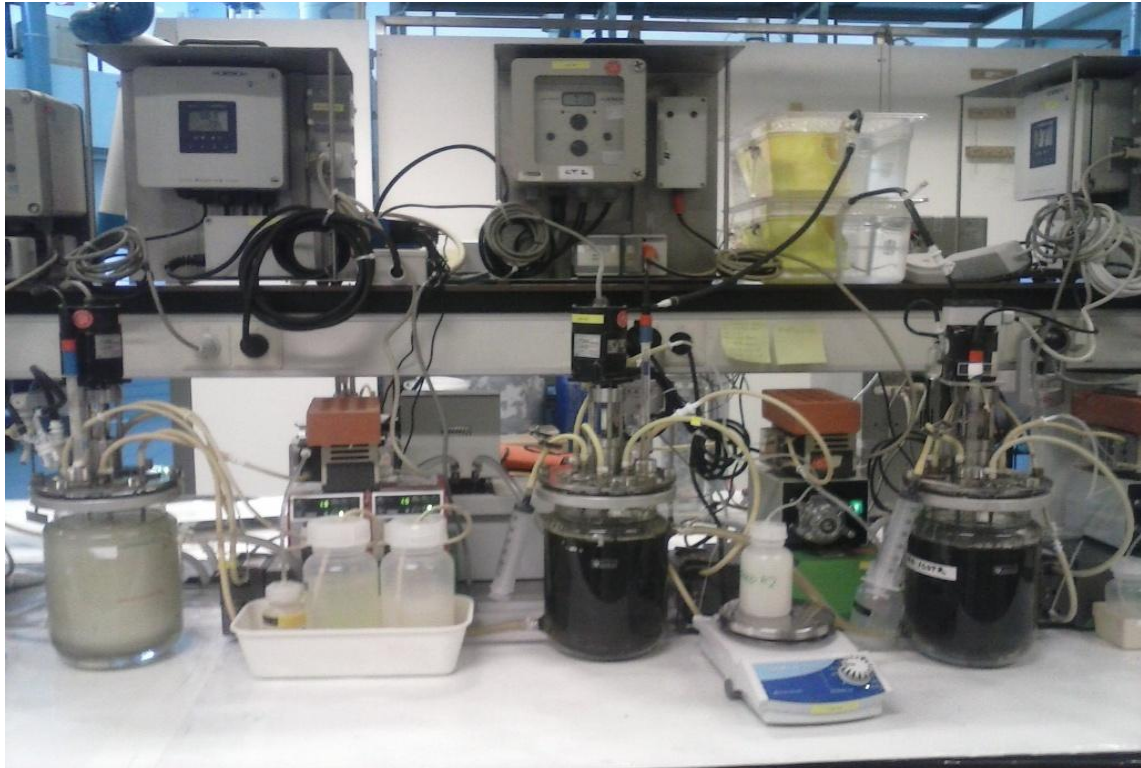


## Effect of acidogenic biomass on sludge filterability



**BIOTHANE**



# Effect of acidogenic biomass on sludge filterability

Loverdou Lefki-Victoria

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Committee:

Prof.ir. J. B. van Lier

Dr.ir. R. Kleerebezem

Dr.ir. F. Van der Zee

Ir. R.K. Dereli

Delft University of Technology  
Sanitary Engineering Section  
Delft University of Technology  
Biotechnology  
Biothane (Veolia Environment)

Delft University of Technology  
Sanitary Engineering Section

Sanitary Engineering Section, Department of Water Management  
Faculty of Civil Engineering and Geosciences  
Delft University of Technology, Delft

## **Abstract**

Anaerobic digestion has been established as the most efficient method for high strength industrial wastewater treatment. Anaerobic membrane bioreactor (AnMBR) is a relatively new reactor configuration that is becoming gradually more attractive for wastewaters under extreme conditions that can hinder granulation or reduce biological activity. Membrane fouling and flux decline impede the widespread use of AnMBRs. Thus, an extensive research has been conducted in order to comprehend the fouling mechanisms and how they could be prevented by manipulating the operational parameters. Recently, it was reported that the presence and the ratio of different microbial populations, can affect the formation of a cake layer on the membrane surface and subsequently reduce the attainable flux.

This thesis investigated the effect of acidogenic biomass on the filtration characteristics of the bulk sludge. Four different lab-scale reactors were installed to cultivate different microbial populations which may or may not include the acidogenic biomass. Sludge characteristics were monitored and compared in order to assess the filterability differences between the different sludges. The results of these measurements indicated that acidogens will indeed affect the filtration characteristics, i.e. specific resistance to filtration and capillary suction time. On the other hand, this observation could not be validated by comparing critical fluxes of different sludges at a cross flow regime and at a short time scale analysis. This indicated that the hydrodynamic regime is of prior importance, at least a short time scale.

*Key words: Anaerobic Membrane Bioreactor, Fouling, Filterability, Acidogenic biomass.*

## **Preface**

This report is the outcome of my master thesis work and is also the termination of my two-year study. The research is conducted to finalize my master program of Sanitary Engineering in water management department in Civil Engineering and Geosciences Faculty of TU Delft, Netherlands. The research work is supported, sponsored and executed in the lab of Biothane (Veolia Environment).

During this thesis, I have acquired precious experience as a researcher but also I had the opportunity to deepen in the knowledge I got during my studies. Of course, all these accomplishments could not have been completed without the support of my supervisors: Jules van Lier, Kaan Dereli, Frank Van der Zee and Robbert Kleerebezem, Biothane colleagues: Kristof Perez, Aurèlie Grèlot and particularly Rewin Pale who played a very important role in making the everyday lab routine a bit more pleasant. Finally, I have to thank my friends, Raluca, Marij and Ioanna for their support, understanding and patience all those unlucky lab days.

Loverdou Lefki  
Delft, Netherlands  
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## List of Abbreviations

AD Anaerobic digestion  
AnMBR Anaerobic Membrane Bioreactor  
BAP Biomass Associated Products  
CF Critical Flux  
CFV Cross Flow Velocity  
COD Chemical Oxygen Demand  
EGSB Expanded Granular Sludge Bed  
EPS Extracellular Polymeric Substance  
FISH Fluorescence In Situ Hybridization  
F/M Food to Mass ratio  
HRT Hydraulic Retention Time  
MBR Membrane Bioreactor  
MLSS Mixed Liquor Suspended Solid  
PCR Polymerase Chain Reaction  
PSD Particle Size Distribution  
PVDF Polyvinylidene Difluoride  
RH Relative Hydrophobicity  
RPM Rotation Per Minute  
SAA Specific Acidogenic Activity

SRF Specific Resistance to Filtration  
SEM scanning electron microscopy  
SMA Specific Methanogenic Activity  
SMP Soluble Microbial Products  
SOC Synthetic Organic Compound  
SRT Solids Retention Time  
SS Suspended Solids  
TKN Total Kjeldahl Nitrogen  
TP Total Phosphorus  
TS Total Solids  
TSS Total Suspended Solids  
TMP Trans- Membrane Pressure  
UASB Upflow Anaerobic Sludge Blanket  
UAF Upflow Anaerobic Filter  
UAP Utilisation Associated Products  
VFA Volatile Fatty Acid  
VS Volatile Solids  
VSS Volatile Suspended Solids

# 1. Introduction

## 1.1. Anaerobic digestion

Anaerobic digestion (AD) process is an excellent collaboration of various microbial species. The microbial populations taking different roles in AD can be divided into three major groups contributing to different processes. Firstly, hydrolysis process, which converts complex particulate material into smaller and simpler organic compounds (Convert carbohydrates to monosaccharides, proteins to aminoacids and lipids to long chain fatty acids). Secondly, acidifiers ferment the basic monomers to even simpler compounds such as volatile fatty acids (VFAs). Third group of bacteria that contribute to AD are the acetogens which convert VFAs with more than two carbon atoms to acetate,  $H_2$  and  $CO_2$ . Finally methanogens are transforming acetate and hydrogen methane (J. B. van Lier et al., 2008).

Hydrolyzing bacteria excrete enzymes called hydrolases (lipases, proteases, cellulases, amylases, etc.) which hydrolyse their respective polymers into smaller molecules, primarily monomeric and dimeric units, which can subsequently cross the cell barrier and be consumed by microbes. Hydrolysis, especially at treatment of wastewaters with high suspended solids concentration, is often considered as the rate limiting step of AD.

Subsequently, fatty acids, sugars and amino acids are diffused inside the bacteria cells through the cell membrane, and are converted by other enzymatic activities into fermentation products like lactate, propionate, acetate, and ethanol. The free energy ( $\Delta G^\circ$ ) of acidifying reactions is the highest of all the reactions that take place during the AD process. This leads up to 20 times higher bacteria yields and conversion rates than methanogenic bacteria. The difference in growth rates and bacteria yields between acidogens and methanogens can provoke, in cases of overloading, a surpass of methanogenic capacity, a VFA increase and a pH decrease that will cause a further inhibition of methanogens (Miyamoto et al., 1997).

During acetogenesis, obligate  $H_2$ -producing acetogenic bacteria are producing acetate and  $H_2$  from short chain fatty acids. These reactions that are taking place and lead to the production of  $H_2$  are energetically unfavorable due to high free energy requirements ( $\Delta G > 0$ ). Therefore this category of bacteria has to act synergetic with  $H_2$  consuming methanogenic bacteria in order for the fatty acids to be converted to acetate and  $H_2$ . Lactate and ethanol, the other two products of acidogenesis are also converted to acetate and  $H_2$ . Especially, propionate degradation requires a very low  $H_2$  concentration. Such a low partial pressure can only be achieved when there is a co-culture, of propionate degraders with  $H_2$ -consumers. From the reaction that is shown in Table 1, propionate conversion to acetate energy deficiency is very high (+76kJ) while when in co-culture with  $H_2$ -consuming methanogens the reaction becomes energetically favorable (-102.4kJ). Methanogenic bacteria usually use molecular hydrogen so rapidly that the partial pressure drops below  $10^{-4}$  atm which is enough in order to ensure the completion of hydrogen producing reaction of acetogenesis (Miyamoto et al., 1997).

The final step in AD procedure is methanogenesis. This step is performed by 2 large categories of methanogens, acetate (acetoclastic methanogens) and  $H_2/CO_2$  (hydrogenotrophic methanogens) consumers. The first group of methanogenic archea has a very low growth rate (at the range of

days). The second category of methanogenic archaea, the hydrogenotrophic, has much higher growth rates of the order of 4 to 12 hours doubling time. The main precursor of CH<sub>4</sub> is acetate (70% of the methane production stems from the conversion of acetate) (Miyamoto et al., 1997).

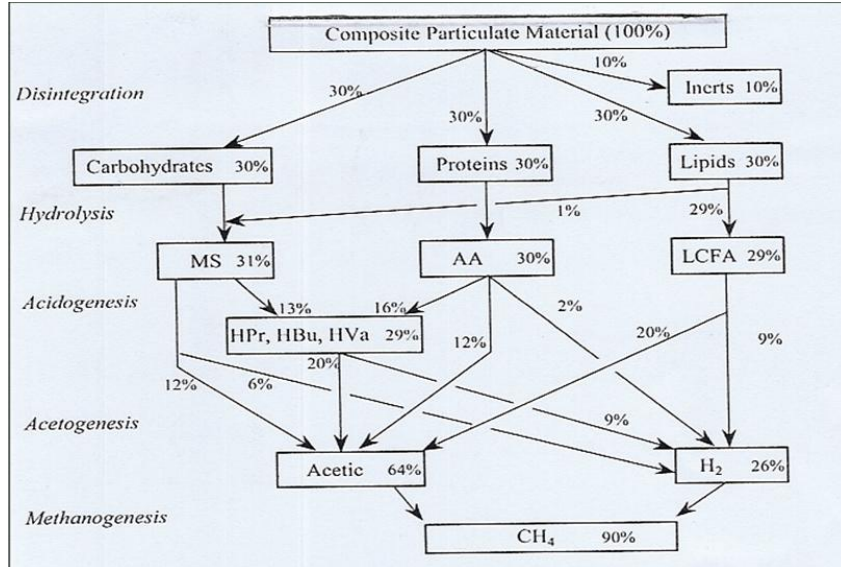


Figure 1 Reactive scheme for anaerobic digestion of polymeric materials MS: monosaccharite AA: aminoacids LCFA: long chain fatty acids HPr, HBu and HVa: propionic, butyric and valeric volatile fatty acids

Table 1 Free-Energy Changes for Reactions Involving Anaerobic Oxidation in Pure Cultures or in Co-Cultures with H<sub>2</sub>-Utilizing Methanogens or Desulfovibrio spp.

Equations	$\alpha^{\circ}G^{0\prime}$ (kJ/reaction)
1. Proton-reducing (H <sub>2</sub> -producing) acetogenic bacteria	
A. $\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \rightleftharpoons 2 \text{CH}_3\text{COO}^- + 2\text{H}_2 + \text{H}^+$	+48.1
B. $\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O} \rightleftharpoons \text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3\text{H}_2$	+76.1
2. H <sub>2</sub> -using methanogens and desulfovibrios	
C. $4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \rightleftharpoons \text{CH}_4 + 3 \text{H}_2\text{O}$	-135.6
D. $4\text{H}_2 + \text{SO}_4^{2-} + \text{H}^+ \rightleftharpoons \text{HS}^- + 4 \text{H}_2\text{O}$	-151.9
3. Co-culture of 1 and 2	
A + C $2 \text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + \text{HCO}_3^- + \text{H}_2\text{O} \rightleftharpoons 4 \text{CH}_3\text{COO}^- + \text{H}^+ + \text{CH}_4$	-39.4
A + D $2 \text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + \text{SO}_4^{2-} \rightleftharpoons 4 \text{CH}_3\text{COO}^- + \text{H}^+ + \text{HS}^-$	-55.7
B + C $4 \text{CH}_3\text{CH}_2\text{COO}^- + 12\text{H}_2 \rightleftharpoons 4 \text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3 \text{CH}_4$	-102.4
B + D $4 \text{CH}_3\text{CH}_2\text{COO}^- + 3 \text{SO}_4^{2-} \rightleftharpoons 4 \text{CH}_3\text{COO}^- + 4 \text{HCO}_3^- + \text{H}^+ + 3 \text{HS}^-$	-151.3

## 1.2. Whey wastewater

Whey, a by-product of cheese manufacturing process, is the liquid remaining following the precipitation and removal of milk casein during cheese production (Siso, 1996).

Whey is characterized by a very high concentration of nutrients and organic matter which can be listed as lactose (4.5-5% w/v), soluble proteins (0.6-0.8% w/v), lipids (0.4-0.5% w/v) and mineral salts (8-10% of dried extract). Main salt composition of whey is NaCl and KCl and calcium salts (primary phosphate). Other components that whey contains at lower concentrations are lactic (0.05% w/v) and citric acids, non-protein nitrogen compounds (urea and uric acid), B group vitamins, etc. Total COD concentration range is 60000-80000 mg/lit (Siso, 1996). The organic matter is around 99% biodegradable. Cheese effluent composition can be approached to the following ratio in terms of carbon/nitrogen and phosphorus C/N/P: 200/3.5/1 which, in principle, can be considered as deficient in nitrogen components for biological processes (Prazeres et al., 2012).

For the biological treatment of whey, aerobic treatment was proven to be ineffective due to high organic load, high energy requirements for oxygen supply as well as excessive sludge production and difficulties in solids settling and thickening. A further problem of the aerobic treatment is that the optimum C/N/P ratio in aerobic processes is roughly 100/5/1 leading to the need of nutrient addition which contributes to a high operational cost. Also, the high COD concentrations of whey can simulate the growth of filamentous microorganisms (bulking) leading to settling difficulties of the sludge (Prazeres et al., 2012). Anaerobic treatment methods offer multiple advantages over aerobic, such as possibility to treat higher organic loads, no energy is required for oxygen supply, on the contrary, energy can be produced due to biogas production. Finally, there is less sludge production, minimizing hence the sludge treatment requirements.

The most common anaerobic reactor types that are used for whey treatment are upflow anaerobic sludge bed reactor (UASB) and upflow anaerobic filter (UAF). The high COD concentrations, though in combination with low alkalinity, would provoke a VFA accumulation in the reactor leading to an unstable operation (Prazeres et al., 2012). An additional problem that was reported was that milk fat as well as its hydrolysis products as oleic acid, may inhibit the biogas production and limit methanogenic activity and also cause sludge flotation (Perle et al., 1994).

### **1.3. Anaerobic Membrane reactors**

Anaerobic membrane reactors are introducing a new way of biomass retention. The most common reactors types that were used for the treatment of high strength industrial wastewater were UASB and expanded granular sludge bed (EGSB) reactors. These two technologies were based on the granulation principal in order to ensure high sludge retention times. Sludge granulation though is depended on the operation conditions of the reactor as well as the properties of the wastewater. The difference that membrane technology is introducing to wastewater treatment is that it is succeeding complete sludge retention independently of the sludge properties (up to 100%) and at the same time produce high quality effluents, free of solids and pathogens (Dereli et al., 2012). The efficiency as well as the cost of membrane reactors is directly dependant on the achievable fluxes. Therefore, wide research is being carried on the parameters that affect attainable fluxes and the fouling mechanisms of membranes.

### **1.4. Objective of this thesis**

This thesis focuses on the effect of acidogenic biomass on the filterability characteristics of sludge. The filterability of the sludge is one of the factors that partly define the attainable flux of an AnMBR therefore. Different microbial consortia lead to different permeabilities of the cake layer that is formed.

Knowing the effect of the acidogens on the formation of the cake layer, can be used for the optimization of the biological operation parameters of AnMBR reactors. The effect of acidogenic biomass was investigated both directly by examining rheological parameters of the sludge, but also indirectly by monitoring secondary parameters that are expected to affect filterability like particle size, hydrophobicity and EPS, SMP material concentrations.

## **1.5. Layout of the report**

This report starts with a short introduction on anaerobic digestion and AnMBR as well as on the whey wastewater that was used as a substrate in this study. Chapter 2 reviews the background knowledge about anaerobic digestion and particularly the different microbial communities; also the membrane fouling mechanisms and factors that affect the sludge filterability properties are revised. In Chapter 3 the aim and scope of the present study are summarized. Chapter 4 presents the main hypothesis and introduces the research. Chapter 5 describes the methodology that was followed during the experiments. Chapter 6 presents the results and Chapter 7 discuss them. Finally, in Chapter 8, conclusions are summarized and in Chapter 9 recommendations for further research are given.

## **2. Literature Review**

### **2.1. Factors that affect sludge filterability**

Membrane fouling is defined as the reduction of permeate flux or increase of filtration resistance due to the clogging of the membrane pores. Fouling mechanisms are associated with sludge characteristics and is distinguished in short and long term fouling depending on the time scale of occurrence. The main fouling mechanisms as described by Meng et al., 2009. are: (1) adsorption of solutes or colloids within/on membranes; (2) deposition of sludge flocs onto the membrane surface; (3) formation of a cake layer on the membrane surface; (4) detachment of foulants attributed mainly to shear forces; (5) the spatial and temporal changes of the foulant composition during the long-term operation (e.g., the change of bacteria community and biopolymer components in the cake layer). If foulants are smaller than or similar in size to the membrane pores, such as colloids and solutes, they can be adsorbed to the pore wall or pore blocking may occur; if foulants are much larger than the membrane pores, such as sludge flocs and colloids, they tend to form a cake layer on the membrane surface (Meng et al., 2009). A further classification of the membrane fouling is according to the reversibility of the fouling. Three types of fouling are therefore defined, removable fouling, irremovable fouling and irreversible fouling (Meng et al., 2009). The mechanism that contributes more to membrane fouling is the cake layer. Lee et al, 2001 reported that the filtration resistances included membrane resistance (12%), cake resistance (80%), blocking and irremovable fouling resistance

(8%). These results though are for an aerobic MBR. For AnMBR different authors had attributed the fouling to different mechanisms. Namely, Choo et al., 1996 and Lee et al. 2001 found the concentration polarization layer responsible for the fouling the while Kang et al., 2002 the internal fouling.

Another factor of major importance that is found out to contribute to the membrane fouling is the deposition of biopolymers. A study conducted by Metzger et al. 2007 on membrane reactors in order to evaluate the biopolymers deposition on the membranes, indicated a stratified cake layer on the membrane surface. The outer layer showed a very high similarity with the sludge flocs and had a more porous composition. The intermediate fouling layer was contributed equally by SMP and bacteria aggregates, and had a high concentration of polysaccharides while the internal layer that is attached to the membrane surface consisted of a high percentage of SMP and bound proteins.

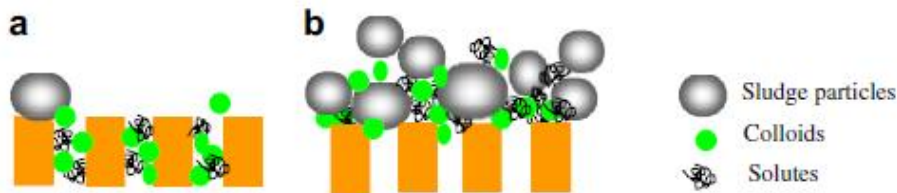


Figure 2 Membrane fouling process in MBRs: (a) pore blocking and (b) cake layer.

From a study of Jeison and van Lier 2007 on cake formation at an AnMBR it was shown that cake formation was mainly reversible in a short scale, while on a long-term operation at fluxes close to critical flux a consolidation of the cake layer was observed. The cake layer formation as the dominant fouling mechanism in AnMBRs was further confirmed by Charfi et al., 2012 who tested different fouling models on several data sets from literature.

The permeability of the cake layer can be affected by *flux*, *electrostatic interactions*, and *particle size* (Le-Clech et al., 2006). As far as the last parameter of particle size is concerned, Le Clech et al., 2006 stated that the permeability of the cake layer passes through a minimum with the increase in the particle size. The explanation suggested was that for very small particles, inter-particle electrostatic repulsion plays a dominant role on the porosity of the cake layer. In this range of particle sizes, the inter-particle repulsion decreases with the increase in particle size resulting in the decrease of permeability. After a certain value of particle size, the effect of the electrostatic force becomes negligible and the permeability increases with the increase in particle size. A different explanation that was used to describe the same phenomenon was that for smaller particle sizes Brownian forces prevail, while for larger particle sizes, hydrodynamic forces dominate (Fane et al., 1983).

Here it should be mentioned that cake formation in membrane bioreactors is expressed as the  $a \cdot K$  product, where  $a$  represents the specific cake resistance and  $K$  is the factor that accounts the cross-flow effect.  $K$  in dead end filtration equals 1. The properties of a cake layer that is formed under dead end filtration are different from those under a cross-flow reactor operation (Wang et al., 2007). During a cross flow membrane operation, the smaller particles of the bulk liquid are less susceptible to back-transport phenomena. This will result in a preferential deposition of fine particles generating a more compact cake layer with higher resistance. The fraction with the

smaller particle size will be the one that will eventually define the cake layer and the permeability of it.

As far as the effect of the concentration of mixed liquor suspended solids (MLSS) is concerned, different observations have been reported in the literature. Some researches support that the increase of MLSS concentration seems to have a mostly negative impact on the membrane performance (Chang et al., 2005, Cicek et al., 1999). Others (Defrance et al., 1999) reported the exact opposite behavior, stating that the increase of the MLSS will result in higher fluxes. Also, some introduced the notion of the existence of a threshold value. Particularly, Rosenberger et al., 2005 stated that a rise at MLSS concentration for low values (<6g/l), seems to decrease fouling while fouling is expected to increase at concentrations higher than 15 g/L while for concentrations between 8-12mg/l the MLSS concentration does not affect the fouling.

## 2.2. Definition and Effect of EPS and SMP on sludge filterability

EPS in either bound or soluble form are considered to play a significant role in membrane fouling. Bound EPS consist of proteins, polysaccharides, nucleic acids, lipids, humic acids, among others that are all located on the cell surface or outside the cell (Drews et al., 2006). SMP has two basic origins. The first one is the organic compounds that are released into the solution due to substrate metabolism (usually this comes with biomass growth) and biomass decay. These two groups of SMP are, substrate-utilisation-associated products (UAP), which are produced directly during substrate metabolism, and biomass-associated products (BAP), which are part of the decay products. There is a complicated interaction between bound EPS and SMP. According to the study of Lapidou and Rittmann, 2002, bacteria when producing new active biomass, they generate bound EPS and UAP in the process. Bound EPS is partly converted with hydrolysis to BAP. BAP and UAP can be used as electron donors for the active biomass and re-converted to bound EPS. The procedure is shown in Figure 3.

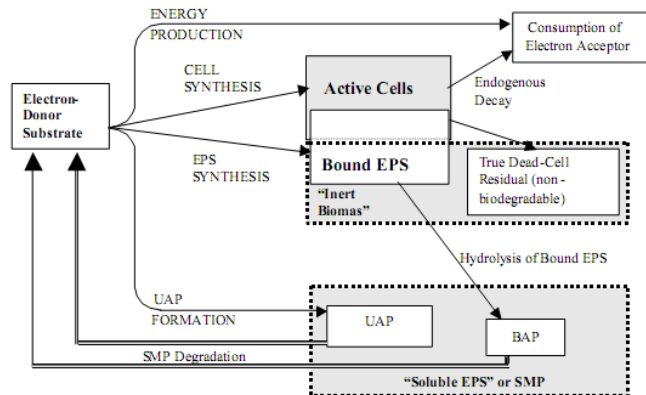


Figure 3 Schematic representation of the unified model for active biomass, EPS, SMP, and inert biomass (Lapidou and Rittmann 2002)

Bound EPS play a major role for floc formation. Cho et al., 2005 indicated that there is a proportional relationship between bound EPS concentration and cake layer resistance. Also, many studies are focusing on the effect of the SMP (soluble EPS) material. Particularly, Rosenberger et al., 2006 examined the impact of SMP in two identical MBRs and concluded that the effect of the

SMP material aggravated the fouling. SMP compared to bound EPS in not only accumulating on the membrane but it also penetrate into membrane pores. Generally, both EPS and SMP are considered to be more important foulants than the microorganisms themselves (Liu et al., 2012).

### **2.3. Effect of Acidifiers in sludge filterability properties**

The effect of acidifying biomass on the performance of AnMBR's was examined by Jeison et al. 2009. The microbial diversity (and thus, their different physical properties, like morphology and adherence capacity) of the anaerobic biomass, which is related to the substrate composition, would influence the physical properties of the sludge like rheology and particle size. A continuous flow AnMBR fed with non-acidified substrate was used in this study Jeison et al., 2009. The biomass was fractionated by centrifuging sludge for 45 min at a RPM of 3500. The supernatant which demonstrated a milky appearance was submitted to specific methanogenic activity (SMA) and specific acidogenic activity (SAA) measurement and the presence of acidogenic bacteria was indicated. Further, epifluorescence observation and FISH analysis showed the existence of rod-shaped microorganisms in the supernatant that were not observed in the pellets. It was therefore inferred by the authors, that the supernatant liquid was composed by acidogenic biomass. The supernatant, the bulk sludge and the re-suspended pellets, after equalizing the TSS concentration (supernatant and pellets had the same TSS concentration 14g/l which was exactly half of the TSS concentration of the sludge) by dilution, were subjected to critical flux (CF) measurement. The CF for the sludge, supernatant and re-suspended pellets were 6.5, 7.5 and 29 L/(m<sup>2</sup>.h), respectively. These results indicated that the supernatant fraction is the one that defines the filterability of the sludge. This behavior was attributed to the small particle size distribution of the supernatant that will lead to increase cake layer resistance that would affect the permeability of the membrane and eventually the applicable flux (Jeison et al., 2009). This observation is in accordance with the findings of Torres et al., 2011 who conducted critical flux measurements to the sludge supernatant and pellets (11 and 31 L/m<sup>2</sup>h respectively). He also attributed this difference to low particle size distribution of microorganisms due to single cell development. However, he states that SMA and SAA activity measurements did not show a specific enrichment of acidogenic or methanogenic microorganisms in the supernatant fraction and therefore no conclusion can be drawn considering a disperse growth of these two microbial populations.

An additional research that was conducted by Jeison et al., 2007 confirmed that the acidification degree of treated wastewater can strongly influence the physical properties of the sludge, determining the applicable flux. A VFA mixture and for a VFA/glucose mixture were fed to two identical reactors. The rheological behavior of the sludges was very different: The VFA-MBR exhibited a behavior, close to that of a Newtonian fluid while the glucose/VFA (with acidogenic biomass) approached the behavior of a Bingham fluid (stress can be applied but it will not flow until a certain value). This difference was attributed to the suspension of fine solid particles. In order to further assess the effect of the acidifiers on the sludge characteristics, 3 series of critical flux measurements were performed:

- S1: performed with the original sludge from the glucose/ VFA-MBR, at a solids concentration of 39 g TSS/L (without centrifugation).

- S2: performed with the original sludge from the glucose/ VFA-MBR, but at 21 g TSS/L, to match the solids concentration of S3 (without centrifugation).
- S3: performed with the glucose/VFA-MBR sludge, after removing the supernatant by the centrifugation. Solids concentration was 21 g TSS/L.

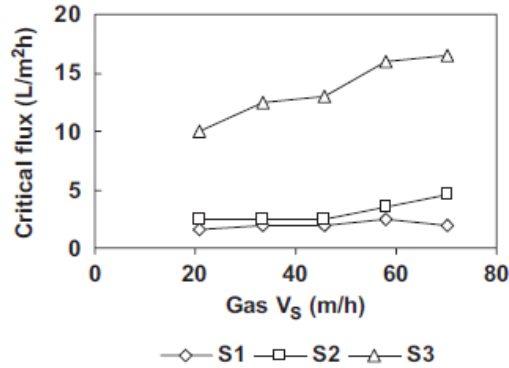


Figure 4 Critical flux of different sludge types

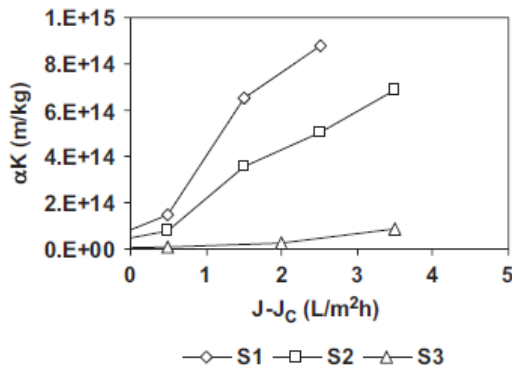


Figure 5 Cake formation rates over the critical flux at  $V_g$  of 70 m/h.

According to the results shown at the graph of Figure 4, the critical flux was found to be much higher for the sludge after the supernatant was removed. It showed a much more intense effect than decreasing the TSS concentration by 50%. Moreover, the cake formation rates were much lower for sludge without the fine particles supernatant (Figure 5).

## 2.4. Microbial populations responsible for the foulant layer

Many studies had examined the effect of the microbial communities on the filterability of the sludge. According to Zhang et al. 2006, the microbial communities on the membrane surfaces are different from the ones in the suspended biomass. Furthermore they reported that some microbial strains showed a tendency to colonize membrane surfaces.

Similar to Jeison et al. 2007 who had indications that the acidogenic biomass led to a significant decrease of the critical flux, different studies that are based on microbial analysis tools identified that some bacteria species had a tendency to attach membrane surfaces and cause a higher degree of fouling (Chen et al., 2004; Jinhua et al., 2006; Zhang et al., 2006c; Miura et al., 2007). The fouling of an AnMBR is related to the relative contributions of microorganisms due to their

differences in the nature and quantity of EPS and SMP, susceptibility to adhesion at different membrane surfaces, and capacity to colonize and form biofilms (Gao et al., 2010). At a lab scale AnMBR fed with artificial sewage, a yellow foulant layer coated the membranes and on top of that layer, a dark cake layer appeared (Gao et al., 2010). The same yellow liquid was also noticed after centrifugation of the sludge. Fluorescent staining showed that the yellow foulant layer was composed of both EPS and cells. Very high percentages of OP11 phylogenetic division were detected in the foulant layer. This division of bacteria is abundant in nature and phylogenetically it is highly diverse. Up till now though, there are no cultivated members of division OP11, so the physiological properties of these organisms are unknown (Gao et al., 2010). Bacteroidetes, even though they were present in the supernatant and the yellow layer that stemmed from centrifugation, they were not detected at the foulant layer. Therefore, it can be deduced that they do not contribute to fouling through attachment on the membrane surface but only through the excretion of proteinaceous EPS. The proteinaceous level of EPS was found to be much higher in the light solids compared to the dark solids (2 times higher) which can be due to the high protein concentration of the feed (30%).

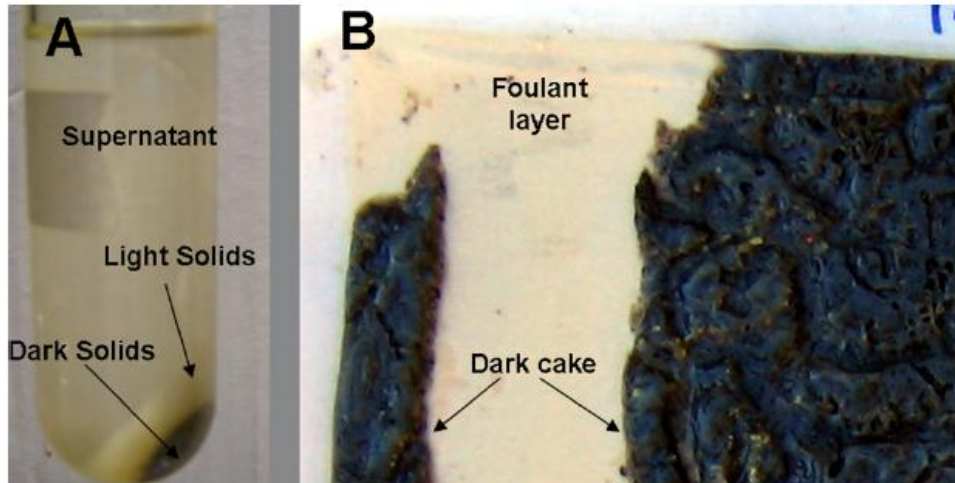


Figure 6 The mixed liquor separated into supernatant, light solids and dark solids after centrifugation at 12,000×g for 15 min. (B) Plan view photograph of the fouled cPVDF membrane. A strip of loosely held dark cake has been removed to reveal a thin yellow foulant layer attached to the membrane (Gao et al., 2010).

## 2.5. Two phase anaerobic reactors

In anaerobic treatment, the overall growth rate of acidogenic biomass exceeds the one of methanogenic biomass. Therefore, anaerobic treatment of highly soluble and easily fermentable substrates at high COD loadings often causes process stability problems such as the accumulation of VFA, pH decrease and inhibition. In order to overcome this problem, two-stage reactor configurations have been proposed and investigated. That is based on the optimal cultivation of the various microbial populations at different reactor stages. Choen et al., 1979 examined the difference between a single and a two stage anaerobic reactor system for synthetic glucose substrate. It was observed that VFA accumulation would take place in both systems under overloaded conditions. But when the feed was ceased, at the single phase system no propionate turnover was observed within one week time period while in the two stage system all

VFAs were converted to acetate. They concluded that two-stage system is more robust at high loading rates.

## **2.6. Correlation between different sludge Filterability factors**

As it was described in the previous paragraphs, filterability of the sludge is a multi-parameter problem. Van der Broeck et al. 2011 conducted a research on activated sludge samples in order to find a correlation of the different sludge characteristics with filterability. The main conclusion of this study was that no clear correlation can be found between only one sludge sample parameter and activated sludge filterability (Van der Broeck et al., 2011). Several parameters in the literature for instance MLSS concentration, as it was stated earlier has shown controversial results on filterability. This controversy is due to interdependencies of biomass, wastewater and operational conditions. One characteristic example is the EPS concentration which affects with two ways the sludge filterability. Firstly, the bound EPS is forming a matrix for microbial aggregates and thus affects sludge morphology. Furthermore, some components of EPS (proteinaceous EPS) contribute to the increase of sludge hydrophobicity. The increase of hydrophobicity decreases the affiliation of the sludge with the membrane surface, and increasing the filterability of the sludge. This example indicates that phenomena related with sludge parameters are associated and that all the factors should be measured and taken into consideration when the filterability is examined.

## **3. Aim and Scope**

AnMBR is considered as a promising technology that combines classical anaerobic treatment and membrane filtration for biomass separation. Conventional anaerobic digesters have the disadvantage that they operate under long hydraulic retention times, thus they require large capacity tanks. One of the advantages that this technology is presenting compared to the most commonly used granular sludge bed reactors, which are often used for the treatment of high strength industrial waste, is the retention of biomass is ensured by membrane filtration regardless of the biomass properties such as settling and granule formation. A very important parameter that defines the operation of an AnMBR is the short term fouling induced by the formation of a cake layer on the membrane surface. This cake layer will eventually affect the attainable permeate flux to a higher extend than the membrane material itself. Therefore, in order to assess the economic feasibility of an AnMBR research on the formation mechanisms of this cake layer is required. One parameter that is expected to have an impact on this layer is the microbial composition of the anaerobic sludge. In this study, the effect of acidogenic biomass on the filterability of the sludge and on the cake formation will be assessed at single stage and at two-stage reactors treating high strength and soluble whey.

## **4. Hypothesis and Research approach**

#### **4.1. Hypothesis**

1. The microbial diversity of the anaerobic biomass, which is related to the substrate composition, will influence the physical properties of the sludge such as rheology and particle size (Jeison et al., 2009).
2. The degree of acidification, can strongly affect the filtration characteristics of the sludge, determining the applicable flux (Jeison et al., 2007).

#### **4.2. Research approach**

In order to testify the stated hypothesis, four lab-scale completely stirred reactors (CSTR) were installed and fed with whey with or without acidification. The sludge filterability characteristics were monitored by applying standard filtration tests and the results obtained for different sludges were compared. The details of the reactor setups and experiments are described in Chapter 5.

### **5. Materials and Methods**

#### **5.1. Reactor configuration**

Four lab-scale reactors were installed to test the effect of acidification on sludge filterability. All reactors consisted of a 2L volume digester and they were operated under mesophilic conditions ( $37\text{ }^{\circ}\text{C} \pm 0.5$ ). In order to maintain the temperature, water was re-circulated through a water bed to double glass water jacket around the reactors. The pH was controlled with stand-alone controllers (and pumps for caustic or acid addition. On the lid of every reactor, a mixing motor was installed and a sampling port connected to a syringe of 60mL volume. A recirculation pump was installed to each reactor in order to simulate the cross flow regime of an AnMBR. Shear stress was applied on large flocs, breaking them into smaller ones. According to the findings of Wisniewski et al 1996, shear stress affects the granulometric distribution of the biological suspension and the particle size leading to different filterability properties. Therefore, the recirculation pump was required to apply shear on the sludge as it is the same case in a normal cross-flow AnMBR configuration. The biogas was measured by wet tipping gas meters and daily biogas production was recorded online. pH was measured both manually and automatically. Methane composition of the biogas was checked daily by washing a certain amount of biogas in 1 M NaOH solution.

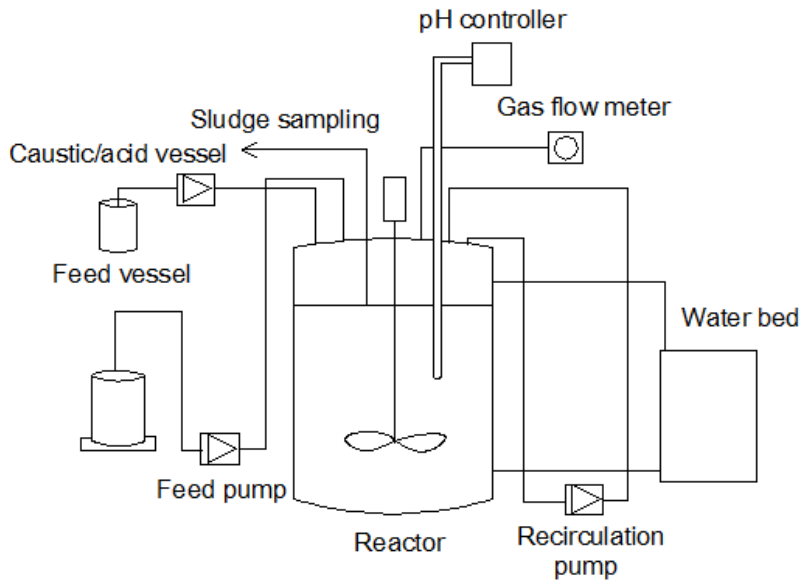


Figure 7 Reactor configuration

## 5.2. Reactor operation

In this section the operation of four reactors operation will be described separately in detail. Figure 8 shows the configuration of the reactors.

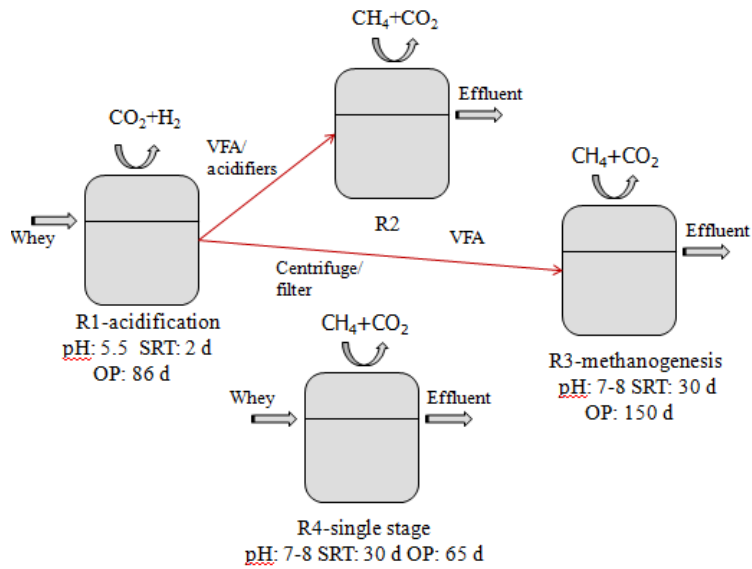


Figure 8 Different substrates for the 4 reactors

### 5.2.1. Reactor 1-acidification

Initially, the operation of R-1 was CSRT but after the 9<sup>th</sup> day it was switched to a sequencing batch reactor (SBR) in order to increase suspended solids concentration to facilitate the execution of filtration tests. The SBR function was executed in 3 steps: feed and react, settle, decant. The first step would begin at 08:00 a.m. with a reactor volume of 1L and will continue until 03:00 a.m. when it would reach 2L in 19 hours. Subsequently, the settling phase for 4 hours would follow (03:00 a.m.-07:00 a.m.). Finally, the last phase would be the decanting, where the extraction pump would discharge 1L from 07:00 a.m. until 08:00 a.m. The SBR operation is summarized in Figure 9.

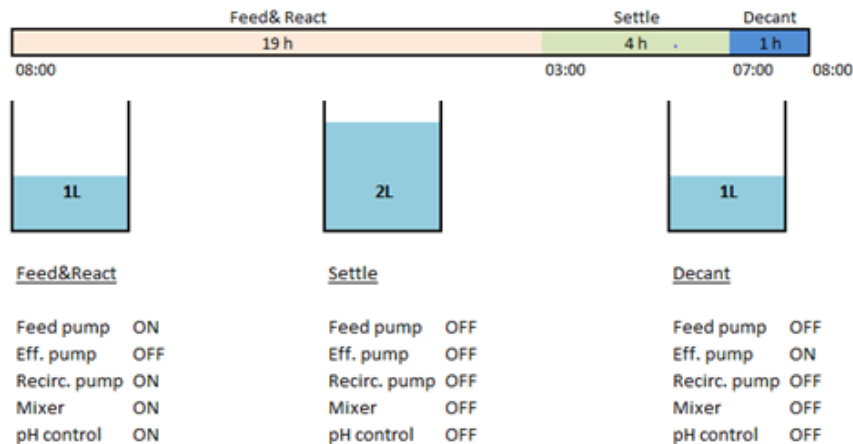


Figure 9 SBR time schedule and equipment operation

Reactor 1 operation started as an SBR with the extraction line made out of rigid material, reaching the depth of 1 L. This led to the extraction of a high amount of biomass resulting in lower VSS concentrations, therefore, on day 26 the rigid line was replaced with an improvised floating decanter made of flexible tube bound to a small floating plastic balloon. The line would always bend following the water level and suck from the water surface so only supernatant would be discharged. The lines before and after the replacement with the supernatant decanter are shown in Figure 10.



Figure 10 Improvised floating decanter

The SBR type of operation may cause a relative choice of sludge with better settleability properties with larger floc size, but this effect is minimized by the operation of the recirculation pump. The VSS concentration was significantly increased under this operation condition.

Hydraulic retention time (HRT) of this reactor was 2 days (feed flow (Q) =1 L/day, reactor volume (V) = 2L). The average VLR during the last and stable phase of the operation was around 29.5 kg COD/(m<sup>3</sup>.day). The pH was monitored with a controller and maintained at 5.5 with the addition of NaOH which would be fed automatically. The pH was adjusted at this value, so the growth of acidogenic species would be exclusively prospered (lower pH would create a toxic environment for the acidogens, while higher would allow the proliferation of methanogens as well). The operational conditions of R-1 are summarized in Table 2 and the reactor setup is shown in Figure 11 Reactor 1.

*Table 2 Reactor 1 operational conditions*

<b>Operation condition</b>	<b>Unit</b>	<b>Phase 1 (day 0-16)</b>	<b>Phase 2 (day 17-87)</b>
<b>SRT</b>	days	2.7±0.5	-
<b>HRT</b>	days	2	2
<b>VLR</b>	kg COD/(m <sup>3</sup> .day)	11.9	29.6±9.0
<b>F:M</b>	kg COD/(kg VSS.day)		1.0±0.2
<b>Temperature</b>	°C	37	37
<b>pH</b>	-	5.5	5.5



*Figure 11 Reactor 1*

### **5.2.2. Reactor 2-acidogenic biomass/ natural VFA mixture**

This reactor was fed with the effluent of Reactor 1 consisting of a mixture of VFA and acidifying biomass. Crushed and sieved (600  $\mu\text{m}$ ) granular sludge from a full scale EGSB reactor treating lactose based wastewater was used as the inoculum. The granular sludge was blended with a kitchen blender and stored at room temperature for several months before use. R-2 was operated as a CSTR. The feed vessel was continuously stirred in order to prevent the biomass from settling. pH was monitored with a pH controller but only at the last phase of operation, acid addition was required. The feeding rate was approximately 70 mL/day. The VLR at the last phase of operation was around 2.5 kg COD/( $\text{m}^3\cdot\text{day}$ ) and the sludge retention time (SRT) was around 30 days. The total operation time was 6 months. The operational conditions of R-2 are summarized in Table 3 and the reactor setup is shown in Figure 12.

*Table 3 Reactor 2 operational conditions*

<b>Operation condition-</b>	<b>Unit</b>	<b>Phase 1 (day 0-28)</b>	<b>Phase 2 (day 29-78)</b>	<b>Phase 3 (day 79-120)</b>	<b>Phase 4 (day 121-140)</b>
<b>HRT</b>	days	32.3 $\pm$ 1.9	32.5 $\pm$ 2.9	31.2 $\pm$ 4.2	32.2 $\pm$ 4.1
<b>VLR</b>	kg COD/( $\text{m}^3\cdot\text{day}$ )	0.6 $\pm$ 0.3	1.5 $\pm$ 0.5	2.1 $\pm$ 0.3	2.5 $\pm$ 0.2
<b>F:M</b>	kg COD/(kg VSS.day)	0.07 $\pm$ 0.02	0.20 $\pm$ 0.08	0.40 $\pm$ 0.1	0.33 $\pm$ 0.04
<b>Temperature</b>	$^{\circ}\text{C}$	37	37	37	37
<b>pH</b>	-	7.5	7.5	7.5	7.5



*Figure 12 Reactor 2*

### **5.2.3. Reactor 3- natural VFA mixture**

The operation conditions of this reactor are the same with R-2 reactor. The feed was the acidified and centrifuged whey from R-1 reactor. It was basically a VFA mixture, mainly composed of butyric acid. The VLR at the last phase of operation was around 3.6 kg COD/( $\text{m}^3\cdot\text{day}$ ) and the SRT was around 26 days. At the last phase of operation the feed was supplemented with acetate

and butyrate in order to boost feed COD and VLR. The total operation time was 6 months. The operational conditions of R-3 are summarized in Table 4 Reactor 3 operational conditions and the reactor setup is shown in Figure 13.

*Table 4 Reactor 3 operational conditions*

<b>Operation condition- Reactor 3</b>	<b>Unit</b>	<b>Phase 1 (day 0-28)</b>	<b>Phase 2 (day 29-78)</b>	<b>Phase 3 (day 79-140)</b>
<b>HRT = SRT</b>	days	27.0±1.5	26.1±2.7	26.1±4.2
<b>VLR</b>	kg COD/(m <sup>3</sup> .day)	0.7±0.3	2.1±0.4	3.6±0.7
<b>F:M</b>	kg COD/(kg VSS.day)	0.07±0.03	0.31±0.08	0.63±0.16
<b>Temperature</b>	°C	37	37	37
<b>pH</b>	-	7.5	7.5	7.5



*Figure 13 Reactor 3*

#### **5.2.4. Reactor 4- single stage AD**

The operation conditions of R-4 reactor were the similar to R-2 and R-3. The main difference was that R-4 was fed with raw whey as substrate. The VLR at the last phase of operation was around 3.2 kg COD/(m<sup>3</sup>.day) (it was increased at day 81 with addition of extra VFA). The total operation time was 2 months. The operational conditions of R-4 are summarized in Table 5.

*Table 5 Reactor 4 operational conditions*

<b>Operation condition- Reactor 4</b>	<b>Unit</b>	<b>Phase 1 (day 0-16)</b>	<b>Phase 2 (day 17-63)</b>
<b>HRT=SRT</b>	days	30.6±2.8	30.6±2.7
<b>VLR</b>	kg COD/(m <sup>3</sup> .day)	2.1±0.2	3.2±0.3

<b>F:M</b>	kg COD/(kg VSS.day)	0.19±0.003	0.35±0.04
<b>Temperature</b>	°C	37	37
<b>pH</b>	-	7.5	7.5

### 5.3. Experimental methods

Frequent analyses were performed to check the characteristics of the feed and sludge. Total solids (TS), suspended solids (SS), total kjeldahl nitrogen (TKN) and ammonium nitrogen were measured according to Standard Methods (APHA, 1998). Chemical oxygen demand (COD), soluble COD, total phosphorus (TP), phosphate ( $\text{PO}_4^{3-}\text{-P}$ ), were measured with Hach-Lange test kits. Soluble COD, calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ) samples were prepared by centrifuging sludge at 17500 g for 10 minutes and then by filtering the supernatant through 0.45  $\mu\text{m}$  membrane filter. The analyses were performed also with Hach-Lange kits.

The VFAs were analyzed by a gas chromatograph (Varian 3900) equipped with a silica column (25 m and 0.53 mm internal diameter) and a flame ionization detector. Injector, column and detector temperatures were 250, 140 and 275 °C, respectively.

Sludge activity tests on different VFAs (acetic, propionic and butyric acid) were done in serum bottles (120 mL) by monitoring the pressure increase with a pressure transducer (Centre Point Electronics PSI-30). The bottles were sealed with butyl rubber stoppers and aluminium caps. The anaerobic medium was prepared by dissolving 3.5 g/L  $\text{NaHCO}_3$  in tap water without the addition of extra nutrients. The head space was flushed with  $\text{N}_2:\text{CO}_2$  (70:30 %) mixture. Methane content in biogas was measured by washing a certain volume of biogas in 1 M sodium hydroxide solution in order to absorb carbon dioxide.

Extracellular polymeric substances were extracted by heat treatment at 100 °C for 1 hour. After heat extraction was centrifuged at 17500 g for 10 minutes and then filtered through 0.45  $\mu\text{m}$  filter. The same procedure was also applied for SMP samples without heat treatment. Total EPS and SMP were determined by measuring proteins (BCA Protein Assay Kit) and polysaccharides (Dubois et al.1956). Bovine serum albumin and d-glucose were used as protein and polysaccharide standards, respectively. Bound EPS and SMP concentrations were calculated as in Equation 1-5 (Laspidou et al., 2002).

$$\text{Total EPS} = \text{Bound EPS} + \text{SMP}$$

$$\text{Bound EPS} = \text{Bound Protein} + \text{Bound Polysaccharide}$$

$$\text{SMP} = \text{Soluble Protein} + \text{Soluble Polysaccharide}$$

$$\text{Bound Protein} = \text{Total Protein} - \text{Soluble Protein}$$

$$\text{Bound Polysaccharide} = \text{Total Polysaccharide} - \text{Soluble Polysaccharide}$$

Sludge relative hydrophobicity (RH) was measured according to microbial adhesion to hydrocarbons (MATH) test (Rosenberg et al., 1980; Rosenberg, 2006; Gaurav, 2010). Dodecane was used as the hydrocarbon phase. The sludge sample was diluted with permeate to achieve 1

g/L TSS concentration. The diluted sample was measured as the initial value at 600 nm ( $Abs_i$ ) in a spectrophotometer (Jenway 7315) with tap water as a blank. 4 mL of diluted sample was transferred into a glass tube and 4 mL of dodecane was added. The sample and the hydrocarbon were shaken vigorously on a whirli-mixer for 2 minutes in order to generate very small hydrocarbon droplets. The sample was allowed to rest for phase separation for 10 minutes. Absorbance of the aqueous phase is then measured at 650 nm ( $Abs_f$ ) and compared to the absorbance of the diluted sample ( $Abs_i$ ). The Sludge RH can then be calculated as follows:

$$RH (\%) = \left(1 - \frac{Abs_f}{Abs_i}\right) \cdot 100$$

The particle size analyses were performed on a Beckman Coulter LS230 laser particle size analyzer, to determine the particle diameter of mixed liquor from 0.4 to 2000 microns. Critical flux measurements were done according to the flux step method of Le Clech et al. 2003 with 2 L/(m<sup>2</sup>.h) step height and 15 minute step length. Critical flux was determined according to its week definition and a slope of  $dP/dt \geq 1$  mbar/min was arbitrary chosen to decide critical flux was reached.

Sludge filtration characteristics were determined by a number of standard analyses performed at room temperature (20-22 °C). Capillary suction time (CST) of the sludge was measured with Triton Capillary Suction Timer (304M) and standard filter paper supplied from Triton Electronics. Resistance to filtration was measured in a dead end filtration cell (Millipore 8050). The sludge was first diluted to 10 g/L TSS concentration with permeate. Then the diluted sample was imposed to a positive pressure (0.5 bar) under non-stirred conditions in order to develop a cake layer on a standard filter paper (0.7 µm). The volume of filtrate versus time was recorded for 30 minutes. The specific resistance to filtration was determined by plotting the ratio filtration time/filtrate volume ( $t/V$ ) versus the filtrate volume ( $V$ ). Using the slope of the line, the SCR was calculated according to the following formula:

$$SCR = \frac{2 \cdot \Delta P \cdot A^2 \cdot \alpha}{\mu \cdot C}$$

$\Delta P$ : Pressure (Pa),  $A$ : Effective filtration area (m<sup>2</sup>),  $\alpha$ : Slope (s/L<sup>2</sup>),  $\mu$ : Viscosity (Pa.s),  $C$ : TSS concentration (kg/m<sup>3</sup>)

Supernatant filterability was determined by a method adapted from Rosenberger et al. 2006. The supernatant was prepared by centrifuging (Hermle Z383 K) the sludge at 17500 g for 10 minutes. The supernatant filterability is determined by filtering through a 0.22 µm membrane filter in a stirred dead end filtration cell (Millipore 8050) under constant pressure (0.5 bars). The supernatant was stirred in order to prevent extensive accumulation soluble macromolecules and immediately block the membrane pores. Permeate was collected for 10 minutes on an analytical balance connected to a PC for data acquisition. The filterability was calculated by averaging the permeate flow rate after 5 minutes.

Viscosity measurements were performed with a viscosity meter (Thermoscientific Haake viscotester 550) coupled with standard PC. A coaxial cylindrical measurement device was used.

The temperature was maintained constant at  $37.6 \pm 0.1^\circ\text{C}$ . A sample volume of 10 mL was used for each measurement. The measurement protocol consists of an exponential increase of the shear stress from 0.01 to 1000 Pa in 3 min. The viscosity was calculated from the slope of the plotted values of shear stress (mPa) against shear rate (1/s).

Critical Flux Measurement was conducted at the beginning and end of the operation of each reactor. The critical flux was measured according to flux step method (Le Clech, 2003) to compare the different filtration performances of the different sludges. The criteria set to determine the critical flux was  $dP/dt < 1 \text{ mbar min}^{-1}$ .

The experimental plan of the study is shown in Table 6

*Table 6 Experimental plan*

<b>Parameter</b>	<b>Feed</b>	<b>Sludge</b>	<b>Frequency</b>	<b>Method</b>
TCOD	X	X	1 per week	Standard Methods
Supernatant COD		X	1 per week	Standard Methods
Soluble COD	X	X	1 per week	Standard Methods
TKN	X	X	1 per 2 weeks	Standard Methods
$\text{NH}_4^+\text{-N}$	X	X	1 per 2 weeks	Standard Methods
TSS/VSS	X	X	1 per week	Standard Methods
TS/VS	X	X	1 per week	Standard Methods
VFA	X		1 per week	GC
$\text{PO}_4^{3-}\text{-P}$	X	X	1 per 2 weeks	HPLC
TP	X	X	1 per 2 weeks	Standard Methods
Cations	X			HPLC
Anions	X			HPLC
pH	X	X		pH meter (Standard Methods)
Alkalinity			1 per week	Standard Methods
Proteins		X	1 per week	BCA
Polysaccharides		X	1 per week	Dubois
Capillary suction time (CST)		X	1 per week	Triton
Specific cake resistance (SCR)		X	1 per week	Amicon Cell
Filterability		X	1 per week	Amicon Cell
Specific methanogenic activity		X	1 per month	Pressure

#### **5.4. Feed characterization**

The reactors were fed with different substrates based on whey as it was mentioned previously. R-1 and R-4 were fed with raw (non-acidified) whey wastewater that was obtained from a cheese production factory. Prior to being fed into the reactor, the whey was diluted to decrease the COD concentration and supplemented with urea ( $\text{CH}_4\text{N}_2\text{O}$ ) as additional nitrogen source for biomass growth. In order to prevent micronutrient deficiency, vithane and  $\text{FeCl}_3$  were also added to the feed.

The effluent of R-1 was extracted and collected in a vessel kept in the refrigerator and fed to R-2. Initially, the VLR of R-2 was low and the sludge concentration rapidly decreased. In order to maintain a sufficient sludge concentration required for the filterability tests, the feed COD concentration was increased by supplementing with extra acidogenic sludge. The acidogenic sludge was obtained by centrifuging R-1 effluent at 17000g pouring the pellets in to R-2 feed. With this method, particulate COD and VSS concentration of R-2 was increased to achieve higher loading rates.

The substrate for R-3 was prepared by centrifuging (17000g for 10 minutes) the effluent of R-1. The supernatant was then passed from a membrane filter (0.45µm) and subsequently from a smaller pore sized filter (0.22 µm). Vithane and FeCl<sub>3</sub> were also added to the feed of R-2 and R-3.

All feeds were prepared in weekly or monthly basis and stored in -4C° before use. The feed characterizations of the reactors during different operational phases are given at Table 7 Table 10 respectively.

*Table 7 Feed characterization during the different operation phases of R1*

Parameters (mg/l)	Feed (day 0-16)	Feed (day 17-87)
TCOD	24700	62786±4725
SCOD	24700	62536±4485
TKN	500	618±29
NH <sub>4</sub> <sup>+</sup> -N	24	64±41
TSS	827	1303±152
VSS	827	1201±298
TS	20000	51648±1795
VS	18000	46571±1586
TP	167	331±44
PO <sub>4</sub> <sup>3-</sup> - P	132	249±105

*Table 8 Feed characterization during the different operation phases of R2*

Parameters (mg/l)	Feed (day 0-28)	Feed (day 28-78)	Feed (day 79-120)	Feed (day 120-140)
TCOD	24455±9911	57153±4414	65873±2496	78662±3817
SCOD	21940±9473	55135±4401	57000	72944±3818
TKN	467±203	976±215	-	-
NH <sub>4</sub> <sup>+</sup> -N	136±62	35±19	-	-
TSS	1770±650	3286±1153	3286±1153	5910±286
VSS	1770±651	3286±1154	3286±1154	5114±248
TS	17658±6988	41849±6989	37058±6449	41363±2007
VS	10601±5078	30042±5079	23357±6596	27036±1311
TP	156±96	410±92	410±92	410±93
PO <sub>4</sub> <sup>3-</sup> - P	120±90	348±14	326±15	326±15

Table 9 Feed characterization during the different operation phases of R3

Parameters (mg/l)	Phase 1 (day 0-28)	Phase 2 (day 29-78)	Phase 3 (day 79-140)
TCOD	20932±5989	55145±2864	96296±3246
SCOD	20932±5990	55145±2865	96296±3247
Acetic acid	2992±1008	1275±825	9124±3314
Propionic acid	530±386	233±103	185
iso-byturic acid	2614±1643	777±302	695
Byturic acid	0	14785±2503	33003±2007
iso-valeric acid	0	0	0
Valeric acid	584±499	1493±2304	4977
Caproic Acid	1226±389	1903±1134	3115

Table 10 Feed characterization during the different operation phases of R4

Parameters (mg/l)	Phase 1 (day 0-16)	Phase 2 (day 17-63)
TCOD	63379	95948±842
SCOD	62672	95362±1194
TKN	629.58	685±31
NH <sub>4</sub> <sup>+</sup> -N	129	488
TSS	1412	2138±19
VSS	933	1412±12
TS	55439	83928±737
VS	49840	75452±663
TP	335	597±91
PO <sub>4</sub> <sup>3-</sup> - P	261	422±29

## 6. Results

### 6.1. Biological Performance

Volumetric loading rate (VLR) and sludge loading rate (F/M ratio) applied to the reactors during the operation period are shown in Figure 14. All the reactors started with an initial low loading rate. This low loading rate didn't allow a significant biomass growth in the reactors, which made it difficult for the measurement of filterability parameters. Therefore, a load increase was considered indispensable. For R-2 this increase was executed by increasing the acidogenic biomass concentration in the feed, by centrifuging the effluent of R-1 and adding the pellets to the feed of R-2. For R-3 the increase of the load was conducted by adding extra acetate and butyrate to the feed. The trend of the F/M follows the same trend with the VLR in all the Reactors, with a few outlier points that can be attributed to operation problems of the feeding pumps.

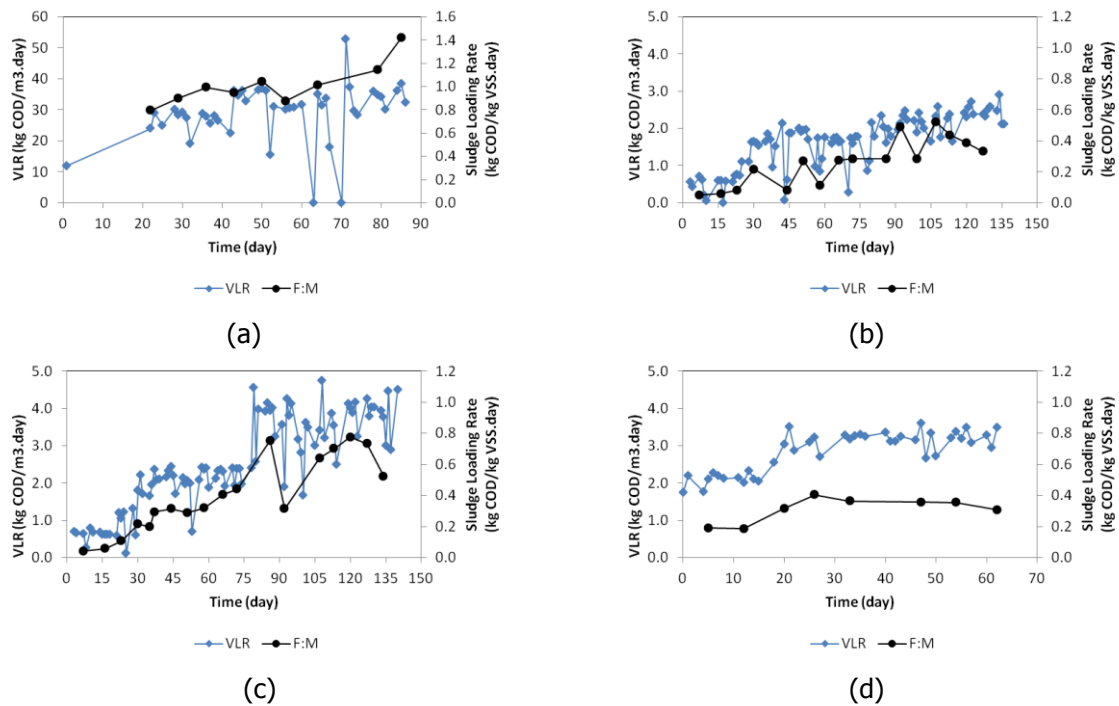


Figure 14 VLR and F/M ratio time evolution for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

#### 6.1.1. TSS/VSS and TS/VS concentrations

The TS-VS and TSS-VSS concentrations in the reactors are presented in Figure 15. The solid matter concentrations gradually decreased in R-2 and R-3 reactors. On the 79<sup>th</sup> day, the VLR increased in order to maintain a sufficient biomass concentration in R-2 and R-3 for filterability analysis. The increase of VLR, led to a stabilization of TSS/VSS for R-2 and even a small increase at R-3. The biomass concentration in R-1 increased after the change of operation from CSTR to SBR at the 9<sup>th</sup> day. It further increased after the increase of VLR at day 43 of operation (Figure

14). For R-4 the sludge concentration slightly decreased after the start up but then it was almost stable until the end of operation period.

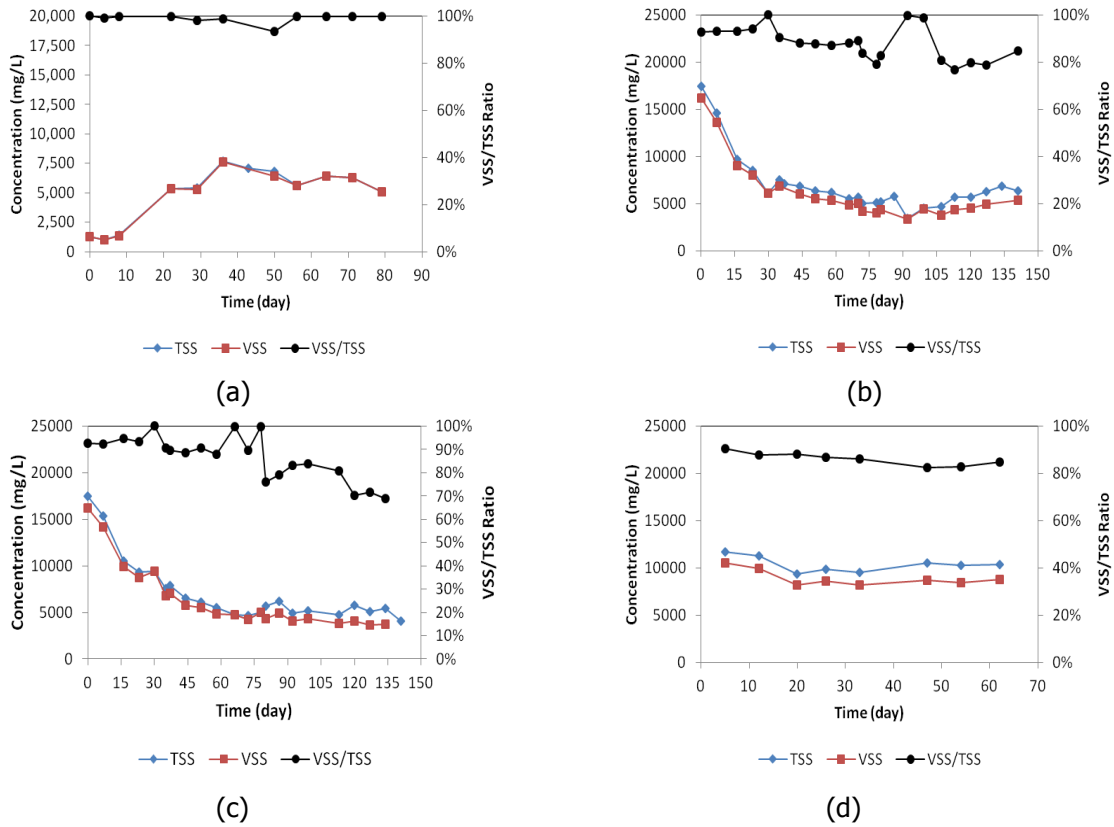


Figure 15 TSS and VSS concentrations for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

The evaluation of TS and VS concentrations in the reactors are shown in Figure 16. Both parameters showed an increase after the conversion of R-1 to a SBR. Similar to the trend of suspended solids concentrations, two peaks appeared the 17<sup>th</sup> and the 43<sup>rd</sup> day of operation. R-2 and R-3 showed a deviant trend of the TS and VS. Specifically, TS showed an increasing trend while VS concentration kept on decreasing until the 90<sup>th</sup> day when both values showed an increasing slope which was induced by the VLR increase. This is probably due to continuous inorganic material precipitation and accumulation in the reactors during operation. Finally, Reactor 4 had an almost constant concentration of TS and VS throughout the operation.

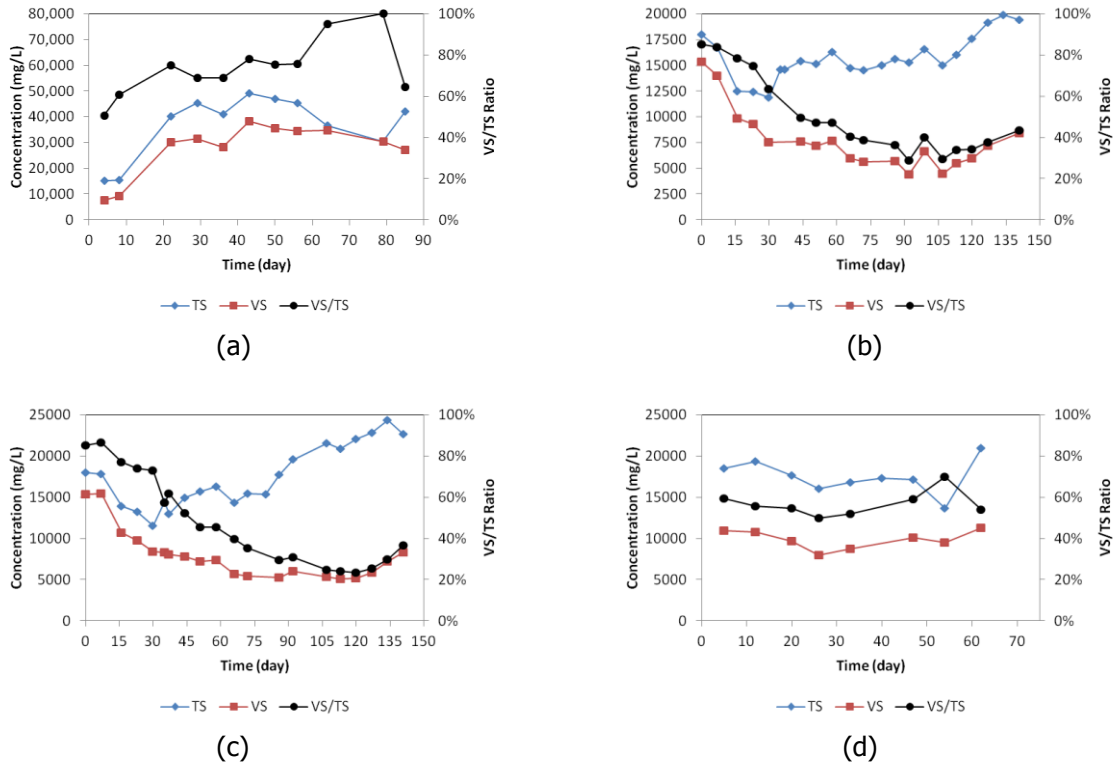


Figure 16 TS, VS concentrations for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

### 6.1.2. COD removal efficiency

The COD removal efficiency ( $E_{COD}$ ) was calculated as:

$$E_{COD}(\%) = \frac{Q_{influent} \cdot TCOD_{influent} - Q_{effluent} \cdot TCOD_{effluent}}{Q_{influent} \cdot TCOD_{influent}} \cdot 100$$

The  $E_{COD}$  for R-1 was approximately 25%. This may be due to  $H_2$  production, which was not measured and included in the COD mass balance, during the acidification of whey. The COD removal for R-2 and R-3 were around 80% and 85-90%, respectively. This is interesting since R-3 was operated even at higher VLR achieved higher COD removal efficiencies. This may be due to the fact that the R-2 reactor was fed with a higher particulate COD concentration compared to the totally soluble feed of R-3. The  $E_{COD}$  of R-4 was similar to R-2 which was around 80% at the stable operation phase (Figure 17).

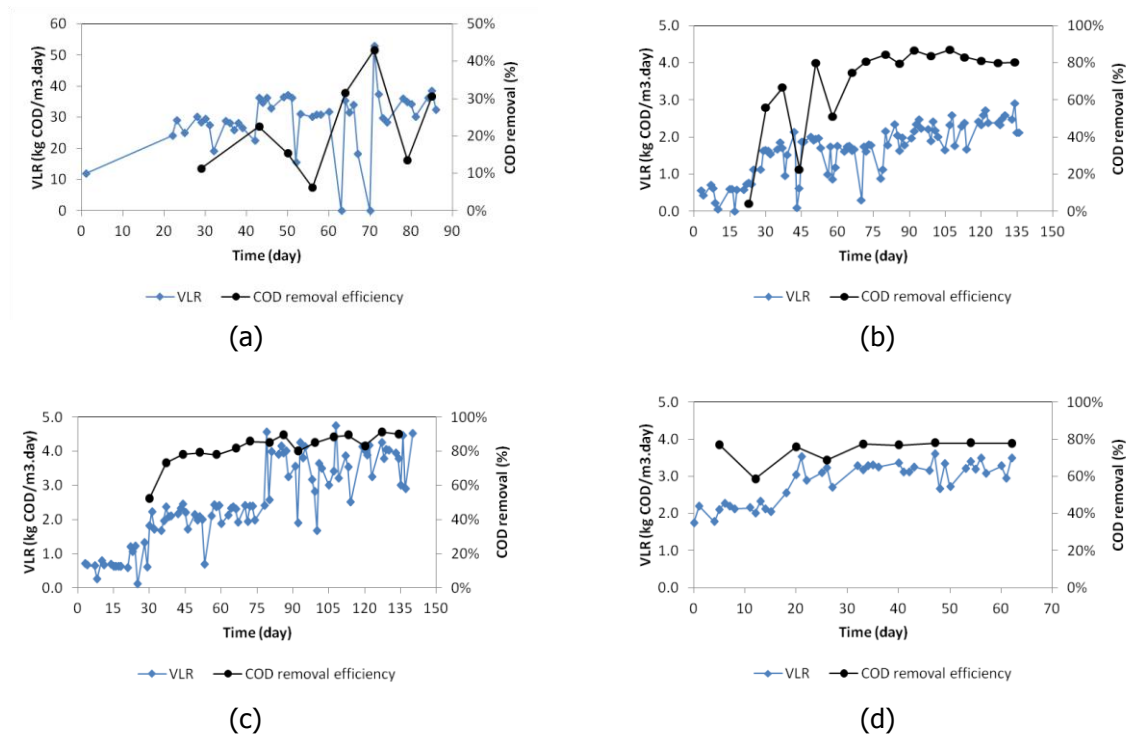
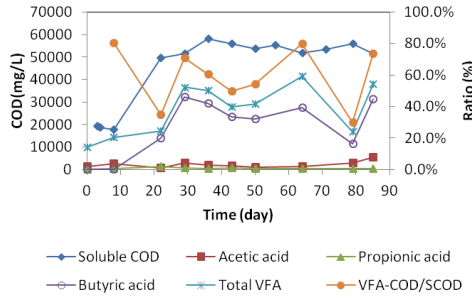


Figure 17 COD removal efficiencies for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

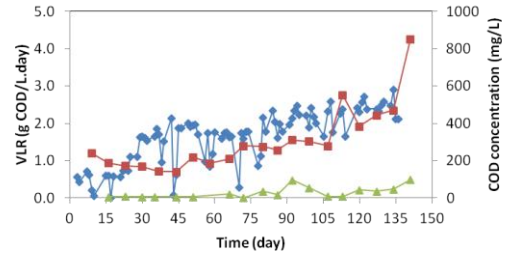
### 6.1.3. VFA concentrations

The VFA concentrations in the reactors are plotted in Figure 18. Butyric acid was the most dominant VFA in R-1, while propionic and acetic were quite low. This indicates that the lactose in raw whey was converted mostly to butyrate. This result is in accordance with the findings of P. Yang et al (2007) who measured the VFA composition of cheese processing wastewater at different F/M ratio and found that in all the different ratios the butyric acid was the main product of the anaerobic fermentation. The soluble COD concentrations were following the same trend with the VLR in R-2 and R-3 reactors. In the first phase of the study the VFA concentrations were quite low due to the low VLR. However when the VLR was increased at around 85<sup>th</sup> day, the VFA concentrations peaked and rapidly decreased.

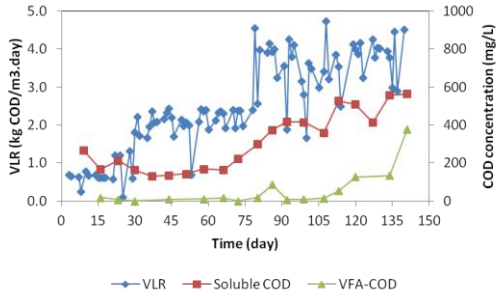
In R-2 and R-3, the difference between SCOD-VFA<sub>COD</sub> demonstrated an increasing trend over time. This increasing trend might be due to an accumulation of SMP in the reactors at higher F:M ratios. The same evaluation method of SMP was also used by Jeison et al., 2009.



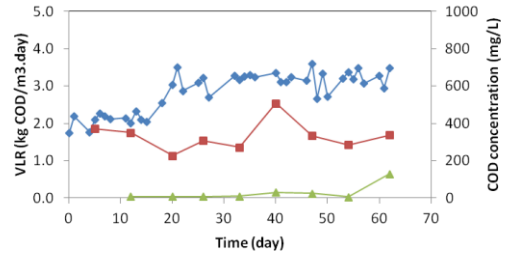
(a)



(b)



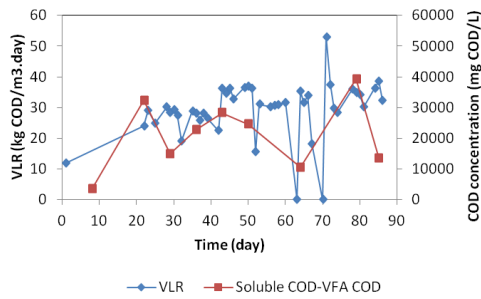
(c)



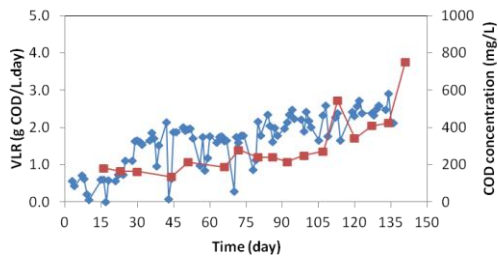
(d)

Figure 18 VFA COD and soluble COD concentrations for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

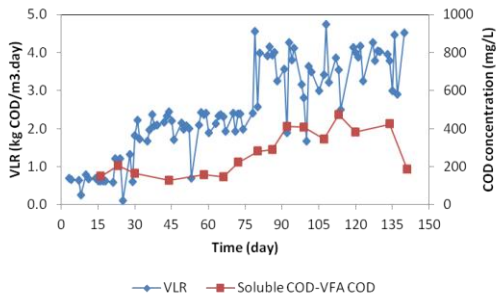
The difference between Soluble and VFA COD is plotted in the graph shown in Figure 19



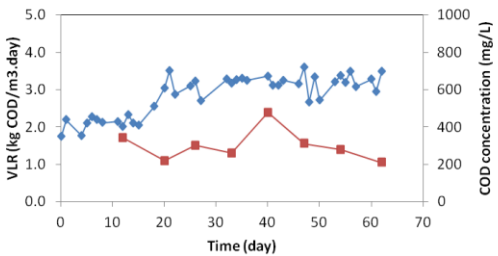
(a)



(b)



(c)



(d)

Figure 19 Soluble COD-VFA COD for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

### 6.1.4. Methane concentration

The methane production for reactors 2 and 3 is given to the Figure 20. For both Reactors it can be seen that the methane production follows the trend of the VLR. During the stable phase of the operation biogas production was 1.1 and 1.5 (Nm<sup>3</sup>/day) for Reactor 2 and 3 respectively. Specific methane production was calculated as the methane produced daily divided by the COD removed. The specific methane production showed a decreasing trend over time to both reactors. The Specific methanogenic production of R-3 had a smaller value during the stable phase of the operation compared to R-2. This result was not expected, since the biomass of R-3 consists of pure methanogenic biomass therefore with higher conversion capacities.

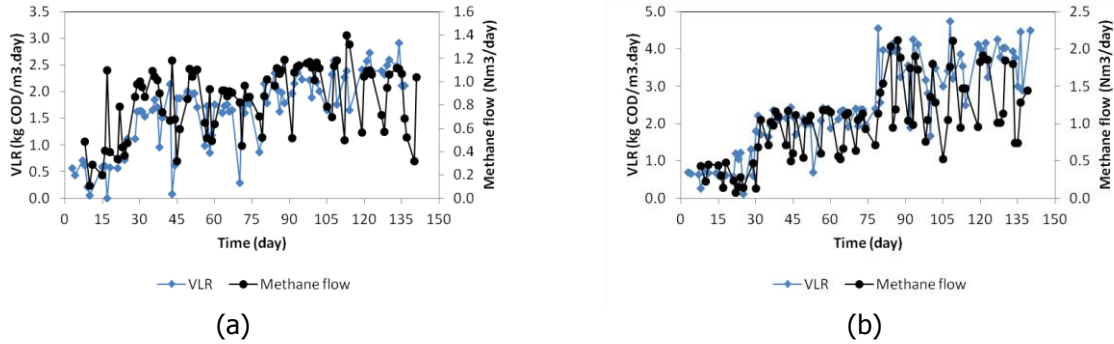


Figure 20 Methane Flow for reactors (a: R-2, b: R-3)

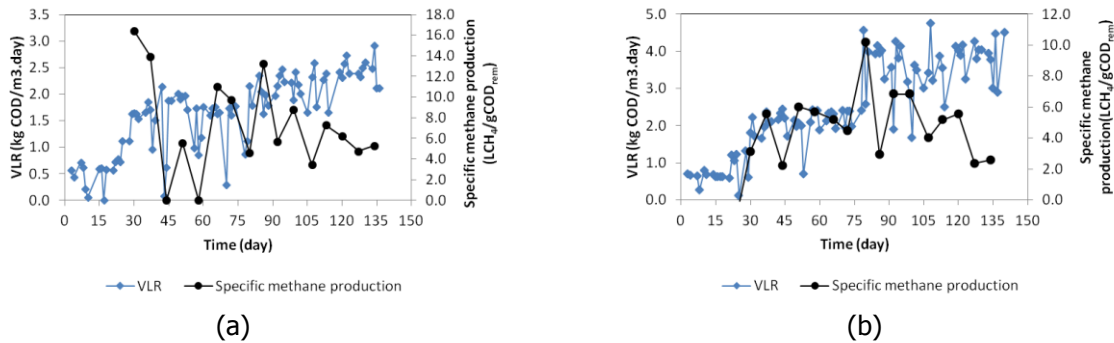


Figure 21 Specific methane production for reactors (a: R-2, b: R-3)

### 6.1.5. Specific Methanogenic activity

Figure 22 shows the activities of sludge on specific substrates such as acetate, propionate and butyrate in the reactors. In R-1, no significant SMA was measured which proves that there is no acetotrophic methanogens present. In R-2, there is an initial increase of all the activities at the 36<sup>th</sup> day of operation which may be due to the acclimatization of the inoculum sludge to the substrate. The inoculum sludge was kept for a long time in room temperature before startup of the reactors, so a part of its activity might be lost during storage. Under proper feeding and temperature conditions, the activity increased. Gradually, the activities dropped due to the dilution of the sludge with acidogenic biomass. The same behavior was observed in the single stage methanogenic R-4. In R-3 acetate activity after an initial decrease showed a constant value

until the end of the operation. Propionate activity showed a small decrease. The feed contained a minor amount of propionate; therefore, this might be due to the wash-out of propionate degraders during the long term operation. Butyrate activity was much higher compared to the other two activities. This fact is justified by the increase in butyrate concentration of the feed. A proliferation of this microbial population may be the reason for the boost of butyrate activity.

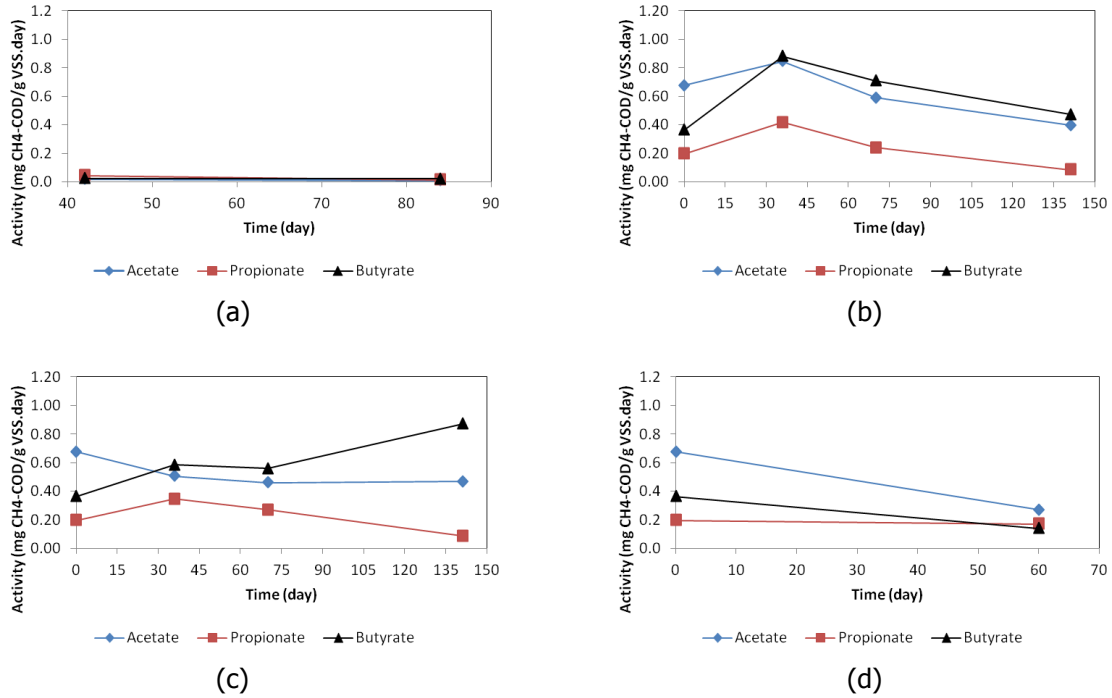


Figure 22 Specific Methanogenic Activity for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

Table 11 Specific methanogenic activity final values

Activity mgCH4- COD/gVSS day	R2	R3	R4
acetate	0.4	0.5	0.3
propionate	0.1	0.1	0.2
butyrate	0.5	0.8	0.2

### 6.1.6. Bacteria Yields

The bacteria yields were calculated in Reactors 2, 3 and 4. The average values during the last operation period are given in Table 12.

Table 12 Bacteria yields

R2	R3	R4
0.06±0.01	0.04±0.01	0.08±0.02

From these results, R-4 yield is 2.31 times higher than this of R-3 and 1.45 times higher than R-2. By comparing R-2 and R-3, it is observed that the yield is of R-2 is 1.59 times higher than R-3.

## 6.2. Filtration Performance

A series of parameters were measured for determining the filtration characteristics of the sludges. Standard filtration tests were executed under controlled conditions in order to monitor directly the filterability of the sludge, and the evolution of it with time, depending on the different operational and loading conditions.

### 6.2.1. Sludge Filtration Characterization

In order to investigate the effect of acidogens on sludge filtration characteristics supplementary indicators such as specific resistance to filtration (SRF) supernatant filterability and capillary suction time (CST), parameters were monitored throughout the study under standard conditions. Although these parameters do not provide direct information on the real membrane filtration process taking place in membrane bioreactors, they are valuable tools to compare the sludge filterability at different reactors and operating conditions.

Figure 23 shows the results of specific resistance to filtration test for the sludges from different reactors.

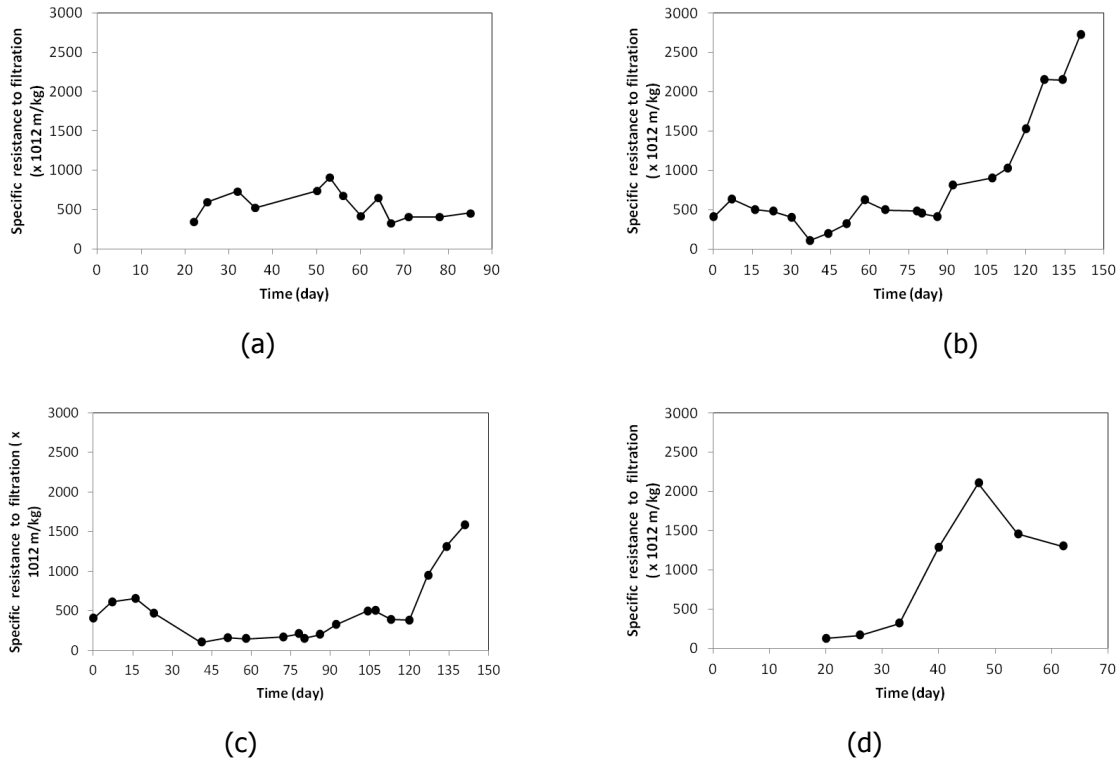


Figure 23 Specific resistance to filtration for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

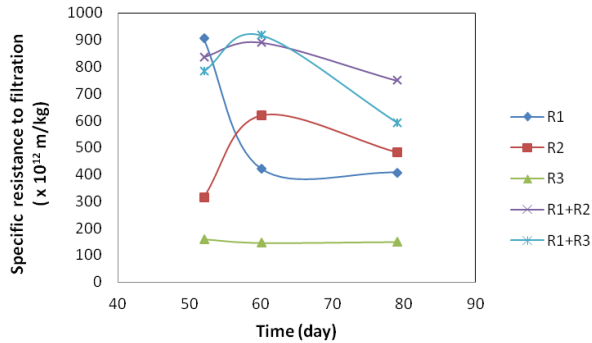
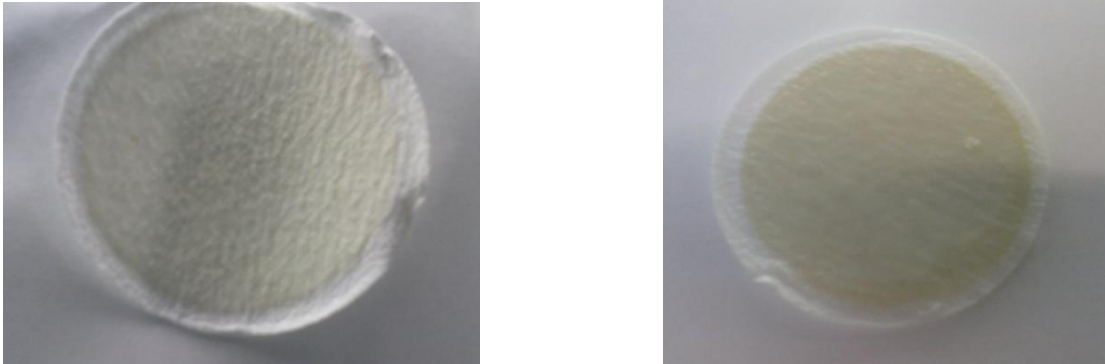


Figure 24 Comparative results for sludge and mixtures

The SRF of R-2 and R-3 showed a similar trend such as an initial increase from  $400 \times 10^{12} \text{m/kg}$  to a peak of maximum at  $600 \times 10^{12} \text{m/kg}$ , within the first 20 days of operation. This may be due to the particle size reduction of the inoculum sludge by the shear provided with the recirculation pump. Subsequently, there is an important decrease of SRF for both reactors until the 44<sup>th</sup> day. At the time period between day 60<sup>th</sup> and 90<sup>th</sup> days, the SRF was significantly higher in R-2 with an average value of  $490 \times 10^{12} \text{m/kg}$  while for R-3 it was only  $200 \times 10^{12} \text{m/kg}$ . Therefore, it can be concluded that the presence of acidogenic biomass had a significant effect on SRF parameter and impaired the filterability of the sludge. After the increase of the VLR at the 80<sup>th</sup> day, both R-2 and R-3 showed a remarkable increase of SRF which continued until the end of the operation period. This increase can be attributed to the increase of the F/M ratio. Liu et al. 2012 compared the cake formation of two AnMBRs operating one in high and the other in low F/M ratio. They found that the reactor with high F/M ratio showed higher concentrations of EPS and SMP and presented higher cake resistances. The increase of SRF ratio R-4, showed an initially lower increasing slope until the 33<sup>rd</sup> day and subsequently a very steep peak at 47<sup>th</sup> day ( $2105 \times 10^{12} \text{m/kg}$ ). By comparison of the data of R-4 and R-2 at similar F/M ratio of  $0.3 \text{kgCOD/kg (VSS.day)}$  the average SRF values were  $1620 \times 10^{12} \text{m/kg}$  and  $448 \times 10^{12} \text{m/kg}$  for R-4 and R-2, respectively. Therefore, it can be concluded that the single stage reactor (R-4) sludge had poorer filterability characteristics. This may be due to the difference in the growth phases of acidogenic biomass in R-2 and R-4 reactors. The acidogenic biomass in R-2 might be in the decay phase due to non-acidified substrate limitation. However in R-4 the acidifiers were at exponential growth phase due to the availability of non-acidified substrate. Hence, it is concluded that the presence of acidogenic biomass at the exponential growth phase had a stronger influence on sludge filterability. In order to examine the effect of living acidogens in R-2 an extra test was executed. Therefore, a mixture of R-1 with R-2 and R-3 were submitted to SRF test for the same period of operation and the results were compared with R-1, R-2 and R-3. It was observed, that the presence of acidogens led to a significant deterioration of the SRF of R-2 and R-3 while the pure acidogenic sludge of R-1 was lower leading to the conclusion that the coexistence of the two populations is worst than pure acidogens. Also, the sludge of R-3 that is completely lacking acidogenic biomass showed the best filterability characteristics than all the examined samples.

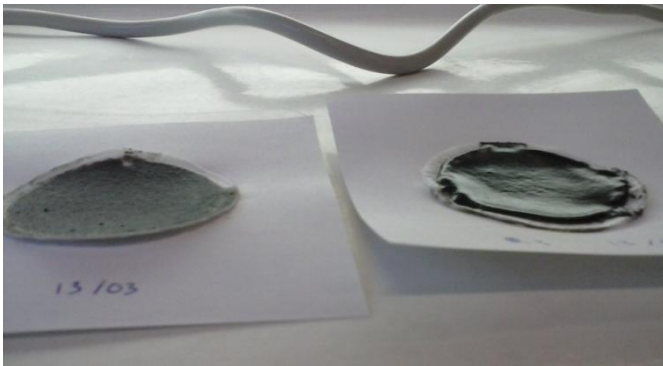
The physical appearance of the cake showed also a difference between the different reactors during the different operation periods (visual observation). During the initial phase of operation which was characterized by a high resistance to filtration, the cake layer that appeared was thin and compact (around day 50). At the second phase of the operation (around day 80), that the

resistance to filtration measured was lower, the cake layer formed on the filter surface was thicker and more watery than before. The two different phase sludges are shown in Figure 25.



*Figure 25 Reactor 1 cake layer appearance on day 50 and day 80 of operation respectively*

Figure 26 and Figure 27 show the appearance of cake layers of R-2 and R-3 on days 58 and 141. As it can be seen from Figure 23, the cake layer of R-3 seems significantly thicker and more porous compared to the one of R-2. On the other hand, at day 141 both cake layers seem to be similar.



*Figure 26 Reactor 2 (left) and 3 (right) cake layers on day 58 of operation*

At the end of the operation, both reactors have acquired a thinner and compacter form (Figure 27).



*Figure 27 Reactor 2 (left) and 3 (right) cake layers on day 141 of operation*

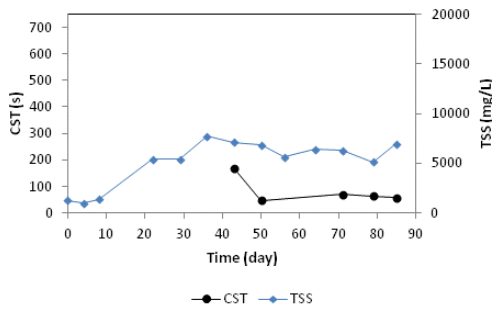
The cake layer of R-4 was impressively thick at the beginning of the operation period (day 20) and at the end of the operation (day 54) a much thinner and a lighter color cake layer occurred.

From visual observations, it is concluded that the thinner and more compact cake layer yields to a higher resistance to filtration.

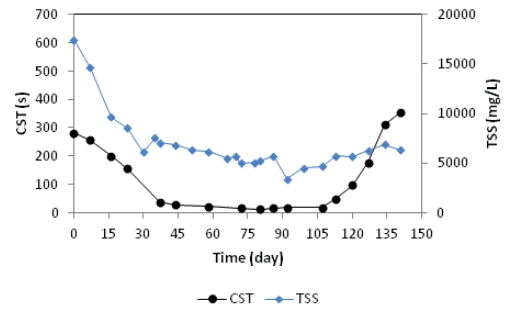


Figure 28 Reactor 4 cake layer appearance on day 20 and day 54 of operation respectively

CST is a common parameter used for sludge dewaterability (Huisman and Kesteren 1998). The evaluation of this parameter for the reactors is shown in Figure 29. It can be noted that CST of R-1 had a high value that coincides with the high sludge concentration and then it reached to a stable value (around 59 s) during the last period. On the other hand, the CST of R-2 and R-3 started from an initially high value of 280 s and presented a decreasing trend within the period of 40 days of operation until it reached to 22 s and 15 s for R-2 and R-3, respectively. After that period, the CST remained stable for both reactors until the day 105, when an increasing trend started to appear. This increasing slope can also be attributed to the increase of the F/M ratio. At the end of operation, the CST of R-3 was significantly higher than the one of Reactor 2 (68 s and 354 s, respectively). The CST of R-4 started with low values, but on the 47<sup>th</sup> day a substantial peak appeared (573s). The graphs presented similar trends with the SRF in all reactors.



(a)



(b)

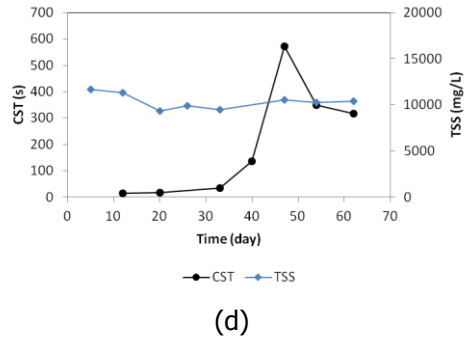
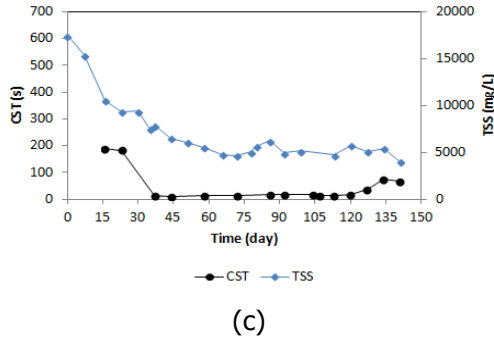


Figure 29 CST for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

The supernatant filterability provides information about the fouling propensity of soluble organic material such as SMP and colloidal particles in the sludge supernatant. Figure 30 presents the supernatant filterability measured in different reactors when the sludge was centrifuged at 1000 rpm. The results of R-2 and R-3 showed a decreasing trend that consists with the increase of SRF. The filterability decrease signifies that more foulants are ending in the sludge fraction, leading to an increase of the filterability resistance of the sludge parameters. This trend though is not very apparent, leading to the conclusion that this test is not sensitive enough to monitor the changes in supernatant filterability.

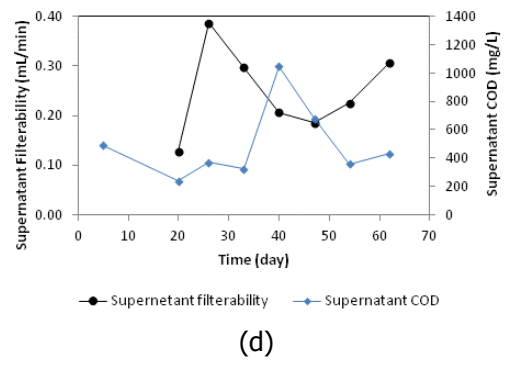
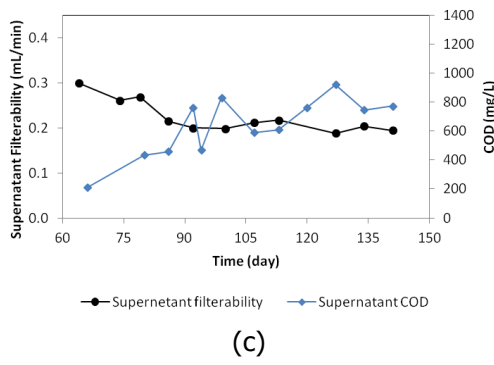
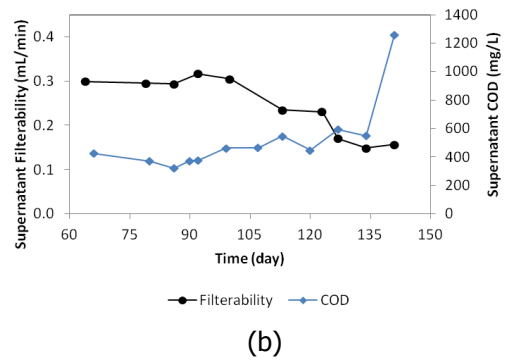
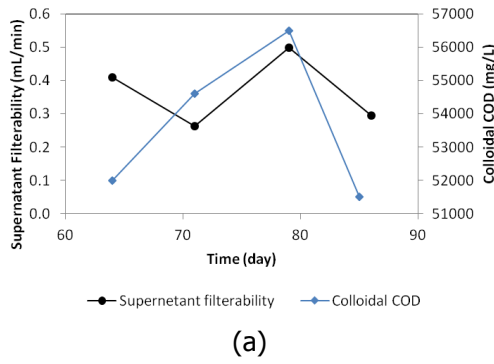


Figure 30 Supernatant filterability for reactors (a: R-1, b: R-2, c: R-3, d: R-4)

### 6.2.2. Critical Flux Measurement

Critical flux, firstly presented by Field et al., 1993, is the key to identify the appropriate operating flux for MBRs. In order to evaluate the effect of acidogenic biomass on the sludge filterability a series of critical flux measurements was conducted. For R-1, critical flux was measured with the sludge that settle at the bottom of SBR (high TSS concentration and larger floc size), the effluent sludge that is pumped out of R-1 (lower TSS concentration and poorer settleability). Also, CF was measured in two different conditions, one when applying shear with a recirculation pump and one without submitting the sludge to shear stress. CF was measured at the inoculum sludge with a high and low TSS concentration in order to match the TSS concentration of R-2 and R-3. At the end of operation, CF was measured for all reactors. The overall results are presented in Table 13 and Table 14 for cross flow velocities of 0.5 and 1 m/s, respectively.

Table 13 Critical flux results for cross flow velocity of 0.5m/s

Sludge	TSS (g/L)	CFV (m/s)	CF (L/(m <sup>2</sup> .h))	Day	Notes
Initial	14.6	0.5	20.4	0	
Initial	5.5	0.5	16.9	0	
R1	6.3	0.5	20.2	73	Without shear
R1	2.2	0.5	14.6	73	Non-settling sludge
R1	6.9	0.5	15.4	87	After shear
R2	5.7	0.5	17.3	70	Low conc. of acidifiers in the feed-low VLR
R3	4.7	0.5	15.2	71	Low VLR
R2	6.3	0.5	11.2	144	High conc. of acidifiers in the feed-Slightly higher VLR
R3	4.0	0.5	11.2	144	High VLR
R4	10.4	0.5	15.8	63	

Table 14 Critical flux results for cross flow velocity of 1m/s

Sludge	TSS (g/L)	CFV (m/s)	CF (L/(m <sup>2</sup> .h))	Day	Notes
Initial	14.6	1	29.2	0	
Initial	5.5	1	35.1	0	
R1	6.3	1	37.9	73	Without shear
R1	2.2	1	29.8	73	Non-settling sludge
R1	6.9	1	29.5	87	After shear
R2	5.7	1	24.4	70	Low conc. of acidifiers in the feed-low VLR
R3	4.7	1	25.8	71	Low VLR
R2	6.3	1	17.5	144	High conc. of acidifiers in the feed-Slightly higher VLR
R3	4.0	1	17.5	144	High VLR
R4	10.4	1	21.3	63	

From these results a series of conclusions can be drawn:

1. Increasing cross flow velocity led to higher critical fluxes in all the examined cases.
2. CF was higher at increased TSS concentrations for the inoculum at CFV of 0.5 m/s. However, at a higher CFV the result was inverse.
3. Reactor 1 (acidogenic sludge) when submitted to shear, led to smaller flocs and a substantial decrease of CF was observed. The decrease was 31% and 22% for CFVs of 0.5 and 1 m/s, respectively.
4. The CF of the non-settling biomass of R-1, that is discharged by the floating decanter and the sludge submitted to shear flow with the recirculation pump had insignificant difference (5.4 % and 1% for CFV of 0.5 and 1 respectively)
5. By comparing of the CF results of R-2 and R-3 before and after the increase of the loading rate and the F/M ratio, it was noticed that the CF decreased by 30% for both reactors.

When the cross flow velocity increased the values of the critical flux decreased. This behavior was consistent to all the examined sludges. Increase of CFV led to a more turbulent flow regime on the area of the membrane surface preventing particle deposition on it. However, Le Clech et al. 2006 observed the opposite behavior. Specifically, he stated that the increase of the CFV tends to wipe out larger particles from the deposits, conducting to cake comprised of smaller particle size, therefore more compact and less permeable.

The highest the TSS concentration the lower the CF was, when the measurements were conducted for low CFV. This fact agrees with the I-Clech et al., 2006 who mention that an increase of the TSS concentration has a negative impact on the performance of a membrane reactor. However, the same trend was not observed when measuring the CF at higher CFV, indicating that the hydrodynamic environment is of primary importance compared with the increase of the TSS concentration.

The decrease of particle size of the flocs when submitting the same sludge to shear stress led to a decrease of attainable fluxes. This result is in accordance with the statement of (Wang et al, 2007), who supported that the smaller the PS is, the less the particles are affected by back transport phenomena, and the more compact the cake layer is.

The results of the CF measurements at 0.5 and 1 m/s CFVs were similar for all reactors. This contradicts with the hypothesis that acidogenic sludge is responsible for low fluxes obtained in AnMBRs. However, the CF experiments are in a short time scale and the real fouling behavior and attainable flux on long term operation can be significantly different.

Finally, impairment of sludge filterability with the increase of the F/M ratio that was observed by the SRF measurements, agreed with the results of the CF. F/M ratio was identified to positively impact to the rate of membrane fouling (Trussell et al., 2006). Thus, it can be deduced that the F/M ratio is one of the most important factors that affect sludge filterability. This may be due to

the release of growth associated microbial products under high loaded conditions. However, at low F:M ratios, the decay products from biomass lysis can also inversely effect sludge filterability.

### 6.2.3. Particle size distribution

The results of PSD of the sludges are presented in Figure 31. The mean and median particle size of the sludges is shown in Table 15.

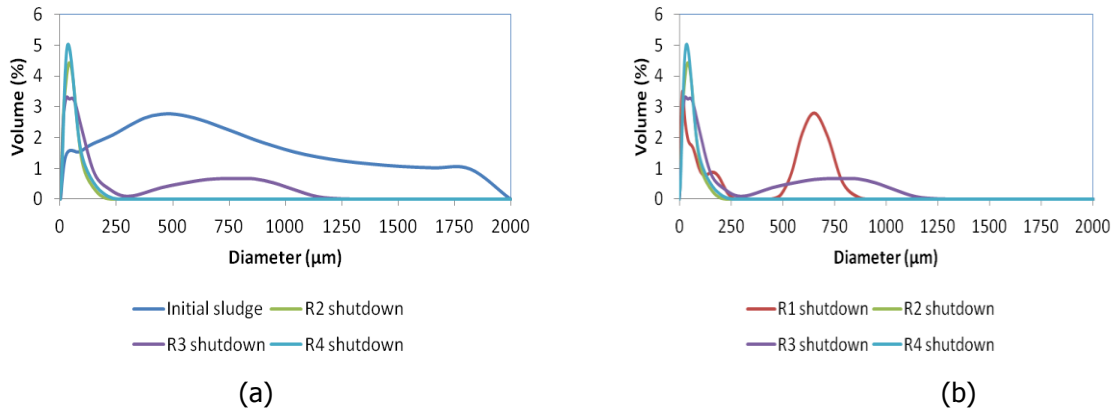


Figure 31 Particle size distribution (a: inoculum and R-2,3,4, b: R-1, R-2, R-3, R-4)

Table 15 Particle size distribution of 4 reactors sludges, inoculum and white solids fraction

Reactor	Date	Day	Mean(µm)	d10(µm)	Median-d50(µm)	d90(µm)
<b>R-2 and R-3</b>		Initial sludge	362	18	202	958
<b>R-1</b>	11/04/2013	Shut-down	91	4	21	198
<b>R-2</b>	06/06/2013	Shut-down	37	7	30	76
<b>R-3</b>	06/06/2013	Shut-down	88	9	37	144
<b>R-4</b>	10/06/2013	Shut-down	41	10	33	81
<b>White solids</b>	26/11/2013		6	2	4	11

The inoculum sludge showed the higher particle size diameter, which was expected since it was acquired from crushed granular sludge. The median particle size in R-3 was significantly larger than R-2 and R-4 reactor sludge which had acidogenic biomass. It can be inferred from this observation that apparently the presence of acidogenic biomass (R-2 and R-4) either in single stage or in separate stages leads to smaller flocs compared to methanogenic biomass. In R-1 pure acidogenic biomass was cultivated, but the average particle size of the sludge was the largest of all the reactors. This observation contradicts with the hypothesis that acidogens form single cell flocs. From a visual observation of the sludge of R-1, large flocs could be identified, and the sludge showed a good settling characteristics. Therefore, it can be deduced that acidogenic biomass do not necessarily form small flocs but this is depended on the hydrodynamic environment of the reactor. In this study R-1 was operated as an SBR, thus only the good settling flocs were kept inside the reactor while the small flocs with poor settling properties would be discharged in the effluent. The white solid fraction that appeared on the surface of the

centrifuge tubes of R-4 and R-3 showed a very small particle size distribution. Jeison et al., 2007 indicated that this fraction defined the filtration characteristics of the sludge and reported that was mainly acidogenic biomass. Gao et al., 2010 identified OP11 species in the whitish material by microbial analysis.

#### 6.2.4. Viscosity

An additional parameter that was measured was viscosity. The results that stem from this measurement for the three reactors showed negligible differences. The values are given in Table 16.

*Table 16 Viscosity values for 4 Reactors*

	<b>R1</b>	<b>R2</b>	<b>R3</b>	<b>R4</b>
<b>Viscosity (mPa.s)</b>	6.2	6.0	6.5	6.7
<b>TSS (mg/L)</b>	5094	5744	6234	10379

#### 6.2.5. Relative hydrophobicity

The membrane surface characteristics were found to play a role in the attainable fluxes. Particularly, hydrophobicity is influencing the flux especially at the beginning of filtration that the membrane is not covered with a thick cake layer. After that first phase, the cake layer characteristics are the ones that affect the fluxes Fang and Shi, 2005.

Fang and Shi, 2005 indicated that membranes of greater hydrophilicity tend to be more vulnerable to deposition of foulants of hydrophilic nature. The EPS material is found to have hydrophilic nature so high percentages of EPS are attaching to the membrane thus decreasing the attainable fluxes.

*Table 17 Relative hydrophobicity values*

<b>Reactor 1</b>	<b>Reactor 2</b>	<b>Reactor 3</b>	<b>Reactor 4</b>
3%	22%	57%	32%

The hydrophobicity of the different sludges that were examined, indicate that the acidogenic sludge of the R-1 is strongly hydrophilic and the methanogenic sludge of R-3 is highly hydrophobic. The sludge of R-2 and R-4 reactors which was a mixture of acidogenic and methanogenic biomass showed similar hydrophobicities. The sludge of R-4 showed a higher relative hydrophobicity compared to the acidogenic biomass (R-1) and the sludge of R-2 but is less hydrophobic than the methanogenic sludge of R-3. According to the observations of Fang and Shi 2005, the more hydrophilic the sludge is, when interacting with a hydrophilic membrane (as the one that was used here for the critical flux measurement), result in faster membrane fouling and lower attainable fluxes. This result could not be validated by short term critical flux measurement. R-2 and R-3 showed similar critical fluxes while R-1 and R-4 had higher values.

The second statement of Fang and Shi 2005, that the EPS material is hydrophilic. Therefore, the sludge that contains that higher percentage of EPS is expected to have a more hydrophilic nature. This is also not proved by the combination of the results of EPS and relative hydrophobicity analysis. Since, sludge of R-1 has the highest percentage of EPS and is more hydrophilic as expected. This relationship between hydrophobicity and EPS was not verified by the hydrophobicity/EPS analysis of the other sludges.

Reactor	Relative Hydrophobicity (%)	EPS (mg/l) at stable phase
1	3	9507±150
2	22	751±80
3	57	737±41
4	32	950±8.62

### 6.2.6. EPS & SMP

The results of EPS were measured throughout the Reactor 2 and 3 operation and the results are shown in Figure 32, Figure 33 respectively.

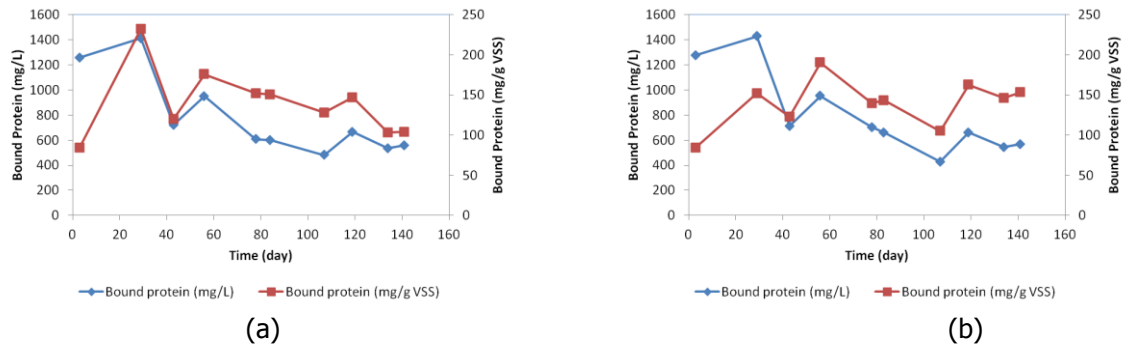


Figure 32 Bound protein concentrations (a: R-2 b: R-3)

For R-2 a decreasing trend is initially observed but it is eventually stabilized at the value of 600mg/l. Since the bound proteins and carbohydrate are on the bacteria membrane surface, these results should be normalized with the bacteria growth. The normalized result for R-2 at the end of the operation is 100mg/g VSS for bound protein. The same results for R-3 are 600mg/l and 150mg/g VSS. This observation shows that the methanogenic sludge contains a relatively high concentration of protein EPS even higher than this of the sludge containing both methanogens and acidogens of R-2. The carbohydrate concentration for R-2 was approximately stable during the operation with an intense peak around day 120. That date a VFA increased was also observed in R-2 concluding that there was probably a disturbance in the reactor operation at that time leading to higher EPS excreting. For R-3 carbohydrate concentration showed an increasing trend during the whole operation period with a steep decrease day 120.

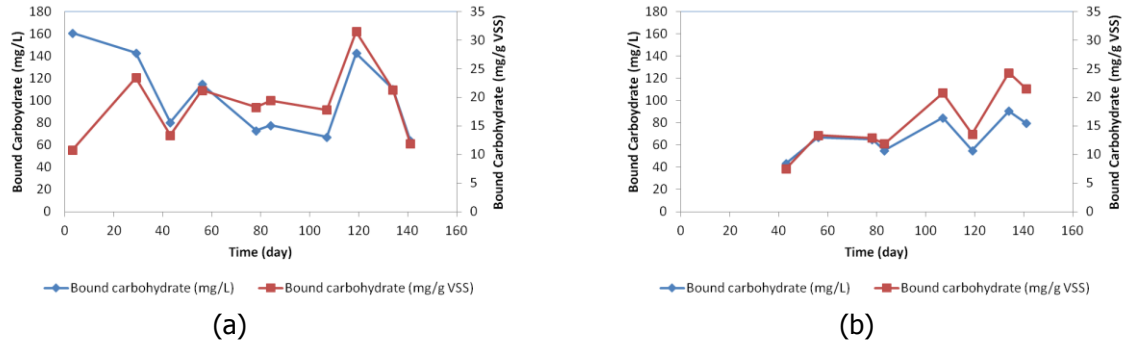


Figure 33 Bound carbohydrate concentrations (a: R-2 b: R-3)

The results of the SMP both proteins and carbohydrate for R-2 and R-3 respectively are shown in Figure 34.

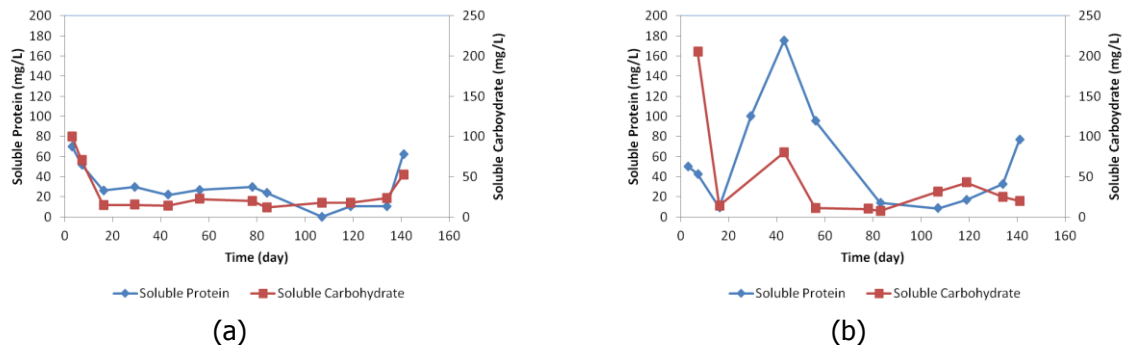


Figure 34 Soluble protein and carbohydrate concentrations (a: R-2 b: R-3)

The trend of soluble protein and carbohydrate concentrations showed an initial steep decrease for both reactors. This is due to the dilution and acclimatization of the inoculum sludge. The concentrations remained stable for most of the operation period until day 120 that an increasing trend reappeared in both parameters for R-2. This result is in accordance with what was noticed for the filterability parameters. It can be concluded that SMP concentration increase is boosted from the increase of the F/M ratio and is related with poorer filterability characteristics of the sludge. For R-3, there was a big concentration peak that might have been caused by an increase of the VLR that would provoke a shock effect and afterwards the peak was absorbed due to acclimatization of biomass at the new operating environment. Even though the same increase of the VLR happened in R-2 this peak was not observed. A possible explanation for that would be that R-2 contains more particulate COD and thus needs more time to hydrolyze. This time eliminates the shock effect that was noticed in R-3. Subsequently, that peak was eliminated and the concentrations remained at a low level until the day 120. After that day, the soluble proteins concentration increased again (the same trend that was noticed in the filterability parameters and F/M ratio). On the contrary, soluble carbohydrate showed a small decrease after day 120. By combining the results of the two reactors, it can be concluded that the soluble protein concentration is related to the F/M ratio in the reactors and is negatively affecting the filterability of the sludge.

### 6.2.7. Summary of filtration performance

All filtration performance and sludge characteristic related with filterability are summarized in Table 18 at the last operational phase of each reactor.

The comparative filtration performances of the reactors were presented in Table 18.

Table 18 Summary of filtration performance and sludge filterability in Reactors during the last operational phase

Parameter	Unit	R-1	R-2	R-3	R-4
<b>Critical flux (CFV=1m/s)</b>	L/(m <sup>2</sup> .h)	30	18	18	21
<b>CST*</b>	s	59	354	68	300
<b>SRF*</b>	10 <sup>12</sup> m/kg	500	2500	1500	1500
<b>Hydrophobicity</b>	%	3	22	57	32
<b>Mean particle size</b>	µm	21	30	37	33
<b>Viscosity</b>	mPa.s	6.2	6.0	6.5	6.7

\*these results correspond to the final period of each reactor

### 6.2.8. Sludge Appearance

During long term operation, the color of the sludge in reactors gradually changed. Specifically, R-2 and R-4 had a blackish sludge while R-3 started to change color until it became ash-grey after the increase of VLR at 90<sup>th</sup> day. This color difference is explained by the lack of acidogenic biomass in Reactor 3 that is increasing the precipitation. R-1 sludge that was composed of acidogenic biomass had a milky (white-yellow) color. The colors of the sludge from different reactors are shown in Figure 35. The effect of substrates with different acidification degrees on the color of the sludge was examined by J. Thaveesri et al., 1995 on granular sludges. In this study, different UASB reactors were fed with different degree of acidification substrates. In one of the reactors the total COD concentration of the influent was composed of VFA. At this reactor black and white granules reactors were formed, with both almost 0 (0.1meqH<sup>+</sup>/g VSS.h) SAA and equally high methanogenic activity (0.52 LCH<sub>4</sub>/g VSS.day). At the other two Reactors that were fed with a mixture of sucrose with VFA contained black white and grey granules. The grey granules were the only ones that indicated a high acidogenic activity. At a suspended growth system, the grey color of the bulk sludge that was observed can be due to the presence of black and white flocs as it was indicated in Thaveesri study. R-4 color also showed a slightly color change as well and it became a bit more light.



Figure 35 Sludge colors (starting from left to right: R-4, R-2, R-3)

A further visual difference was the occurrence of a whitish layer on top of the sludge pellets when R-3 and R-4 sludge was centrifuged (Figure 36). This fraction seemed to be similar to Gao et al. 2010.



*Figure 36 Centrifuged sludge of R-4*

Furthermore, the observation that acidogens form single cell flocs reported by Jeison et al., 2007 was contradictory to the findings of this study in R-1. Thus, pure acidogenic biomass, cultivated in R-1, formed visible flocs with good settling properties. However, the reason for that may be also due to SBR type of reactor operation.

## **7. Discussion**

In this section, an extended discussion on the overall process performance and filterability characteristics will take place by comparing the 4 different reactors.

The acetogenotrophic and syntrophic activities of sludge in reactors fed with non-acidified substrates are expected to decrease due to the proliferation of acidogenic bacteria. Thus, the fraction of methanogens in sludge decreases which inevitable leads to less acetate to methane conversion capacity. When the observed biomass yield was compared, R-4 which was fed with no acidified whey showed the highest yield. This is indeed was expected due to the higher bacterial yields of acidogenic biomass compared to methanogenic archea. This, in combination with the lowest methanogenic activity observed in R-4 reactor, signifies the proliferation of acidogenic bacteria fed with non-acidified substrate such as lactose present in whey. The biomass yield of R-2 was slightly higher compared to R-3. R-2 reactor was fed with acidified whey and a substantial amount of acidogenic bacteria. In this reactor due to the less availability of non-acidified substrate, the acidogenic bacteria may undergo a decay phase, being consumed by methanogens as slowly degradable particulate substrate, or may be washed out from the reactor. Therefore, it is not clear that the difference observed in the sludge yields of R-2 and R-3 is due the growth of acidogens or accumulation of slowly degradable particulate substrate. Moreover, the acetotrophic activities of both reactors were comparable and not significantly different which may be due limited accumulation of acidogenic bacteria in R-2. In the study of A.P Alphenaar, 1994 that was conducted on UASB reactors fed with acidogenic biomass, a percentage of 95% of the suspended

acidogens was converted to methane in a period of 3 days. R-3 had the lowest bacteria yield but the highest acetate and butyrate activities, and the highest COD removal efficiency. This indicates that the bacteria population of this reactor indeed consists of mainly methanogenic archaea and syntrophic biomass.

When the sludge filterabilities were compared, it is noticed that R-4 showed poorer filterability characteristics in terms of CST and SRF than R-2 and 3 at the same loading and operational conditions (and subsequently at the same F/M ratio). By comparison of the SRF data of R-2 and R-4 at similar F/M ratio of 0.3kgCOD/kg (VSS.day) the average values were  $1620 \times 10^{12}$  m/kg and  $448 \times 10^{12}$  m/kg for R-2 and R-4, respectively. Thus, it can be concluded that a two stage operation leads to a better sludge filterability. It can be proposed here, that a two stage membrane reactor with a pre-acidification tank can increase the filterability of the second step and increase the attainable fluxes. However, a cost and benefit analysis for the extra reactor for two stage system and increased sludge filterability for the membrane operation has to be made in order to propose it as a feasible option. By comparing R-2 and R-3, we noticed that the SRF for R-2 under the same loading rate and F/M was significantly higher. Thus, the acidification degree of the feed is playing a role in the filterability characteristics of the sludge. A fully acidified wastewater can generate sludge with improved filterability characteristics. An additional conclusion that can be drawn is that when the F/M ratio is increased, then the filterability parameters showed a very steep increasing trend for both of the reactors. The increase of the F/M ratio may lead to an increase of supernatant COD, provoking a higher accumulation of SMP in R-2 and R-3. From the results of soluble protein and polysaccharide measurements in R-2 and R-3, it was observed that there is an increase in the soluble protein with the increase of F/M ratio. On the other hand, soluble polysaccharides increased only in R-2 with the increase of the F/M ratio. Capillary suction time analysis verified the previous conclusion that the increase of F/M ratio, and as a result the increase of SMP accumulation, will reduce the sludge filterability. The particle size distribution analysis of the different sludges showed that R-3 sludge had the largest particle median particle size. This observation is in accordance with the general conclusion that R-3 had better filterability, (the higher the particle sizes the more porous and permeable cake layer and eventually lesser filtration resistance). When R-2 and R-4 were compared, the results had an insignificant difference (37 $\mu$ m and 41 $\mu$ m respectively). Finally, R-1 that is composed of acidogenic biomass and not any presence of methanogens showed higher PS. This may be due to the different operation regime of R-1 (SBR) that caused a selection of the bigger, well settleable flocs during the settling phase.

When the sludges from different reactors were submitted to centrifugation, on the surface of R-4 sludge pellets a white slurry biomass fraction was observed. This was attributed to different microbial populations in different studies from the literature (D. Jeison et al., 2009, Torres et al., 2011, Cohen et al., 1979). PS analysis was conducted at this sludge fraction and the mean diameter of the particles in this fraction was significantly lower than the bulk sludge. In several studies this fraction was attributed to acidogens which grow as single cells. However, unexpectedly in our study a similar sludge fraction was also observed in R-3 sludge which was normally expected to be composed of methanogenic archaea and syntrophic biomass. If the initial assumption of this white fraction as acidogenic biomass, it should not appear in R-3 as well. Of course in order to be completely sure about the fact that this white sludge fraction is of the same origin, and is not just a visual coincidence, further analysis should take place with the help of

microbial tools. Moreover, the different sludges also showed a difference in the relative hydrophobicity. Particularly, R-1 sludge was mainly hydrophilic. On the contrary, the sludge of R-3 that is mainly methanogenic sludge was highly hydrophobic. Sludges of R-2 and R-4 were both less hydrophobic than R-3. It can be concluded that the presence of acidogens led to a more hydrophilic sludge.

Overall, the acidogenic biomass did affect the filterability characteristics of the bulk liquid. This effect though was minimal compared to the effect of the SMP accumulation due to the increase of the F/M ratio. The critical flux measurements that were conducted did not show any significant difference between the different sludges. The effect of the presence of acidogens was not verified by a decline in the critical flux values. This can be attributed to the short term interaction of the sludge with the membrane surface during the critical flux tests. On the other hand, the increase of the F/M ratio affected so intensely the filterability of R-2 and R-3 and the CF decrease at long term operation was notable.

## **8. Conclusions**

The results of the standard filtration tests such as SRF and CST showed that there was an impairment of the filterability of the sludge when acidogenic biomass was present. Moreover, the sludge from single stage reactor (R-4) was worse than the sludge from R-2 reactor which was operated as the methanogenic stage of a two-staged reactor configuration. Overall, the R-3 reactor fed with only VFA (without the acidifiers) had the best filterability characteristics. However, these measurements were conducted at short time scale and the real filtration dynamics in an AnMBR can be significantly different from the test conditions. Moreover, CF measurements, conducted under cross-flow membrane operation mode, showed that the presence of acidogenic biomass in the sludge did not affect the CF results significantly. However, it should be noted that the real fouling behavior and attainable flux on long term operation can be explicitly different than CF experiments.

Finally, it should be remarked that the increase of the F/M ratio had a substantial impact on the deterioration of filterability characteristics of the anaerobic sludge.

## **9. Recommendations**

In order to gain a better understanding on the effect of the presence of acidogenic biomass on filterability, its effect on the long term cake formation and fouling on a real AnMBR setup should be investigated. The consolidation of the cake layer that was observed by Jeison and van Lier, et al., 2007 is considered to be an effect that is aggravated with time. Thus, a long term analysis is required in order to investigate this phenomenon.

Identification of the microbial populations cultivated in different reactors and sludge fractions, i.e. supernatant and sludge pellet, would help to solve the riddle about the effect of acidogenic biomass on sludge filterability. Therefore, some microbial tools should be applied to define

microbial species in the sludge supernatant which seems to be the most important fraction determining the overall filterability of the sludge.

Finally, it was observed that the SBR environment of R-1 strongly affected the floc formation, and thus the results. If the whole experimental procedure was repeated, a new idea would be used for the increase of the VFA concentration rather than changing the hydrodynamic regime. In order to have comparable filterability results between R-1 and the other Reactors, the same exactly hydrodynamic regime should have been applied.

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