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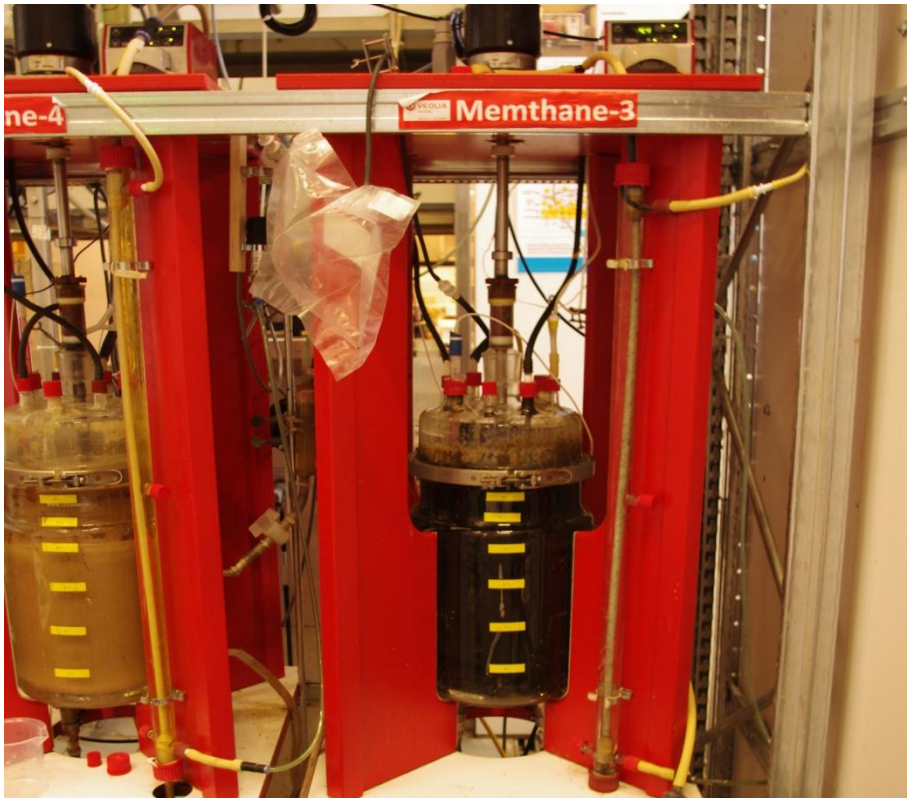

Biological and Filtration Performance Research on Cheese Whey Treatment by Lab-scale Anaerobic Membrane Reactor (AnMBR)

Master of Science Thesis in the Master's Program Environmental Measurement and Assessment

Xiaofei Wang

Department of Civil and Environmental Engineering

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Abstract

Nowadays, with the development of dairy industry, the production of cheese whey has a dramatically increase as well. Even though cheese whey is the raw material of various products, small or medium cheese manufactures cannot afford the high cost of the valorization technologies. It is necessary to find a proper way to treat whey efficiently. For this kind of easily degradable wastewater with high organic load, anaerobic treatment is the optimum method.

This thesis investigated the possibility of a new method, anaerobic membrane bioreactor (AnMBR), to treat cheese whey through a lab-scale reactor. For economical concern, digested municipal sludge was used as the inoculums. The highest VLR applied in this study was 4.5 kg COD/m^3·day with a maximal flux at 18 LMH. And the average COD removal efficiency was 96%. The poor methanogenic activity of municipal sludge seemed to limit the biological performance. The addition of crushed granular sludge was proven not to be efficient in the activity improvements. The further experiment showed that nitrogen was the limiting element for biomass growth. Inorganic precipitation was the crucial cause of membrane fouling. The cleaning process with stronger acid or longer soaking time should be applied to improve the cleaning efficiency.

Key words: whey, AnMBR, VLR, COD, flux, filtration, fouling

Content

1. Introduction

Whey, a by-product of cheese-making process, is the liquid remaining after the precipitation and removal of fat and casein (Siso 1996). According to the investigation in 2008, over 180 billion kilograms liquid whey was produced globally,about 70% of which was produced in Europe and US (Affertsholt 2009). Figure 1 shows the production process of semi-hard cheeses, which could represent almost all types of cheese manufacturing. Whey basically represents 85–95% of milk volume and 55% of milk nutrients (Siso 1996). About 93-94% of cheese whey is water. The major nutrients in whey are lactose (4.5-6.0%), soluble proteins (0.6-1.1%), minerals (0.8-1.0%), lactic acid (0.05-0.9%) and fats (0.06-0.5%)(Prazeres, Carvalho et al. 2012). More than 50% of whey salts are NaCl and KCl and the rest are calcium salts (mainly phosphate) (Siso 1996). The composition of whey varies depending on the different cheese milk. Gouda cheese whey is an example to represent the nutrients composition in most cheese whey Table 1.

According to the processing techniques of casein removal from liquid milk, whey could be separated into two types, sweet whey and acid whey. Sweet whey is produced at approximately pH 6.5 based on rennet-induced coagulation of casein, and almost all the whey products available on the market are of this type. The process to produce acid whey relies on fermentation or addition of organic or mineral acids to coagulate the casein at pH <5. Fresh cheese and most industrial casein are of this type(Kennedy, Panesar et al. 2007). The typical compositions of these two types of whey are shown inTable 2. Compared with sweet whey, acid whey generally has lower protein content and higher salinity.

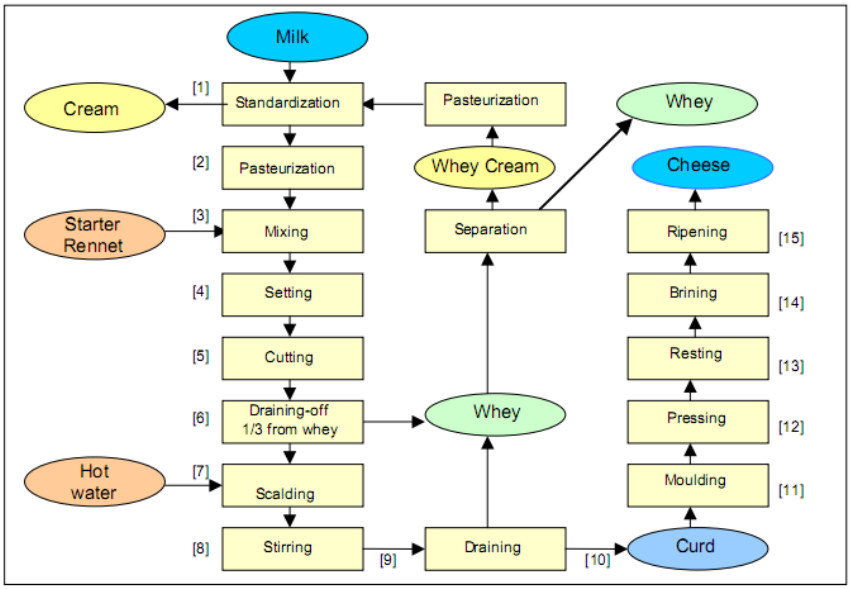


Figure 1 Flow sheet for the manufacture of semi-hard cheese (Wit 2001)

Table 1 Approximate composition of Gouda cheese whey (Wit 2001)

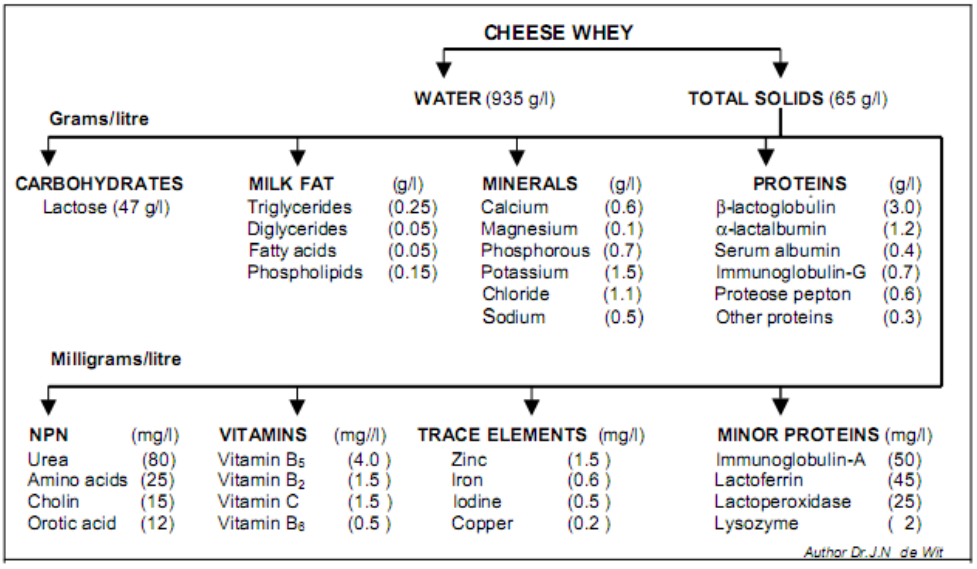


Table 2 Typical composition of sweet and acid whey(Kennedy, Panesar et al. 2007)

Components	Sweet whey (g/l)	Acid whey (g/l)
Total solids	63–70	63–70
Lactose	46–52	44–46
Protein	6–10	6–8
Calcium	0.4–0.6	1.2–1.6
Phosphate	1–3	2–4.5
Lactate	2	6.4
Chloride	1.1	1.1

In the past, the most common way to treat cheese whey was to discharge it directly to receiving waters or to dispose it by land application without any pre-treatment. However, these methods threatened the environment since the cheese whey could cause an excess of oxygen consumption, impermeabilization, eutrophication, toxicity, etc.(Prazeres, Carvalho et al. 2012). Then, for environmental and recycle concern, cheese whey was used as the liquid base of animal feed. However, the connection of cheese factories and pig farms broke down due to some unknown reasons (Malaspina, Cellamare et al. 1996). Another alternative to reduce the impact of whey was to treat it together with domestic wastewater. But it failed since the unique composition of whey impaired the efficiency and stability of microorganisms of municipal wastewater treatment (Prazeres, Carvalho et al. 2012).

It is necessary to find an efficient way for the treatment of whey. Today, there are basically three main stream options (Prazeres, Carvalho et al. 2012). The first one relies on valorization technologies. It is possible to recover many voluble products from whey through valorization, such as condensed or powdered whey, whey protein concentrate, lactose and its derivatives and single cell protein, however, many small to medium scale factories do not have the economical power or market dimension to apply these technologies (Malaspina, Cellamare et al. 1996; Siso 1996; Prazeres, Carvalho et al. 2012). The second choice is based on the application of physicochemical treatments,

such as thermal precipitation, electrochemical coagulation, acid precipitation, membrane separation, protein precipitation with coagulant/flocculant agents, etc. By these methods, contaminant load such as organic matter, suspended solids and turbidity can be reduced. Valuable products like proteins and lactose can also be recovered (Siso 1996; Souza, Bergamasco et al. 2010; Prazeres, Carvalho et al. 2012). The third option is the application of biological treatment methods. Some valorization technologies are also based on biological methods, for instance, lactose hydrolysis (Kosaric and Asher 1985), fermentation to ethanol (Sansonetti, Curcio et al. 2009) and anaerobic digestion (Prazeres, Carvalho et al. 2012).

When considered as a wastewater source, the cheese whey is a very concentrated effluent characterized by high COD (60–80 g/L) and BOD (30–50 g/L), low pH (4 – 5) and bicarbonate alkalinity (~50 meq l^{-1}). The whey is highly biodegradable (~99%) and the main portion of the COD can be attributed to lactose content inherited from the milk (Malaspina, Cellamare et al. 1996; Siso 1996). Hence, biological treatments are the best options for this kind of substrate(Prazeres, Carvalho et al. 2012). Aerobic processes, such as activated sludge, trickling filterswere proven to be not very effective since the organic load of whey was too high. Even after dilution, there were still other difficulties like extensive energy requirement, oxygen transfer limitations, and large amount of sludge production(Prazeres, Carvalho et al. 2012). In comparison to aerobic treatment, anaerobic processes are more effective for easily biodegradable wastewater with high organic load. Less amount of sludge is produced and no energy is required for oxygen supply. In addition, the products of anaerobic treatment, methane rich biogas, can also be applied as an energy source(Saddoud, Hassairi et al. 2007; Chen, Cheng et al. 2008).

Prazeres (2012)made a review of the main parameters of anaerobic digestion processes applied to whey (Table 3). Among these processes, UASB and UAF reactors are the most common used reactor types. They provided higher than 95% COD removal efficiency and comparatively high methane yield (>0.28 m^3kg^{-1}COD). Anaerobic upflow fixed film loop reactor (AUFFLR), anaerobic semicontinuous digester with flocculant addition (ASDFA), downflow-upflow hybrid reactor (DUHR) and two-stage mixed anaerobic membrane digester (TSMAMD) could also achieve high COD removal efficiency (>95%) with raw cheese whey as the influent. On the contrary, anaerobic upflow fixed film reactor (AUFFR), two-stage unmixed anaerobic digester(TSUAD), anaerobic rotating biological contact reactor (ARBC), and contact process (CP)did notshow acceptable efficiency even with the influent of diluted cheese whey. Despite anaerobic processes were proven with the possibility to get satisfying treatment results, some authors stated that the high COD concentration, low bicarbonate alkalinity and rapid acidification were limitations for stable reactor operation and effective anaerobic digestion(Malaspina, Cellamare et al. 1996; Saddoud, Hassairi et al. 2007).

For wastewater with high organic load like cheese whey, biomass retention is an essential factor determining the treatment efficiency. Membrane bioreactors (MBR) can achieve high biomass retention by applying membrane separation technologies. The four key separation processes are reverse osmosis (RO), nanofiltration (NF), ultra filtration (UF) and microfiltration (MF) (Jeison, Días et al. 2008; Judd 2011). Thus, for cheese whey treatment, anaerobic membrane bioreactor (AnMBR) can be an alternative option.

Generally, there are two types of membrane operation. The first one is “dead-end” filtration. The feed flows against the membrane surface, and all the feed flow is converted to permeate product. Another type is called “cross-flow” filtration. The feed flow goes on parallel to the membrane. Only part of it is transferred to permeate. For dead-end filtration process, it is easy to form thick cake layers on the membrane. Regular backwash is needed to sustain a stable permeability. In comparison, cross-flow process works better for continuous filtration (Judd 2011).

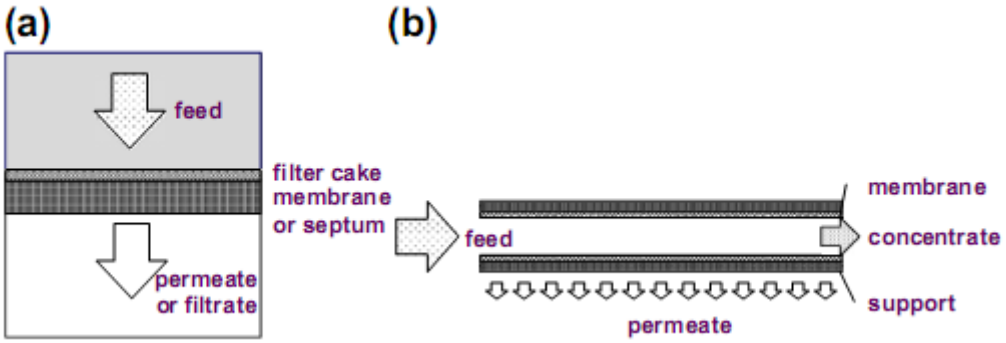


Figure 2 Dead-end (a) and cross-flow (b) membrane operation(Judd 2011)

Based on these membrane operations, there are two types of membrane bioreactors distinct by the position of the membrane (Figure 3). The first one is side-stream MBR, with a membrane module outside the bioreactor. Sludge is circulated in a recirculation loop through the membrane module. Another one is submerged MBR, with a membrane module immersed in the sludge inside the bioreactor. Compared with submerged MBR, the side-stream MBR requires more energy to sustain the designated cross-flow velocity and higher operational trans-membrane pressure. Indeed, the high levels of membrane surface shear of side-stream MBR ensure operation with higher permeate flux under anaerobic conditions. In addition, the cleaning and regular check of MBR module is much easier than the other one since the side-stream position is more convenient to remove. (Jeison 2007; Judd 2011; Skouteris, Hermosilla et al. 2012)

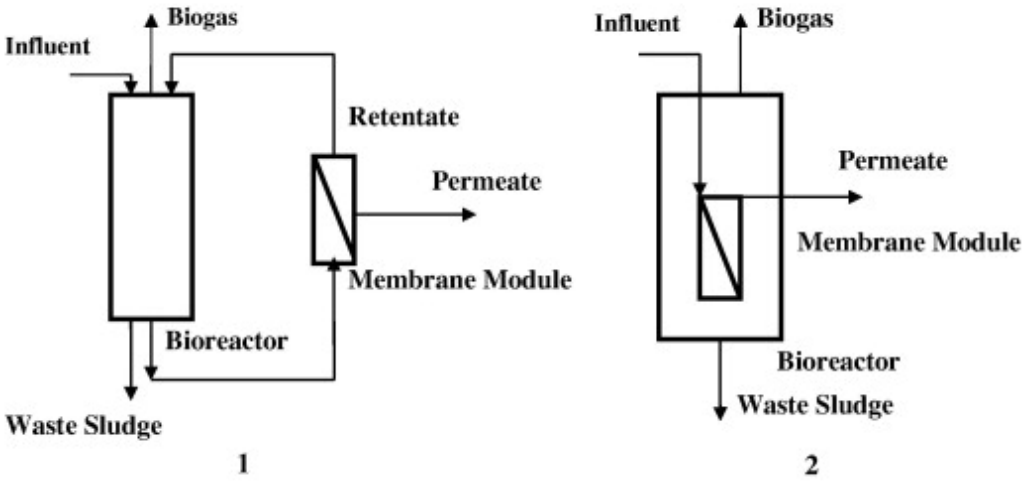


Figure 3 Side-stream MBR (1) and submerged MBR (2) (Skouteris, Hermosilla et al. 2012)

Table 3 Bibliographic compilation: anaerobic digestion of cheese whey(Prazeres, Carvalho et al. 2012)

Experimental conditions						Results					Residual concentration ($kg\ m^{-3}$)
Substrate	Reactor type	COD_i ($kg\ m^{-3}$)	pH	T (°C)	HRT (day)	Loading ($kg\ m^{-3}\ d^{-1}$)	Gas production ($m^3\ d^{-1}\ m^{-3}$)	% CH_4	CH_4 yield ($m^3\ kg^{-1}$ COD)	COD removal (%)	COD
SCW	AUFFLR	79	6.7	35	5	14	5.6	79	0.4	95	3.9
CW	ASDFA	70	–	–	–	16.1	–	–	–	99	0.7
Diluted CW	UASB	4.5–38.1		–	5	–	–	–	–	97	–
CW	AUFFR	70	7.0	37	2	35	6.7	72	–	81	13.4
CW	DUHR	68.8	6.5–7.5	mesophilic	9	10	10	53	0.33 nL g^{-1} COD	98	1.4
CW	TSUAD	72.22	7.0	35	20	10 L d^{-1}	125.6 L d^{-1}	70.9	0.23 COD	36	33
Diluted CW	Vertical ARBC	30	7	37	3	–	3.3	73	0.33 $m^3\ kg^{-1}$ TS d^{-1}	78	6.6
Diluted CW	Horizontal ARBC	30	7	37	3	–	3	73	0.30 $m^3\ kg^{-1}$ TS d^{-1}	77	6.9
Diluted CW	UASB	55.7–58.4	4–7	35	2.06–2.46	22.6–24.6	23.4 LCH_4 L^{-1}	77	0.424	95–97	1.7–2.7
CW	CP	60.3–66.7	–	36	7	4.3–18.3	5.5–20 L d^{-1}	76	(0.28–0.59) × 10^{-3}	83	4.7
CW	UASB		6.5–7.5	36	2.5	0.5–9.0	0.2–18.5 L d^{-1}	78	–	98	4.6
CW powder solution	ASBR	0.5–4.0	7.6–84	30	–	0.6–4.8 mg L^{-1} d^{-1}	–	–	–	>90	0.1–0.6
CW	TSMAMD	68.6	6.5	37	5	19.78	10	>70	0.3	98.5	1.03
Pre-treated CW[a]	UAF	5–20	7.2	35	2–5	4	1.3 LCH_4 L^{-1} d^{-1}	–	0.28	98	–
Pre-treated CW[a]	UAF	15	7.2	35	2–5	3	3.2 L d^{-1}	–	0.28–0.38	95	0.75

[a] Lactic fermentation of diluted CW and lime neutralization.

AUFFLR: Anaerobic upflow fixed film loop reactorASDFA: Anaerobic Semicontinuous digester with flocculant addition

UASB: Up-flow anaerobic sludge blanket DUHR: Downflow-upflow hybrid reactor

TSUAD: Two-stage unmixed anaerobic digester ARBC: Anaerobic rotating biological contact reactor

CP: Contact processASBR: Anaerobic sequencing batch reactor

TSMAMD: Two-stage mixed anaerobic membrane digesterUAF: Up-flow anaerobic filter

AUFFR: Anaerobic upflow fixed film reactor

2. Aim and Scope

Since cheese whey is a concentrated wastewater source, it is meaningful to find a cheap way to treat it efficiently. Anaerobic membrane bioreactor (AnMBR) is considered as analternative anaerobic technology for the treatment of whey. The aim of this thesis is to operate an AnMBR for whey treatment, and try to optimize membrane performance and treatment efficiency. In this study, the digested sewage sludge was used as inoculum to start-up the AnMBR reactor for economical concerns since inoculation of full scale reactors requires a high amount of sludge and digested sewage sludge is the most abundant anaerobic sludge that can be found very cheap and easily.

The lab-scale cross-flow AnMBR reactor was operated for 6 months. Throughout this period biological treatment performance and membrane performance were followed with regular experiments performed twice a week. All the experiments were conducted in the Research & Development Laboratory of Biothane System International in the Netherlands. The period of thesis work was from Jan, 2012 to Jun, 2012.

3. Materials & Methods

3.1 Lab-scale Reactor construction

The lab-scale reactor consists of 3 main units (Grélot, Dereli et al. 2012): a continuously mixed feed vessel kept at 4-5 °C in a fridge, a 10L anaerobic reactor with continuous stirring, and a side-stream tubular cross-flow ultrafiltration membrane with a length and diameter of 69.5cm and 5.2mm, respectively. The membrane used in the study is a hydrophilic tubular polyvinylidene fluoride (PVDF) membrane cast on a polyester carrier supplied by Norit X-flow. The properties of the membrane are given in Table 4. The membrane was operated in continuous filtration mode. The anaerobicreactor was operated under mesosphilic conditions (37 ˚C± 0.5). In order to keep membrane fluxes at a high level, permeate is partially recycled back to the reactor, and an overflow line is constructed to make sure the reactor volume is kept at 10L. All the sensors and pumps in the AnMBR system could be monitored and controlled through a PLC system and a computer program developed with Labview Software running on a standard PC. The pH in the reactor was controlled with a stand-alone controller (HACH LANGE SC 1000) and two KNF pumps for acid and caustic. The detailed technical properties of the equipment (pumps, gas meters, etc.) used in the lab-scale reactor set up are given in Table 5.

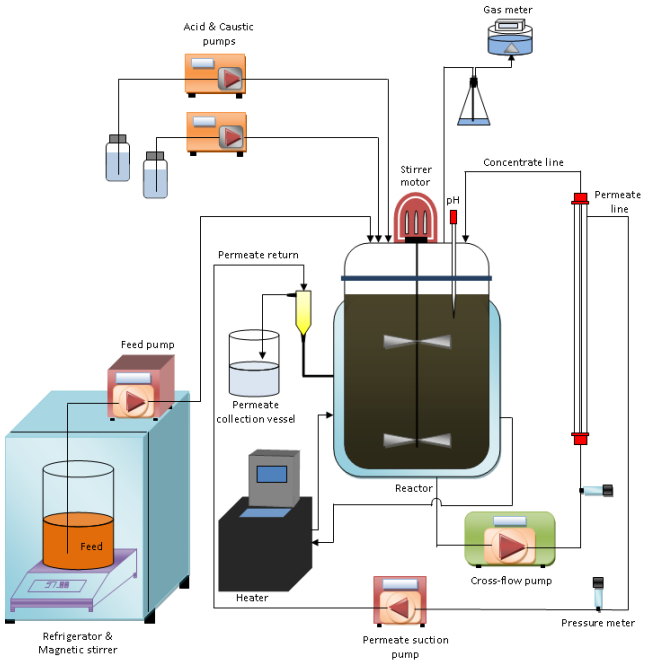


Figure 4 Lab-scale reactor units(Grélot, Dereli et al. 2012)

Table 4 Membrane and module properties

Parameter	Unit	Membrane (F 4385)	Remarks
Clean water flux	$L/m^2.h.100$ kPa	>1000	RO-water at 25 ^0C
Transmembrane pressure	kPa	-100 .. + 500	-
Mean pore size	nm	30	-
pH	-	2 - 10	at 25 C
Chlorine exposure	ppm.h	250000	at 25 ^0C
Temperature	^0C	1 - 70	pH 7 and 100 kPa
Membrane material	-	Polyvinylidene fluoride	-
Carrier material	-	Polyester woven/non-woven	-
Structure	-	Asymmetric	-
Hydrophobicity	-	Hydrophilic	-
Membrane geometry	-	Tubular	-
Inner diameter	mm	5.2	-
Length	cm	69.5	-
Membrane area	m^2	0.0114	-

Table 5 Parameters of equipments in this study

Equipment	Type	Manufacture	Description
Feed Pump	120U	Watson-Marlow	Perisaltic pump 120U/DV manual; control with digital display; 114DV pumphead; 90-264V; 110mm x 196mm x 112mm (w x d x h); 0.1rmp to 200rpm; 0.002mL/min to 170mL/min
Permeate Suction Pump	120U	Watson-Marlow	
Recirculation (Cross-flow) Pump	520 U	Watson-Marlow	Peristaltic pump; 230V 50/60Hz; 158mm x 276mm; 4μL to 3.5L; 0.1 to 220rpm; accuracy 0.1%; analogue/manual
Acid & Caustic Pumps		KNF	
Heater (water bath)	SAHARA PPO S5P Heated Bath Circulator (152-3058)	Thermo Scientific	SC100-S5P heating circulator; Ambient +13 to 100°C; 115V/60Hz; 5L
Pressure meter (sensor)		ATM	-600 to 800 mbars
Gas meter	Wet tipping gas meter		
pH controller	SC 1000 Controller	HACH LANGE	Consisted of a display module (model LXV402) and one or more probe modules (model LVX400); Ambient -20 to +55°C

3.2 Experimental Methods and Procedures

Daily biogas production, pH, and membrane feed, permeate and trans-membrane pressures were recorded online. Feed flow, permeate flow and methane content of biogas were checked manually every day. Frequent analyses (once or twice per week) were performed to check the characteristics (Total and Volatile Suspended Solids, TS/VS, COD, soluble COD, pH, alkalinity, TKN, NH_4^+-N, TP, PO_4^{3-}-P) and protein (BCA method), polysaccharides (Dubois method)of the raw wastewater, permeate and sludge according to Standard Methods(APHA 2005). Other parameters were also measured as indicators of anaerobic treatment stability and membrane performance, such as volatile fatty acids (VFAs), cations, anions, protein and polysaccharides. In addition, filterability, capillary suction time (CST), specific cake resistance (SCR) and particle size distribution were checked as well to evaluate the sludge filterability. The experimental plan of the study is shown in Table 6.

Table 6 Experimental plan

Parameter	Feed	Sludge	Sludge Supernatant	Permeate	Frequency	Method
TCOD	X*	X		X	2 per week	Standard Methods
SCOD (0.45µm)			X		2 per week	Standard Methods
TKN		X		X	1 per week	Standard Methods
NH_4^+-N			X	X	1 per week	Standard Methods
TSS/VSS		X			2 per week	Standard Methods
TS/VS		X		X	2 per week	Standard Methods
VFA (0.45µm)	X*		X	X	2 per week	GC
PO_4^{3-}-P (0.45µm)			X	X	2 per week	HPLC
TP		X		X	1 per week	Standard Methods
Cations (0.45µm)			X	X	1 per week	HPLC
Anions (0.45µm)			X	X	1 per week	HPLC
PH		X		X	2 per week	pH meter (Standard Methods)
Alkalinity			X	X	1 per week	Standard Methods
Proteins (0.45µm)		X**	X	X	1 per week	BCA
Polysaccharides (0.45µm)		X**	X	X	1 per week	Dubois
Capillary suction time (CST)		X			1 per week	Triton
Specific cake resistance (SCR)		X			1 per fortnight	Amicon Cell
Filterability		X			1 per fortnight	Amicon Cell
Specific methanogenic activity		X			1 per fortnight	Pressure

**TCOD and VFA of feed sample from feed vessel connected to the reactor were measured once per week to make sure there was no big change of the feed components. All the parameters were measured aftera new batch of feed was prepared.*

*** In proteins and polysaccharides measurements, sludge samples were used to measure total proteins and polysaccharides, and samples from supernatant and permeate are for soluble ones.*

When trans-membrane pressure (TMP) increased up to400mbar, to get better performance, membrane cleaning was conducted by flushing the membrane with tap water and chemical cleaning afterwards.Critical flux was measured periodically in order to operate the membrane with an optimized flux. Critical flux measurements were also carried out several times at the beginning to evaluate the optimum operating flux under different cross-flow velocities.

Except for the parameters following standard methods and those measured by GC and HPLC, the others were performed according to the protocols prepared by the research engineers of the company. Details of these protocols are explained in the following sections.

3.2.1 Sample preparation for Extracellular Polymeric Substances (EPS) and Soluble Microbial Products (SMP)

The measurements of both protein(BCA Method) and polysaccharides(Dubois Method) according to standard methodsare for EPS and SMP. Details of the conversion from protein and polysaccharides to EPS and SMP are presented in Section 4.2.2. Only the preparation protocol is described in this part.

Sample preparation for EPS:

One sample of anaerobic sludge is first heated at 100 °C for 1hour and 15minutes;
The supernatant of the anaerobic sludge samples is then separated from solid phase by centrifugation at 14000 rpm for 10 min at 16 °C;
The supernatant must then be passed through a rough filter;
Pass the supernatant through the 0,45 µm filter;
Measure the sample for protein and polysaccharides following the protocol of each one.

Sample preparation for SMP:

The supernatant of the anaerobic sludge samples is then separated from solid phase by centrifugation at 14000 rpm for 10 min at 16 °C;
The supernatant must then be passed through a rough filter;
Pass the supernatant through the 0,45 µm filter;
Measure the sample for protein and polysaccharides following the protocol of each one.

3.2.2 Protocol of the Capillary Suction Time (CST)

The CST equipment is a practical method for the determination of sludge dewaterability, providing a rapid comparison of the effects of different agents and dosages in waste water.

The rate at which the filtrate passes through the paper filter is influenced by the characteristics of the sludge. The Capillary Suction Time (CST) is calculated by the time that the water from the sample takes to travel from one electrode to another.

The equipment is formed by, the reader apparatus, two different cells, the filter support and the upper plate (Figure 5).

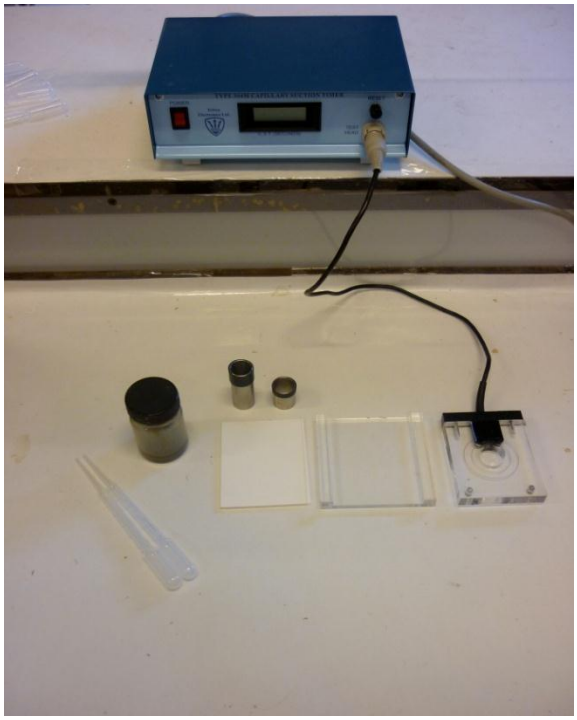


Figure 5 CST equipment (model CST), reader apparatus, two different cells, the filter support, the upper plate, one sample, paper filter and 2ml pipette tips

Procedure to assemble and operate the CST equipment:

The equipment should be disconnected (inOff signal)
Connect the signal reader to the upper plate (with the electrodes)
Put the filter in the paper filter support. Take care to put the filter on the right side (weaving part on the filter support)
Assemble the upper plate on top of the filter, with the electrodes down touching the paper filter
Put the chosen cell in the upper plate, making sure that is touching the paper filter completely
Pour the sample into the cell (should be totally full and present a meniscus, see picture 9)
Turn the switch to ON
Depending on the type of sample the measurement will take more or less time, a beep signals when the filtrate reaches the first electrode and also when the second set of electrodes is reached.

After the measurement, the equipment should be clean, the filter support (if necessary), the upper plate, and the cell, so the filter used in the next measurement will not be contaminated.

3.2.3 Protocol for the Amicon cell to measure SCR and filterability

The Amicon Filtration Cell is commonly used for filterability tests and specific cake resistance. A gas pressure is applied directly to the cell. The solids are retained inside the cell, while the permeate passes through the filter and out of the cell. The maximum operating conditions are:

Pressure: 75 psi
Volume: 50ml
Temperature: Although brief exposure to higher temperatures is possible, do not operate cell continuously above 85°C (185°F).

The Amicon cell is composed by the following components: clamp, permeate line, filter holder, base of the cell, cap, O-ring, cell body and finally the stirrer (Figure 6).

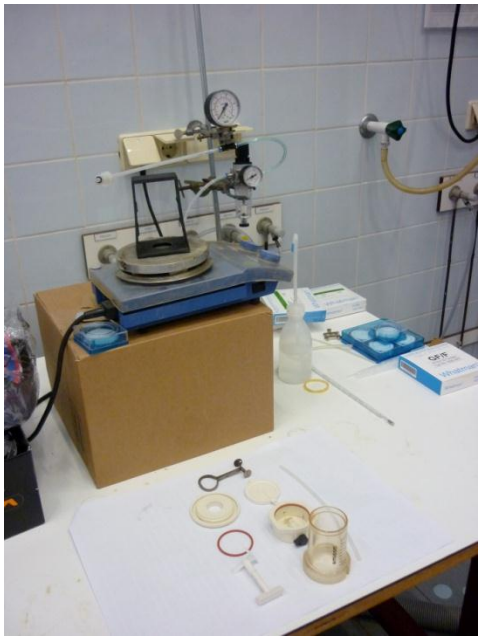


Figure 6Amicon cell components, stand, magnetic stirrer, pressure valve and 0.22µm filters

Procedure to assemble and operate the Amicon cell:

Place the filter in the holder; place O-ring on top of the filter. Gently push O-ring down to seal the filter against the bottom of the holder. Apply the O-ring gently to avoid scratches and contamination, **in filterability tests a 0.22 µm pore size filter is recommended, for specific cake resistance is preferable to use 0,7 µm filter.** Connect the line to the filter holder and clamp it.

Fit the filter holder into the cell body and screw the base of the cell firmly.
Place the stirrer into cell body (depending on the analysis). When properly installed, the arms of the stirrer will be held by the inside ridge on top of the cell body.
Introduce sample into cell.
Push cap down into the cell body, with the gas inlet oriented to the opposite side of the permeate line.
Set pressure-relief valve to horizontal (open) position, see picture 8.
Slide cell into retaining stand, it will ensure the proper position of the Amicon cell and prevents the rotation of the cell while measuring.
Attach gas pressure line, for filterability tests the recommended Pressure is 0,5 mbar.
Place cell on magnetic stirring table.
Turn on stirring table and adjust stirring rate to level 5. Stir the sample solution for 20 min before beginning the measurements (in filterability tests).
Turn pressure-relief valve to vertical (closed) position see picture 9, the cap moves upward, forming a secure lock with retaining stand once the system is pressurized.
Unclamp the permeate line.
When finished, turn off the pressure and stirring table.
Release the pressure inside the cell by slowly turning pressure-relief knob to horizontal position. Push cap down, then slide cell out of the retaining stand.
Use a twisting motion to remove cell cap and the magnetic stirrer assembly. Always remove the cell top with the pressure-relief valve set to the horizontal (open) position.
Pour out the solution.
Disassemble the cell, wash all components with a mild detergent/water solution, and then rinse thoroughly. Leave the cell disassembled until it is necessary to use it again.

3.2.4 Specific Cake resistance:

Measurement procedure:

Data acquisition frequency for the test is15 sat minimum.
The test takes 30 minutes
Prepare the sample:
Measure the temperature of the sample before beginning the test, and the TSS concentration
Use preferably a minimum amount of 30/40 ml of final volume to do the test.
Dilute the sample to 10 g/L adding permeate.

Notes: Do not mount the stirrer part of the Amicon cell for specific cake resistance tests. Always adjust the pressure before beginning the test.

Data treatment:(Jeison 2007)

In the filtration process, the relation of flux and cake and membrane resistance follows equations Eq1~Eq3:

$$J = \frac{1}{A}\frac{dV}{dt} = \frac{TMP}{\eta}\frac{1}{R_M + R_C} \quad \text{Eq1}$$

J- Flux; A- membrane area; V- permeate volume; t- time; η: permeate viscosity, R_M- apparent membrane resistance; R_C- cake resistance.

In a dead end filtration like this, R_C is related to specific cake resistance (α) directly:

$$R_C = \frac{V}{A}\alpha \cdot C \quad \text{Eq2}$$

C- Solids concentration.

Combine the 2 equations and another equation would be obtained assuming that the flux is constant:

$$TMP = \frac{\eta \cdot \alpha \cdot C \cdot J}{A} V + \eta \cdot R_M \cdot J \quad \text{Eq3}$$

The specific resistance could be gotten as the slop of TMP against permeate volume. The volume is recorded by on-line scale under the amicon cell.

3.2.5 Supernatant filterability:

- Measurement procedure:

  Preparation of the supernatant
  Collect the sample and centrifuge it.
  Optimal conditions: 14000 RPM
  70ml of sample=2tubes=nearly 30ml of supernatant (this result depends on the type of sludge)
  Use preferably a minimum amount of 20 ml of sample to do the test.
  Data acquisition frequency for the test is 15 s.
  The test takes 10 minutes.
  Do not forget to mount the stirrer part of the Amicon cell for filterability.
  Always adjust the pressure before beginning the test.
  MEASURE THE COD CONCENTRATION OF THE SUPERNATANT BEFORE THE TEST.

- Data treatment:

The accumulated weight of supernatant filtered is recorded by on-line scale under the amicon cell. The filtration flow rate is calculated as ml/ min

The filterability measurement corresponds to obtained stable filtration flow rate and the specific filterability is this filtration flow rate divided by the COD concentration of the supernatant.

3.2.6 Membrane cleaning protocol

Operate the membrane during 20 min – note TMP and measure membrane inst. flux
Rinse the membrane with water
Operate the membrane during 20 min – note TMP and measure membrane inst. flux
Rinse again with water. Put the chemical solution (NaOCl 500 ppm) in the membrane module for 1 h
Remove the chemical solution and collect it to analyze it
run the membrane for 20 min – note TMP and measure membrane inst. flux
Rinse with water and put the citric acid (1%) in the membrane module for 1h
Remove the chemical solution and collect it to analyze it
run the membrane – note TMP and measure membrane inst. Flux

3.3 Reactor Operation

3.3.1 Equipment calibration

Before the reactor start up, equipments like pumps, pH meter and gas meter were all calibrated.

The calibration methods for the 3 primary pumps: feed pump, recirculation pump and permeate suction pump, were the same. Feed pump, for instance, was operated at different rotation frequency, expressed as revolutions per minute (rpm). Collect feed pumped for 10 min manually at each rotation frequency. A calibration line with feed (ml/min) and rotation frequency (rpm) is obtained. The slope of this calibration line was set in controlling program. When feed flow was changed during operation, the pump would automatically work at the rotation frequency according to the line.

For on-line pH meter calibration, 2-point manual method was conducted following the instruction displayed on the sensor controller screen. The standard solutions for pH calibration were with pH 4 and 7. For off-line pH meter calibration, 3-point automatic method was applied according to the installed program. The pHs of standard solutions were 4, 7 and 10.

3.3.2 Membrane preparation

The membranes modules used in the study were constructed in the laboratory by gluing a single straw of Norit X-flow tubular membrane with epoxy based glue and sealing it in a glass module. The membranes were deconditioned by soaking them into 500 ppm NaOCl solution for 1 hour and 10-20% ethanol solution for half an hour. After deconditioning the initial clean water permeability of the membranes were determined in a similar setup to the lab-scale cross-flow AnMBRs by using tap water at 37 ^{0}C.

3.3.3 Operational parameter set up

Cross-flow velocity set up:

In the beginning of the study, several trials were done for operating the membrane at high cross-flow velocity to limit membrane fouling. However, due to equipment limitation and

very high pressure fluctuation at high velocities, a high cross flow velocity such as 2 m/s could not be maintained in this study.This is mainly due to the peristaltic pump used as the cross-flow pump which was causing a high pressure fluctuation. In order to limit the pressure variation, several options such as pressure dampener and buffering bottles (Figure 7) were tried out. The maximum cross flow velocities (CFVs) that could be applied were 1.5 m/s and 1 m/sfor a short period of time. However, in the end all the attempts had failed. Therefore, due to equipment limitations a cross-flow velocity of 0.5 m/s was chosen for long term sustainable operation.

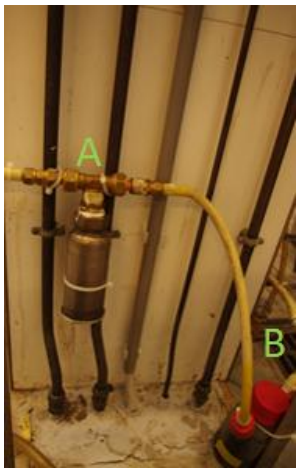


Figure 7 Buffering system (A: pressure dampener; B: buffering bottle)

Permeate flux set up:

Higher permeate flux is preferable to operate the reactor at shorter hydraulic retention time.However, when the permeate flux is set too high, the membrane could be fouled rapidly. Critical flux measurements were conducted to determine highest operational flux that leads to lowest membrane fouling. The operational flux was set under critical flux. The permeate flux was set as 10 LMH at the beginning of the operation. The operational flux was changed in accordance to the critical flux experiments conducted during long term study. The details will beexplained in the Section “Critical flux”.

Sludge retention time (SRT) set up:

Anaerobic digestion needs longer sludge (solid) retention time than aerobic biodegradation (Prazeres, Carvalho et al. 2012). In the first phase of the experiment, SRT was set as 50 days. However, after 22 days of continuous operation, very poor reactor performance indicated by high VFA concentrations was obtained which may be due to low activity of the seed sludge or due to inappropriate SRT for this wastewater type. Therefore at day 45, daily sludge extraction was ceased to operate the reactor at high (infinite) sludge age. As a result, no sludge was extracted except for sampling to measure regular parameters. The anticipated sludge retention time would be 300 days under this situation.

3.3.4 Overall operation

The whole experiment was divided into several phases according to the actions taken in reactor operation.

The 1st phase, from day 0 to day 57, after the adjustment of cross-flow velocity and permeate flux, the sludge retention time (SRT) was set as 50 days. The 2nd phase, from day 58 to day 85, 644mL crushed granular sludge with high methanogenic activity from a full scale EGSB treating lactose based wastewaterwas added to boost the activity of biomass in reactor. At the same time, to keep as much biomass as possible in the reactor, nosludge wasextracted except for samples for regular measurements. The 3rd phase, from day 86 to day 114, another batch of 800ml crushed granular sludge was added into the reactor since VFA concentrations were still high (>1000 mgCOD/L) at VLR around 4 kg COD/m^3.day in the previous phase. The operation conditions are shown in Table 7.

Table 7 Operation condition of 3 phases

Operation condition	Unit	Phase 1 (day 0-57)	Phase 2 (day 58-85)	Phase 3 (day 86-114)
SRT	days	50	-	-
HRT	days	17	8	6
VLR	kg COD/m^3.day	2.1±1.0	3.4±1,0	4.5±0,6
F:M	kg COD/kg VSS.day	0.53±0.1	0.47±0.12	0.30±0.09
Temperature	^{0}C	37	37	37
CFV	m/s	0.5	0.5	0.5
Flux*	LMH	5	18	10

*day 17-62 with flux 5 LMH, day 63-99 with flux 18 LMH, day 100-114 with flux 10 LMH.

3.4 Characterization of whey

The whey used as the feed in this study was obtained from a cheese production facility. The characterization of the whey used in the study was similar to the whey treated in a full scale AnMBR plant (Table 8).

Table 8Feed characterization

parameters	unit	Whey from full-scale plant	Whey used in this study
TCOD	mg/L	27860	26180±1290
SCOD	mg/L	26440	25690±720
TKN	mg/L	370	130±7
SKN	mg/L	290	120±20
NH_4^+-N	mg/L	50	25±7
TSS	mg/L	1620	620±220
VSS	mg/L	2120	560±170
TS	mg/L	14780	26060±630
VS	mg/L	11340	21750±530
VFA	mg/L	630	260±90

TP	mg/L	340	360±20
PO_4^{2-}- P	mg/L	360	220±80
SO_4^{2-}	mg/L	250	60±20
Cl^-	mg/L	1950	2080±310
Ca^{2+}	mg/L	740	580±80
Mg^{2+}	mg/L	720	80±10
K^+	mg/L	1130	840±200
Na^+	mg/L		480±60
pH	-	3.4	5.1±0.3
Soluble protein	mg/L	1250	1440±300
Total protein	mg/L		1630±380
Soluble polysaccharide	mg/L	1420	11130±1240
Total polysaccharide	mg/L		10770±690

3.5 Inoculums Characterization

The digested municipal sludge is from Delft wastewater treatment plant, the Netherlands. Before putting the sludge in to the lab-scale reactor the sludge was screened through a 0.6 mm mesh filter to remove any fibers and large particles that may clog the tubes. 8.5L sludge was put into the reactor at the very beginning as the inoculums. Because of the poor activity of municipal sludge, 2 batches of crushed granular sludge with high methanogenic activity were added into the anaerobic reactor to enhance the activity of biomass during the operation. Details of the addition were stated in the section "Reactor operation". The characteristics of the inoculums are listed in Table 9.

Table 9 Characteristics of inoculums

Parameter	Unit	Inoculum
TCOD	mg/L	34900
TSS	mg/L	30480
VSS	mg/L	20900
TS	mg/L	32900
VS	mg/L	21180
Soluble proteins	mg/L	328
Soluble polysaccharides	mg/L	106
CST	s	550
Specific cake resistance	10^{12} m/kg	500
Filtrability	mL/min	0.27
Acetic Activity	g CH4-COD/g VSS.day	0.19
Propionic Activity	g CH4-COD/g VSS.day	0.06
Butytic Activity	g CH4-COD/g VSS.day	0.23

4. Results and discussion

4.1 Biological performance

Volumetric loading rate (VLR) and sludge loading rate (F: M ratio) applied during the operation period are shown in Figure 8. The reactor was started up with a relatively low organic loading rate (1 kg COD/m^3.day) to acclimatize the sludge to wastewater. After 1 week, the VLR was gradually increased up to 4 kg COD/m^3.day. However, VFA started to build up in the reactor at this VLR and the load was first decreased to 3 kg COD/m^3.day then to 1.5 kg COD/m^3.day.Accordingly, acetic acid concentrations decreased and propionic acid concentrations were stable. A second attempt to increase the VLR to 3 kg COD/m^3.day resulted in a sharp increase of propionic acid. The effluent COD which was mainly propionic acid reached up to3500mg/L which corresponds to 87%COD removal efficiency.The average VLR in the 1st phase was 2.1 kg COD/m^3.day which was very low compared to those VLRs reported by other authors for the anaerobic treatment of whey shown in Table 3. At this point, several measures were undertaken to improve reactor performance. First, daily sludge discharge was stopped to retain more biomass in the reactor to improve degradation capacity. Secondly, to immediately boost biomass activity and sludge concentration, highly active crushed granular sludge was added (65 g as VSS) into the reactor. After adding extra sludge and stopping regular sludge extraction, the VLR was started to increase gradually. This time VLR could be increased up to 4 kg COD/m^3.day without any VFA accumulation. However, at a slightly higher VLR than that the VFA concentrations started to increase once again. The average VLR in 2nd phase was calculated as 3.4 kg COD/m^3.day. In order to test, whether the active biomass concentration was limiting the biological performance a second portion of crushed granular sludge was added (112g as VSS) to the reactor and the VSS concentration was boosted up to 33 g/L. In the 3rd phase of the study, VLR was kept in between 4-5 kg COD/m^3day; however a stable reactor performance could not be achieved. Although the acetic acid concentrations were generally low (<100 mg COD/L), the propionate tend to accumulate and fluctuate wildly in the reactor.

During the operation,it was found out that the nitrogen concentration measured as ammonia in permeate was very low. This brought the idea that nitrogen limitation may be the real reason of instable reactor performance. In order to investigate the effect of nitrogen on the biological performance the nitrogen concentration in the reactor was booted to 112 mg/L by manually adding NH4Cl as shots in two consecutive days (day 94 and 95). The result was quite remarkable since the VFA concentration in reactor decreased to zero next day after the second addition of nitrogen. However, this performance boost was not permanent and the VFA concentrations started to increase after the nitrogen addition was ceased. However, this result was quite interesting since nitrogen limitation is generally not pronounced for anaerobic process due to the small growth yield of anaerobic bacteria. Nitrogen and phosphorus are the two essential macro-nutrients for biomass growth. Therefore, the bacteria, which have a higher cell yield and specific growth rate, have an important advantage over the other bacteria when competing for nutrients in a nitrogen limited environment. In this specific case, acid producing bacteria which are converting lactose in whey into propionate had an advantage for nitrogen due to their faster metabolism and

growth yield over the propionate utilizing bacteria which are known as the most sensitive and slow growing microorganisms in an anaerobic consortium. Therefore, the acidogens were consuming most of the nitrogen fed to the reactor and limiting the growth of slow growing microorganisms. The poor and unstable performance of AnMBR in this study may be explained by this phenomenon. All in all, the reactor could not be operated with VLR higher than 5 kg COD/m^3.day by only increasing the active biomass concentration in the reactor.

Compared to VLR, F:M ratio was more stable. Even though VLR increased after adding extra sludge, VSS was also higher due to the addition. The average SLR was calculated as 0.14±0.04 kg COD/kg VSS.day.

Figure 10 shows the variation of VFAconcentrations together with VLR in reactor. In the initial phase of the study both acetic and propionic acids accumulated in the reactor when VLR was increased. However, in the longer operation period propionic acid was the main VFA building up in the reactor. The butyric acid concentrations were always low during the whole operation time.The results indicate that propionate conversion plays a key role and it determines the stability of the reactor for whey treatment. The low activity or the concentration of syntrophic bacteria may be the reason for instable bioprocessand reactor performance.

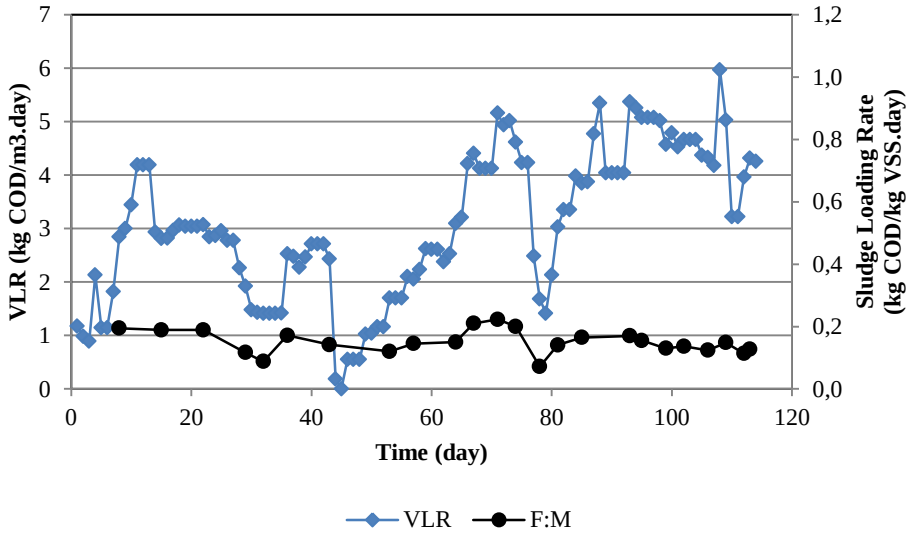


Figure 8 Volumetric and sludge loading rates change with time

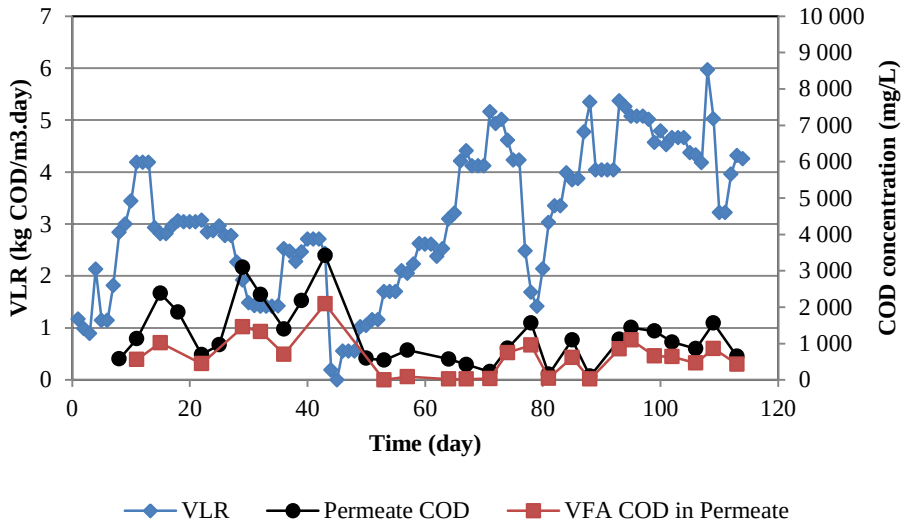


Figure 9 VLR, permeate COD and permeate VFA COD change with time

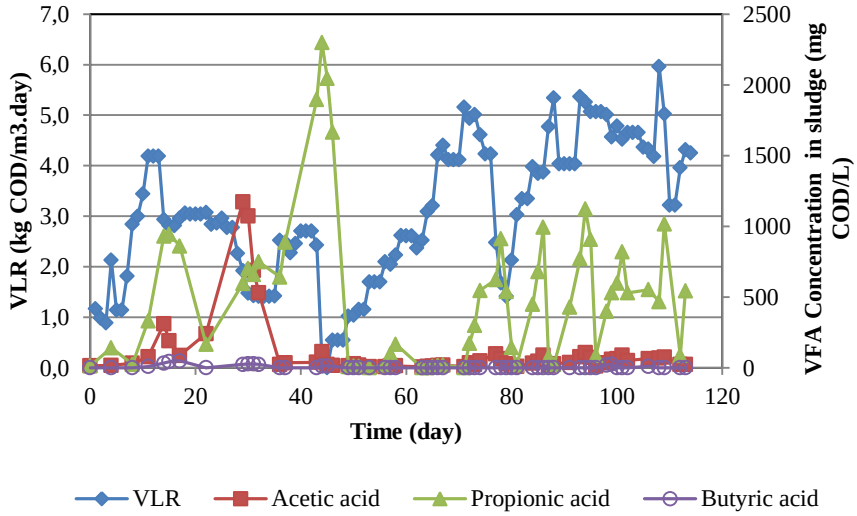


Figure 10 VLR and VFA concentrations in reactor

The TS-VS and TSS-VSS concentrations in the reactors are presented in Figure 11and Figure 12. The solids concentrations increased by adding crushed granular sludge. The average solids concentrations at different operation phases are given in Table 10. The VS/TS and VSS/TSS ratios were around 60% and 70%, respectively, during the whole operation time andtheyincreased slightly by adding crushed granular sludge.

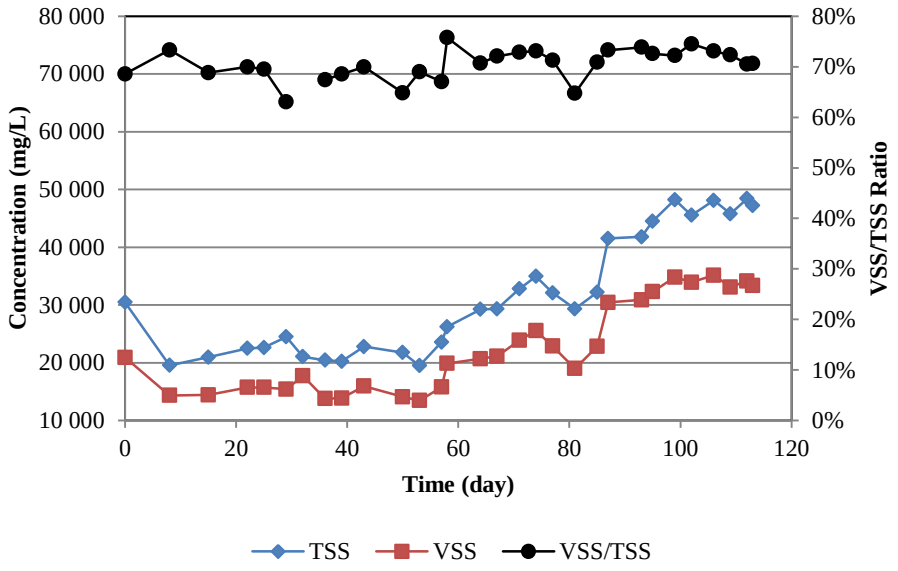


Figure 11 TSS and VSS concentrations in reactor

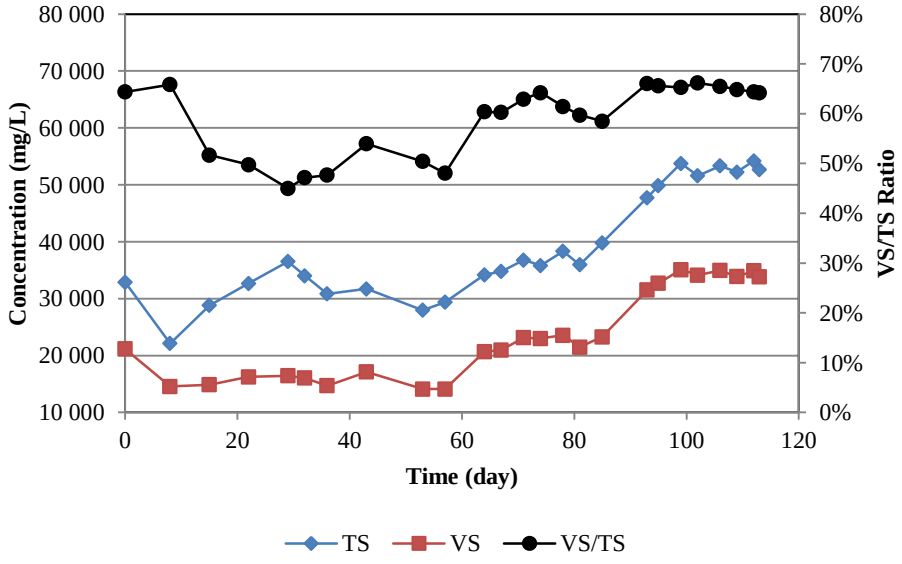


Figure 12 TS and VS concentrations in reactor

Table 10 Average concentrations and standard deviation of solids of different phases

Parameter	Unit	1st phase (day 0-57)	2nd phase (day 58-85)	3rd phase (day 86-114)
TSS	mg/L	22300±2890	30770±2740	45680±2640
VSS	mg/L	15480±2020	21980±2190	33130±1650
VSS/TSS	%	69.6±5.1	71.4±3.1	72.6±1.3
TS	mg/L	30700±3950	36530±1970	51930±2180
VS	mg/L	15950±2120	22300±1220	33910±1240
VS/TS	%	52.4±7.2	61.1±1.9	65.3±0.8

Figure 13 shows the activities of sludge on specific substrates such as acetate, propionate and butyrate. There is almost no change in the activity of the sludge during the long term operation. After the second addition of crushed granular sludge, the activities were boosted for a while. However, this could not be maintained in the long term and the activities decreased to the same levels as before sludge addition.

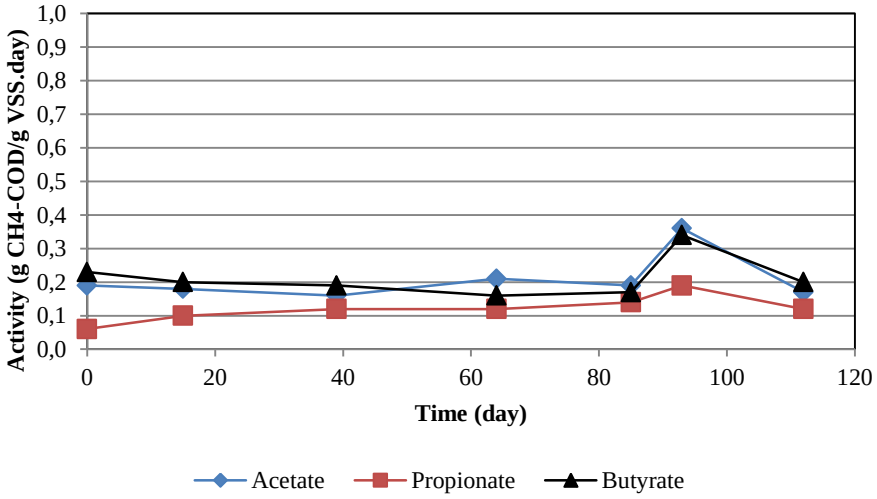


Figure 13 Biomass activity on individual VFAs

Figure 14shows the COD removal efficiency of the reactors together with applied VLR.Due to the unstable reactor performance, COD removal efficiency fluctuated between 92% and99%.The average COD removal efficiency was 96.6% for the whole operation period, which is still high due to membrane filtration which retains the suspended solids in the reactor. VFA accumulation was a key factor affecting the COD removal efficiency in this study. It can be observed from Figure 15that the fluctuation of VFA was larger than other components of COD when VLR was increased. The COD removal efficiency would be improved without VFA accumulation.

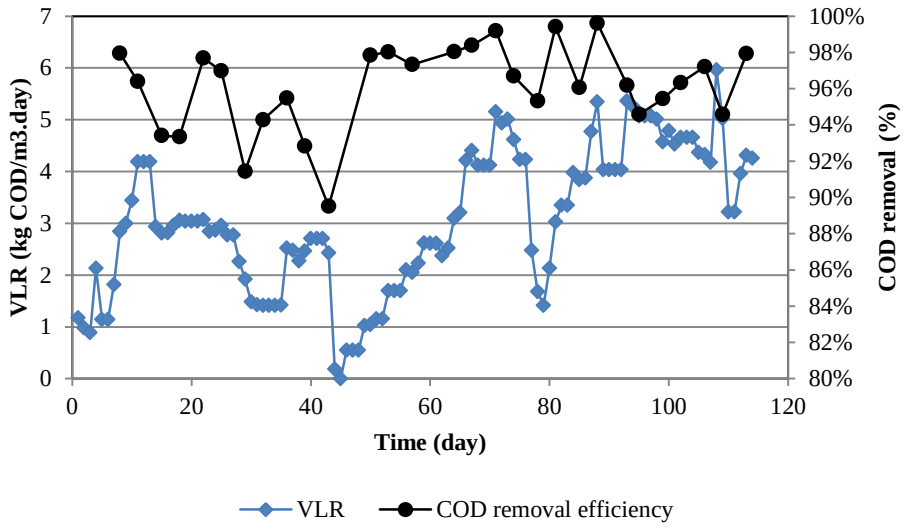


Figure 14 VLR and COD removal efficiency

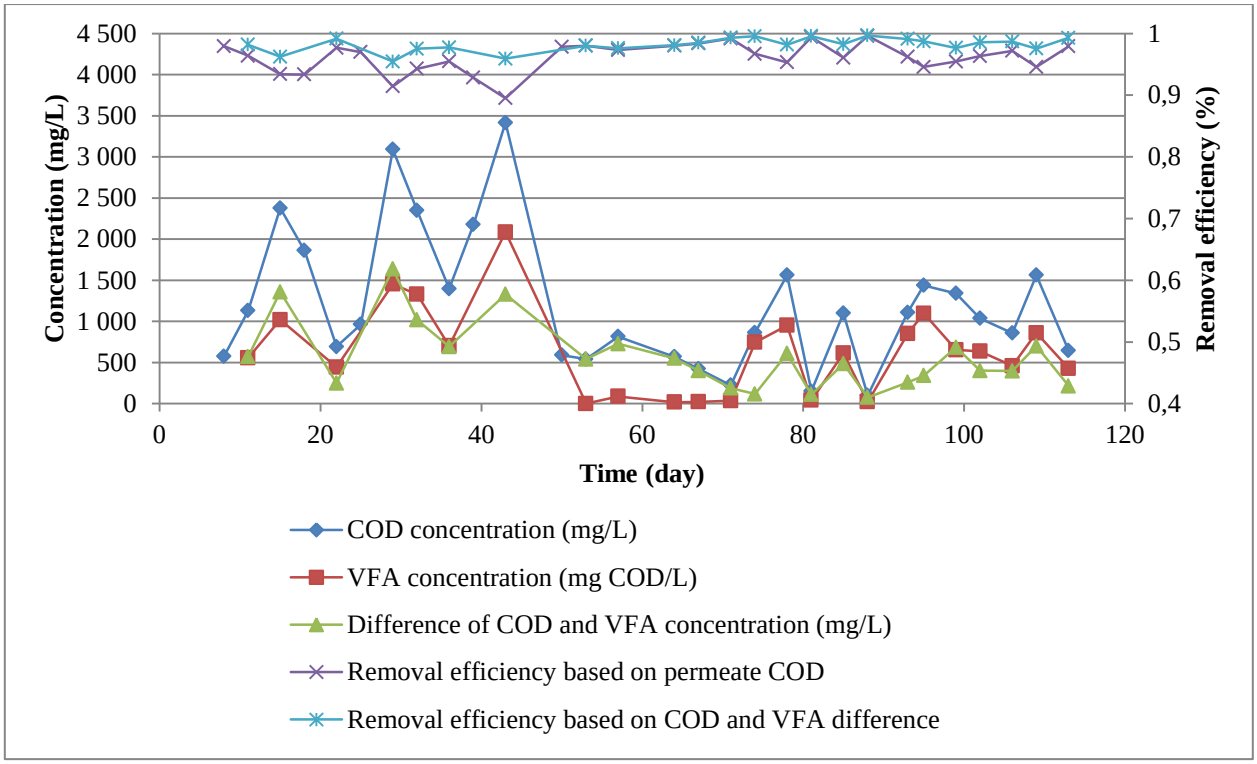


Figure 15 COD and VFA concentrations in permeate and COD removal efficiency

As expected, the methane production followed the same trend with the applied organic load (Figure 16). Figure 17 illustrates the fractions of COD present in different streams such as

permeate, wasted sludge, and generated methane gas. The digestion efficiencies in the reactors were calculated asEq4:

$$Digestionefficiencybasedon COD_{influent} = \frac{COD_{methane}}{COD_{influent}} \quad \text{Eq4}$$

The average digestion efficiencies of the 3 phases were calculated as 87%, 91% and 96% respectively. In general, the digestion efficiencyhasimprovedby enlarging sludge retention time (stop sludge extraction) and adding sludge with high methanogenic activity. On the other hand, due to unstable reactor performance the digestion efficiency was sometimes higher than 100%. This situation occurred mainly when VFA accumulated in the reactorandfeed flow was reduced to let the biomass convert the accumulated VFA. At this time, even though the influent was very low, the digestion of those accumulated VFA in reactor was still going on and this resulted in the production of moremethane than expected for the subsequent day. Based on the formula above, the digestion efficiency could be higher than reality sometimes. Those high values were omitted out when getting the average values.

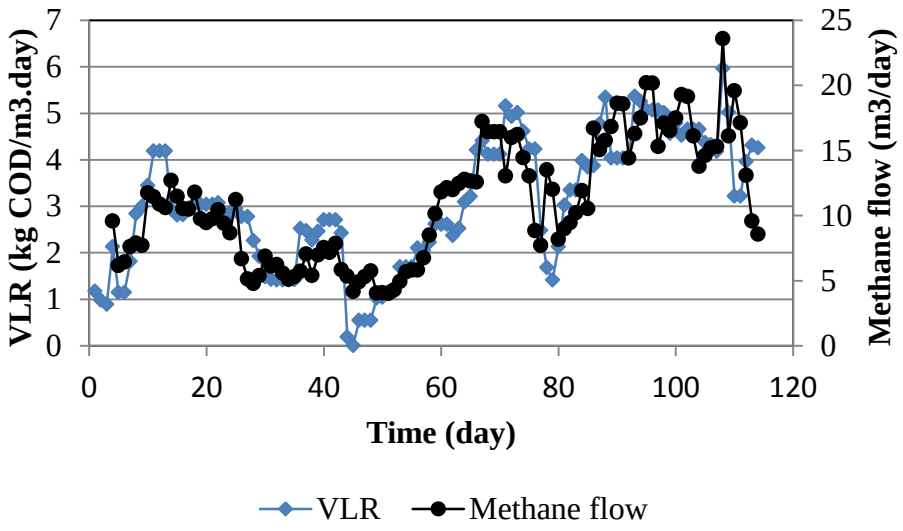


Figure 16 VLR and methane generation

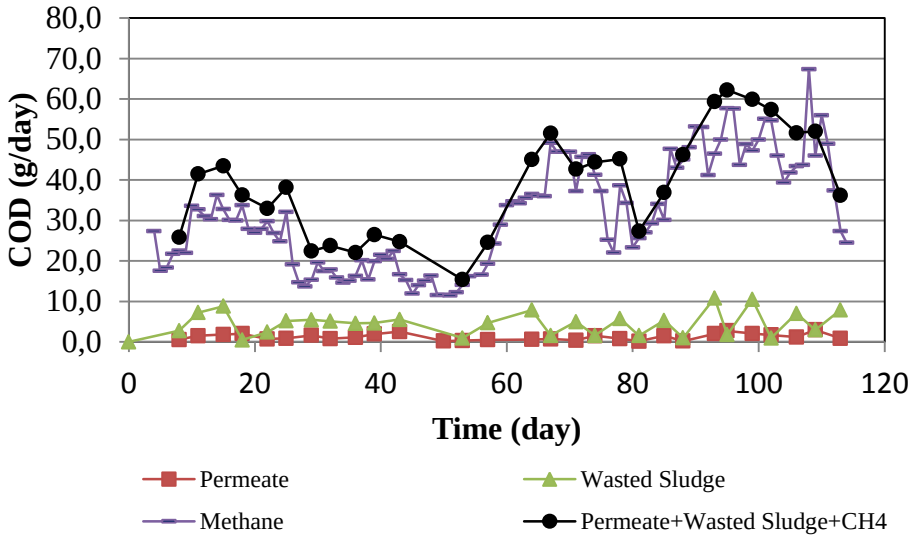


Figure 17 Fractions of COD in different streams

Table 11 COD mass balance (average+standard deviation)

Stream	Phase 1(day 0-57)		Phase 2(day 58-85)		Phase 3(day 86-114)	
	g COD/day	%	g COD/day	%	g COD/day	%
Influent	22.9±5.8	100	38.1±8.9	100	47.1±3.2	100
Permeate	1.1±0.5	4.8±1.6	0.9±0.6	2.2±1.6	1.6±0.8	3.3±1.7
Wasted sludge	4.6±1.1	21.2±7.8	3.6±1.9	9.4±4.5	5.3±3.8	11.3±8.2
Biogas	19.5±2.3	87.5±17.2	34.4±7.0	91.1±9.7	45.3±6.6	95.6±8.7

The COD mass balance results of the 3 phases are shown in Table 11.The average conversion rate from whey wastewater to biogas increased greatly from 87.5% to 95.6%.

Table 12 Biological performance in the reactor

Parameter	Unit	Phase 1 (day 0-57)	Phase 2 (day 58-85)	Phase 3 (day 86-114)
VLR	kg COD/m^3.d	2.1±1.0	3,4±1,0	4.5±0,6
F/M ratio	kg COD/kg VSS.d	0.15±0.04	0.17±0.05	0.14±0.02
TSS in reactor	g/L	22.3±2.9	30.8±2.7	45.7±2.6
VSS in reactor	g/L	15.5±2.0	22.0±2.2	33.1±1.6
Permeate COD	mg/L	1570±975	699±508	1011±477
COD removal efficiency based on permeate quality	%	95.7±2.1	97.6±1.6	96.5±1.7

Methane production	L/d	12.0±3.6	22.1±6.4	29.4±5.0
Digestion efficiency based on influent COD	%	87±17	91±10	96±9
Methane yield	m^3 CH_4/kg $COD_{removed}$	0.36±0.08	0.44±0.22	0.37±0.07
Sludge yield	g VSS/ g COD fed	0.11±0.06	0.05±0.05	0.08±0.06

The overall biological performance of the 3 phases is presented in Table 12. To sum up, the volumetric loading rate could be slightly increased with the addition of extra sludge, but the biological performance of the reactor was not satisfying. The VLR achieved in this study was significantly low compared with the successful operations achieved by other authors with VLR higher than 10 kg COD/m^3.d (Table 3).The average methane yield about 0.4m^3 CH_4/kg $COD_{removed}$ is at a high level compared with other authors' results (Table 3), which means that the conversion of COD to CH4 was efficient. However, the average permeate COD was relatively high due to the frequent VFA accumulation.

The poor methenogenic bacteria activity of municipal sludge which was used as inocculum may be one of the reasons of the poor biological performance of the reactor in this experiment. The addition of extra sludge could only increase the amount of biomass but not boost the individual activity. Although it is preferable to have higher sludge concentration in the reactor for better biological treatment performance, the sludge concentration had to be decreased due to the limitation of the cross-flow pump for pumping high concentration sludge (>40 g TSS/L). Therefore, 2.7L of sludge in the reactor was replaced with permeate to reduce solids concentration at the end of phase 3.

In anaerobic digestion, nitrogen is an important element for microorganism growth. The ration of carbon, nitrogen and phosphorus should be 500/5/1(Prazeres, Carvalho et al. 2012). Almost no ammonia could be observed in the effluent which is an indication of all the nitrogen fed to the reactor was either used for biomass growth or inorganic precipitation reactions such as struvite formation. However, the pH in the reactor was generally around 6.7 which was obviously not the optimum pH for struvite precipitation. Therefore the loss of a significant part of nitrogen with chemical precipitation is unlikely in this study, however this must be proven by conducting elemental mass balance calculations. The lack of nitrogen might be a limitation for biomass growth and treatment efficiency in this experiment. A continuous experiment following this one with increased nitrogen concentrations in the feed showed that adding nitrogen could improvereactor stability and biological performance. However, the results of that complementary experiment were not presented in this thesis.

4.2 Filtration Performance

4.2.1 Membrane Performance

According to the TMP changes with time (Figure 18), the whole operation period could be easily divided into 3 phases, which are exactly the same as those separated according to the

different actions taken in the reactor operation. At the end of the 2nd and 3rdphases, membrane was chemically cleaned with NaHOCl and citric acid, which was presented as a sharp drop of TMP in Figure 18.

In the 1st phase, a backwash was done to clean the cake layer accumulated on membrane in order to decrease the high TMP on day 24.The TMP dropped for a short term and then increased even more rapidly. This phenomenon indicated that the membrane was fouled due to the unstable operation such as changing the flux and CFV frequently to optimize at the beginning of the reactor. On day 28, the membrane was cleaned with chemicals. The flux was set to 5 L/m^2.h and the TMP was stable till the end of this phase. The details of chemical cleaning were explained in Section3.2.6.

After stopping regular sludge extraction, the 1st batch of crushed granular sludge with high methanogenic activity was added to the reactor. After the addition of extra sludge the critical flux was checked and it was measured as 22 L/m^2.h. This may be due to change of sludge particle size due to the addition of crushed granular sludge which still contains larger particles compared to the sludge inside the reactor. Therefore, the operational flux was set to 18 L/m^2.h accordingly in continuous filtration mode (Figure 18). The trend of TMP change is exactly as expected from a continuous filtration process, such as a linear increase of TMP followed by an exponential TMP jump. The increase of TMP indicated that cake layer accumulated on membrane and became compactedwith filtration time, eventually causing membrane fouling. After chemical cleaning, irreversible fouling was removed with oxidation of organic foulants by chlorine and dissolving of inorganic foulants at low pH of citric acid solution. Hence, TMP dropped to a very low level, which was only a little bit higher than that after the 1stchemical cleaning. One cause of this difference could be that this cleaning process was incomplete; another one, which is more credible, could be explained as the irrecoverable fouling which inevitably occurs on the long term operation and cannot be recovered or removed by any means.

The 3rd phase, characterized by a decrease of flux and huge increase of TMP, is also shown in Figure 18. Due to the operation at a flux as high as 18 L/m^2.h, membrane was easier to be fouled compared to operation at low fluxespecially with a relatively low cross-flow velocity which actually provides the shear force to prevent the deposition of the particles on the membrane. Another chemical membrane cleaning was conducted on day 99 and the operational flux was reduced to 10 LMH according to a new critical flux measurement. However, theseactionscould not stop TMP from increasing. Moreover, the sludge recirculation system in the membrane loop was not stable and easily blocked, and peristaltic pump tubes sticked together. The increase of suspended solids concentration and sludge viscosity after adding a new batch of granular sludge was supposed to be the main reason of these problems. Therefore, TSS concentration in the reactor was reduced manually in the next phase.

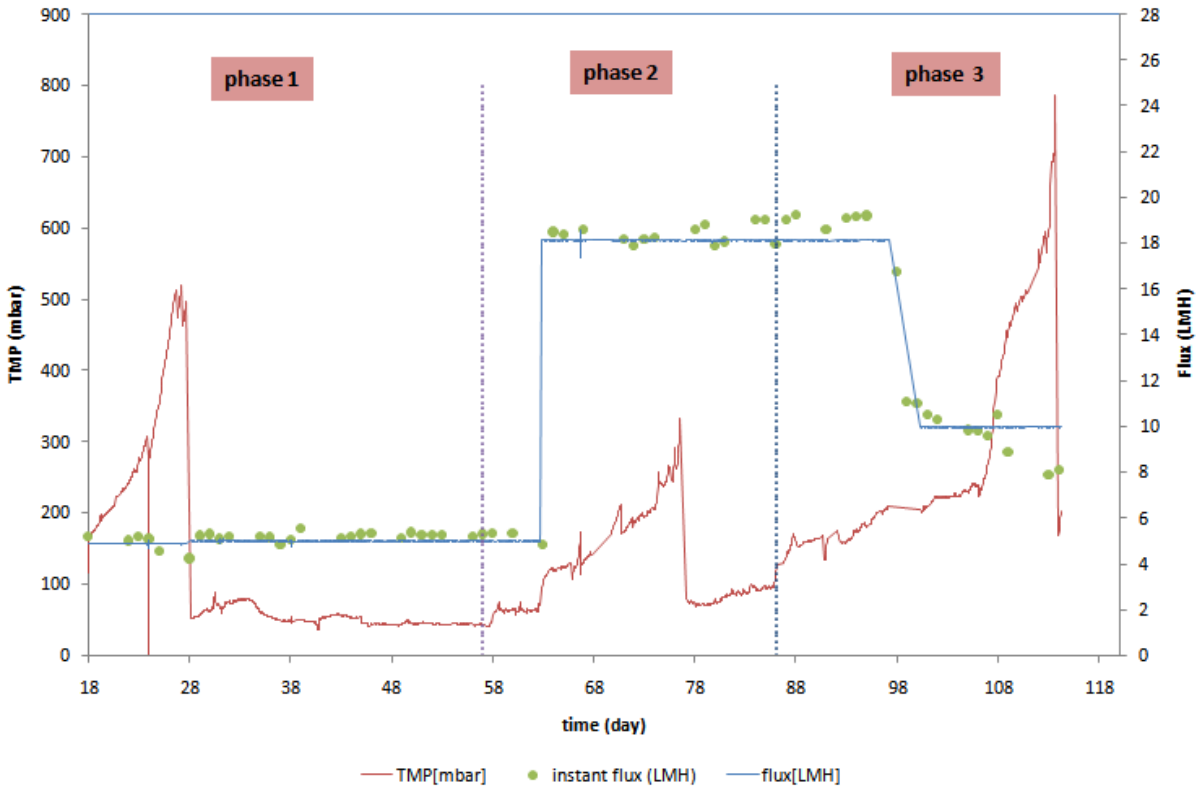


Figure 18TMP, flux and instant flux change during operation

The conversion of permeate flux (J) from the operation temperature 37 °Cto normal temperature 20 °C (J20) depending on the viscosity followsEq5. Permeability, which is described as flux per unit pressure (Eq6), could be converted to the normal temperature according to the same principle. The filtration resistance is correlated to the permeability (Eq7). After deconditioning the membrane with 500 ppm NaOCl solution, the initial tap water permeability of the membrane was measured as 101.4 L/m^2.h.bar and the membrane resistance was calculated as 3.54*10^{12}m^{-1}at 20 °C. The change of permeability and resistance are illustrated in Figure 19, respectively.

$$J = J_{20} \times 1.025^{(T-20)} \text{(Fan, Zhou et al. 2006)} \quad \text{Eq5}$$

$$\text{Perm} = \frac{J}{\text{TMP}} \text{(Jeison 2007)} \quad \text{Eq6}$$

$$R_M = \frac{\text{TMP}}{\eta.J} = \frac{1}{\eta.\text{Perm}} \text{(Jeison 2007)} \quad \text{Eq7}$$

η represents the permeate viscosity, which was regarded the same as pure water viscosity, 1.002 mPa.Sat 20°C.

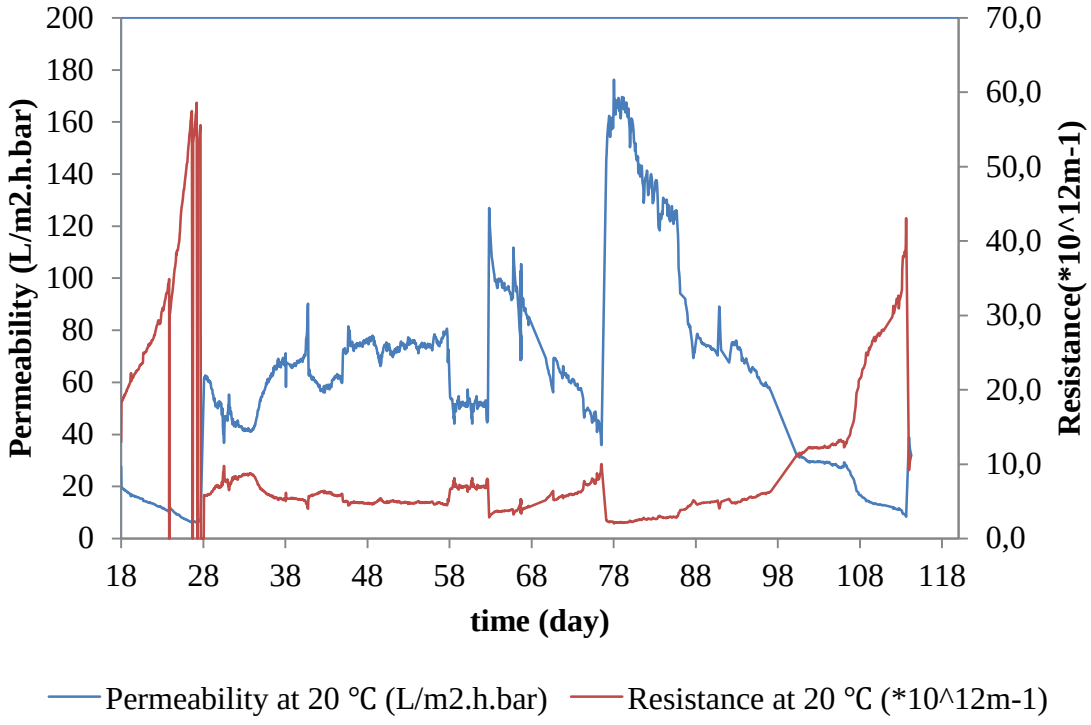


Figure 19 Permeability and resistance during operation

Table 13 Critical flux and operational flux

Day	CFV (m/s)	TSS (mg/l)	Critical flux (LMH)	Operational flux (LMH)
0	1	19600	10	10
0	0.5	21000	6	5
10	0.5	24500	8	5
45	0.5	29300	22	18
77	0.5	32100	30	18
91	0.5	41800	22	18
100	0.5	48200	14	10
112	0.5	48400	10	10
114	0.5	47200	14	10

Critical flux, firstly presented by Vandevivere(1999), is the key to identify the appropriate operatingflux for MBRs. In this study critical flux was measured according to flux step method(clech 2003)to determine the operational permeate flux. The criteria set to determine the critical flux was dP/dt<1 mbar min^{-1}.The results of critical flux experiments and operational flux are shown in Table 13. The improvement of critical flux after 45^{th} day may be due to the modification of sludge characteristics after crushed granular sludgeaddition

and the recovery of membrane permeability after chemical cleaning. Moreover, the significant decrease of critical flux on day 100 may be caused by the addition of NH_4Cl on day 94 and 95. As mentioned in Section 4.1, the acidogenic bacteria grow faster compared to methanogens in the system and these single cell organisms can play a role in membrane fouling due to cake compaction. Jeison (2007)showed that acidogenic bacteria grew as individual cells with high density through microscopy observations, which could be a great problem causing poor filtration performance and low operational fluxes. Additionally, the high level of TSS concentration in the 3rd phase could also affect membrane filtration. Consequently, both the sludge quantity and quality may be effective on membrane fouling.

4.2.2 Sludge Filtration Characterization

In addition to reactor operation and membrane performance following, the changes in sludge filterability were regularly monitored with additional parameters. Sludge supernatant filterability, capillary suction time (CST) and specific cake resistance (SCR) are measured to identify the sludge filterability under standard conditions. The trends of these parameters are shown in Figure 20, Figure 22 and Figure 23. The results from day 0 to 44 may not be representative since the reactor was unstable during the starting-up period.

The supernatant filterability provides information about the fouling propensity of soluble organic material such as SMP and colloidal particles in the sludge supernatant. From Figure 20 and Figure 21, it can be observed that supernatant filterability improved with the increase of SMP and decrease of colloidal COD (CCOD). The trend with CCOD was indeed in accordance with the expectations. However, the relation between SMP and filterability was somehow unexpected. In literature it is generally reported that the filterability was reduced with the accumulation of SMP inside the reactor(Pan, Su et al. 2010).

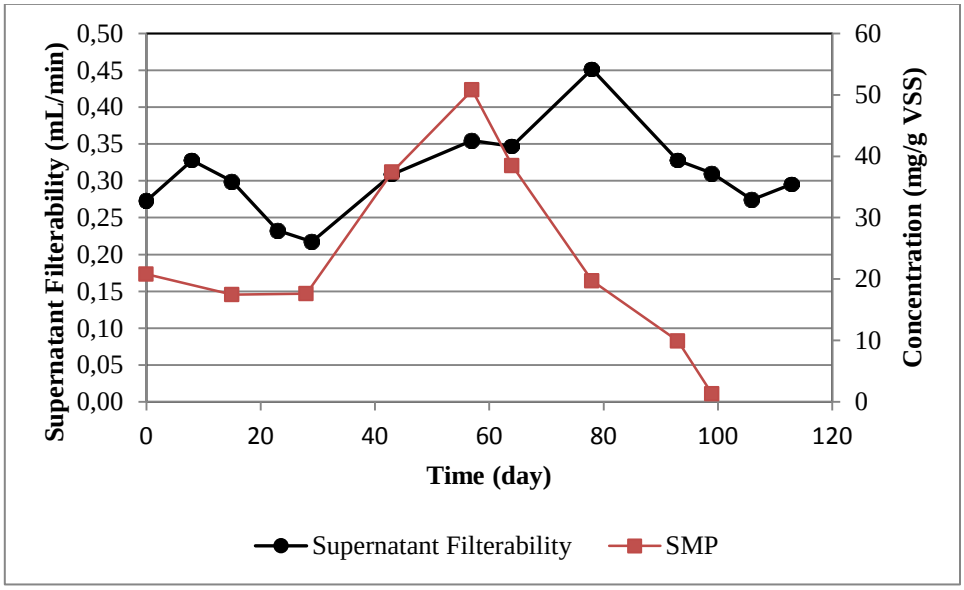


Figure 20 supernatant filterability and SMP

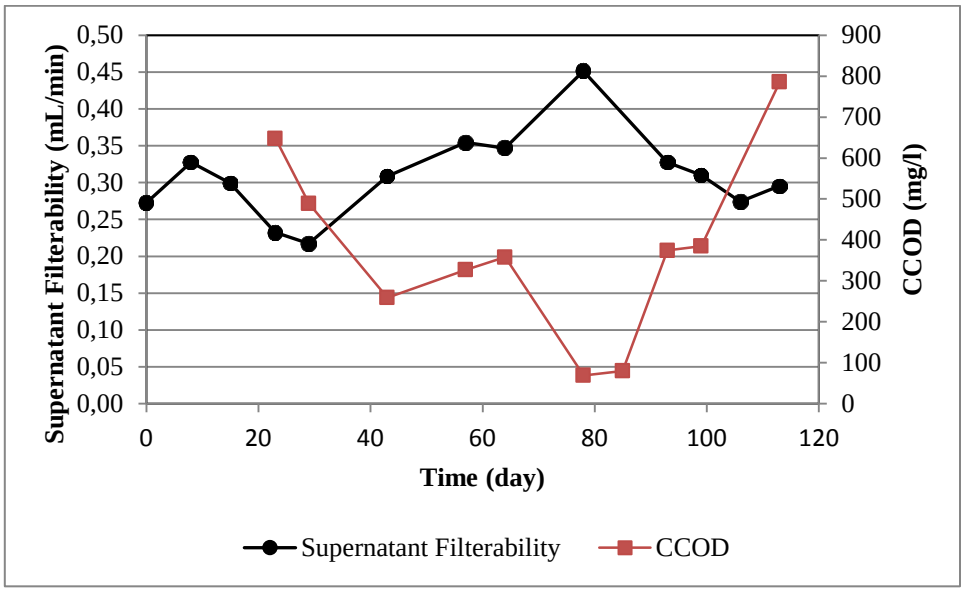


Figure 21 Supernatant filterability and CCOD

CST has been an indicator of sludge dewaterability since 1967 (Huisman and Kesteren 1998). In Figure 22, CST decreased with little fluctuation from day 44 to day 93. This trend may due to the increase of solids by adding crushed granular sludge. However, after the second addition of extra sludge, CST increased with TSS increase. Especially after the addition of NH_4Cl on day 94 and 95, CST was observed to increase by more than 100 seconds. It seems that the addition of ammonia and too high concentrations of TSS (>40 g/l) made sludge dewaterability worse.

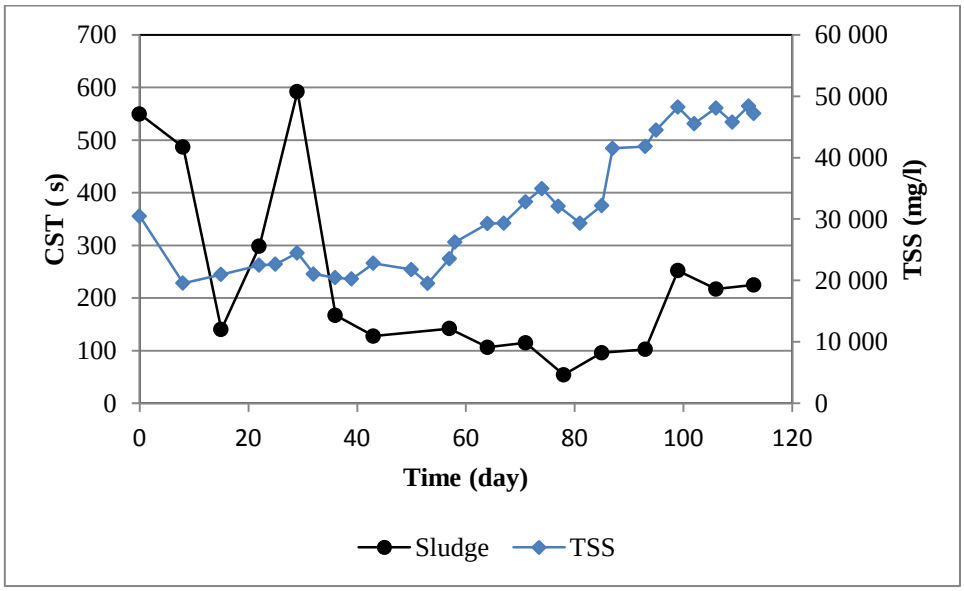


Figure 22capillary suction time (CST) and TSS

Cake layer formation is generally regarded as the most important fouling mechanism in AnMBRs (Jeison 2007). Therefore, SCR parameter gives an indication about the quality of cake accumulating on the membrane surface. According to the SCR calculations Eq1to Eq3, it could be concluded that a lower SCR means a compact and less porous cake layer formed with small size particles whereas a higher SCR indicates a cake layer with high porosity. In the first 2 phases, except for the unstable condition at the very beginning, SCR decreased greatly. In the 3rd phase, SCR didn't change much. The SCR in fact is directly related to the sludge quality in terms of particle size. The decreasing trend of SCR in this study is remarkable and somehow contradictory to the results reported by other authors which indicate that the mean particle size in AnMBR reactors decreases during long term operation due to the shear rate applied with pumps or gas recirculation(Jeison 2007). On the other hand the cross-flow velocity applied in this study was relatively lower compared to the other studies.

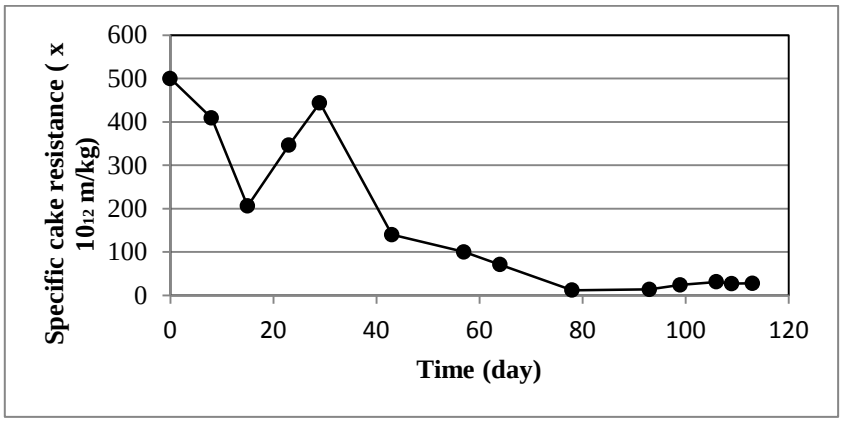


Figure 23specific cake resistance (SCR) change with time

In general, sludge filtration characteristics improved in phase 1 and phase 2, but reduced after the second addition of crushed granular sludge in phase 3. In the first two phases, sludge in the reactor was adapting to whey wastewater. The amount of biomass and the size of flocs increased with the addition of new sludge, hence the sludge got easier to filter. However, after the second addition, as shown in Table 10, solids concentrations were too high, which is not good for filterability. After the addition of NH_4Cl sludge filterability decreased.

Extracellular polymeric substances (EPS) are located at or outside the cell surface, which can sustain a cooperative and commutative surrounding for microorganisms to survive(Laspidou and Rittmann 2002). The main components of EPS are polysaccharides, proteins, nucleic acids, and humic substances (Drews, Leeb et al. 2006). Soluble microbial products (SMP) are soluble components released by cells, which could be a major part of COD in the effluent (Laspidou and Rittmann 2002). SMP contain polysaccharides, proteins, humic and fulvic acids, nucleic acids, amino acids(BARKER and STUCKEY 1999). There are two independent mechanisms of SMP production: erosion of floc-associated EPS and decay of active cells(Menniti and Morgenroth 2010).

Laspidou et al.(2002) presented a critical review of the relationships among EPS, SMP and active and inert biomass based on the different or even contradictory opinions by former researchers. In this review, a unified model was sketched (Figure 24).

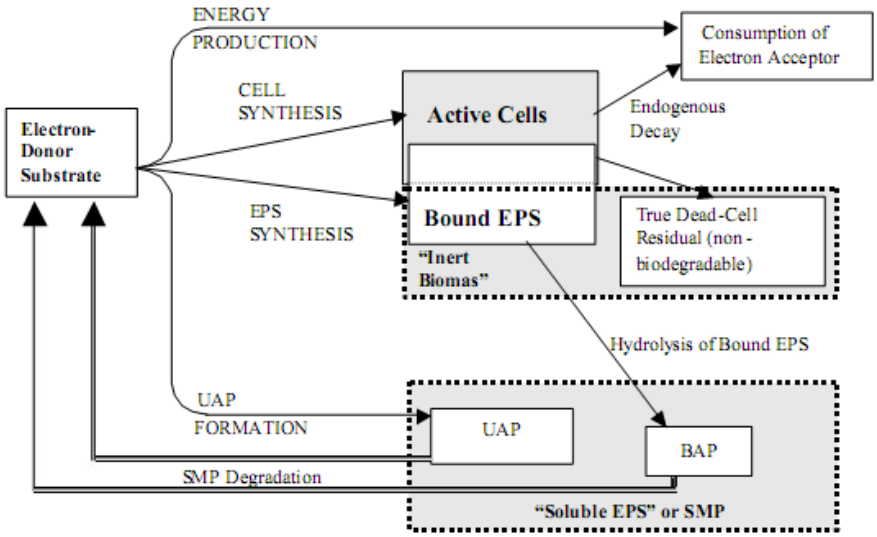


Figure 24 Schematic representation of the unified model for active biomass, EPS, SMP, and inert biomass (Laspidou and Rittmann 2002)

In this study, for simplification, only proteins and polysaccharides were measured to indicate the concentration of SMP and EPS. Eq8,Eq9 andEq10 show the conversion.

$$\text{SMP} = \text{Soluble Proteins} + \text{Soluble Polysaccharides} \quad \text{Eq8}$$

$$\text{EPS} = \text{Total Proteins} + \text{Total Polysaccharides} \quad \text{Eq9}$$

$$\text{Bound EPS} = \text{EPS} - \text{SMP} \quad \text{Eq10}$$

For SMP measurement, 5 samples were tested in the first phase and 2 samples in the following phases. And for EPS, 2 samples were checked in each phase. The concentrations of SMP, bound EPS, together with VLR are shown in Figure 25 and Figure 26. Soluble polysaccharides concentration was very low (<10 mg/g VSS) during the whole study. The variation of SMP was mainly due to changes in soluble protein concentrations. During the unstable first phase, SMP increased due to soluble protein accumulation. But in the second and third phases, SMP concentration showed a trend of decreasing even though VLR increased. On the other hand, bound EPS increased gentlely. Bound polysaccharides accumulated faster than bound protein in the reactor. After the addition of NH_4Cl on day 94 and 95, bound EPS showed a sharp increase. Maybe the boost of microorganism growth led to this result.

Mikkelsen (2002) stated that bound EPS accumulation was good for the stability of sludge floc structure thereby the dewaterability and filterability, meanwhile, some other authors

found it in the opposite way(Drews, Leeb et al. 2006). Houghton (2001) found optimum levels of EPS to get maximum sludge dewaterability at 10 mg EPS/g SS for digested sludge. However, from the results of this study, there is no direct connection of EPS, SMP and filterability.

Both EPS and SMP are important substances related membrane fouling (Drews, Leeb et al. 2006), The effects of EPS and SMP on membrane fouling are always on debate. Lin (2010) found that SMP and EPS might act as "glue" to form an apparent slime layer, which could prompt fouling. Charfi (2012)stated that bound EPS have a positive effect on flocculation. When large flocs are accumulating, surface fouling of membrane would occur. On the other hand, when bound EPS concentration is low, the dispersed microorganisms could enter membrane pores and lead to pore constriction fouling.

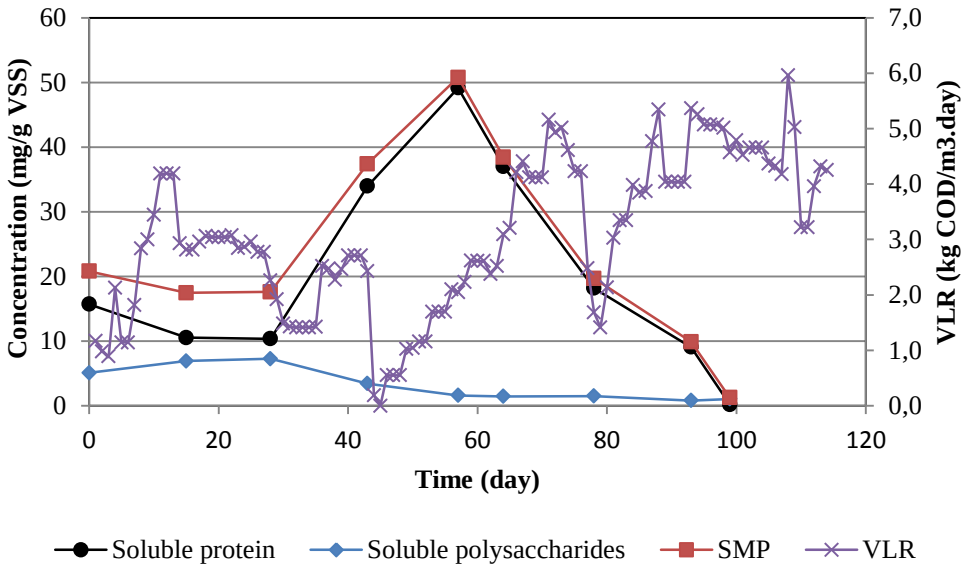


Figure 25SMP concentrationin sludge and VLR

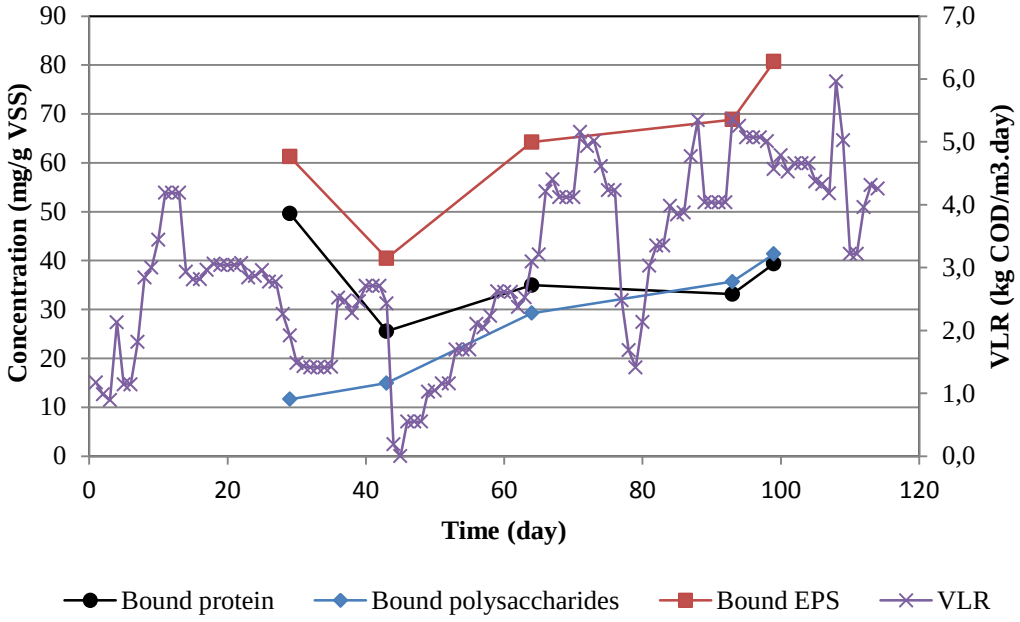


Figure 26BoundEPS concentration in sludge and VLR

4.2.3 Membrane cleaning

Membrane cleaning was done when the membrane fouled indicated with a high TMP, and when new operation was conducted, which needed a clean membrane to get more representative results. In this study, membrane was cleaned 4 times: once in phase 1 and 2 and twice in phase 3 (Figure 27). The cleaning solutions, water, NaOCl (500ppm) and citric acid (1%) were used successively to remove cake layer, organic and inorganic deposits, respectively. After the whole cleaning procedure, the membrane permeability increased to varying degrees. On day 77 and 99, the membrane was operated at higher permeate fluxes (Table 14), hence, the permeability before cleaning were comparatively higher. During the first 3 cleaning process, permeability increased higher by water rinsing than by NaOCl cleaning. However, the 4th one showed the opposite trend. This result indicates that cake layer played more important role than organic depositin membrane fouling in the first 3 cases, and the opposite in the last one. In all processes except for the 3rd one, citric acid removed a high amount of inorganic fouling.Except for the cleaning efficiency, sludge property determined the permeability as well. The highest permeability shown in Figure 27 may be due to the high sludge supernatant filterability (Table 14) on that day. Overall, the permeability at 114th day was the worst. It indicated that more irrecoverable fouling was formed. The membrane resistance trend in Figure 28 shows the same result gotten from permeability measurements. The high resistance during the first cleaning may be due to the poor sludge filterability, and the high values of the last time may due to the irrecoverable fouling of membrane.

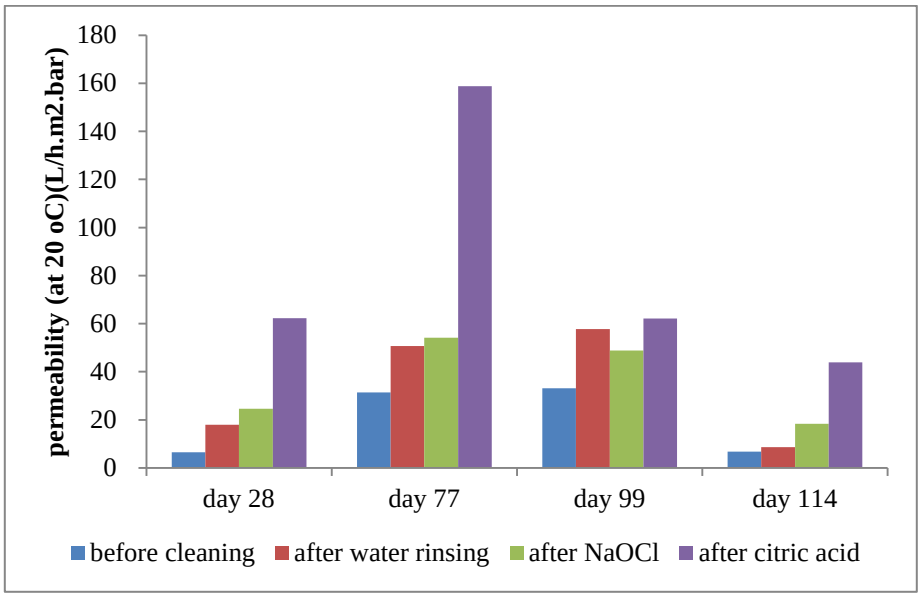


Figure 27 Permeability change in membrane cleaning

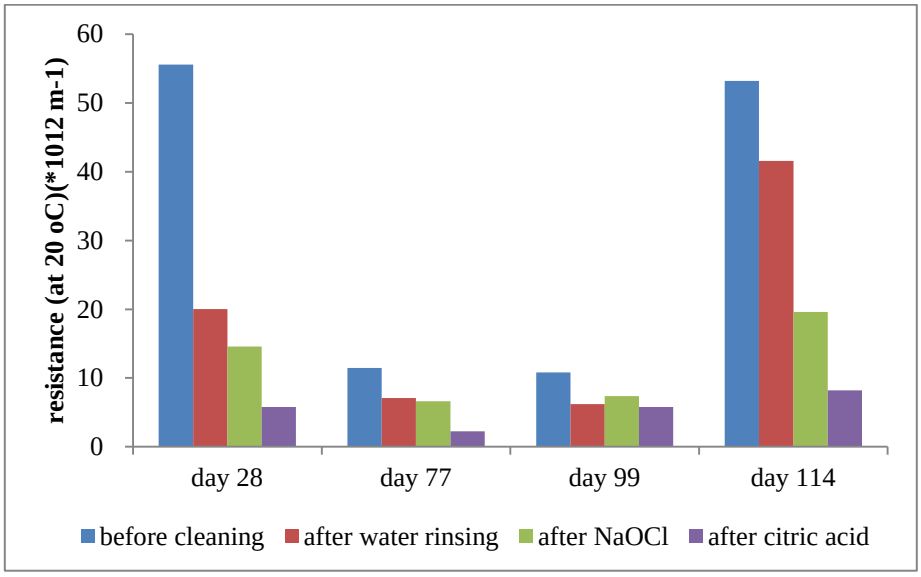


Figure 28 Membrane resistance change in membrane cleaning

Table 14 TMP, flux and permeability and membrane resistance in membrane cleaning

Procedure	Parameter	Unit	Day			
			28	77	99	114
before cleaning	TMP	mbar	496	380	220	786
	instant flux	L/h.m2	4.9	18.1	11.1	8.1
	permeability (at 20 ^{o}C)	L/h.m^{2}.bar	6	31	33	7
	resistance (at 20 ^{o}C)	*10^{12} m^{-1}	56	11	11	53

after water rinsing	TMP	mbar	180	235	130	660
	instant flux	L/h.m2	4.9	18.1	11.4	8.7
	permeability (at 20 ^{o}C)	L/h.m2.bar	18	51	58	9
	resistance (at 20 ^{o}C)	*10^{12} m^{-1}	20	7	6	42
after NaOCl	TMP	mbar	134	220	150	340
	instant flux	L/h.m2	5.0	18.1	11.1	9.5
	permeability (at 20 ^{o}C)	L/h.m2.bar	25	54	49	18
	resistance (at 20 ^{o}C)	*10^{12} m^{-1}	15	7	7	20
after citric acid	TMP	mbar	53	75	120	155
	instant flux	L/h.m2	5.0	18.1	11.4	10.3
	permeability (at 20 ^{o}C)	L/h.m2.bar	62	159	62	44
	resistance (at 20 ^{o}C)	*10^{12} m^{-1}	6	2	6	8
supernatant filterability		mL/min	0.22	0.45	0.31	0.3

One ICP analysis was conducted on the collected NaOCl solution and citric acid after cleaning on day 99. The membrane was severe fouled on day 99. From the permeability change shown in Figure 27, the membrane could not be recovered totally by cleaning. Hence the results of ICP analyses (Table 15) are not representative for the exact concentrations of inorganic foulants. But they are indicators of foulants composition. Ferrum and Calcium precipitation were the major causes of fouling according to the difference concentrations in NaOCl solution and citric acid. The high amount of Sodium was from the cleaning solution itself after NaOCl cleaning.

Table 15 ICP analyses of cleaning solutions

Sample	Unit	Fe	Ca	K	Mg	Mn	Cu	Zn	Al	S	Total P	Na
NaOCl solution	mg/l	0.31	6.7	43.9	1.4	<0.002	0.09	0.01	0.02	0.8	1.3	205
Citric acid	mg/l	34.7	23	46.8	2	0.02	0.38	0.12	0.16	< 0.6	9.4	93.5

4.2.4 Membrane autopsy

Since the membrane was heavily fouled after the experiment, the membrane samples after each cleaning process were sent to have SEM (Scanning Electron Microscopy) and EDX (Energy Dispersive X-ray) analyses to investigate the reasons of fouling.

The 5 samples sent for SEM analysis are list in Table 16 and the autopsy pictures of them are shown in Figure 29 to Figure 33. The fluffy layer upon the dark black layer shown in Figure 29 is the so-called cake layer in this study. It was easily removed by water rinsing,

since this layer is totally disappeared in Figure 30. The dark black layer appeared in each figure may be a density and compacted layer which caused fouling of membrane. This layer cannot be removed by water, NaOCl and citric acid. The cracks in Figure 31, Figure 32 and Figure 33 were due to the drying process before this analysis. Some crystals shown in Figure 31 were the inorganic precipitates like $Ca_3(PO_4)_2$ and $CaCO_3$, which could not be removed by NaOCl but could be removed by citric acid since no such crystals appearing in Figure 32. Those white spots in Figure 33 may be precipitates that cannot be removed by both NaOCl and citric acid, like Fe_2S_3 and CuS. This deduction will be explained in the following EDX analysis section.

Table 16 Membrane samples for SEM analysis

Sample 1	Membrane with cake layer before membrane cleaning
Sample 2	Membrane without cake layer after rinsing with water
Sample 3	Membrane after NaOCl
Sample 4	Membrane after 1% citric acid and soaking for 1 hour
Sample 5	Membrane after NaOCl and then 1% ciric acid

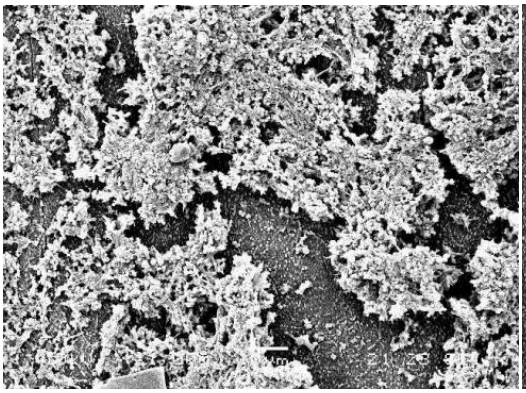


Figure 29 Membrane with cake layer

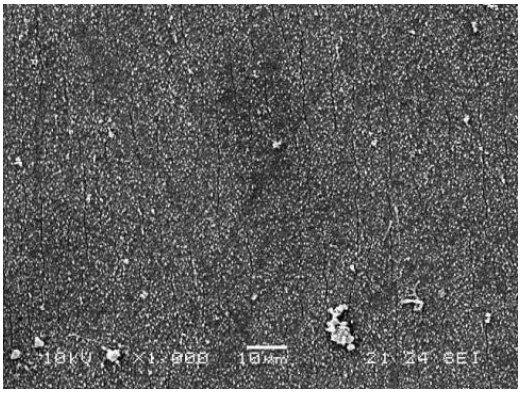


Figure 30 Membrane without cake layer after water rinsing

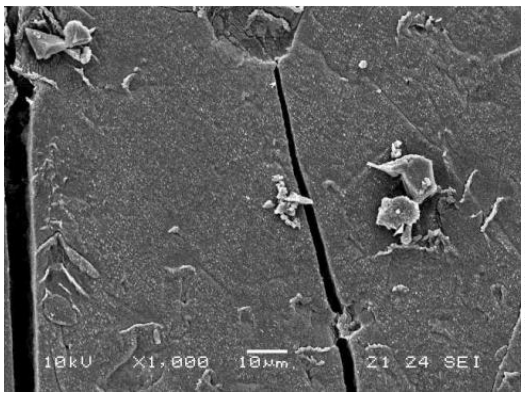


Figure 31 Membrane after NaOCl

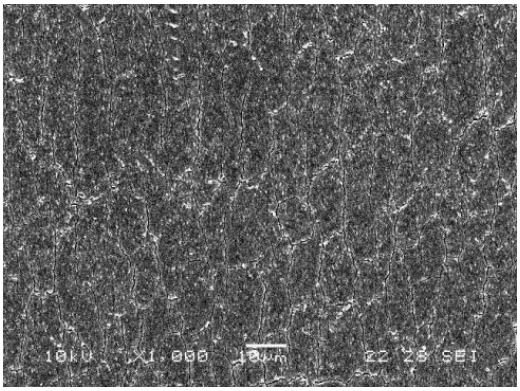


Figure 32 Membrane after 1% citric acid soak for 1hour

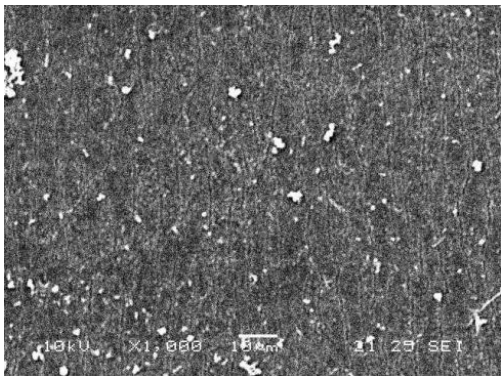
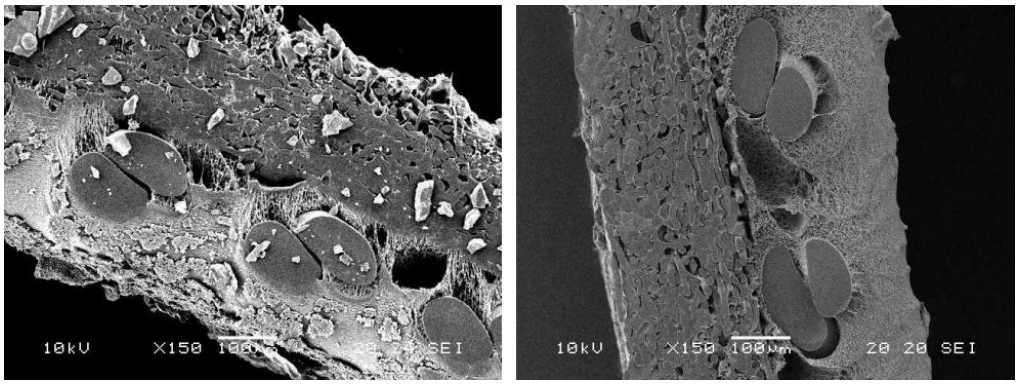


Figure 33 Membrane after NaOCl and 1% citric acid

To make a better interpretation of the membrane fouling, the cross sections of a fouled membrane and a new one were both analyzed by SEM. The comparison of them is shown in Figure 34. It is obvious that the membrane was totally blocked after this experiment. There were some crystals even observed in the supporting material of this membrane.



(a) (b)

Figure 34The cross section of membrane: (a) fouled membrane; (b) new membrane

The EDX analysis provided the elemental composition of the membrane surface. In this case, the elemental composition of fouling can be identified. The results of EDX analysis are shown in Table 17. The order of samples is the same as that of SEM analysis inTable 16.For all the values of elementals, the uneven distribution should be taken into consideration. It is reasonable to have distinctive values since the detective points were random. Fluorine was detected in sample 2,4,5, which indicated that the dense dark black layer was very thin and had not a clear boundary with the membrane, since the only source of fluorine is the PVDF membrane itself.Almost no Calcium and Phosphorus was detectedin sample 4 and 5 after soaking in citric acid. Hence, Calcium precipitation like $Ca_3(PO_4)_2$ and $CaCO_3$ could be easily removed by citric acid in this case. Ferrum, Copper and Sulfur appeared in all the five samples. Both NaOCl and citric acid could not remove them. The obstinate precipitation could be Fe_2S_3 and CuS.

Table 17 Elemental composition of fouling by EDX analysis

Parameter	C	N	O	F	Mn	S	Ca	P	Fe	K	Cu
Sample 1	55	-	25	-	5	4	4	4	4	-	5
Sample 2	27	-	11	43	-	5	2	-	6	-	7
Sample 3	22	-	26	-	-	7	20	11	7	-	8
Sample 4	27	-	-	42	-	9	1	-	9	-	12
Sample 5	29	-	-	44	-	8	-	-	7	-	12
New membrane inner surface	68	6	3	23	-	-	-	-	-	-	-

The results from SEM and EDX analyses showed that the membrane was fouled by inorganic precipitation. 1% citric acid was effective at Calcium precipitation removal but cannot remove Fe_2S_3 and CuS. To improve the cleaning efficiency, higher percentage of citric acid or more strong acid should be applied. To prolong the soaking time could also be an alternative.

5. Conclusion

This study investigated a new method, anaerobic membrane bioreactor, to treat cheese whey.In this operation, the highest VLR applied was 4.5 kg COD/m^3.day, and the highest flux could be achieved was 18 LMH. The average COD removal efficiency is as high as 96%.

Compared with other anaerobic cheese whey treatment conducted by other people, this result is not satisfying. The main reason was the poor activity of municipal sludge. Adding crushed granular sludge was proven to be not efficient to boost the biomass activity. In addition, the high concentration of TSS that brought by this addition made membrane filtration worse. An alternative to improve the sludge quality could be adding nitrogen source. The following experiment after this study showed nitrogen was the limiting elemental of biomass growth. And it was also proved that a higher VLR could be applied after increase the nitrogen concentration of whey.

Inorganic precipitation was the main cause of fouling. The cleaning process cannot remove the dense layer on the membrane. Stronger acid or longer soaking time could be applied to improve the cleaning protocol for better results. On the other hand, the sources and distribution of inorganic elementals were not very clear. Mass balance of these elementals should have been done for better interpretation.

Even though this study was not a completed one ending with the optimum biological and filtration performance, it was worthwhile to explore the alternatives to treat cheese whey in a more efficient and economical way.

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