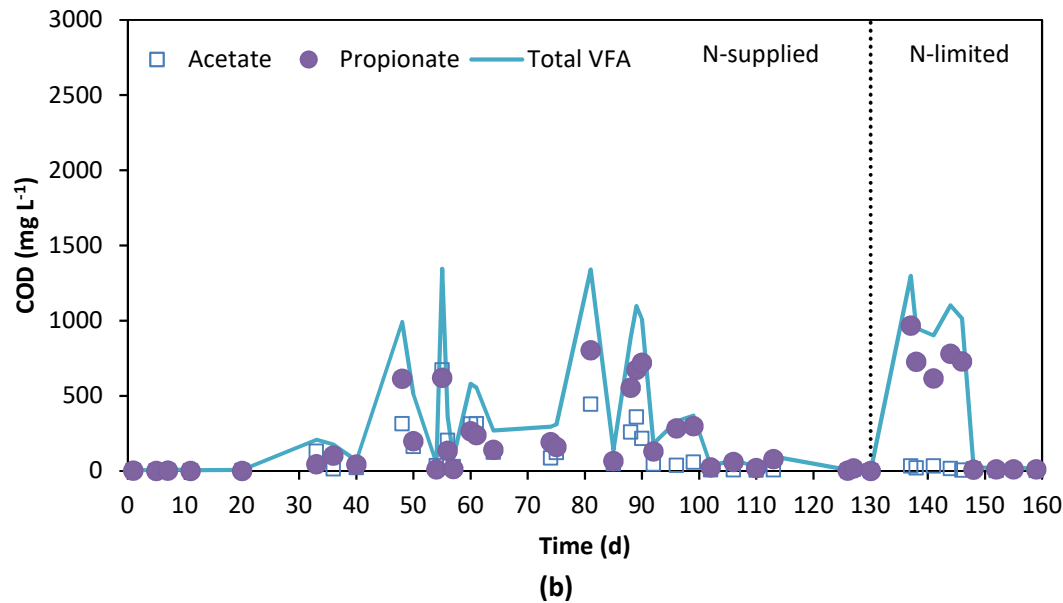
Accumulation of VFAs, especially propionate, under nitrogen limited conditions was observed by several other researchers (Zinder et al., 1984; Sam-Soon et al., 1990; Astals et al., 2011; Qiao et al., 2013). Recently, Xu et al. (2018) reported that the C:N ratio of the substrate determines the microbial community structure in anaerobic digesters. They observed a decrease in the abundance of hydrogenotrophic methanogens, i.e. *Methanothermobacter* and *Methanoculleus* under high C:N ratio. Sam-Soon et al. (1990) reported that the growth of the hydrogenotroph *Methanobacterium* Strain AZ is limited if there is insufficient nitrogen. Efficient propionate conversion requires strict collaboration of syntrophic bacteria in order to maintain sufficiently low hydrogen partial pressures for propionate oxidation to occur. Thus, the accumulation of propionate under nitrogen limited conditions may be due to growth limitation of hydrogenotrophic methanogens. Results of several hydrogen production studies also indicate that the  $\text{H}_2$  yield increases under nitrogen limited conditions, which may be due to suppression of the growth of hydrogenotrophic methanogens (Argun et al., 2008; Anzola-Rojas et al. 2015). However, it should be noted that the conditions in methanogenic reactors and the fermenters used for hydrogen production studies are significantly different. At the last stage of

R-1 operation, the volumetric loading rate (VLR) was fixed to  $2 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$  and the TKN concentration of the feed was increased to  $340 \text{ mg}\cdot\text{L}^{-1}$  between 134<sup>th</sup> and 158<sup>th</sup> days (Figure 1). As a result of the decreased organic load, a stable performance was observed and permeate COD concentrations of  $80\pm 20 \text{ mg}\cdot\text{L}^{-1}$  could be achieved without any VFA accumulation. It seems that under low organic loading conditions, biomass growth is much less and the recycled nitrogen from decaying biomass may be sufficient for efficient bioconversion. However, when the load is increased, the growing biomass simply lacks nitrogen and an unstable performance is observed.

A more stable performance was observed in R-2 (Figure 2). Compared to R-1, a higher volumetric load could be applied with occasional VFA peaks. Nevertheless, the VFA concentrations were much lower than R-1. In the last period of the study, the TKN concentration of the R-2 feed was gradually decreased to  $110 \text{ mg}\cdot\text{L}^{-1}$  between 130<sup>th</sup> to 169<sup>th</sup> days (Figure 1). Interestingly, VFA concentrations in the permeate gradually increased up to  $1000 \text{ mg COD}\cdot\text{L}^{-1}$  within one week (Figure 3). Propionate was the only VFA accumulating in the reactor at this period. Consequently, the applied volumetric load had to be decreased to  $2 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$  and the reactor regained its stability. This result clearly confirms that feed COD:TKN ratio has a strong effect on biological treatment performance and stability of anaerobic reactors (Speece, 1996; Chernicharo, 2007).







**Figure 3.** VFA concentrations in R-1 (a) and R-2 (b).

The main operational problem in R-2 was severe sludge foaming, which was never observed in R-1. Several studies mentioned foaming problems during anaerobic treatment of carbohydrate rich wastewaters such as cheese whey (Brooks et al., 2008; Suhartini et al., 2014; Moeller et al., 2015). Kougias et al. (2014) reasoned that to the dominance of acidogenic bacteria such as *Lactobacillus*, *Bacillus*, *Thermotoga*, *Micrococcus* and *Pseudonocardia*. These species are known to produce foam promoting compounds such as proteins, lactic acid, extracellular water-soluble bio-surfactants and lipo-peptides which may be extensively released to the bulk liquor under high shear conditions of an AnMBR.

The operating conditions and treatment performance of R-1 and R-2 are summarized in Table 2. A high COD removal efficiency could be achieved in both reactors thanks to membrane filtration, which removed all particulate matter from the effluent. Nitrogen supplementation remarkably increased the biomass yield in R-2 compared to R-1. Moreover, due to increased biomass synthesis a lower methane yield per gram COD fed in to the reactor was observed in R-2. During the methanogenic conversion process, part of the energy in the substrate is used for cell synthesis and the rest is transferred to methane as an end product of anaerobic metabolism (e.g. Rittman and McCarty, 2001). Although the biomass yield of anaerobic sludge is in general very low, it is reported that for readily biodegradable carbohydrate-type substrates, the yield can reach up to  $0.2 \text{ g VSS} \cdot \text{g}^{-1} \text{ COD}_{\text{removed}}$  (Kalyuzhnyi and Davlyatshina, 1997; Fernandez et al., 2011). This is mainly due to growth of acidogenic microorganisms that are characterized by a higher biomass yield compared to methanogens (Batstone et al., 2002). As a result of the higher biomass yield, the anaerobic treatment of complex substrates would logically require a substantial amount of nitrogen and phosphorus not to limit biomass growth. This is generally determined by the substrate type (biodegradability and COD:N ratio) and organic load applied to a reactor. Especially at high organic loads there must be sufficient nutrients available for biomass growth, whereas under low loading conditions the nutrients can be efficiently recycled by biomass decay to sustain the limited growth.

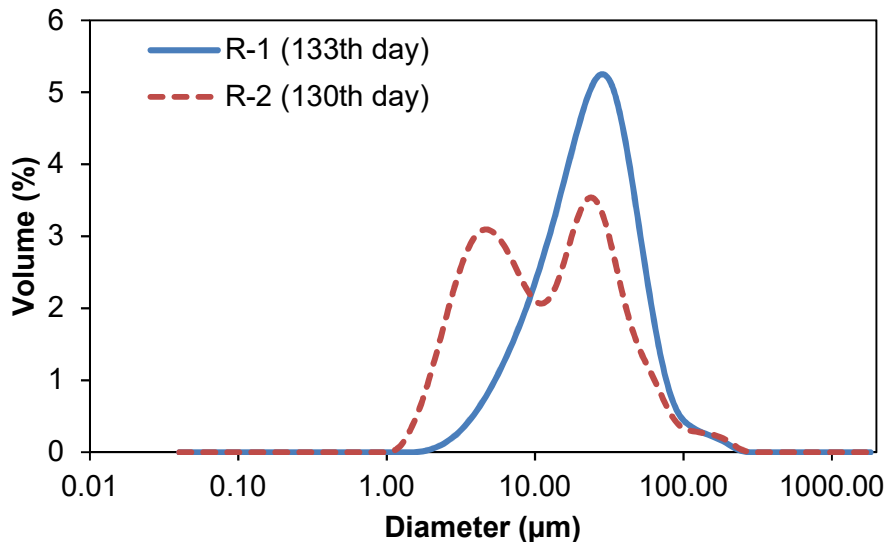
**Table 2.** Operating conditions and performance comparison of the reactors (mean±standard deviation).

Parameter	Unit	R-1	R-2
Solids retention time (SRT)	d	300	50
VLR	kg COD·m <sup>-3</sup> ·d <sup>-1</sup>	3.0±1.3	5.0±0.8
Hydraulic retention time (HRT)	d	10.6±5.1	6.0±1.3
Food to mass (F:M) ratio	kg COD·kg <sup>-1</sup> VSS·d <sup>-1</sup>	0.14±0.04	0.18±0.02
Permeate COD	mg·L <sup>-1</sup>	1260±990	380±360
Permeate VFA	g COD·L <sup>-1</sup>	600±590	320±390
COD removal efficiency based on permeate quality	%	96±3	98±2
Methane yield	Nm <sup>3</sup> CH <sub>4</sub> ·kg <sup>-1</sup> COD <sub>fed</sub>	0.33±0.05	0.28±0.02
Sludge yield	g VSS·g <sup>-1</sup> COD <sub>removed</sub>	0.03±0.01	0.19±0.03

## 3.2 Sludge Characteristics and Filterability

### 3.2.1 Particle Size Distribution

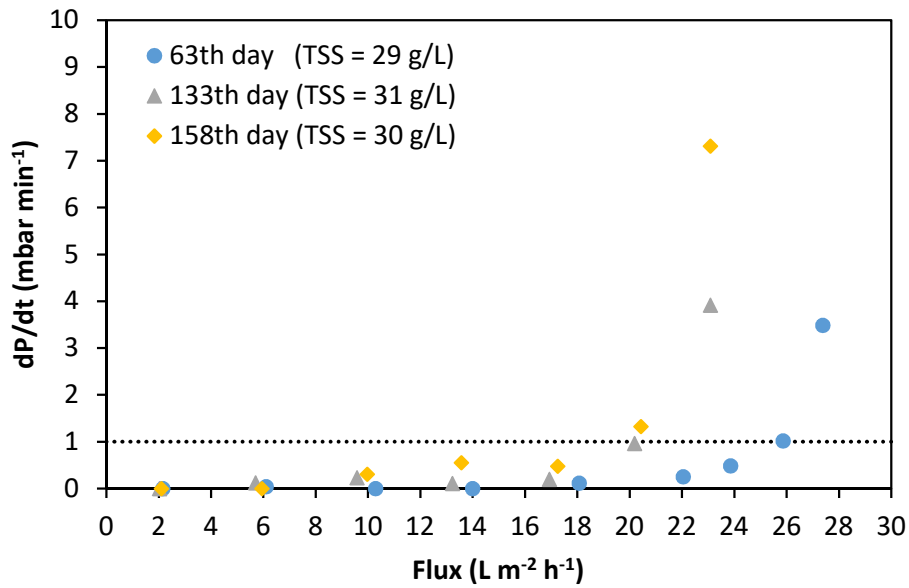
Interestingly, R-1 sludge showed a unimodal PSD, whereas the PSD of R-2 sludge was bimodal (Figure 4). The median particle size in R-1 and R-2 was 25 µm and 13 µm under nitrogen limited and supplemented conditions, respectively. Ye et al. (2011) reported that the mean floc size of activated sludge decreased when the feed C:N ratio was reduced. Similarly, Hao and Liao (2015) observed that the floc size of the mixed liquor shifted to lower sizes in aerobic MBRs when operated at decreasing COD:N ratios. Under anaerobic conditions, the decrease in particle size may be due to the proliferation of acidogens, which prefer dispersed growth. Jeison and van Lier (2007b) reported that acidogens grew to a large extent as individual single cells. The substrate, whey, used in this study contains rapidly fermentable compounds such as lactose which promotes acidogenic growth when nutrients are sufficiently present.



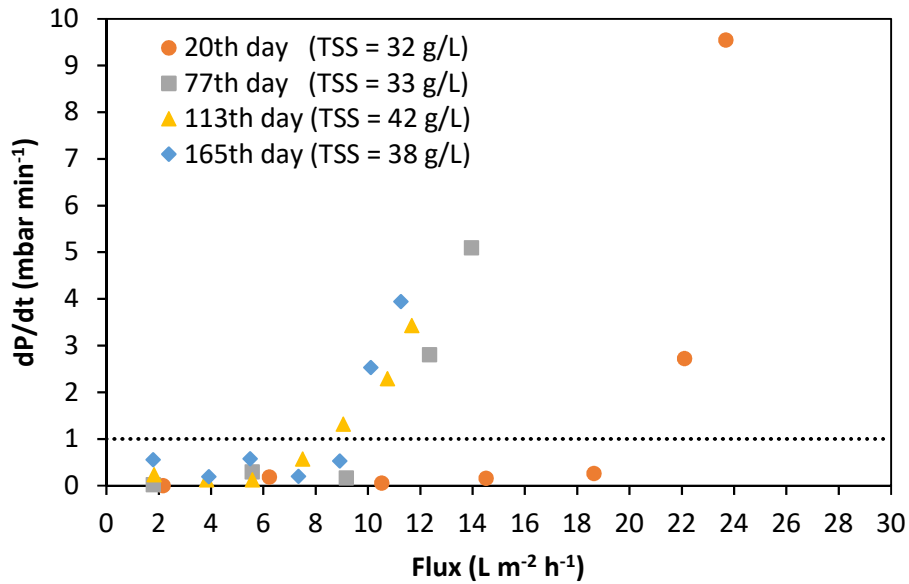
**Figure 3.** Particle size distribution in the reactors.

### 3.2.2 Sludge Filterability

During operation, the critical flux of R-2 decreased from 20 to 9 L·m<sup>-2</sup>·h<sup>-1</sup> within 2.5 months and remained stable until the end of the experimental study (Figure 5). In contrast, the steady state critical flux measured in R-1 was 20 L·m<sup>-2</sup>·h<sup>-1</sup>, which was two times higher than that of R-2. Short term critical flux tests revealed that the COD:TKN ratio has an important effect on sludge filterability.



(a)



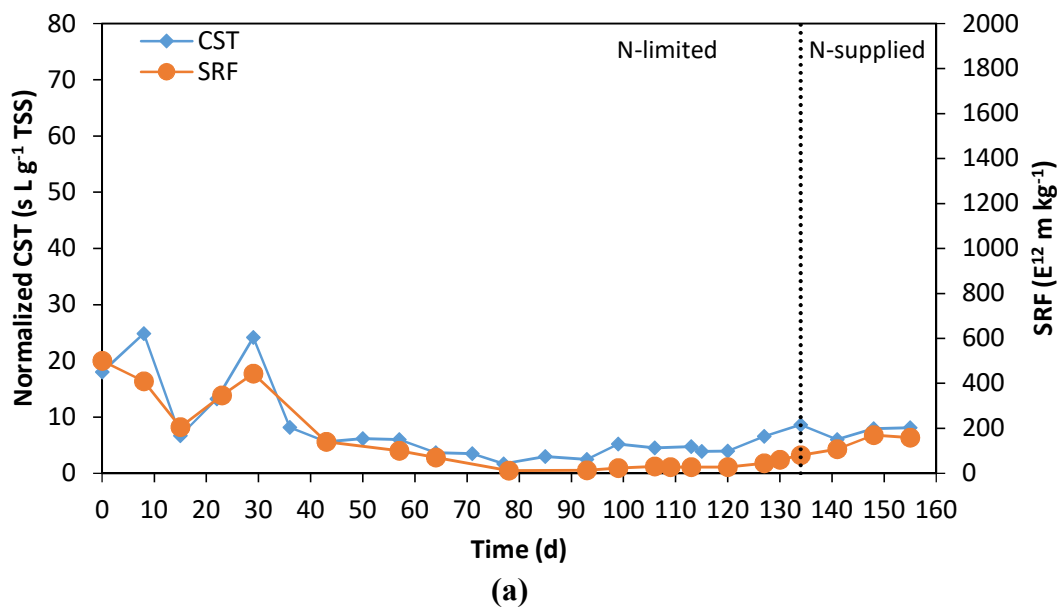
(b)

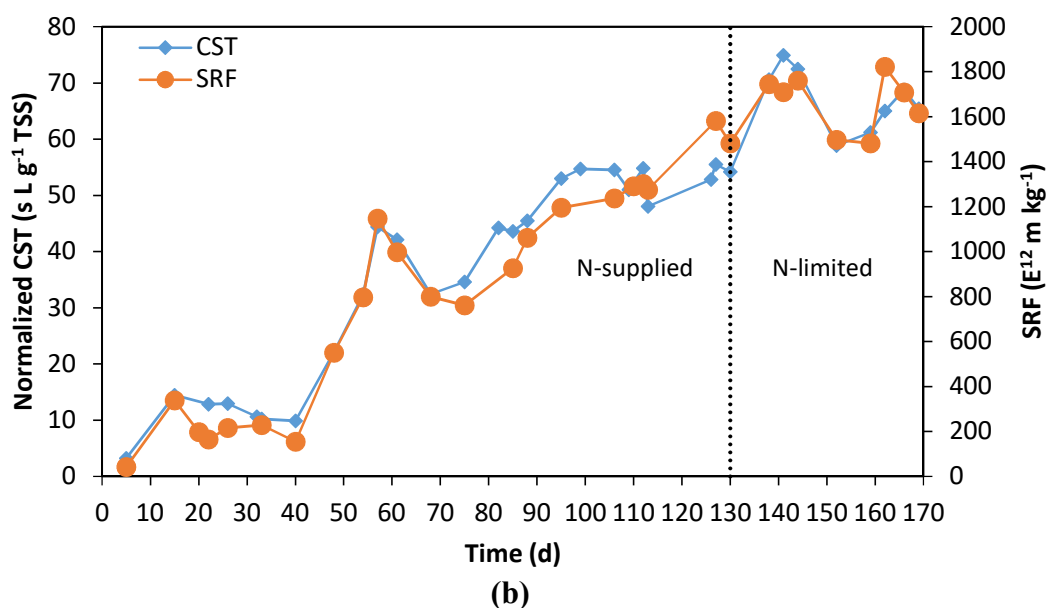
**Figure 5.** Fouling rate evolution, expressed as TMP increase rate, in critical flux tests of R-1 (a) and R-2 (b).

In order to investigate the effect of the COD:TKN ratio on sludge filtration characteristics, supplementary indicators such as CST and SRF were monitored throughout the study (Figure 6). These standard parameters allow an objective comparison of sludge filterability at different operating conditions (Dereli et al. 2014b; Ersahin et al., 2014). CST and SRF parameters were clearly linked and showed a very high positive correlation in both reactors (Pearson correlation coefficient  $> 0.92$ ,  $\alpha = 0.05$ ). Similarly, good correlation between these parameters was observed in Dereli et al. (2015a). Since CST is a rapid and easy to measure parameter, it is suggested to use it as an indicator of sludge filterability. This was also recommended by several authors (Pollice et al., 2008; Laera et al., 2009).

The increase in CST and SRF in R-1 between 15<sup>th</sup> and 30<sup>th</sup> days was attributed to uncontrolled caustic dosing due to the failure of the pH meter (Supplementary document Figure 1). In general, the CST and SRF parameters decreased until day 80 and remained stable till day 115. A decrease in the VLR after day 115, led to a slight increase of CST and SRF values in R-1. This may be due to a sudden change of the F:M ratio from 0.14 to 0.09 kg COD·kg VSS<sup>-1</sup>·d<sup>-1</sup>. The F:M ratio was reported as an important parameter that affects sludge filterability and membrane fouling (Liu et al., 2012).

The sludge filterability in R-2 continuously deteriorated throughout operation (Figure 5). A pH shock of 8.6 on day 55 (Supplementary document Figure 1) led to a rapid increase in CST and SRF, meanwhile the system performance recovered within 10 days. Moreover, the decrease in the feed nitrogen concentration between 130<sup>th</sup> and 169<sup>th</sup> days seemed not to improve sludge filterability, however a further worsening was not observed. At the end of operation, the normalized CST and SRF parameters reached to 70 s·L·g<sup>-1</sup> TSS and 1700 E<sup>12</sup> m·kg<sup>-1</sup>, respectively. These values were several times higher compared to what was measured in R-1. The SRF of a poly-dispersed solution is mainly determined by small sized particles, which form a dense cake layer with low porosity on the membrane (Endo and Alanso, 2001). The lower median particle size in R-2 correlated well with the higher SRF observed in this reactor compared to the one operated with nitrogen limited feed. Ye et al. (2011) reported that the CST and SRF of activated sludge increased when the C:N ratio decreased, indicating a deterioration of flocculation, filterability and dewaterability.





**Figure 6.** Evolution of CST and SRF in R-1 (a) and R-2 (b).

The particle size analysis of R-2 sludge showed a bimodal distribution (Figure 4). Indeed, after centrifugation of bulk sludge at 17500g for 10 minutes, two distinct fractions in the sludge pellet were observed: a whitish milky top layer and a darker bottom layer. In fact, a similar observation was previously made by Gao et al. (2010a) who investigated the microbial species distribution of sludge fractions and cake layer of an AnMBR. They reported that *Bacteroidetes*, *OP11* species were distinctly dominant in light solids compared to dark solids. However, it should be noted that the microbial composition in an anaerobic reactor is strongly dependent on the substrate type, seed sludge, environmental and operational conditions.

In order to investigate the filterability of different sludge fractions, bulk sludge was separated into light and dark solids by centrifugation. The SRF of bulk sludge, light solids and dark solids were measured as 1150, 6000 and 770 E<sup>12</sup> m·kg<sup>-1</sup>, respectively. The cake resistance of light solids was much higher than the original sludge and black solids found at the bottom of the pellet. The PSD analysis of light solids revealed that the median particle size (4 μm) was lower than the bulk sludge. The latter likely contributed significantly to the high cake resistance, since the SRF parameter is directly linked to the sludge particle size (Endo and Alanso, 2001). The SRF of dark solids was remarkably lower than the original sludge. This means that when the light solids are selectively removed, an improvement in filterability may be achieved. Interestingly, the light solids were found to be rich with polysaccharide and poor with proteinaceous type EPS compared to bulk sludge and dark solids (Table 3). On the contrary, Gao et al. (2010a) reported a higher proteinaceous EPS content compared to polysaccharide type EPS for light solids fraction in the sludge. This may be attributed to the differences in substrate composition used in both studies. They used a protein rich substrate, whereas in our case it was mainly a disaccharide, lactose. The protein to polysaccharide (PN:PS) ratio was also low in the harvested light solids. The protein to polysaccharide ratio is known to affect sludge hydrophobicity and the membrane fouling propensity (Drews, 2010). Gao et al. (2010b) reported a reciprocal correlation between PN:PS ratio and membrane fouling rate.

**Table 3.** EPS content and specific acidogenic activity of different sludge fractions in R-2 (mean±standard deviation).

Parameter	Unit	Bulk Sludge	White Solids	Black Solids
Protein	mg·g <sup>-1</sup> VSS	37±2	30±1	37±3
Polysaccharide	mg·g <sup>-1</sup> VSS	25±3	35±0.1	22±0.3
Protein:Polysaccharide	-	1.5	0.9	1.7
Acidogenic activity	g COD <sub>VFA</sub> ·g <sup>-1</sup> VSS·d <sup>-1</sup>	2.67±0.2	2.64±0.01	2.65±0.08

Acidogenic bacteria are known to exert an adverse effect on sludge filterability. Jeison and van Lier (2007b) reported an increased rate of fouling and decreased critical flux in an AnMBR fed with partially acidified wastewater compared to the control reactor operated with VFA based wastewater. They attributed this to the single cell growth of acidogenic biomass, which results in a decrease in sludge overall particle size. In a further study, they validated that acidogens dominate the supernatant fraction of sludge which determines the overall filtration behavior and fouling propensity of bulk liquor (Jeison et al., 2009). In Dereli et al. (2015a) the effect of substrate acidification degree on sludge filterability was systematically evaluated and it was reported that the acidogenic biomass also decreased sludge particle size and negatively affected filterability. In the present case, rapid deterioration of CST and SRF parameters was observed, immediately following the start-up of the AnMBR treating non-acidified cheese whey with nitrogen supplement (R-2). To investigate whether the acidogenic bacteria were dominant in light solids, batch acidification tests to different fractions of the sludge was conducted. Interestingly, the results showed that there was not a specific enrichment of acidogenic bacteria in the light solids fraction (Table 3). A similar observation was also made by Torres et al. (2011) who found no significant difference in acidogenic activities of the pellet and supernatant fractions of sludge from an AnMBR treating brewery wastewater. Tools such as real time qPCR would certainly help to identify which species are more dominant in different fractions of the sludge and understand their relationship with membrane fouling. Nevertheless, according to our results rapidly fermentable substrates with low COD:TKN ratio seem to promote dispersed growth in AnMBRs.

#### 4 Conclusions

The results presented in this study clearly show that COD to nitrogen ratio of wastewaters affects the biological performance and sludge filterability of AnMBRs. Nitrogen deficiency limited the biomass growth which from an engineering point of view may be beneficial due to less sludge post-processing. However, nitrogen limited conditions led to a feeble reactor stability, as reflected by the accumulation of propionate and the impossibility to operate the AnMBR at VLRs higher than 2 kg COD·m<sup>-3</sup>·d<sup>-1</sup>. On the other hand, supplementation of nitrogen adversely affected sludge filterability in terms of CST, SRF and critical flux. The median particle size of sludge decreased and two distinct fractions of sludge with different filterability characteristics became visually apparent. The abundance of nitrogen seems to promote dispersed growth, which adversely effects sludge filterability. Nevertheless, for the treatment of wastewaters with high COD:N ratio, operating AnMBRs at an optimised nitrogen supplement may ensure adequate treatment performance and process stability and at the same time maintain sludge filterability by suppressing dispersed growth.

#### Acknowledgements

This research was funded and supported by Veolia Water Technologies, Biothane Systems International under MEMTHANE® development project. R.K. Dereli would specially thank to Prof. Dr. Eoin Casey from University College Dublin for proof reading the manuscript.

## References

- Anzola-Rojas, M.P., da Fonseca, S.G., da Silva, C.C., de Oliveira, V.M., Zaiat, M. (2015). The use of the carbon/nitrogen ratio and specific organic loading rate as tools for improving biohydrogen production in fixed-bed reactors. *Biotechnology Reports* 5, 46–54.
- APHA (1998). *Standard Methods for the Examination of Water and Wastewater*, 1995. 20th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.
- Argun, H., Kargi, F., Kapdan, I.K., Oztekin, R. (2008). Biohydrogen production by dark fermentation of wheat powder solution: Effects of C/N and C/P ratio on hydrogen yield and formation rate. *International Journal of Hydrogen Energy*, 33, 1813-1819.
- Astals, S., Ariso, M., Galí, A., Mata-Alvarez, J. (2011). Co-digestion of pig manure and glycerine: Experimental and modelling study. *Journal of Environmental Management* 92, 1091-1096.
- Babatsouli, P., Costa, C., Palogos, I., Kalogerakis, N. (2015). Evaluation of a MBR pilot treating industrial wastewater with a high COD/N ratio. *Journal of Chemical Technology and Biotechnology*, 90, 26-33.
- Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin. V.A. (2002). *Anaerobic Digestion Model No.1. Scientific and Technical Report No.13*, IWA Publishing, London.
- Brooks, L., Parravicini, V., Svardal, K., Krois, H., Prendi, L. (2008). Biogas from sugar beet press pulp as substitute of fossil fuels in sugar beet factories. *Water Science and Technology* 58, 1497-1504.
- Charfi, A., Ben Amar, N., Harmand, J., 2012. Analysis of fouling mechanisms in anaerobic membrane bioreactors. *Water Research* 46 (8), 2637-2650.
- Chernicharo, C.A.D. (2007) *Biological Wastewater Treatment: Volume 4 – Anaerobic Reactors*. IWA Publishing, UK.
- Demirel B, Scherer P. (2011) Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass Bioenergy* 35, 992-998.
- Dereli, R.K., Ersahin, M.E., Ozgun, H., Ozturk, I., Jeison, D., van der Zee, F., van Lier, J.B. (2012). Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters. *Bioresource Technology* 122, 160-170.
- Dereli, R.K., van der Zee, F.P., Heffernan, B., Grelot, A. and van Lier, J.B. (2014a) Effect of sludge retention time on the biological performance of anaerobic membrane bioreactors treating corn-to-ethanol thin stillage with high lipid content. *Water Research* 49, 453-464.

Dereli, R.K., Grelot, A., Heffernan, B., van der Zee, F.P., van Lier, J.B. (2014b). Implications of changes in solids retention time on long term evolution of sludge filterability in anaerobic membrane bioreactors treating high strength industrial wastewater. *Water Research* 59, 11-22.

Dereli, R.K., Heffernan, B., Grelot, A., van der Zee, F.P., and van Lier, J.B. (2015a). Influence of high lipid containing wastewater on filtration performance and fouling in AnMBRs operated at different solids retention times. *Separation and Purification Technology*, 139, 43-52.

Dereli, R.K., Loverdou, L., van der Zee, F.P., van Lier, J.B. (2015b). A systematic study on the effect of substrate acidification degree and acidogenic biomass on sludge filterability. *Water Research*, 82, 94-103.

Drews, A. (2010) Membrane fouling in membrane bioreactors - Characterisation, contradictions, cause and cures. *Journal of Membrane Science* 363(1-2), 1-28.

Endo Y., Alonso, M. (2001) Physical meaning of specific cake resistance and effects of cake properties in compressible cake filtration. *Filtration & Separation*, 38(7), 42-46.

Ersahin, M.E., Ozgun, H., Dereli, R.K., Ozturk, I. (2011). Anaerobic Treatment of Industrial Effluents: An Overview of Applications. In *Waste Water Treatment and Reutilization*, Ed: Fernando S. García Einschlag, ISBN 978-978-953-307-249-4, pp:3-28, InTech, India.

Ersahin, M.E., Ozgun, H., Tao, Y. and van Lier, J.B. (2014) Applicability of dynamic membrane technology in anaerobic membrane bioreactors. *Water Research* 48(0), 420-429.

Feng, S., Zhang, N., Liu, H., Du, X., Liu, Y., Lin, H. (2012). The effect of COD/N ratio on process performance and membrane fouling in a submerged bioreactor. *Desalination* 285, 232–238.

Fan, X., Li, H., Yang, P., Lai, B. (2014). Effect of C/N ratio and aeration rate on performance of internal cycle MBR with synthetic wastewater. *Desalination and Water Treatment*, 57(27), 12763-12769.

Fernández, F.J., Villaseñor, J. and Infantes, D. (2011) Kinetic and stoichiometric modelling of acidogenic fermentation of glucose and fructose. *Biomass and Bioenergy* 35(9), 3877-3883.

Gao, D.-W., Zhang, T., Tang, C.-Y.Y., Wu, W.-M., Wong, C.-Y., Lee, Y.H., Yeh, D.H. and Criddle, C.S. (2010a) Membrane fouling in an anaerobic membrane bioreactor: Differences in relative abundance of bacterial species in the membrane foulant layer and in suspension. *Journal of Membrane Science* 364(1-2), 331-338.

Gao, W.J.J., Lin, H.J., Leung, K.T. and Liao, B.Q. (2010b) Influence of elevated pH shocks on the performance of a submerged anaerobic membrane bioreactor. *Process Biochemistry* 45(8), 1279-1287.

Hao, L, Liao, B.Q. (2015). Effect of organic matter to nitrogen ratio on membrane bioreactor performance. *Environmental Technology* 36(20), 2674-2680.

Hao, L., Liss, S.N., Liao, B.Q. (2016). Influence of COD:N ratio on sludge properties and their role in membrane fouling of a submerged membrane bioreactor. *Water Research* 89, 132-141.



Hendriks, A.T.W.M., van Lier, J.B., de Kreuk, M.K. (2017). Growth media in anaerobic fermentative processes: The underestimated potential of thermophilic fermentation and anaerobic digestion. *Biotechnology Advances*, <https://doi.org/10.1016/j.biotechadv.2017.08.004>

Jeison, D., van Lier J.B. (2007a) Cake formation and consolidation: main factors governing the applicable flux in anaerobic submerged membrane bioreactors (AnSMBR) treating acidified wastewater. *Separation and Purification Technology* 56, 71-78.

Jeison, D. and van Lier, J. (2007b) Thermophilic treatment of acidified and partially acidified wastewater using an anaerobic submerged MBR: Factors affecting long-term operational flux. *Water Research* 41(17), 3868-3879.

Jeison, D., Plugge, C., Pereira, A. and Lier, J. (2009) Effects of the acidogenic biomass on the performance of an anaerobic membrane bioreactor for wastewater treatment. *Bioresource Technology* 100(6), 1951-1956.

Kalyuzhnyi, S. V. and M. A. Davlyatshina (1997) Batch anaerobic digestion of glucose and its mathematical modeling. I. Kinetic Investigations. *Bioresource Technology* 59: 73-80.

Kougias, P.G., De Francisci, D., Treu, L., Campanaro, S., Angelidaki, I. (2014) Microbial analysis in biogas reactors suffering by foaming incidents. *Bioresource Technology* 167, 24-32.

Laera, G., Pollice, A., Saturno, D., Giordano, C., Sandulli, R., (2009) Influence of sludge retention time on biomass characteristics and cleaning requirements in a membrane bioreactor for municipal wastewater treatment. *Desalination* 236(1-3), 104-110.

Le-Clech, P., Chen, V. and Fane, T.A.G. (2006). Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science* 284(1-2), 17-53.

Le-Clech, P., Jefferson, B., Chang, I.S. and Judd, S.J. (2003) Critical flux determination by the flux-step method in a submerged membrane bioreactor. *Journal of Membrane Science* 227(1-2), 81-93.

Liu, Y., Liu, H., Cui, L. and Zhang, K. (2012) The ratio of food-to-microorganism (F/M) on membrane fouling of anaerobic membrane bioreactors treating low-strength wastewater. *Desalination* 297(0), 97-103.

Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S. and Yang, F. (2009) Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research* 43(6), 1489-1512.

Moeller, L., Lehnig, M., Schenk, J., Zehndorf, A. (2015). Foam formation in biogas plants caused by anaerobic digestion of sugar beet. *Bioresource Technology* 178, 270–277.

Ozgun, H., Karagul, N., Dereli, R.K., Ersahin, M.E., Coskuner, T., Altinbas, M., Ozturk, I. (2012) Confectionery industry: A case study on treatability based effluent characterization and treatment system performance. *Water Science and Technology* 66(1), 15-20.

- Qiao, W., Takayanagi, K., Shofie, M., Niu, Q., Yu, H.Q., Li, Y. (2013). Thermophilic anaerobic digestion of coffee grounds with and without waste activated sludge as co substrate using a submerged AnMBR: System amendments and membrane performance. *Bioresource Technology*, 150, 249-258.
- Pollice, A., Laera, G., Saturno, D., Giordano, C., 2008. Effects of sludge retention time on the performance of a membrane bioreactor treating municipal sewage. *Journal of Membrane Science* 317, 65-70.
- Punal, A., Trevisan, M., Rozzi, A., Lema, J.M. (2000). Influence of C:N ratio on the start-up of up-flow anaerobic filter reactors. *Water Research*, 34(9), 2614-2619.
- Rittmann, B.E., McCarty, P.L. (2001). *Environmental Biotechnology: Principles and Applications*. Tata McGraw Hill Education Private Limited.
- Sam-Soon, P.A.L.N.S., Loewenthal, R.E., Wentzel, M.C., Marais, G.R. (1998). Effect of nitrogen limitation in upflow anaerobic sludge bed (UASB) systems. *Water SA*, 16(3), 165-170.
- Sari Erkan, H., Onkal Engin, G., Ince, M., Bayramoglu, M.R. (2016). Effect of carbon to nitrogen ratio of feed wastewater and sludge retention time on activated sludge in a submerged membrane bioreactor. *Environmental Science and Pollution Research* 23, 10742–10752.
- Speece, R.E. (1996). *Anaerobic Biotechnology for Industrial Wastewaters*. Archae Press
- Suhartini, S., Heaven, S., Banks, C.J. (2014). Comparison of mesophilic and thermophilic anaerobic digestion of sugar beet pulp: Performance, dewaterability and foam control. *Bioresource Technology* 152, 202–211.
- Torres, A., Hemmelmann, A., Vergara, C., Jeison, D. (2011). Application of two-phase slug-flow regime to control flux reduction on anaerobic membrane bioreactors treating wastewaters with high suspended solids concentration. *Separation and Purification Technology*, 79(1), 20-25.
- van den Broeck, R., Krzeminski, P., van Dierdonck, J., Gins, G., Lousada-Ferreira, M., van Impe, J.F.M., van der Graaf, J.H.J.M., Smets, I.Y., van Lier, J.B., 2011. Activated sludge characteristics affecting sludge filterability in municipal and industrial MBRs: unrevealing correlations using multi component regression analysis. *Journal of Membrane Science* 378, 330-338.
- van Lier, J.B., N. Mahmoud and G. Zeeman (2008). *Anaerobic Wastewater Treatment*. In: M. Henze, M.C.M. van Loosdrecht, G.A. Ekama, D. Brdjanovic (eds.), *Biological Wastewater Treatment, Principles, Modelling and Design*, Chapter 16, ISBN: 9781843391883, IWA Publishing, London, UK, p. 415-456.
- Xu, R., Zhang, K., Liu, P., Khan, A., Xiong, J., Tian, F., Li, X. (2018). A critical review on the interaction of substrate nutrient balance and microbial community structure and function in anaerobic co-digestion. *Bioresource Technology* 247, 1119-1127.

Ye, F., Ye, Y., Li, Y. (2011). Effect of C/N ratio on extracellular polymeric substances (EPS) and physicochemical properties of activated sludge flocs. *Journal of Hazardous Materials* 188, 37-43.

Zinder, S.H., Cardwell, S.C., Anguish, T., Lee, M., Koch, M. (1984). Methanogenesis in a thermophilic (58°C) anaerobic digester: *Methanotherix* sp. as an important acetoclastic methanogen. *Applied and Environmental Microbiology*, 47(4), 796-807.