

**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY**

**DELFT UNIVERSITY OF TECHNOLOGY ★ GRADUATE SCHOOL OF CIVIL
ENGINEERING AND GEOSCIENCES**

**TREATMENT OF INDUSTRIAL WASTEWATERS BY
ANAEROBIC MEMBRANE BIOREACTORS:
IMPLICATIONS OF SUBSTRATE CHARACTERISTICS**

Ph.D. THESIS

Recep Kaan DERELİ

Department of Environmental Engineering

Environmental Sciences and Engineering Programme

OCTOBER 2015

**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY**

**DELFT UNIVERSITY OF TECHNOLOGY ★ GRADUATE SCHOOL OF CIVIL
ENGINEERING AND GEOSCIENCES**

**TREATMENT OF INDUSTRIAL WASTEWATERS BY
ANAEROBIC MEMBRANE BIOREACTORS:
IMPLICATIONS OF SUBSTRATE CHARACTERISTICS**

Ph.D. THESIS

**Recep Kaan DERELİ
(501052706)**

Department of Environmental Engineering

Environmental Sciences and Engineering Programme

**Thesis Advisor: Prof. Dr. Izzet OZTURK
Co-Advisor: Prof. Dr. Jules B. van LIER**

OCTOBER 2015

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

**DELFT TEKNOLOJİ ÜNİVERSİTESİ ★ İNŞAAT MÜHENDİSLİĞİ VE YER
BİLİMLERİ ENSTİTÜSÜ**

**ENDÜSTRİYEL ATIKSULARIN ANAEROBİK MEMBRAN
BİYOREAKTÖRLER İLE ARITIMI: SÜBSTRAT
KARAKTERİZASYONUNUN ETKİSİ**

DOKTORA TEZİ

**Recep Kaan DERELİ
(501052706)**

Çevre Mühendisliği Anabilim Dalı

Çevre Bilimleri ve Mühendisliği Programı

**Tez Danışmanı: Prof. Dr. İzzet OZTURK
Eş Danışman: Prof. Dr. Jules B. van LIER**

EKİM 2015

Recep Kaan Dereli, a Ph.D. student of ITU Graduate School of Science Engineering and Technology student ID 501052706, successfully defended the thesis/dissertation entitled “TREATMENT OF INDUSTRIAL WASTEWATERS BY ANAEROBIC MEMBRANE BIOREACTORS: IMPLICATIONS OF SUBSTRATE CHARACTERISTICS”, which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor: **Prof. Dr. Izzet OZTURK**
Istanbul Technical University

Co-advisor: **Prof. Dr. Jules B. van LIER**
Delft University of Technology

Jury Members: **Prof. Dr. Isabel ARENDS**
Delft University of Technology

Prof. Dr. Mehmet KITIS
Suleyman Demirel University

Prof. Dr. Patricia OSSEWEIJER
Delft University of Technology

Prof. Dr. Timo HEIMOVARAA
Delft University of Technology

Dr. Henri SPANJERS
Delft University of Technology

Date of Submission : 08 September 2005
Date of Defense : 20 October 2015

*To my dear wife, Emine,
and my family*

FOREWORD

Life is a collection of decisions you take. I have never taken life so serious but it was the most difficult decision for me to move Netherlands to restart my PhD research from scratch. Not because of its unknown outcomes but to leave my most beloved ones for such a long time. In the end, it turned out to be the most challenging and successful adventure of my life that I'll never regret. When I look back to this 4 years period, many people have been involved in my research and personal life. Without their help, it would never be possible to realize this dissertation.

First, I would like to thank my two promoters, Prof. Izzet Ozturk (ITU) and Prof. Jules van Lier (TUD) for their support and guidance throughout my PhD. I feel myself very lucky to have two very reputable researchers at my back that I can always consult when I need. Prof. Ozturk: thanks for giving me responsibility to make my own decision, for your unconditional support and for all the things you thought me. Prof. van Lier: thank you for guiding me in to scientific world, for always questioning results, and for your prompt and valuable feedback. Without your guidance, I would be definitely lost in my research.

I should also express my gratitude to Prof. Cumali Kinaci (ITU) who was the originator of the idea to pursue a PhD in abroad and devoted himself to our cause. I'll be lifelong in debt to Prof. Izzet Ozturk, Prof. Ismail Koyuncu (ITU), Tincel Foundation and TUBA for the funding I received at the initial stages of my PhD studies.

This research was carried out in cooperation with Biothane Systems International (the Netherlands) and Veolia Water Systems (France) under MEMTHANE® development program. Without their funding, it would be impossible to accomplish this research study. The experimental part of this study was completed in Biothane R&D laboratory. A sincere "thank you" goes to all Biothane staff for their friendship, kindness and helpfulness which made my life in the company much easier. Thank you all for providing such a stimulating and enjoyable environment. Even though I worked very hard, I enjoyed every single moment of it. Besides, it was a great opportunity for me to conduct research in cooperation with one of the most respectable companies in anaerobic wastewater treatment market. I will always be proud of being a humble member of the team which took part in the development of MEMTHANE®. I can say that I was in the right place at the right time.

In particular, I would like to express my sincere appreciation to some people; Jan Pereboom, Barry Heffernan, Frank van der Zee, Aurelie Grelot, Paul Steevenweg and Rewin Pale, from Biothane.

I am very grateful to Jan for his support during my adaptation period to the Netherlands, Dutch culture and to the company. I will never forget the international Christmas dinners with your nice family at your home. It was a lot of fun with the delicious food, gifts and Sinterklaas poems.

Thank you Barry for always being available to talk, your understanding and trust to me, for your practical solutions and critical decisions which some of them were the turning points of this study. I still remember your words when I was depressed with thinking how I am going to operate those reactors "Don't worry Kaan, in a few months time you'll be flying these things". It happened exactly as you said when the sludge was erupting through the tube up to the ceiling. Thank you for helping me to recover the sludge from the ground several times.

I know it drives you mad but thank you, Frank, for reading my extra-long manuscripts, for your prompt comments, sometimes quite harsh criticism and extraordinary sense of humor. I would like to thank you for sharing your knowledge and expertise with me. For me you are a real scientist, always questioning and reasoning, that I will always admire and take as an example. You also have a very important role in speeding up my experimental studies by ordering all the instruments for the AnMBR set-ups before I came to the Netherlands.

Aurelie, thanks for your friendship, always backing me up when I need and teaching me a lot about membranes. You and me had very hard times in the lab and had to overwork sometimes but I think what we achieved and the amount of information we produced in such a short time is admirable. Without your experience and help it would be impossible for me to operate the membranes and to establish all those filterability tools used in this study.

During the course of my research, I received great help and support for analyses from Paul Steewenweg and Revien Pale, the technical people at Biothane lab. Thanks Paul for sharing your office with me and making me addicted to coffee “Wiener Melange”. Without your help Revien, it would be impossible for me to produce that much data. I always enjoyed our coffee breaks and talks about Dutch weather, food, politics and life. Without you guys, it would be impossible to create such a synergy and enjoyable work environment. Additionally, thanks to Dutch radio stations “Sky Radio, Q-music and Radio Veronica” for playing the same songs every single day for 2.5 years, and making our life in the lab even more fun by tormenting us.

I would like to thank my Spanish, Portuguese, Greek, Chinese, Romanian and French friends; Lefki, Xiaofei, Ana, Monica, Joao, Jose, Elisa, Raluca, Yago, Ruben and Hakeem, who came as trainees to Biothane lab and contributed to my research from all over the world. Together, we created a very good spirit and an international atmosphere which I enjoyed a lot. I hope our paths will cross once again somewhere in time.

A few words for my companions in this PhD journey, Evren and Hale. You have always been brother and sister to me. If you were not by my side, it would be impossible for me to find the strength and courage to start this journey. Life will be as hard and unjust as it has always been but we’ll stand together to overcome the problems. I also owe a big “Thank you” to my best friend Salih, his wonderful wife Nazli and Tilburg gang. I always felt at home when I was with you and collected very nice memories.

I want to express my gratitude to Mehmet Ağabey, owner of “Erciyes Slagery” in Delft, who helped me, Evren and Hale during this long PhD period. I feel very luck to know that there are still some people on Earth with a pure golden heart like him and his family.

I can’t forget to thank my colleagues from TUDelft Sanitary Engineering Section; Maria, Dara, Xuedong, Julian, Ran, Troy, Haoyu, Annelies who I enjoyed talking to. In particular, I would like to thank Maria for her valuable advices about both research and life. Then, my compliments go to Mieke Hubert and Jennifer Duiverman for their support in all the administrative and bureaucratic stuff.

The final thanks go to my family, my parents and brother, for their unconditional support and encouragement to pursue my academic career. A few words for my dear wife, Emine: Thank you for your understanding and sacrificing your precious years for me. We have been through very hard times, but I have never stopped loving you for a single moment. This dissertation would be meaningless to me if I didn’t have you in my life.

Thank you all!

October 2015

Recep Kaan DERELI
Engineer

TABLE OF CONTENTS

	<u>Page</u>
FOREWORD	ix
TABLE OF CONTENTS	xi
ABBREVIATIONS	xv
SYMBOLS	xvii
LIST OF TABLES	xix
LIST OF FIGURES	xxi
SUMMARY	xxiii
ÖZET	xxv
1. INTRODUCTION	29
1.1 Background	29
1.2 Problem Statement	32
1.3 Research Objectives	34
1.4 Thesis Outline.....	36
2. LITERATURE REVIEW	39
2.1 Introduction	39
2.2 Limitations to Traditional, Granular Sludge Based, Anaerobic Treatment Technology	40
2.2.1 High SS content	41
2.2.2 FOG content.....	41
2.2.3 Thermophilic conditions	42
2.2.4 Toxicity.....	42
2.2.5 High salinity.....	43
2.2.6 OLR and HRT shocks.....	44
2.2.7 Calcium scaling.....	44
2.3 AnMBR Applications for the Treatment of Industrial Wastewaters.....	46
2.3.1 Lab-scale.....	48
2.3.1.1 Temperature	51
2.3.1.2 Sludge retention time (SRT)	52
2.3.1.3 Organic loading rate (OLR)	53
2.3.1.4 Shear rate.....	54
2.3.1.5 Substrate composition	56
2.3.1.6 Membrane properties	57
2.3.1.7 Cake layer.....	58
2.3.2 Full and pilot scale	59
2.4 Problems Encountered and Future Perspectives.....	62
2.5 Conclusions	64
3. EFFECT OF LIPIDS ON BIOLOGICAL PERFORMANCE	65
3.1 Introduction	65
3.2 Materials and Methods	67
3.2.1 Wastewater characterization	67
3.2.2 Reactor configuration and operation.....	68

3.2.3 Experimental methods	69
3.3 Results	71
3.3.1 Reactor Performance.....	71
3.3.2 COD mass balance	74
3.3.3 LCFA precipitation with calcium and magnesium	76
3.3.4 LCFA accumulation.....	78
3.3.5 Sludge activity loss and restoration	80
3.4 Discussion.....	83
3.5 Conclusions	85
4. EFFECT OF SRT AND LIPIDS ON FILTRATION PERFORMANCE	87
4.1 Introduction	87
4.2 Materials and Methods	90
4.2.1 Reactor set-up and operation	90
4.2.2 Experimental methods	91
4.2.2.1 Analytical methods.....	91
4.2.2.2 Sludge filtration characteristics	92
4.2.2.3 Sludge relative hydrophobicity	93
4.2.2.4 Membrane cleaning	93
4.2.2.5 Membrane autopsy	94
4.2.3 Wastewater characterization	95
4.2.4 Statistical analysis	95
4.3 Results	95
4.3.1 Treatment performance	95
4.3.2 Long term filtration performance.....	96
4.3.3 Sludge characteristics.....	99
4.3.3.1 Particle size distribution	99
4.3.3.2 EPS and SMP	101
4.3.3.3 Relative hydrophobicity	101
4.3.4 Sludge filterability	102
4.3.4.1 Specific resistance to filtration.....	102
4.3.4.2 Capillary suction time	103
4.3.4.3 Supernatant filterability.....	105
4.3.5 Relationship between sludge characteristics and real membrane performance	107
4.3.6 Membrane cleaning.....	110
4.3.7 Membrane autopsy.....	112
4.3.7.1 FT-IR analysis of membrane foulants	112
4.3.7.2 SEM-EDX	114
4.4 Discussion.....	116
4.5 Conclusions	121
5. EFFECT OF SUBSTRATE ACIDIFICATION DEGREE	123
5.1 Introduction	123
5.2 Materials and Methods	125
5.2.1 Reactor setup and operation.....	125
5.2.2 Experimental methods	126
5.2.2.1 Analytical methods.....	126
5.2.2.2 Sludge filterability	127
5.2.3 Substrate characteristics.....	127
5.3 Results and discussion	129
5.3.1 Biological performance.....	129

5.3.2 Sludge characteristics	131
5.3.2.1 Particle size distribution.....	131
5.3.2.2 Soluble microbial products	133
5.3.2.3 Relative hydrophobicity	133
5.3.3 Sludge filterability	135
5.3.3.1 CST and SRF	135
5.3.3.2 Supernatant filterability.....	137
5.3.3.3 Critical flux	137
5.3.4 Single stage versus two-stage AnMBRs.....	138
5.4 Conclusions	139
6. EFFECT OF NITROGEN DEFICIENCY	141
6.1 Introduction	141
6.2 Materials and Methods	144
6.2.1 Reactor setup and operation.....	144
6.2.2 Experimental methods	144
6.2.2.1 Analytical methods	144
6.2.2.2 Substrate characteristics	145
6.2.2.3 Sludge filterability.....	145
6.3 Results and Discussion.....	145
6.3.1 Biological performance.....	145
6.3.2 Sludge characteristics	149
6.3.2.1 Particle size distribution.....	149
6.3.2.2 Soluble microbial products	150
6.3.3 Sludge filterability	151
6.4 Conclusions	156
7. CONCLUSIONS	157
7.1 Introduction	157
7.2 Conclusions from the Various Research Steps.....	157
7.2.1 Effect of SRT	157
7.2.2 Effect of substrate composition	158
7.2.2.1 Lipids	158
7.2.2.2 Degree of substrate acidification.....	159
7.2.2.3 Nitrogen limitation	159
7.3 Perspectives and Recommendations for Future Research.....	160
REFERENCES.....	165
CURRICULUM VITAE.....	183

ABBREVIATIONS

ADUF	: Anaerobic digestion ultrafiltration
AF	: Anaerobic filter
AnMBRR	: Anaerobic membrane bioreactor
ANOVA	: Analysis of variance
AOX	: Absorbable organic halides
BCA	: Bicinchoninic acid
CF	: Critical flux [$\text{L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$]
CFD	: Computational fluid dynamic
COD	: Chemical oxygen demand
CST	: Capillary suction time [second]
CSTR	: Completely stirred tank reactor
CUMAR	: Cross-flow ultrafiltration membrane anaerobic reactor
DFCm	: Delft filtration characterization method
EGSB	: Expanded granular sludge bed
EPS	: Extracellular polymeric substances
F:M	: Food to mass ratio
FISH	: Fluorescence in situ hybridization
FOG	: Fat, oil and grease
FT-IR	: Fourier transform infra-red spectrometer
GC	: Gas chromatograph
HEMA	: 2-hydroxyethyl methacrylate
HRT	: Hydraulic retention time [day]
IC	: Internal circulation
LCFA	: Long chain fatty acid
MARS	: Membrane anaerobic reactor system
MATH	: Microbial adhesion to hydrocarbons
MBR	: Membrane bioreactor (generally aerobic)
MF	: Microfiltration
MLSS	: Mixed liquor suspended solids
MWCO	: Molecular weight cut-off [kDa]
OLR	: Organic loading rate [$\text{kg COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$]
P:C	: Protein to carbohydrate ratio
PES	: Polyethersulfone
PHA	: Polyhydroxyalkanoate
PLC	: Programmable logic controller
PN:PS	: Protein to polysaccharide ratio
PSD	: Particle size distribution
PVDF	: Polyvinylidene fluoride
RH	: Relative hydrophobicity
RPM	: Rotations per minute
SBR	: Sequencing batch reactor
SCOD	: Chemical oxygen demand of soluble organics

SEM-EDX	: Scanning electron microscopy with energy dispersive X-ray spectroscopy
SF	: Supernatant filterability [$\text{mL} \cdot \text{min}^{-1}$]
SMA	: Specific methanogenic activity [$\text{mg CH}_4\text{-COD} \cdot \text{g VSS}^{-1} \cdot \text{d}^{-1}$]
SMP	: Soluble microbial product
SRF	: Specific resistance to filtration [$\text{m} \cdot \text{kg}^{-1}$]
SRT	: Solids residence time [day]
TKN	: Total Kjeldahl nitrogen
TMP	: Transmembrane pressure [mbar]
TN	: Total nitrogen
TP	: Total phosphorus
TS	: Total solids
TSS	: Total suspended solids
UASB	: Upflow anaerobic sludge bed
UF	: Ultrafiltration
VFA	: Volatile fatty acids
VS	: Volatile solids
VSS	: Volatile suspended solids

SYMBOLS

μ	: Dynamic viscosity of water [Pa·s]
A	: Effective filtration area [m ²]
ABS_f	: Absorbance of final sample at 600 nm after hydrocarbon treatment
ABS_i	: Absorbance of initial sample at 600 nm before hydrocarbon treatment
b	: Slope of time/filtrate volume (t/V) versus. filtrate volume (V) line [s·L ⁻²]
J	: Flux [L·m ⁻² ·h ⁻¹]
P	: Permeability [L·m ⁻² ·h ⁻¹ ·bar ⁻¹]
R_{intrinsic}	: Intrinsic membrane resistance [m ⁻¹]
R_{irrecoverable}	: Resistance caused by foulants that cannot be removed by neither physical nor chemical cleaning [m ⁻¹]
R_{irreversible}	: Resistance caused by organic and inorganic foulants, which can be removed by chemical cleaning [m ⁻¹]
R_{removable}	: Cake resistance which can be physically removed by water jet [m ⁻¹]
R_{total}	: Total filtration resistance [m ⁻¹]
ΔP_T	: Transmembrane pressure (TMP) [Pa]

LIST OF TABLES

	<u>Page</u>
Table 2.1 : Advantages and disadvantages of AnMBRs for the mitigation of problems induced by industrial wastewaters at extreme conditions.	45
Table 2.2 : Treatment performance of lab-scale AnMBRs used for the treatment of various industrial wastewaters.	49
Table 2.3 : Membrane performance of lab-scale AnMBRs used for the treatment of various industrial wastewaters.	50
Table 2.4 : Treatment and membrane performance of pilot- and full-scale AnMBRs used for the treatment of various industrial wastewaters.	61
Table 3.1 : Wastewater characterization (mean±standard deviation).	68
Table 3.2 : Comparison of the steady state performance of reactors at different SRTs (mean±standard deviation).	73
Table 3.3 : LCFA fed to the reactors and removal efficiency of calcium and magnesium (mean±standard deviation).	74
Table 3.4 : COD mass balance (mean±standard deviation).	75
Table 3.5 : Elemental composition of LCFA precipitates from R-20.	77
Table 3.6 : LCFA composition of fat deposits from R-20 (mean±standard deviation).	78
Table 3.7 : LCFA concentrations in different reactors at the end of operation (mean±standard deviation).	80
Table 4.1 : Membrane and module properties.	91
Table 4.2 : Operating parameters and treatment performance of each reactor.	96
Table 4.3 : SMP and bound EPS concentrations at the end of operation.	101
Table 4.4 : Correlations between selected filterability parameters for R-50.	108
Table 4.5 : Permeability and filtration resistance of reactors R-20 (at day 60 and day 83) and R-30, measured after physical cleaning of the membranes.	110
Table 4.6 : The results of EDX analysis on fouled and cleaned membranes.	115
Table 4.7 : Comparison of sludge characteristics between AnMBRs and MBRs.	120
Table 5.1 : Substrate composition fed to the reactors (mean ± standard deviation).	128
Table 5.2 : Operating conditions and biological performance (mean ± standard deviation).	129
Table 5.3 : Biomass activity in g CH ₄ -COD·g VSS ⁻¹ ·d ⁻¹ on different VFAs.	130
Table 5.4 : Granulometry of the sludge from each reactor.	132
Table 5.5 : Critical flux (L·m ⁻² ·h ⁻¹) of different sludges.	138
Table 6.1 : Feed characterization of R-1 and R-2 (mean±standard deviation).	145
Table 6.2 : Operating conditions and performance comparison of the reactors (mean±standard deviation).	149
Table 6.3 : EPS content of different sludge fractions.	156

LIST OF FIGURES

	<u>Page</u>
Figure 1.1: The increase in installed number of high rate anaerobic reactors between 1972 – 2006 (van Lier, 2008).....	30
Figure 1.2: The number of scientific papers on AnMBRs between 1978 and 2014 (June) (This graph was generated from SCOPUS database as a result of a search with keywords “anaerobic” and “membrane” in the title of publications. The relevance of the results was further refined by manual check.)	31
Figure 1.3: Relationships between different parameters and membrane fouling	33
Figure 1.4: Typical TMP profile and fouling under constant flux operation: Stage I: Initial TMP increase due to adsorption of solutes; Stage II: Long term TMP rise due to cake layer development; Stage III: TMP jump.	34
Figure 2.1: Different configurations of AnMBRs (a) Pressure-driven external cross-flow membrane, (b) Vacuum-driven submerged membrane with the membrane immersed in to the reactor, (c) Vacuum-driven submerged membrane with the membrane immersed in an external chamber (Liao et al., 2006).....	47
Figure 2.2: Factors affecting membrane fouling in AnMBRs.	48
Figure 3.1: Lab-scale cross-flow AnMBR set-up.	69
Figure 3.2: OLR, permeate COD COD _{VFA} and COD removal efficiency at different SRTs (a: R-20, b: R-30, c: R-50).	72
Figure 3.3: LCFA precipitates	76
Figure 3.4: Methane production during incubation of LCFA precipitates with different particle sizes, and corn oil with an initial concentration of about 10 g COD·L ⁻¹ . Inoculum concentration was about 6.6 g VSS·L ⁻¹ . The methane production data are corrected for the methane production in the substrate-free controls. All values are expressed in mg COD per liter liquid phase.	78
Figure 3.5: Lipid concentrations in each reactor.....	79
Figure 3.6: Methanogenic activity on individual VFAs at different reactors (a: R-20, b: R-30, c: R-50).....	81
Figure 3.7: Activity of the sludge from R-50 reactor.	83
Figure 4.1: Filtration performance of the reactors (a: R-20, b: R-30, c: R-50).....	97
Figure 4.2: Sludge particle size distribution in the reactors, a: R-20, b: R-30, c: R-50.	100
Figure 4.3: Evolution of SRF of the sludge during reactor operation.....	103
Figure 4.4: Evolution of CST together with TSS concentration (a: R-20, b: R-30, c: R-50).....	104
Figure 4.5: Supernatant filterability at different SRTs.	105
Figure 4.6: COD fractions of supernatant from the reactors.....	106
Figure 4.7: Filterability of different supernatant fractions of R-20 and R-30.....	107
Figure 4.8: Relationship between TSS concentration and SRF parameter.	109

Figure 4.9: Relationship between CST/TSS and SRF parameter.	109
Figure 4.10: The proportions of different fouling resistances in total filtration resistance at the end of operation of reactors (a): R-20 and (b): R-30. .	110
Figure 4.11: Efficiency of cleaning on permeability and filtration resistance recovery for the R-50 membrane (a) Permeability after cleaning, (b) Resistance after cleaning.	111
Figure 4.12: FT-IR spectra of the membranes from reactors (a): R-20, (b): R-30, and (c): R-50.	113
Figure 4.13: SEM photographs of the membranes from the reactors before and after physical cleaning, (a): R-20 before cleaning, (b): R-20 after cleaning, (c): R-30 before cleaning, (d): R-30 after cleaning, (e): R-50 before cleaning, and (f): R-50 after cleaning.	115
Figure 4.14: SEM-EDX analysis of precipitates found on membrane surface (a-c: SEM photographs, b-d: EDX analysis).	116
Figure 5.1: Schematic representation of reactors.	126
Figure 5.2: Substrate fed to each reactor.	128
Figure 5.3: Volume based particle size distribution of the sludges.	132
Figure 5.4: Evolution of SMP in reactors (a: R-2, b: R-3).	134
Figure 5.5: The evolution of SRF and CST in reactors R-1 (a), R-2 (b), R-3 (c), and R-4 (d).	136
Figure 6.1: Feed TKN and permeate ammonium nitrogen concentrations of R-1 (a) and R-2 (b).	146
Figure 6.2: Applied load and permeate COD concentrations in R-1 (a) and R-2 (2).	147
Figure 6.3: Particle size distribution in different reactors.	149
Figure 6.4: Soluble microbial products measured in R-1 (a) and R-2 (b).	151
Figure 6.5: Fouling rate evolution, expressed as TMP increasing rate, in critical flux tests of R-1 (a) and R-2 (b).	152
Figure 6.6: Evolution of CST and SRF in R-1 (a) and R-2 (b).	154
Figure 6.7: Particle size distribution of bulk sludge and white solids in R-2.	155

TREATMENT OF INDUSTRIAL WASTEWATERS BY ANAEROBIC MEMBRANE BIOREACTORS: IMPLICATIONS OF SUBSTRATE CHARACTERISTICS

SUMMARY

The success of anaerobic digestion relies on the presence of highly active methanogenic biomass, requiring effective retention of slow growing anaerobic microorganisms inside bioreactor by decoupling the hydraulic retention time (HRT) from solids residence time (SRT) or the employment of long SRTs in fully mixed systems. So far, flow through systems, i.e. completely stirred tank reactor (CSTR) digesters, and granular sludge bed reactors have been commonly applied for anaerobic treatment of slurries and low particulate matter containing streams, respectively. Physical separation of particulates by membranes is an efficient tool to uncouple SRT from HRT. Anaerobic membrane bioreactor (AnMBR) is a combination of an anaerobic process with membrane units located either inside or outside the reactor. These systems are expected to fill the gap between low loaded slurry digesters and high rate granular sludge bed reactors. AnMBRs are especially suitable when efficient biomass retention cannot be achieved due to wastewater characteristics, inappropriate reactor operation and/or design. AnMBRs provide an excellent treatment efficiency, thanks to the membrane filtration which ensures particulate free effluent.

On the other hand, membrane fluxes in AnMBRs are naturally limited by the effectiveness of the filtration process which is impacted by the build-up of a fouling layer in and on the membrane surface. The degree of fouling can be described by the increase in transmembrane pressure (TMP) or by the reduction of filtrate flux due to clogging of membrane pores or dense cake-layer later build-up. The reasons and mitigation possibilities of membrane fouling have received a significant scientific interest for decades. However, thus far, membrane fouling remains the focal point of membrane bioreactor (MBR) studies due to the complexity of phenomenon. The purpose of this thesis is to further increase the understanding of fouling phenomena in AnMBR systems by evaluating the impact of substrate composition and the effect of factors such as SRT on biological performance and sludge filterability. In order to mitigate fouling in AnMBRs, it is of crucial importance to understand the impact of operational conditions on sludge characteristics and filterability. Standard parameters such as capillary suction time (CST), specific resistance to filtration (SRF), and critical flux (CF) was used to evaluate the effect of different operation conditions. Moreover, the influence of high lipid content, acidified and non-acidified wastewaters and nitrogen deficiency on sludge characteristics and filterability was investigated.

The SRT had an effect on both biodegradation efficiency and filterability. A higher degree of substrate bioconversion to methane was observed at increased SRT. Controversially, the increase in SRT led to poor filterability due to accumulation of colloids and soluble microbial products (SMP) in the bulk sludge. The filterability parameters such as CST and SRF were found as valuable tools for subjective comparison of operational changes. However, it was difficult to evaluate the

relationships between filterability parameters and membrane fouling due to the complexity of fouling and non-linearity of correlations. Thus, it was suggested to evaluate a set of parameters about sludge characteristics and filterability in order to establish a link with membrane fouling.

For corn thin stillage and cheese whey permeate as the substrates we observed high COD removal efficiencies, exceeding 95%, which confirms the excellent performance of AnMBRs. However, the hydrolysis products of lipids, i.e. long chain fatty acids (LCFAs), caused reversible inhibition by forming a layer on the bioflocs that retards the transfer of substrate and nutrients. The biomass activity decrease due to LCFA adsorption was almost completely recoverable when the feed was stopped. However, the LCFA adsorption had other consequences on sludge surface characteristics. The results of this thesis showed that LCFA adsorption on biomass increased its hydrophobicity which in turn decreased its fouling propensity. Very likely, owing to the increased hydrophobicity of the flocs there is less interaction with the hydrophilic membrane.

Feeding non-acidified substrate to the AnMBR led to a rapid deterioration of the sludge filterability, which was attributed to the proliferation of acidogenic microorganisms on the rapidly fermentable carbohydrates. The abundant presence of single cell acidogens decreased the median particle size of the sludge. Additionally, the food to microorganism ratio (F:M) was found an important parameter impacting sludge filterability. An increase in this parameter resulted in accumulation of soluble microbial products (SMP) which led to deterioration of the supernatant filterability.

The experiments carried out with cheese whey revealed that nitrogen content effected both reactor stability and bulk liquid filterability. Nitrogen deficiency limited biomass growth and caused volatile fatty acids (VFA) accumulation, especially propionic acid, even at low organic loads. This was ascribed to metabolic changes which likely caused an adverse effect on syntrophic propionate oxidation. On the other hand sludge filterability rapidly deteriorated when AnMBR was fed with nitrogen supplied whey at a COD:TKN ratio of 50. We observed two distinct fractions of biomass with different particle size distribution, SRF and extracellular polymeric substances (EPS) content. The reduction of sludge filterability was attributed to induced growth of acidogenic biomass on lactose with surplus of nitrogen.

ENDÜSTRİYEL ATIKSULARIN ANAEROBİK MEMBRAN BİYOREAKTÖRLER İLE ARITIMI: SÜBSTRAT KARAKTERİZASYONUNUN ETKİSİ

ÖZET

Anaerobik arıtmanın verimi ve performansı, çok yavaş çoğalabilen anaerobik mikroorganizmaların reaktördeki kalış süresine bağlıdır. Biyoreaktördeki aktif biyokütle miktarının artırılabilmesi ancak çok uzun bekletme süreleri ya da hidrolik bekletme süresi (HBS) ile çamur yaşının birbirinden ayrılması ile mümkündür. Bu zamana kadar yüksek katı maddeli çamurların ya da düşük partiküler madde içerikli atıksuların arıtımında tam karışımli (ör: anaerobik çamur çürütücü) ve çamur yataklı anaerobik reaktörler (ör: havasız çamur yataklı reaktör (HÇYR)) başarıyla kullanılmıştır. Özellikle çamur yataklı anaerobik reaktörlerde kullanılan ve katı-sıvı-gaz fazlarının ayrımını sağlayan deşarj yapıları çamur yaşı ile HBS'ni başarılı bir şekilde ayırarak mikroorganizmaların reaktördeki kalış süresini arttırabilmektedir. Katı-sıvı-gaz ayrımı yapan deşarj yapılarının mikroorganizmaları tutma verimi mikroorganizmaların bira araya gelerek çok hızlı çökebilen granüller oluşturmaya bağlıdır. Granül oluşumu birçok faktörün etkili olduğu (sübatrat kompozisyonu, 2 değerlikli katyonlar, hücre dışı polimerik maddeler, sıcaklık v.b.) çok karmaşık bir süreç olup günümüzde halen tam olarak anlaşılmamıştır. Çamur yataklı reaktörlerde granül oluşumu, floküler biyokütlenin düşük HBS kullanılarak sistemden uzaklaştırılması ile gerçekleştirilebilir. Ancak, özellikle çok yüksek sıcaklık, tuzluluk, inhibitör, yağ-gres ve partiküler madde içeren atıksuların anaerobik arıtımında granülüzasyonun olumsuz etkilendiği ve arıtma veriminin düştüğü belirlenmiştir.

HBS ve çamur yaşının birbirinden etkili bir şekilde ayrılmasını sağlayabilmek için partiküler maddeleri fiziksel olarak tutabilen membran sistemleri de kullanılabilir. Anaerobik membran biyoreaktörler (AnMBR), anaerobik arıtma prosesleri ile membran sistemlerinin başarılı bir şekilde bir arada kullanılması ile teşkil edilir. Bu tip reaktörlerin düşük yüklü anaerobik çamur çürütücüler ile yüksek yüklü granüler çamur yataklı sistemler arasındaki boşluğu doldurması beklenmektedir. AnMBR'ler özellikle granülüzasyonun sağlanmadığı atıksu tipleri ve koşullar için oldukça uygundur. Bu tür durumlarda membranlar partiküler madde ve biyokütlenin reaktör içerisinde tutulması amacıyla kullanılabilir.

AnMBR prosesinin en önemli dezavantajı membran tıkanmasıdır. Membran tıkanması, zamanla membran porları ve membran üzerinde biriken maddelere bağlı olarak transmembran basıncının artması ya da akının azalması olarak tanımlanabilir. Membran tıkanmasının nedenleri ve azaltma yolları bu zamana kadar birçok bilimsel araştırmanın konusu olmuştur. Ancak, konu oldukça karmaşık olması sebebiyle günümüzde de halen önemini korumaktadır. Bu tezin amacı; sübatrat kompozisyonunun ve çamur yaşı gibi işletme parametrelerinin AnMBR sistemlerinde oluşan membran tıkanmasına etkilerinin incelenmesi ve bu konudaki bilgi birikiminin artırılmasıdır. AnMBR'lerde membran tıkanmasının azaltılabilmesi için işletme şartlarının çamur filtre edilebilirliğine etkisinin belirlenmesi gereklidir. Bu amaçla,

pratikte özellikle çamur susuzlaştırma işlemlerinde uzun zamandır kullanılan özgül kek direnci (ÖKD) ve kapiler emme süresi (KES) gibi standart parametreler kullanılmıştır. Aynı zamanda, yüksek yağ konsantrasyonunun, asidifiye edilmiş ve edilmemiş atıksuların ve düşük azot konsantrasyonlarının membran tıkanmasına etkileri sistematik olarak araştırılmıştır.

AnMBR’lerde çamur yaşı hem biyolojik giderim verimi hem de filtre edilebilirlik üzerinde etkili bir parametredir. Yüksek çamur yaşlarında yavaş ayrıışan organik maddenin stabilizasyon derecesi ve biyolojik olarak metana dönüşüm verimi artmaktadır. Ancak, yüksek çamur yaşlarında kolloidlerin ve çözünmüş mikrobiyal ürünlerin (ÇMÜ) reaktörde birikimi artacağından çamurun filtre edilebilirliği azalmaktadır. Bu çalışmada, ÖKD ve KES gibi parametrelerin, işletme şartlarındaki değişimlerin çamur filtre edilebilirliğine etkisini objektif olarak karşılaştırabilmek için çok önemli araçlar olduğu anlaşılmıştır. Ancak gerek membran tıkanması prosesinin karmaşıklığı gerekse parametreler arasındaki non-lineer ilişkiler nedeniyle çamur filtre edilebilirliği ile membran tıkanması arasında net bir ilişki kurabilmek her durumda mümkün olmamıştır. Bu nedenle tek bir parametre üzerine yoğunlaşmak yerine çamur karakterizasyonu ve filtre edilebilirliği ile ilgili parametrelerin gruplar halinde ele alınması ve membran tıkanması ile ilişkilendirilmesi daha çok tavsiye edilmektedir.

Bu çalışmada, mısırdan biyo-etanol üretimi atıksuları ve peynir altı sularının AnMBR prosesi ile arıtımı incelenmiştir. Biyo-etanol üretimi özellikle son yıllardaki petrol fiyatlarının dengesizliği, politik karmaşalar ve yenilenebilir enerji kaynaklarının kullanımına olan ilgi nedeniyle oldukça artmıştır. Biyo-etanol; mısır, buğday, kassava gibi bitkilerdeki 6 karbonlu şekerlerinin fermentasyon yolu ile etanole dönüştürülmesi ile üretilmektedir. Biyo-etanol endüstrilerinde bu proses sonucunda partiküler madde içeriği yüksek oldukça kirli bir atıksu oluşmakta olup, genelde kurutularak elde edilen ürün hayvan yemi olarak satılmaktadır. Ancak kurutma işlemi sırasında oldukça fazla enerji harcanmakta ve sistemin fizibilitesini olumsuz yönde etkilemektedir. Bu bakımdan, biyo-etanol atıksularının hem biyogaz üretimi sağlayan hem de arıtılan atıksuyun endüstri içerisinde yeniden kullanımına olanak tanıyan AnMBR prosesi ile arıtımı oldukça avantajlıdır.

Peynir altı atıksuyu ise peynir üretimi sırasında açığa çıkan ve karbonhidrat (laktoz) içeriği çok yüksek, partiküler madde konsantrasyonu düşük atıksulardır. Günümüzde peynir altı atıksularından birçok yan ürün geri kazanımını sağlayan teknolojiler mevcut olmasına rağmen, özellikle küçük ve orta büyüklükteki işletmeler de genellikle bu teknolojilerin uygulanması tekno-ekonomik olarak fizibil olmamaktadır. Bu bakımdan, peynir altı sularının AnMBR ile arıtımı hem biyogaz/enerji geri kazanımı hem de deşarj standartlarının sağlanmasında büyük avantaj sağlamaktadır.

Yapılan çalışmada, her iki atıksuda %95’in üzerinde kimyasal oksijen ihtiyacı (KOİ) giderme verimi elde edilmiştir. Ancak özellikle biyo-etanol üretimi atıksularında bulunan yüksek konsantrasyonlu yağlar ve hidroliz ürünleri uzun zincirli yağ asitleri (UZYA) biyolojik arıtma verimini olumsuz etkilemiştir. UZYA mikroorganizma flokları üzerine adsorbe olarak etkileri geri döndürülebilir inhibisyona neden olmaktadır. Bu nedenle biyokütle aktivitesi düşmektedir. Ancak, atıksu beslemesi durdurulduğunda biyokütle yavaş da olsa aktivitesini tamamen geri kazanabilmektedir. Atıksu içerisindeki kalsiyum ve magnezyum gibi 2 değerlikli katyonların UZYA ile birleşerek onları sabunlaştırdığı ve iri taneli çökelekler oluşturduğu gözlemlenmiştir. Bu sayede UZYA’lerinin mikroorganizma üzerindeki inhibisyonu azaltılabilir. Ancak, UZYA sabunları suda çok zor çözündüklerinden

metana dönüşümleri sınırlı olmakta ve metan üretimi azalmaktadır. UZYA'lerinin biyokütle üzerine adsorpsiyonu çamurun yüzey karakteristiklerini de değiştirdiğinden filtre edilebilirlik ve membran tıkanması üzerinde de etkili olmaktadır. UZYA'leri çamura adsorbe olduğunda flokların hidrofobisitesi artmaktadır. Bu çalışmada, membran tıkanmasının çamurun hidrofobisitesi ile ters orantılı olduğu gözlemlenmiştir.

Asidifiye olmamış ve çözünmüş karbonhidrat içeriği yüksek peynir altı atıksuların AnMBR ile arıtımında çamurun filtre edilebilirliğinin çok hızlı azaldığı gözlemlenmiştir. Bu durum özellikle çözünmüş karbonhidrat türü kolay ayrışabilen organik maddeleri kullanan asidojen bakteriler neden olmaktadır. Asidojen bakterilerin fazla çoğalması çamurun partikül dağılımının daha düşük boyutlara kaymasına neden olmaktadır. Bu durum çamurun ÖKD'nin artmasına neden olmaktadır. AnMBR'lerdeki en önemli tıkanma mekanizmasının kek oluşumu olduğu bir çok çalışmada gözlenmiştir. ÖKD'nin artması membran üzerinde porozitesi düşük ve hızlı bir şekilde konsolide olan kompakt bir kek tabakasının oluşacağını göstermektedir. Buna ek olarak, sübstrat:biyokütle (S:B) oranının çamur filtre edilebilirliği için önemli bir parametre olduğu belirlenmiştir. Bu parametrenin artması durumunda ÇMÜ'lerin arttığı ve özellikle süpernetanın filtre edilebilirliğinin düştüğü gözlemlenmiştir.

Peynir altı suyu ile yürütülen çalışmada, atıksuyun azot içeriğinin biyoreaktör stabilitesi ve çamur filtre edilebilirliği için çok önemli olduğu belirlenmiştir. Azot eksikliği mikroorganizma çoğalma hızını sınırlandırmış ve reaktörde özellikle propiyonik asit gibi uçucu yağ asitlerinin biriktiği gözlemlenmiştir. Öte yandan, laboratuvar ölçekli AnMBR yüksek azot içerikli (KOİ:TKN oranı 50) peynir altı suyu ile beslendiğinde çamur filtre edilebilirliği düşmektedir. Bunun nedeni yüksek azot konsantrasyonlarında asidojen bakterilerin çamur içerisinde aşırı çoğalması ve çamur partikül boyutunun düşmesi olarak belirlenmiştir.

Membran teknolojilerinin atıksu arıtma proseslerinde kullanılmaya başlanması birçok avantaj ve fırsat yaratmıştır. Bu tez çalışması kapsamında AnMBR'lerin özellikle mikroorganizma granülüzasyonunun sağlanamadığı endüstriyel atıksuların arıtımında hem biyogaz hem de partiküler madde içeriği çok düşük, yüksek kalitede arıtılmış su geri kazanımı sağlanabilen yenilikçi bir proses olduğu gösterilmiştir. Gelecekte, endüstrilerde su geri kazanımı ve yeniden kullanımının artacağı düşünüldüğünden üretilen atıksuların karakterlerinin de değişmesi ve arıtımının zorlaşması beklenmektedir. Bu bakımdan AnMBR gibi yenilikçi arıtma teknolojilerine olan ihtiyaç artacaktır.

1. INTRODUCTION

1.1 Background

Anaerobic treatment can be viewed as an excellent collaboration of different microbial species with unique characteristics at different trophic levels. Since the biomass yield under anaerobic conditions is very low compared to aerobic, biomass retention has always been an important driver for developing efficient designs and operational strategies of anaerobic reactors. The invention of the upflow anaerobic sludge blanket (UASB) reactor in early 70s by Lettinga and his colleagues was a great leap for the acceptance of high rate anaerobic reactors for industrial wastewater treatment (Lettinga et al., 1980). The self-aggregation of biomass, so called granulation, in UASB reactors enabled the efficient decoupling of hydraulic and solids residence times and extended the application of anaerobic treatment to high strength and mainly soluble industrial wastewaters. By time, the UASB and its successors, i.e. the expanded granular sludge bed (EGSB) reactor and internal circulation reactor (IC), received a worldwide acceptance and they are now state of the art technologies for high rate anaerobic treatment of agro-industry wastewaters (Figure 1.1). It is reported that 2400 units of registered high rate anaerobic reactors were in operation worldwide in 2006 (van Lier, 2008). Now, high rate anaerobic treatment with granular sludge bed reactors is a mature technology.

On the other hand, the granulation process which was the key mechanism behind the success of these reactors is still not fully understood. Sludge granulation is a very complex process which is affected by many factors such as reactor design, dilution rate, substrate characteristics and biomass properties. However, several substrate characteristics such as high fat oil and grease (FOG), salt and particulate matter concentrations were identified as adversely affecting biomass granulation (Dereli et al., 2012a). This limits the application of high rate granular sludge bed reactors to mainly soluble carbohydrate and protein based wastewaters.

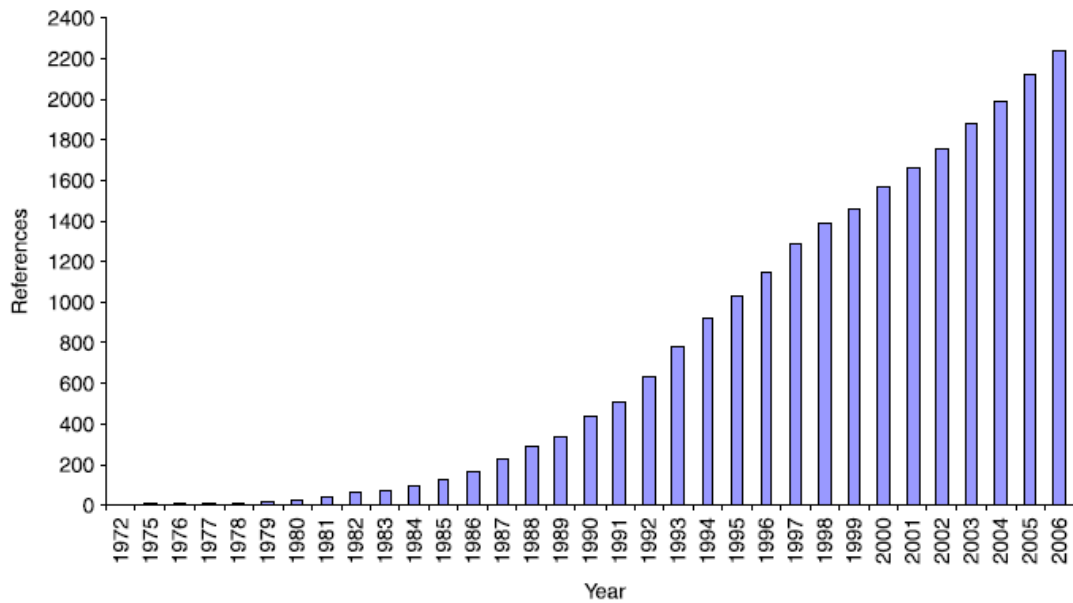


Figure 1.1 : The increase in installed number of high rate anaerobic reactors between 1972 – 2006 (van Lier, 2008).

The idea of using membranes as a physical separation tool for biomass retention in anaerobic reactors is actually not new. The first attempt on this was done by Grethlein (1978) who applied membrane filtration to septic tank effluent. In early 80s, the Dorr-Oliver Company developed the first anaerobic membrane bioreactor (AnMBR) for the treatment of high strength cheese whey permeate. In the 90s, investigations were carried out on biological and filtration performances of this new reactor concept by several research groups (Kataoka et al., 1992; Bailey et al., 1994; Cadi et al., 1994; Ince et al., 1995). These reactors were referred to with different names such as Membrane Anaerobic Reactor System (MARS), Anaerobic Digestion Ultrafiltration (ADUF) and Cross-flow Ultrafiltration Membrane Anaerobic Reactor (CUMAR). The Japanese Aqua Renaissance program, launched in 1990 was one of the most important research initiatives on AnMBRs (Kimura, 1991). Several pilot and full scale reactors with different configurations were operated for the treatment of various substrates. The final conclusion of the cited research was a very high treatment efficiency in contrast to low membrane fluxes and high fouling rates under anaerobic conditions. Therefore, the energy costs as well as the costs for membrane installation and replacement would be too high, preventing that AnMBRs would become a competitive technology. As a result, the on-going research on AnMBRs was limited until 2005 (Figure 1.2).

The reasons for the recently increased scientific interests in AnMBRs might be ascribed to decreasing membrane prices, the development of new membranes with

good anti-fouling properties, the gradual increase in energy prices, water reuse initiatives, and the more stringent discharge standards. The number of journal papers about AnMBRs published since 2005 showed an exponential increase (Figure 1.2) thus 86 percent of the whole literature was developed in the last decade. In parallel to scientific developments and market needs, AnMBRs has also received an ever growing commercial interest. Some companies such as Kubota Corporation (Japan), ADI Systems Inc. (Canada) and Biothane Systems International (the Netherlands) have developed their own patented AnMBR solutions.

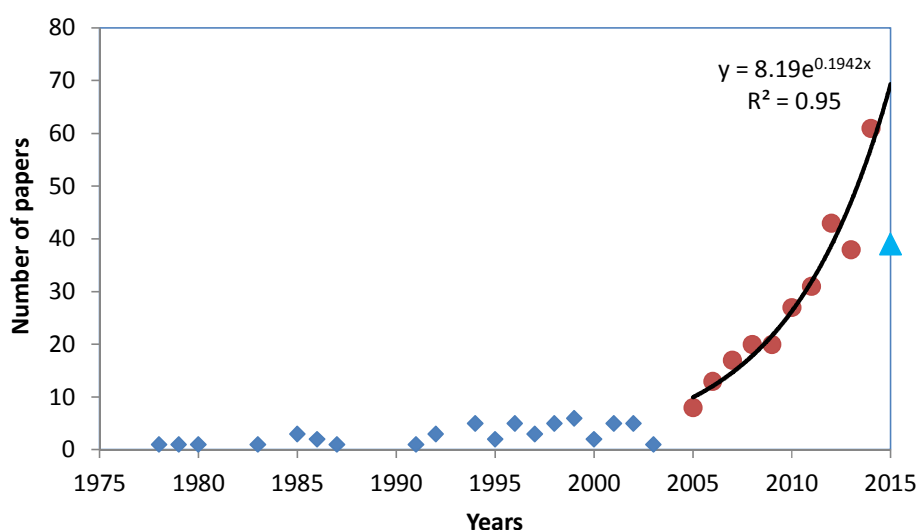


Figure 1.2 : The number of scientific papers on AnMBRs between 1978 and 2014 (June) (This graph was generated from SCOPUS database as a result of a search with keywords “anaerobic” and “membrane” in the title of publications. The relevance of the results was further refined by manual check.).

Membranes present an alternative solution for biomass retention when granulation cannot be achieved due to substrate characteristics, poor reactor design and/or operation. In AnMBRs, the retention of slow growing anaerobic biomass is assured by physical separation with microfiltration or ultrafiltration membranes. This allows an ultimate control over solids retention time (SRT) which enables to operate the reactor at long SRTs independent from the hydraulic residence time (HRT). Due to the size exclusion of the membranes, any particulate, colloid or solute with a larger size than the membrane pores is retained inside the reactor. Therefore, AnMBRs offer complete retention of biomass regardless of its settling/granulation properties, superior treatment efficiency and high quality effluent free of solids and pathogens. Furthermore, AnMBRs can be used to retain special biomass types that degrade

specific pollutants in the wastewater. In that sense, AnMBRs are very promising and offer several opportunities.

Liao et al. (2006) suggested that AnMBRs have a great potential for high strength particulate wastewaters whereas, for high strength soluble wastewaters granular sludge bed reactors already offer a good performance and will continue to dominate the market. Indeed, AnMBR is an emerging treatment technology for industrial wastewaters/slurries with extreme properties, which often cause biomass retention problems in conventional anaerobic granular sludge bed reactors. These extreme conditions can be high salinity, FOG, toxicity and particulate matter concentrations, fluctuating hydraulic and organic loads. In the future, this type of wastewaters with extreme characteristics are expected to increase due to water reuse approaches and zero discharge initiatives in industries. Thus, AnMBRs can provide an alternative reactor technology with a high treatment performance and permeate quality that enables water reuse for industrial wastewaters. Therefore, AnMBRs are expected to bridge slurry digestion and high rate treatment, filling the gap between low loaded sludge digesters and granular sludge bed reactors.

1.2 Problem Statement

Membrane fouling is the most important problem, that increases the installation and operation costs of membrane bioreactors (MBRs), hampering their wide spread application. Membrane fouling causes reduction in permeate flow or increase in transmembrane pressure (TMP) due to clogging of membrane pores or formation of a dense cake or gel layer on the membrane surface during filtration. It is a very complex phenomenon depending on different variables such as sludge characteristics, bioreactor operating parameters, membrane properties and operation (Figure 1.3). In order to mitigate membrane fouling, it is crucial to understand the inter-relation of these parameters. The adverse effects of fouling on the design and operation of MBRs can be summarized as:

- High energy requirement for membrane scouring and permeate extraction
- Decrease of operation flux and thus increase in required membrane area
- High chemical consumption for membrane cleaning
- Decrease in membrane life time and increase in replacement frequency

Membrane fouling starts with the adsorption of solutes on to the membrane surface and into the pores at the preliminary stages of filtration. It was shown that adsorption of solutes take place even at very low fluxes (Le-Clech et al., 2003). Thus, membrane characteristics such as pore size, hydrophobicity and surface charge are important in the initial adsorption of solutes. As the filtration continues, the surface of the membrane is covered with a gel layer due to concentration polarization. The gel layer is generally considered to be composed of extracellular polymeric substances secreted by bacteria. It forms a suitable surface for the attachment of bacteria and sludge flocs. The deposition of particulate matter leads to cake layer build up on the membrane surface. It was reported that cake layer formation is the most important fouling mechanism in AnMBRs (Jeison et al., 2007; Charfi et al., 2012). At the final stage of constant flux filtration, TMP increase accelerates, which is generally referred as “TMP jump” (Figure 1.4). This may be due to cake compaction which leads to a less porous layer on the membrane or non-even distribution of permeate drag force on the membrane and local flux transients which is caused by blockage of membrane pores.

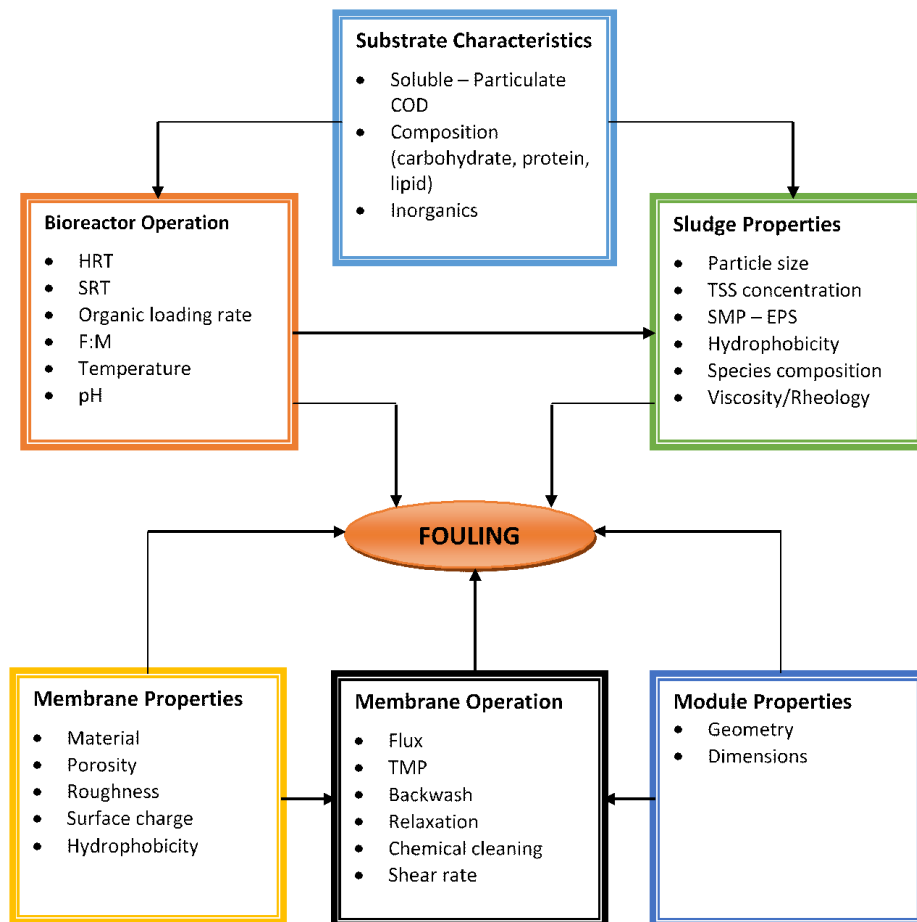


Figure 1.3 : Relationships between different parameters and membrane fouling.

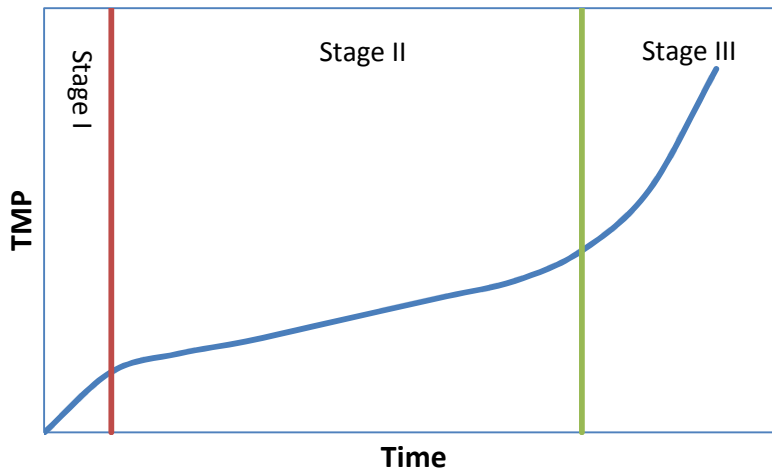


Figure 1.4 : Typical TMP profile and fouling under constant flux operation: Stage I: Initial TMP increase due to adsorption of solutes; Stage II: Long term TMP rise due to cake layer development; Stage III: TMP jump.

1.3 Research Objectives

The first objective of this thesis is to understand the influence of substrate characteristics on filterability and membrane fouling. The effects of substrate characteristics on sludge filterability have been generally overlooked in MBR research. However, substrate composition determines the microbial species distribution, soluble microbial product (SMP) concentration and characteristics, floc stability, thus in return indirectly effects filterability and fouling (Le-Clech et al., 2006).

In this study, two different wastewaters with unique characteristics were used:

- **Thin stillage from corn-to-bioethanol production:** High lipids and particulate matter content
- **Whey permeate from cheese production:** High carbohydrate content, almost completely soluble and rapidly fermentable

Lipids and their hydrolysis products, i.e. long chain fatty acids (LCFAs), often cause problems to biomass retention and activity in anaerobic reactors. AnMBRs represent a suitable candidate for the treatment of lipid-rich wastewaters, since there would be no biomass retention problems. Lipids are also regarded as problematic for MBRs since they may cause rapid fouling of polymeric membranes through hydrophobic interactions. Interestingly, the information about the biological and membrane

performance of MBRs/AnMBRs treating high lipid containing wastewaters is very limited. Thus, this research seeks to present the impacts of high lipid containing wastewaters on the biology and membrane performance of AnMBRs.

The poor sludge filterability in AnMBRs has been attributed to the rapid proliferation of acidogenic biomass, which grows as single cells and decreases the sludge particle size, when treating non-acidified carbohydrate based wastewaters by several researchers (e.g. Jeison and van Lier, 2007a; Jeison et al., 2009a). Moreover, with the help of recently developed microbial investigation tools it was determined that some species in mixed cultures have a higher tendency to accumulate on membranes and play a pioneering role in membrane fouling (Calderon et al., 2011; Lin et al., 2011). The microbial species composition in an AnMBR is directly related to operating conditions and parameters, i.e. temperature, pH and SRT, and substrate characteristics. Therefore, it is considered imperative to systematically investigate the effects of acidogenic biomass on sludge filterability when treating acidified and non-acidified wastewaters.

Solids retention time is commonly accepted as a fundamental parameter for the design and operation of any biological treatment system. In order to maintain a high SRT, conventional granular sludge bed systems rely on the granulation mechanism of biomass and three phase separators which may not work efficiently at all times due to several reasons. Therefore, sludge washout is inevitable in some cases which hamper the treatment performance of these reactors. SRT can be controlled much easier in AnMBRs since there will be no washout of particulates with the effluent due to the membrane rejection.

SRT is also one of the most important parameters which effects the filterability and fouling propensity of sludge in AnMBRs (Le-Clech et al., 2006). SRT affects many parameters in AnMBRs such as;

- Applicable organic load
- Food to mass ratio
- Biomass concentration
- Inorganic precipitates accumulation
- EPS and SMP concentration and characteristics
- Biomass activity

Therefore, understanding the relation of SRT with these parameters and its optimization for each specific case to improve reactor performance and mitigate fouling is of crucial importance. On the other hand, it is a very difficult task since many parameters related to sludge characteristics change with the variation in SRT. Thus, contradictory results have been reported on the effect of SRT on sludge filterability and fouling (Meng et al., 2009). Therefore, the second objective of this study is investigate the effect of SRT on sludge filterability with standard tools, which allows a better comparison of different operating conditions. Moreover, implications of SRT on biological parameters such as sludge activity and treatment performance were also investigated.

1.4 Thesis Outline

The remaining chapters of this thesis are structured as follows:

Chapter 2 extensively reviews the potential of AnMBRs for the treatment of industrial wastewaters. It summarizes the granulation problems faced in high rate granular sludge bed reactors and how membrane assisted biomass separation can help to improve the performance. Moreover, limitations of AnMBRs such as fouling and low flux are comparatively discussed both at laboratory and full scale.

Chapter 3 focuses on the biological performance of AnMBRs for the treatment of high strength lipid rich wastewaters. It is shown that although membrane provides an ultimate solution for biomass retention, the process still suffers from LCFA inhibition. The mechanisms of LCFA removal such as biodegradation, adsorption and precipitation with divalent cations and their effect on anaerobic biomass activity were discussed in details.

Chapter 4 evaluates the effects of SRT and lipids on sludge filterability in AnMBRs. The sludge filterability at different SRTs was compared by standard analysis such as capillary suction time, specific resistance to filtration (SRF) and supernatant filterability (SF). A link between sludge physicochemical characteristics, such as TSS, supernatant COD and SMP, and sludge filterability was tried to be established. Moreover, the effects of LCFA inhibition and accumulation on biomass were discussed in the perspective of sludge filterability and membrane fouling.

Additionally, the impact of inorganic precipitates on membranes was researched with cleaning assays and SEM-EDX analysis.

Chapter 5 focuses on the influence of substrate acidification degree and food to mass ratio on sludge filterability. The effect of substrate characteristics on microbial species composition, especially acidogenic biomass, and its indirect impact on sludge filterability was investigated. Standard filterability tests were applied in order to evaluate the differences in the filterability of sludges fed with acidified and non-acidified substrates.

Chapter 6 focuses on the influence of nitrogen limitation on the treatment performance and sludge filterability of AnMBRs. The impact of nitrogen on acidogenic biomass growth and process stability was investigated. Besides, sludge filterability was systematically evaluated with standard parameters in order to achieve an unbiased comparison under nitrogen deprived and supplemented conditions.

Chapter 7 is the final chapter of this thesis. It summarizes the results and presents a perspective on the future of AnMBRs in the light of the knowledge acquired in this thesis work.

2. LITERATURE REVIEW ¹

2.1 Introduction

High rate anaerobic treatment of industrial wastewaters is a proven technology that offers many advantages such as high organic matter removal efficiency, recovery of energy, and excess sludge reduction (Rajeshwari et al., 2000; van Lier et al., 2001; van Lier, 2008). The success of high rate anaerobic treatment depends on the retention of slow growing methanogenic bacteria in the reactor, i.e. efficient decoupling of solids retention time (SRT) and hydraulic retention time (HRT). The three commonly used mechanisms for biomass retention are settling, attachment and granulation. The latter is the most commonly applied mechanism, as reflected by the abundance of upflow anaerobic sludge bed (UASB), expanded granular sludge bed (EGSB) and internal circulation (IC) reactors for the treatment of industrial wastewaters (van Lier et al., 2001; van Lier, 2008). Granulation, defined as the formation of well-settleable microbial aggregates with various functionalities (Hulshoff Pol et al., 2004), depends on several aspects, such as hydraulic conditions, wastewater characteristics, physico-chemical parameters, etc. Successful granulation can be accomplished by bacterial selection mechanisms. As a rule of thumb, successful granulation in anaerobic high rate reactors can be expected at short hydraulic retention times, i.e., <2 days. Although granular sludge based technology is feasible for the anaerobic treatment of a wide range of wastewaters, there are certain limitations, which are further discussed in Section 2.

The most recent development in high rate anaerobic treatment is using membranes to separate biomass from the effluent. Anaerobic membrane bioreactors (AnMBRs) offer high quality effluents free of solids and pathogens due to their superior treatment efficiencies and complete retention of biomass, regardless its settling and/or

¹ This chapter was published as:

Dereli, R.K., Ersahin, M.E., Ozgun, H., Ozturk, I., Jeison, D., van der Zee, F., van Lier, J.B. (2012) Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters. *Bioresource Technology* 122, 160-170.

granulation properties. Furthermore, AnMBRs can be used to retain special microbial communities that can degrade specific pollutants in the wastewater (van Lier, 2008; Tao et al., 2012). Therefore, this technology may present an attractive option for treating industrial wastewaters and/or slurries at extreme conditions, such as high salinity, high temperature, high concentrations of suspended solids (SS) and presence of toxicity, that hamper granulation and biomass retention or reduce the biological activity (van Lier et al., 2001). Industrial wastewaters at extreme conditions will likely occur more often in the future as cleaner industrial production processes require reduction of water consumption, water reuse and resource recovery (van Lier et al., 2001; van Lier, 2008).

Although AnMBRs offer many advantages, membrane fouling presents one of the main bottlenecks for their application. Membrane fouling reduces the flux due to the accumulation of organic and inorganic particles in/on the membrane pores or on the surface. Membrane fouling is a multivariable process that is affected by the influent characteristics, reactor operation, membrane features and biomass properties. Cake layer formation was identified as the most important fouling mechanism in AnMBRs (Choo and Lee, 1998; Jeison and van Lier, 2007b; Xie et al., 2010). Although membrane fouling is inevitable at least on the long term, efforts focus on how to reduce its build up with time by efficient operation and control measures (Jeison and van Lier, 2006a, b).

The aims of this paper are to determine the problems encountered in the high rate anaerobic treatment of industrial wastewaters and to present AnMBR technology as a possible solution by addressing the most recent examples of applications from the literature. Moreover, the most important factors that affect the filtration performance of AnMBRs for the treatment of industrial wastewaters are also discussed.

2.2 Limitations to Traditional, Granular Sludge Based, Anaerobic Treatment Technology

Anaerobic granular sludge is the major success factor of high rate anaerobic processes. However, various industrial wastewaters characteristics negatively impact the sludge granulation process or even lead to de-granulation and loss of biomass. High suspended solids (SS), high temperature, fat, oil and grease (FOG) content, toxicity, high salinity, drastic changes in organic loading rate (OLR), and significant HRT

fluctuations can be given as examples of extreme situations that have negative impacts on the performance of anaerobic reactors. For successful anaerobic high-rate treatment, adequate retention of all required slow growing biomass is of crucial importance. Therefore, when sludge granulation is hampered or even lacking, membrane assisted biomass separation can be the ultimate tool to achieve full bacterial retention. The advantages and disadvantages of AnMBRs for the mitigation of problems induced by industrial wastewaters with extreme characteristics are summarized in Table 2.1.

2.2.1 High SS content

Industries such as potato processing, meat processing and slaughterhouses generate wastewaters with high SS concentrations. The accumulation of slowly degradable particulate matter and inert solids in the sludge bed of UASB reactors deteriorates the sludge methanogenic activity during long term operation. Thus, a pretreatment step to decrease high SS concentration is usually required before high rate anaerobic reactors (Rajeshwari et al., 2000). On the other hand, the removal of suspended organic matter in the pretreatment step lowers the methane production potential, possibly affecting the feasibility of the system. Kalyuzhnyi et al. (1998) reported problems, such as excessive foaming, sludge flotation, accumulation of undigested ingredients at elevated OLRs in an UASB reactor treating high SS containing potato-maize wastewater.

2.2.2 FOG content

Problems associated with the anaerobic treatment of lipid containing wastewaters are the toxicity of long chain fatty acids (LCFA) to methanogens and acetogens (Hwu, 1997) and the adsorption of a lipid layer around biomass particles which limits the transport of substrate and nutrients (Pereira et al., 2005) resulting in biomass flotation (Rinzema et al., 1989). Although it is evident that the granular sludge is more resistant to LCFA inhibition than the flocculent sludge due to its lower specific surface area (Hwu, 1997), many researchers have reported operational problems during the treatment of high lipid containing wastewaters by high rate anaerobic reactors, such as impairment of the granulation (Hawkes et al., 1995), flotation and wash-out of biomass (Rinzema et al., 1989; Zhang et al., 2008), suppression of methanogenic activity (Pereira et al., 2002) and foam/scum accumulation on top of the reactor (Zhang et al.,

2008). Hawkes et al. (1995) compared the performance of different reactors such as UASB, EGSB, anaerobic filter (AF) and anaerobic contact reactor for the treatment of ice cream wastewater containing high lipid concentration. They concluded that the anaerobic contact reactor had a higher COD removal efficiency due to the good mixing of the substrate and biomass. Hence, a pretreatment step for lipid removal is usually required for the treatment of wastewaters with high lipid content by anaerobic high rate reactors (Rajeshwari et al., 2000).

2.2.3 Thermophilic conditions

Difficulty in the immobilization of anaerobic biomass is often considered one of the most important problems encountered in the anaerobic treatment of industrial effluents at high temperature. This problem may be attributed to the decrease in the production of extracellular polymeric substances (EPS), which play a major role in biomass aggregation at high temperatures (Quarmby and Forster, 1995). For instance, stillage process effluents generated from ethanol production (distillery wastewaters) are typical examples of high temperature wastewaters. Since the temperatures may be as high as 90 °C, cooling is required: 35 °C for mesophilic treatment or 55-60 °C for thermophilic treatment. Thermophilic anaerobic treatment of distillery wastewaters improves process economics since less cooling is required and similar methane yields can be achieved, at twice the organic loading rate compared to mesophilic treatment (Wilkie et al., 2000). However, Soto et al. (1992) concluded that immobilization of anaerobic biomass is more difficult under thermophilic conditions than under mesophilic conditions due to the formation of dispersed sludge under high temperature which has poor settling characteristics. Similar phenomena in UASB reactors at thermophilic conditions were reported by van Lier et al. (1992) treating acidified synthetic wastewaters.

2.2.4 Toxicity

The retention of slow growing specific organisms is crucial for efficient treatment of toxic and recalcitrant wastewaters. On the other hand, the low HRTs applied as a selection mechanism to promote the granulation process in granular bed reactors may cause the wash out of the specific types of bacteria that is required for the degradation of toxic organic pollutants. In other words, effective retention of the required bacteria can only be guaranteed if their attachment properties are similar to the bacteria

constituting the methanogenic granular consortia. If adherence properties are insufficient, membrane separation certainly assists in retaining the specific bacteria that can degrade biodegradable toxic compounds present in wastewaters.

Anaerobic technology can be applied for the treatment of toxic wastewaters such as textile wastewaters (dos Santos et al., 2007), solvent containing pharmaceutical wastewaters (Enright et al., 2005), polymer synthesis effluents (Araya et al., 1999), pulp and paper industry wastewaters including absorbable organic halides (AOX) (Savant et al., 2006). For instance, the treatability of phenolic wastewaters by anaerobic technology in UASB reactors and upflow sludge bed filters is well documented in literature (Fang et al., 1996). Long acclimation time, small granule size and low phenol removal efficiency at high loadings are the most common drawbacks associated with the treatment of phenol at relatively high concentration. Phenol concentrations over a range of 500-750 mg·L⁻¹ are generally inhibitory for the treatment of phenolic wastewaters by UASB processes (Tay et al., 2000).

2.2.5 High salinity

High salinity conditions occur in various waste streams such as fish and sea food-processing, chemical, petroleum and leather industries (Lefebvre and Moletta, 2006). The presence of high salt concentrations is regarded as one of the serious limiting factors for anaerobic systems with the inhibitory/toxic effects on non-adapted biomass mainly due to cations (Ismail et al., 2010; Rinzema et al., 1988; Lefebvre et al., 2006). Salt stress on microbial species results in the inhibition of many enzymes, a decrease in cell activity and plasmolysis (Ismail et al., 2010). Rinzema et al. (1988) proved the inhibitory effect of sodium on acetoclastic methanogens at various acetate concentrations and pH levels. Lefebvre et al. (2006) attributed the absence of granular sludge in a UASB reactor inoculated with granules to the high salinity of the wastewater (tannery soak liquor) and showed that proper performance was limited to very low organic loadings. The concentration of ions determines the level of inhibition together with the antagonist/synergistic effects of other ions, additional effect of the adaptation period and nature of the sludge, substrate and reactor configuration (Chen et al., 2008). For instance, Omil et al. (1995) showed that anaerobic treatment of highly saline fish-processing wastewater was possible without any toxic effects in case a suitable strategy is applied for adapting the biomass to high salinity. Recent research

of Ismail et al. (2010) shows that long term adaptation periods result in high sodium tolerance, only limitedly impacting the specific methanogenic activity (SMA) between 10 and 20 g Na⁺·L⁻¹. However, the sludge was rather dispersed with a low settleability and prone to rinsing from the reactor system.

2.2.6 OLR and HRT shocks

Daily variations in the production of industries can result in hydraulic and organic shock loads causing short or long term detrimental effects on anaerobic treatment systems. Whereas organic shock loads can cause deterioration of anaerobic reactor performance due to accumulation of volatile fatty acids (VFA), drop in pH, and flotation of granular sludge, hydraulic shocks are characterized by the increase in the effluent SS concentrations due to the wash out of biomass (Blaszczyk et al., 1994). Chua et al. (1997) investigated the stability of an anaerobic fixed-film reactor treating a simulated food-processing wastewater under critical hydraulic shock loadings. The hydraulic shocks up to five fold of the regular flow resulted in a temporary reduction in COD removal efficiency and affected pH of the treated effluent and biogas production, whereas under ten times hydraulic shock loading a drastic deterioration was observed in treatment performance, resulting in reactor souring and failure. However, the system performance recovered when the hydraulic shock loadings were alleviated, which can be attributed to the immobilized biofilm design of anaerobic fixed-film reactors.

2.2.7 Calcium scaling

It is well known that calcium up to 150 mg·L⁻¹ can promote granulation and improve the stability of granular sludge. However, many industrial wastewaters such as effluents generated from paper processing industries, whey, sugar and olive oil factories can contain high concentrations of calcium. Calcium precipitation in granular bed reactors may lead to formation of too heavy sludge, sludge bed cementation and methanogenic activity loss due to scaling of the granules which limits the substrate and nutrient transfer (van Langerak et al., 2000).

Table 2.1 : Advantages and disadvantages of AnMBRs for the mitigation of problems induced by industrial wastewaters at extreme conditions.

Extreme Conditions	Effects on anaerobic process	Advantages/Disadvantages
High SS content	- Problems at influent distribution systems in granular bed reactors. - Clogging in anaerobic filters. - Less efficient granulation. - Accumulation of slowly degradable organic matter in the sludge bed, particularly in UASB reactors.	- Biomass retention is not dependant on granulation. - There is no need for a special influent distribution system if a CSTR is used as the bioreactor. - All particulate matter is retained in the reactor. This may provide more complete digestion for the slow degradable organic matter and improve digestion efficiency. - Inert particulate material can accumulate in the reactor and this may necessitate sludge discharge.
FOG content	- Impairment of granulation. - Biomass flotation and wash out. - LCFA inhibition. - Suppression of methanogenic activity due to mass transfer limitations.	- No biomass washout. - The effects of FOG on both biomass and membrane in AnMBRs are not well documented yet.
Thermophilic conditions	Difficulty in granulation of anaerobic biomass.	- Biomass retention is not dependant on granulation. - Inoculating the reactor with non-adapted mesophilic sludge can cause serious membrane fouling due to the decay of mesophilic bacteria under thermophilic conditions. - Temperature fluctuation can cause stress on biomass and increase membrane fouling. - A higher flux may be achieved due to reduced viscosity of the sludge at thermophilic conditions. - Lower fluxes were reported compared to mesophilic conditions due to more compact cake layer under thermophilic conditions.
Toxicity	- Inhibition of activity or decay of biomass depending on the type of the toxic compound. - Long acclimation time. - Problems in granulation and biomass loss.	- May provide a better dilution under a toxic shock load if a CSTR is used as the bioreactor. - No biomass loss. - All bacteria are retained in the reactor regardless of their settling/granulation properties. This may provide better adaptation to toxic compound. - Bioaugmentation of specialized bacteria for a specific compound is possible.
High salinity	- Reduced activity. - Long adaptation time. - Biomass decay.	- Bacterial products due to osmotic pressure stress may negatively affect filtration performance. - All bacteria are retained in the reactor regardless of their settling/granulation properties. This may provide better adaptation to salinity.
OLR and HRT Shocks	- Biomass loss. - Reduction in treatment performance. - Acidification.	- No biomass washout due to a hydraulic shock. - A stable performance can be maintained under a HRT shock since the particulate COD would be retained in the reactor. - An AnMBR is a bioreactor which has its own organic loading capacity determined by many factors such as biomass concentration, activity and substrate properties. Therefore, under an organic shock load system performance would be similar to a conventional anaerobic reactor. Moreover, shock loadings can stimulate the release of EPS/SMP and increase membrane fouling.
Calcium Scaling	- Activity loss. - Sludge bed cementation.	- Calcium scaling would not be effective on flocculent biomass. - Calcium and other ions can increase inorganic membrane fouling.

2.3 AnMBR Applications for the Treatment of Industrial Wastewaters

There are mainly three types of AnMBR configurations depending on the location of the membrane and the application of permeate driving force such as pressure or vacuum (Liao et al., 2006) (Figure 2.1).

The shear force needed to scour the membrane surface can be applied by liquid and/or biogas recirculation in AnMBRs. In external cross-flow AnMBRs, the sludge is pumped to the membranes with a high velocity in order to limit cake formation and fouling (Figure 2.1a). Moreover, the cross-flow pump creates a driving pressure to push the water through the membrane pores. In submerged configuration the membrane can be either immersed directly into the reactor or in an external chamber. A vacuum pump is used to suck the permeate through membrane pores and the membrane surface is scoured by biogas sparging. Both configurations have their own advantages and disadvantages. Membrane replacement and cleaning is much easier in external cross-flow AnMBRs. On the other hand energy consumption for liquid recirculation may be much higher in cross-flow mode. In submerged AnMBRs, the biogas recirculation lines must be leak and fire proof which can increase the construction costs.

To overcome the problems frequently encountered in the treatment of industrial wastewaters by conventional anaerobic treatment processes, membrane assisted separation of biomass offers a possible alternative. Recent studies about the application of this technology at lab-, pilot- and full-scale reactors are summarized in the following sections.

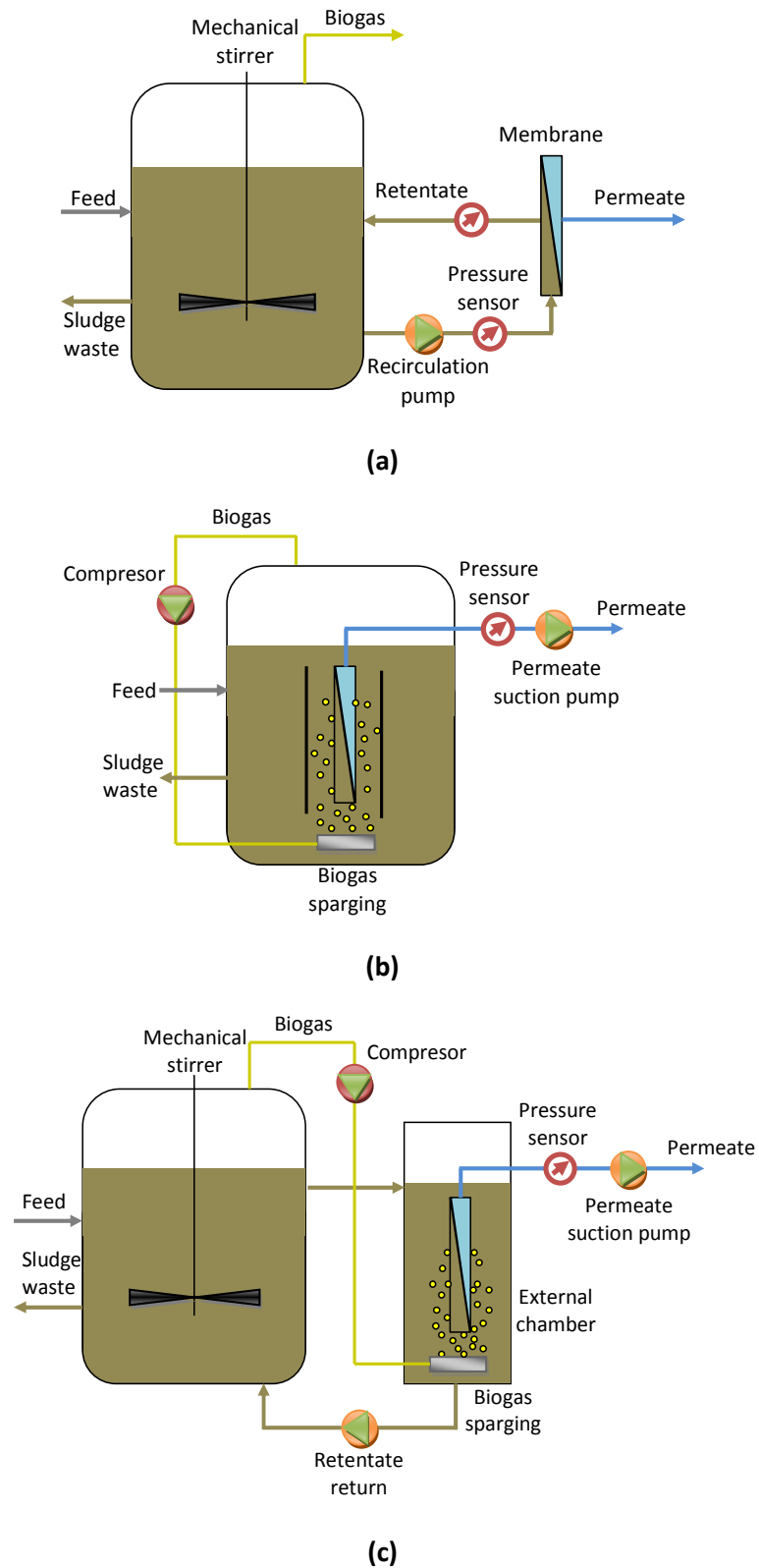


Figure 2.1 : Different configurations of AnMBRs (a) Pressure-driven external cross-flow membrane, (b) Vacuum-driven submerged membrane with the membrane immersed in to the reactor, (c) Vacuum-driven submerged membrane with the membrane immersed in an external chamber (Liao et al., 2006).

2.3.1 Lab-scale

Various studies have investigated the treatability of industrial wastewaters by AnMBRs at lab-scale. Table 2.2 and 2.3 present performance data of lab-scale AnMBRs used for the treatment of miscellaneous industrial wastewaters in terms of treatability and filterability, respectively. As can be seen in the tables, high OLRs (up to $24 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$), and fluxes (up to $140 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) could be achieved.

Several parameters such as temperature, SRT, OLR, shear rate, etc. are likely to affect both treatability and filterability. While factors such as substrate composition and bioreactor operating conditions have an indirect effect on fouling by influencing the sludge characteristics, factors related to membrane material and operation have a direct relation to membrane fouling (Figure 2.2). Besides, the shear rate has both a direct effect, by preventing membrane fouling due to scouring the membrane surface, and an indirect impact, by disrupting the bioflocs and creating fine particles, on fouling. These parameters and their effects are discussed in the following sub-sections.

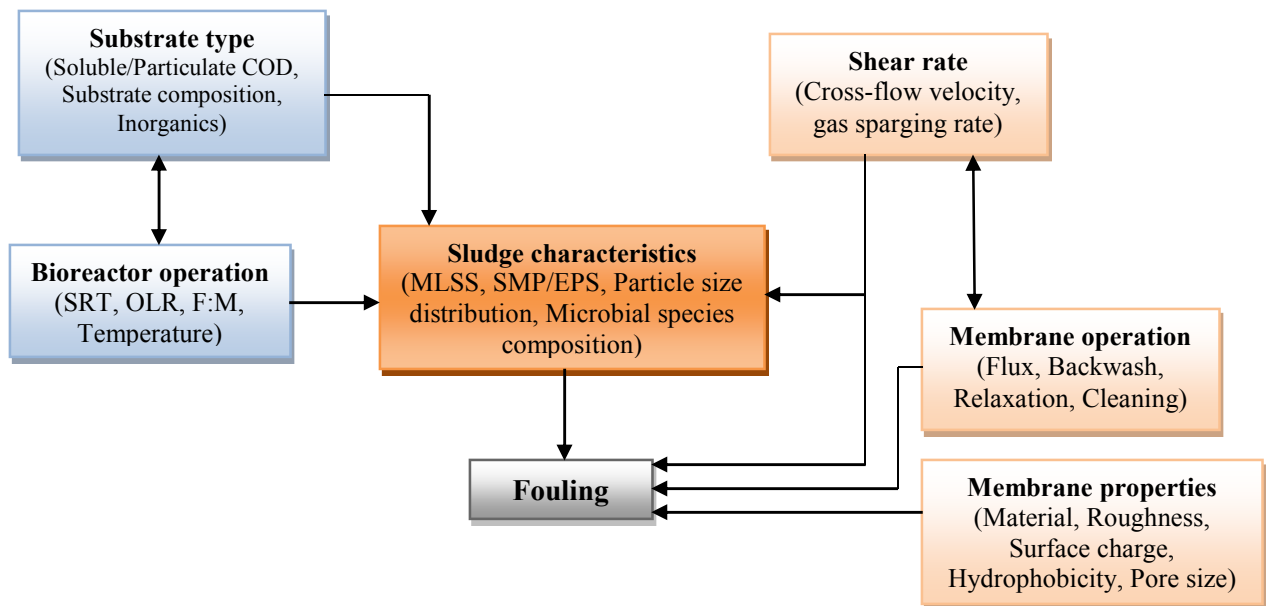


Figure 2.2 : Factors affecting membrane fouling in AnMBRs.

Table 2.2 : Treatment performance of lab-scale AnMBRs used for the treatment of various industrial wastewaters.

Wastewater Type	Reactor Volume(L)/ Temperature (°C)	OLR (kg COD.m ⁻³ .d ⁻¹)	HRT (d)	SRT (d)	TSS (g.L ⁻¹)	COD Removal (%)	Specific CH ₄ Production (m ³ .kg ⁻¹ COD _{removed})	Reference
Palm oil mill	50/-	1-11	6.8-600	12.1-1000	11.8-20.8	96-99	0.25-0.57 ^a	Abdurrahman et al., 2011
Thermomechanical pulping pressate	10/37-45-55	2.59 ± 0.53	-	350	10.9±0.5	76-83	0.21 ^b	Gao et al., 2011a
Thermomechanical pulping whitewater	10/37	2.6-4.8	-	280	6.7-9.1	90	0.25-0.30	Lin et al., 2011
Brewery with surplus yeast	4.5/30	1-12	-	-	25 ^c	>97	-	Torres et al., 2011
Thermomechanical pulping whitewater	10/37	2.4±0.4	-	280	5.7-10	30-90	0.35-0.41 ^b	Gao et al., 2010a
Kraft pulp mill evaporator condensate	10/37	-	-	230	3.7-5.7	95-99	-	Lin et al., 2010
Kraft evaporator condensate	10/37	1-24	0.5-1.0	∞ ^d	5-10	93-99	0.35±0.05	Xie et al., 2010
Kraft evaporator condensate	10/37	12.2±1.1	-	230	9	97-99	0.35±0.05	Lin et al., 2009
Kraft evaporator condensate	10/55	3.1±0.8	-	230	9	97-99	0.35±0.05	Lin et al., 2009
Simulated petrochemical	58(23) ^e /37	<25	0.71	175	30-36	97	-	Van Zyl et al. (2008)
Acidified cheese whey	20(15) ^e /37	5-20	4	30-79	6.4-10 ^c	98.5	0.3	Saddoud et al. (2007)
Slaughterhouse	100(50) ^e /37	4.4-13.3	1.25-3.33	-	2-10 ^c	62-98.8	0.13-0.33	Saddoud and Sayadi (2007)
Food processing	500(400) ^e /37	0.88-4.52	2.5	50	6-8	81-94	0.136 ^f	He et al., 2005
Slaughterhouse	17/37	5-17	35	30-40	-	>90	-	Siegrist et al., 2005
Sauerkraut brine	7/30	2-8.6	-	-	25-60	>90	0.2-0.34 ^g	Fuchs et al. (2003)
Slaughterhouse	7/30	1-8	1.2	-	20-28	>90	0.12-0.32 ^g	Fuchs et al. (2003)
Alcohol fermentation	5/55	3-3.5	1	∞ ^d	2	93-97	-	Kang et al. (2002)
Alcohol-distillery	4/53-55	1.5	-	-	1-3.2	-	-	Choo and Lee (1998)
Palm oil mill	50/35	14.2-21.7	2.82-3.15	77-161	50.8-56.6	91.7-94.2	0.24-0.28	Fakhru'l-Razi and Noor (1999)

^a Reported as m³.kg⁻¹ COD.d⁻¹ ^b Reported as biogas, ^c Reported as g VSS.L⁻¹, ^d No sludge wastage except sampling, ^e Effective volume, ^f Reported as biogas: m³.kg⁻¹ COD, ^g Reported as m³ CH₄.kg⁻¹ COD_{fed}

Table 2.3 : Membrane performance of lab-scale AnMBRs used for the treatment of various industrial wastewaters.

Wastewater Type	Membrane Type and Properties	Membrane Configuration	TMP (bar)	Cross-Flow Velocity (m.s ⁻¹)	Gas Sparging Rate (L.min ⁻¹)	Flux (L.m ⁻² .h ⁻¹)	Reference
Palm oil mill	UF (MWCO ^a : 200 kDa, 0.1 µm, tubular, 0.024 m ²)	Side-stream cross-flow	1.5-2	-	-	-	Abdurrahman et al., 2011
Thermomechanical pulping pressate	UF (MWCO:70 kDa, flat sheet, 0.03 m ²)	Submerged		-	1.5	5.7-6.9	Gao et al., 2011a
Thermomechanical pulping whitewater	MF (MWCO:70 kDa, flat sheet, 0.03 m ²)	Submerged	<0.4	-	0.75	4.8-9.1	Lin et al., 2011
Brewery with surplus yeast	MF (0.2 µm, tubular) UF (0.03 µm, tubular)	Gas-lift	-	-	0.2-0.35 ^b	6-20 <10	Torres et al., 2011
Thermomechanical pulping whitewater	MF (MWCO:70 kDa, flat sheet, 0.03 m ²)	Submerged	<0.3	-	0.75	4.3-5.2	Gao et al., 2010a
Kraft pulp mill evaporator condensate	MF (MWCO:70 kDa, flat sheet, 0.03 m ²)	Submerged	<0.3	-	0.4	5.3±1	Lin et al., 2010
Kraft evaporator condensate	MF (MWCO:70 kDa, 0.3 µm, flat sheet, 0.03 m ²)	Submerged	<0.3	-	0.3-0.75	5.6-12.5	Xie et al., 2010
Kraft evaporator condensate	MF (MWCO:70 kDa, flat sheet, 0.03 m ²)	Submerged	<0.3	-	0.75	7.2±0.9 ^b	Lin et al., 2009
Kraft evaporator condensate	MF (MWCO:70 kDa, flat sheet, 0.03 m ²)	Submerged	<0.3	-	0.75	2.4±0.6 ^c	Lin et al., 2009
Simulated petrochemical	MF(0.45 µm, flat sheet, 0.351 m ²)	Submerged	0.005	-	-	1.5-4.5	Van Zyl et al. (2008)
Acidified cheese whey	MF (0.2 µm, 0.4 m ²)	Side-stream cross-flow	1.25-2.25	5	-	137-140	Saddoud et al. (2007)
Slaughterhouse	UF (MWCO: 100kDa, 1 m ²)	Side-stream cross-flow	1	3	-	2-8	Saddoud and Sayadi (2007)
Food processing	UF (MWCO:20-70 kDa, flat sheet, 0.32 m ²)	Side-stream cross-flow	2	1.02-1.09	-	13.1-18.9	He et al., 2005
Slaughterhouse	UF (0.06-3 µm)	Side-stream cross-flow	-	-	-	40-100	Siegrist et al., 2005
Sauerkraut brine	MF (0.2 µm, 0.126 m ²)	Side-stream cross-flow	-	2-3	-	5-10	Fuchs et al. (2003)
Slaughterhouse	MF (0.2 µm, 0.126 m ²)	Side-stream cross-flow	-	2-3	-	5-10	Fuchs et al. (2003)
Alcohol fermentation	MF (0.14 µm, tubular, 0.0113 m ²) MF (0.2µm, tubular, 0.0129 m ²)	Side-stream cross-flow	0.6	3	-	70-85 95-130	Kang et al. (2002)
Palm oil mill	UF (MWCO: 200kDa)	Side-stream cross-flow	1.5	2.3	-	26.4-30.3	Fakhru'l-Razi and Noor (1999)
Alcohol-distillery	UF (MWCO:20kDa, flat sheet, 0.0168 m ²)	Side-stream cross-flow	0.5-3	0.5 – 1.25	-	10-40	Choo and Lee (1998)

^a MWCO: Molecular Weight Cut-off, ^b Reported as m/s, ^c Mesophilic, ^d Thermophilic

2.3.1.1 Temperature

Membrane flux can be improved by rising temperature due to the reduced viscosity of liquid at high temperatures. A lower sludge viscosity means that lower shear rates will be required in order to obtain the same shear stress, which results in lower energy requirements (Jeison and van Lier, 2006b). A lower permeate viscosity also increases the membrane permeability by decreasing the trans-membrane pressure (TMP). Jeison and van Lier (2006b) observed higher critical flux values for thermophilic AnMBRs compared to mesophilic ones, which supported the idea that better filtration performance can be achieved under thermophilic conditions due to the reduced viscosity of the sludge. However, in a subsequent study (Jeison and van Lier, 2007a) the advantage of thermophilic operation in the short term tests turned to be a disadvantage on long term continuous operation. Long term fluxes observed in the thermophilic submerged AnMBR were unexpectedly 2-3 times lower than those attained under mesophilic conditions. Thus, it was concluded that the physiological effects of temperature on the properties and composition of the sludge on the long term were more important for membrane filtration than the physical effect of temperature on sludge rheology and liquid viscosity.

Lin et al. (2009) investigated the influence of sludge properties on membrane fouling in submerged AnMBRs treating kraft evaporation condensate. The systems were operated under both mesophilic and thermophilic conditions, at varying OLRs but under similar hydrodynamic conditions and mixed liquor suspended solids (MLSS) concentrations. The results showed that the cake layer resistance was significantly higher for the thermophilic AnMBR, resulting in a very low long-term flux ($1.8 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) in comparison to the mesophilic AnMBR ($7.4 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$). Furthermore, the effluent quality of the mesophilic reactor was significantly better than the thermophilic one. The thermophilic sludge showed bimodal particle size distribution consisting of small size flocs (1-15 μm) and macroflocs (60-200 μm), whereas the mesophilic sludge had a unimodal distribution. The significantly higher resistance in thermophilic AnMBRs was attributed to the increased concentration of small size particles. Besides, the cake layer in the thermophilic AnMBRs was found to be more compact and less porous than that in the mesophilic AnMBRs (Lin et al., 2009; Jeison and van Lier, 2007a). Lin et al. (2009) also reported that the bound EPS and protein content in

thermophilic sludge cake layer was remarkably higher in comparison to that in mesophilic sludge cake layer, possibly due to the elevated temperature which generally increases the decay rate of bacteria. Sludge with high protein/polysaccharide ratios in bound EPS is usually considered to have a high stickiness, thus favoring cake formation. Moreover, sludge cake layer in the thermophilic AnMBR were found to be more compact and less porous than that in the mesophilic AnMBR.

On the other hand, thermophilic anaerobic wastewater treatment can have advantages in comparison to mesophilic processes, e.g. higher organic loading potentials and elimination of cooling requirements. Furthermore, granulation and biofilm formation, which is usually required to retain the biomass in high rate anaerobic systems, are less easily achieved under thermophilic conditions than under mesophilic conditions. From the biological point of view, the microbial growth and decay rates increase gradually with increasing temperature. The higher decay rate of thermophilic bacteria may be associated with the formation of small particles, i.e. decay products and/or cell debris, which are held responsible for membrane fouling. Fine particles in the sludge can also lead to cake compaction, which reduces the reversibility of flux loss in membranes (Jeison and van Lier, 2007b). Moreover, the history of the inoculum plays an important role (van Lier et al., 1992) and a significant amount of time might be necessary for developing a new bacterial consortium completely adapted to thermophilic temperatures from mesophilic seed sludge. Jeison and van Lier (2007a) observed a cake layer which is composed of extremely small particles with almost no bacterial cells in a thermophilic AnMBR. This result was attributed to the presence of mesophilic cell fragments still remaining from the inoculum. Gao et al. (2011a) observed a decrease in the particle size distribution and an increase in the permeate COD in an AnMBR in which the operating temperature was shifted from 37 to 55 °C. This may be attributed to the decay of mesophilic inoculum under thermophilic conditions.

2.3.1.2 Sludge retention time (SRT)

Generally, as a rule of thumb, SRTs in high-rate bioreactors are equal or more than 3 times the doubling time of the rate limiting biomass. Therefore and considering doubling times of 4-10 days of acetotrophic methanogenic biomass, an SRT exceeding 20 days is generally applied in mesophilic anaerobic high-rate reactors, whereas

industrial sludge bed reactors are often characterized by SRTs of 100-200 days or even more. An SRT completely independent from HRT can be controlled relatively easier in AnMBRs compared to other types of anaerobic reactors, irrespective the sludge quality. The SRTs applied in AnMBRs are generally in the range of 30-300 days, similar to industrial high-rate sludge bed reactors. In principle, high SRTs are more desirable since it corresponds to less sludge production and higher sludge concentrations in the reactor. However, long SRTs may also affect methanogenic sludge activity owing to a decrease in viable microorganism concentration. Moreover, Jeison and van Lier (2006b) showed that sludge concentration affected the critical flux in AnMBRs and lower flux rates were obtained at increasing sludge concentrations. Moreover, the accumulation of inorganic solids (Ca, Mg, PO₄ salts and silt) at high SRTs may increase inorganic fouling, a parameter of concern in AnMBR operations (Kang et al., 2002).

The effect of high SRTs especially on membrane filtration performance is still a research topic that needs to be further investigated. High SRTs may stimulate cell lysis, which also increases the release of inert decay products and soluble microbial products (SMP). On the other hand, high SRT means high sludge concentrations leading to a rapid cake layer build up being a protective layer for membrane pore blocking. The negative part is that cake compaction is more pronounced leading to flux decline.

2.3.1.3 Organic loading rate (OLR)

High OLRs and short HRTs can theoretically be applied in AnMBRs. However, the OLR of the system is not a standalone parameter and it should be evaluated together with SRT and activity of the sludge. Therefore, high sludge concentrations do not always necessarily translate into high applicable OLR. The activity of the biomass plays a key role in applicable OLRs.

Van Zyl et al. (2008) studied the treatment of simulated petrochemical industry wastewater by a submerged AnMBR equipped with flat sheet MF membranes and achieved a very high COD removal efficiency at organic loading rates up to 25 kg COD·m⁻³·d⁻¹ during the long term operation. Saddoud and Sayadi (2007) investigated the treatability of slaughterhouse wastewater by an AnMBR at relatively high organic loading rates between 4.4 and 13.3 kg COD·m⁻³·d⁻¹. They experienced a process failure at an OLR of 16.3 kg COD·m⁻³·d⁻¹ due to VFA accumulation. An AnMBR

treating palm oil mill wastewater achieved very high COD removal performance (>96%) at OLRs of 1-11 kg COD·m⁻³·d⁻¹ and HRTs of 7-600 days (Abdurrahman et al., 2011).

2.3.1.4 Shear rate

Liquid recirculation and/or gas sparging are the most common ways to provide shear over the membrane surface in order to remove foulants or to restrict their interaction with the membrane (Liao et al., 2006; Torres et al. 2011). The cake layer formation rate was found to be positively correlated with the fraction of small size particles and flux, whereas it was negatively correlated with the gas sparging rate (Lin et al., 2010). The magnitude of back transport that prevents the particles to be dragged to the membrane surface by the convective permeate flux depends mainly on the particle size: basically it decreases with decreasing particle size (Choo and Lee, 1998). Therefore, the particle size distribution in a suspension affects the attainable permeate flux in membrane filtration, with the smallest particles determining the filterability. Torres et al. (2011) concluded that the small size particles, which correspond to less than only 10% of the total sludge mass, assigned the critical flux in an AnMBR. Similar results were also presented by other researchers (Jeison et al., 2009a; Lin et al., 2009).

Torres et al. (2011) demonstrated that the liquid recirculation rate of a gas-lift AnMBR treating brewery wastewater was more effective in membrane fouling control than the gas sparging rate. They reported a minor improvement of the critical flux with increased gas recycle flows, i.e., from 0.16 to 1 m·s⁻¹, and concluded that further measures such as modification of sludge properties should be undertaken in order to boost the flux. Xie et al. (2010) reported that the membrane critical flux of a submerged AnMBR increased and the fouling rate decreased when the biogas sparging rate was increased from 0.3 to 0.75 L·min⁻¹. However, they indicated that there was a practical limit above which further increasing the biogas sparging rate provides little added benefit. Similar results, also for submerged AnMBRs, were presented by Jeison and van Lier (2006b). Choo et al. (2000) have shown that the resistance due to cake layer formation can be decreased by increasing the cross-flow velocity. However, at a Reynold's number of 2000 a plateau was reached, beyond which increasing the cross-flow velocity did not further reduce the resistance.

Adding more complexity to the situation, high shear rates may stimulate the breakdown of microbial flocs and increase the cake layer resistance due to the selective deposition of fine particles in the cake layer and membrane pores during long term operation. As a result, even higher shear rates are required, which in turn form a dense consolidated cake layer that is very hard to remove. This phenomenon has been called the notorious shear rate dilemma (Jeison et al., 2009b).

Zhang et al. (2007) determined that maintaining high cross-flow velocities is critical to limit the fouling rate in AnMBRs. However, even at high cross-flow velocities ($2 \text{ m}\cdot\text{s}^{-1}$), the membrane resistance continues to increase at a low rate due to the deposition of small particles on the membrane surface or inside the membrane pores. Frequent chemical cleaning could not prevent the development of irreversible fouling. Furthermore, high cross-flow velocities may lead to increased internal fouling of the membrane by reducing the cake layer thickness, which acts like a barrier against the fine particles (Choo and Lee, 1998).

Padmasiri et al. (2007) studied the long term methanogenic population dynamics and performance of an AnMBR treating swine manure at high cross-flow velocities ($0.9\text{-}2 \text{ m}\cdot\text{s}^{-1}$). They concluded that sudden changes in shear rate can have a negative effect on biomass activity in spite of improved membrane performance due to decreased cake layer resistance.

Fakhru'l-Razi and Noor (1999) reported that cross-flow velocities over $1.5 \text{ m}\cdot\text{s}^{-1}$ would be desirable to limit solids deposition on the membrane surface. However, this contrasts the negative effect of shear rate on anaerobic biomass activity and particle size as reported by many researchers (Brockmann and Seyfried, 1997; Akram and Stuckey, 2008a). Declining biomass activities may be due to the lysis of the cells under high shear or due to disrupting the juxtapositioning of bacterial species in syntrophic microbial consortia, hampering intercellular hydrogen transfer. Thermodynamically, acetogenic conversions, i.e., the oxidation of VFA to acetate under anaerobic conditions is only possible at low hydrogen concentrations, thus requiring syntrophic association of acetogens and hydrogen consuming methanogens. As the hydrogen concentrations are always lowest at the close perimeter of hydrogen consuming methanogens, VFA oxidizers should be close to hydrogenotrophic methanogens to maintain a thermodynamically feasible interspecies electron transfer distance. Rigorous mixing may impair the syntrophic association between acetogenic bacteria

and hydrogen utilizers (Kim et al. 2002; Stroot et al. 2001) by changing the juxtaposition of the cells. On the other hand, Padmasiri et al. (2007) propounded that the syntrophic interactions involving the acetogens and hydrogenotrophic methanogens remained intact regardless of the degree of shear in the AnMBR. Jeison et al. (2009a) reported a significant specific methanogenic activity (SMA) reduction for propionate in a cross-flow AnMBR but the value was still comparable with the values given for granular methanogenic sludge in the literature. The contradictory reports about the effect of shear conditions on activity may be attributed to the differences in the configuration of the experimental setups with different types of pumps and valves, which can provide different levels of shear. Moreover, the loss of biomass activity can also be a scale problem in laboratory systems where the reactor content has to be recirculated around the membrane loop several times in an hour to provide a sufficient cross-flow velocity. In addition, the shear effect of full-scale pumps and valves can be significantly different from those used in lab-scale setups. For single species conversions, such as acetotrophic methanogenesis, size reduction of microbial flocs lowers the mass transfer resistance and thus leads to an increased activity, which was confirmed in the studies of Jeison et al. (2009a). Although the actual impact of shear and subsequent floc size reduction on microbial activity has yet not been elucidated, it is very plausible to claim that the small sized particles enhance membrane fouling due to the compaction of the cake layer (Jeison and van Lier, 2007b).

2.3.1.5 Substrate composition

Anaerobic digestion is a multistep process that requires a balanced ecosystem of a number of different microbial species. Both the quantity and physical features of these microorganisms determine the physical properties of the sludge such as rheology and particle size (Jeison and van Lier, 2007a; Jeison et al., 2009a). Substrate composition, one of the most important factors determining microbial diversity in anaerobic reactors, can indirectly affect the filtration performance of the AnMBR systems. For instance, feeding non-acidified substrates to an AnMBR can promote the proliferation of single cell acidogenic bacteria growing dispersedly and thus, yielding small sized particles. Jeison et al. (2009) have clearly shown that the small sized particles that were apparently acidogenic biomass, determined the attainable critical fluxes in an AnMBR.

Moreover, the inorganic species present in different industrial effluents should also be evaluated in respect to inorganic membrane fouling (Kang et al., 2002).

2.3.1.6 Membrane properties

The membrane properties, including surface characteristics, material and pore size, play a role in their fouling. Although hydrophobic membranes are more durable under harsh chemical and thermal conditions, hydrophilic membranes are normally favored because they are less prone to organic fouling. Modification of the surface characteristics of hydrophobic membranes by coating or grafting is one of the means to obtain hydrophilicity (Stuckey, 2010). Choo et al. (2000) reported that surface modification of the organic membrane by grafting with 2-hydroxyethyl methacrylate (HEMA) altered its properties to hydrophilic and led to a 35% flux increase. Sainbayar et al. (2001) reported 13.5% flux enhancement in a surface modified polypropylene membrane having 70% degree of grafting with HEMA. However, albeit observed flux enhancement, the intrinsic membrane resistance increased, which was attributed to the reduction in membrane pore size depending on the graft polymerization. Apparently, there exists an optimal degree of grafting in order to maximize flux enhancement.

Kang et al. (2002) have investigated the effect of the membrane material on the fouling mechanism and compared the fouling characteristics of organic, i.e. polypropylene, and inorganic, i.e. zirconia skinned, microfiltration membranes coupled to a thermophilic anaerobic reactor treating high strength distillery wastewater. The organic and inorganic membranes had completely different fouling characteristics. In the pores of the inorganic membranes, struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) accumulated therewith playing a key role in the flux decline. They concluded that the inorganic membranes are more prone to inorganic fouling due to their surface characteristics, such as charge and functional groups, and they are more severely affected by the ligand exchange reactions or complex formation between metal ions and VFAs present in anaerobic reactors. In contrast, the organic membranes were characterized by the formation of a thick cake layer composed of biomass and inorganic compounds, which resulted in distinct flux decline. It was also determined that back flushing with alkaline instead of acid was more effective in flux recovery for both the organic and inorganic membranes (Kang et al., 2002, Choo et al., 2000).

In an effort to determine the effect of membrane pore size on the filtration performance and fouling mechanism in AnMBRs, He et al. (2005) have studied the behavior of polyethersulfone (PES) membranes with different molecular weight cut-off (MWCO) ranging from 20-70 kDa. It turned out that all membranes, regardless of their pore sizes, exhibited similar high removal efficiencies in terms of COD, SS, color and bacteria, i.e., 90%, >99.9%, 98%, and 5 logs, respectively. Thus, the pore size had little impact on removal efficiencies.

2.3.1.7 Cake layer

Although the cake layer on the membrane surface originates from the digestion broth in the reactor, it seemingly differs from the bulk sludge in terms of particle size distribution, EPS content and microbial population. Lin et al. (2011) observed that the cake layer contained a high fraction of fine particles and it exhibited a bimodal particle size distribution. The filtration resistance of the fine particles in the bulk sludge was similar to the cake layer, which was over 6 and 4 times higher than those of large flocs and bulk sludge liquor, respectively (Lin et al., 2011). The elemental analysis of the cake sludge showed that a significant amount of inorganics accumulated in the cake layer, probably due to the charge neutralization and bridging effects between metal ions and flocs or biopolymers. It was also concluded that the cake sludge had significantly higher bound EPS and protein content, which increased the sludge adhesion on the membrane surface to a higher extent compared to the bulk sludge (Lin et al., 2011; Vyrides and Stuckey, 2010).

PCR-DGGE analyses showed that some species detected in the bioreactor were not present in the cake layer and the intensity of some other species was higher in the cake layer (Lin et al., 2011). Most recently, Vyrides and Stuckey (2010) demonstrated that the biofilm/cake biomass was apparently more active than the suspended biomass in an AnMBR treating saline wastewater (35 g NaCl·L⁻¹) with high levels of chromium (200 mg·L⁻¹). Gao et al. (2010b) have shown that mechanisms and rates of microbial attachment differ for different species and membranes with distinct surface properties. However, additional research should be conducted to explain these differences.

2.3.2 Full and pilot scale

The performances of pilot and full scale AnMBRs used for the treatment of various industrial wastewaters in terms of treatability and filterability are presented in Table 2.4. Most of the full scale AnMBR installations were established in Japan (Kanai et al., 2010). Kanai et al. (2010) reported that there were fourteen AnMBR implementations in Japan, equipped with flat sheet membranes, which treat a wide spectrum of substrates such as alcohol production stillage, organic wastes, wastewater treatment sludge and food processing residues (dairy, potato, confectionary, etc.). However, the performance data in terms of removal efficiency and operating flux of these plants are still limited. Kanai et al. (2010) reported a full scale AnMBR treating distillery stillage (COD: $101 \text{ g}\cdot\text{L}^{-1}$, TS: 6%, TN: $3.7 \text{ g}\cdot\text{L}^{-1}$) that achieved high COD removal efficiencies (72-92%). However, no information about the operational flux and fouling was presented. Another full scale AnMBR treating the effluents of a food industry producing salad dressings and barbeque sauces (COD: $34 \text{ g}\cdot\text{L}^{-1}$, TS: 1.1%) was located in the United States (US). It was reported that the plant achieved an average COD removal efficiency of 99.4% and a flux rate ranging between 2.5 and $4.2 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ during the first two years of operation (Christian et al., 2010). Moreover, Ewing et al. (2008) reported a full scale AnMBR utilizing tubular cross-flow membranes in the US. This plant has been operating more than two years and treating acid cheese whey with very high COD removals (99%).

Two pilot scale AnMBR installations treating stillage from tequila production (COD: $38 \text{ g}\cdot\text{L}^{-1}$, TSS: $17.4 \text{ g}\cdot\text{L}^{-1}$) (Grant et al., 2010) and potato processing wastewater (COD: $56.9 \text{ g}\cdot\text{L}^{-1}$, TSS: $14.8 \text{ g}\cdot\text{L}^{-1}$) (Singh et al., 2010) were reported to achieve more than 95% COD removal efficiencies without serious fouling problems. Operational fluxes obtained in AnMBR treating potato wastewater was reported as $0.83\text{-}5 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ (Singh et al., 2010). Most recently, Dereli et al. (2012b) reported a pilot-scale AnMBR equipped with flat sheet membranes treating ethanol thin stillage at OLRs ranged between $4.5\text{-}7 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. They obtained more than 98% COD removal efficiency. Moreover, a flux of $4.3\pm 1.1 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ could be maintained at MLSS concentrations of about $24 \text{ g}\cdot\text{L}^{-1}$ and a TMP of 0.1-0.2 bar. Diez et al. (2012) operated an AnMBR, consisting of a UASB reactor equipped with hollow fibre membranes, for the treatment of snacks factory wastewater with high FOG content up to $6000 \text{ mg}\cdot\text{L}^{-1}$. The reactor achieved COD removal efficiency of 97% at an OLR of $5.1 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$.

$^3 \cdot d^{-1}$. Successful operation is attributed to the membranes for the retention of biomass that otherwise would be washed out due to the poor settling property of FOG accumulated sludge. Authors optimized membrane operation by adjusting the filtration, relaxation and backwash cycles and by doing so they obtained long term fluxes between 6.5 and 8 $L \cdot m^{-2} \cdot h^{-1}$. Diez et al. (2012) observed an increased fouling rate at slightly higher fluxes exceeding 8 $L \cdot m^{-2} \cdot h^{-1}$ and reported a fouling rate of 26.5 $mbar \cdot d^{-1}$ at the operational flux of 8 $L \cdot m^{-2} \cdot h^{-1}$.

So far, most of the AnMBR research is conducted in laboratory scale and only a few studies report large scale AnMBR applications. Although lab-scale studies provided the invaluable information on treatability and membrane fouling mechanisms with scientific insight, the hydraulics and shear forces acting on a small membrane module will significantly differ from a full scale membrane module (Dereli et al., 2012b). Moreover, it is very hard to extrapolate membrane data from one application to another due to the differences in reactor/membrane configuration, operation, sludge characteristics and substrate composition. In general, similar fluxes (1-5 $L \cdot m^{-2} \cdot h^{-1}$) even at higher MLSS concentrations (23-40 $g \cdot L^{-1}$) were reported for large scale submerged AnMBRs in comparison to lab-scale studies. Moreover, and interestingly, serious membrane fouling problems were not reported in pilot and full scale studies.

Table 2.4 : Treatment and membrane performance of pilot- and full-scale AnMBRs used for the treatment of various industrial wastewaters.

Parameter	Dereli et al., 2012b	Diez et al., 2012	Grant et al., 2010	Singh et al., 2010	Christian et al., 2010	Brockman and Seyfried, 1997
Wastewater Type	Ethanol thin stillage	Snacks factory wastewater	Stillage from tequila production	Potato processing	Food processing	Potato starch
Reactor Volume(m ³)/ Temperature (°C)	12/37	0.76/35	1.3/37	3.3/35	8700/33	4/-
OLR (kg COD·m ⁻³ ·d ⁻¹)	4.5-7	5.1	4.8	2-12	1.2	1.5-5
HRT (d)	16	-	12.4	3.5-14	29	-
TSS (g·L ⁻¹)	24	7.9-10.4	-	40	23	-
SRT (d)	200	-	70	80	-	-
COD Removal (%)	98	97	95	99	99.4	-
Specific CH ₄ Production (m ³ ·kg ⁻¹ COD _{removed})	0.31	-	-	0.34	-	-
Membrane Type and Properties	Flat sheet (0.08 µm, 18 m ²)	Hollow fibre (0.4 µm, 2 m ²)	Flat sheet	Flat sheet (0.4 µm)	Flat sheet (0.4 µm)	Tubular (0.1 µm, 5.3 m ²)
Membrane Configuration	Submerged	Submerged	Submerged	Submerged	Submerged	Side-stream Cross-flow
TMP (bar)	0.1 - 0.2	-	-	0.03-0.04	0.03	1
Cross-Flow Velocity (m·s ⁻¹)	0.003-0.013	-	-	-	-	1.5-2
Gas Sparging Rate (m ³ ·m ⁻² ·h ⁻¹)	47-65	17.6	-	-	-	-
Flux (L·m ⁻² ·h ⁻¹)	4.3±1.1	6.5-8	-	0.83-5	2.5-4.2	-
Membrane Cleaning	NaOCl and HCl	500 mg·L ⁻¹ NaOCl	- ^a	%10 Citric acid (2 h)	- ^b	-

^a No cleaning was done until the end of study., ^b Membranes were cleaned in situ when the TMP reached 0.1 bar.

2.4 Problems Encountered and Future Perspectives

AnMBRs are somehow more prone to inorganic fouling by the precipitates of calcium, phosphorus and sulphur than their aerobic counterparts. This is because of the high concentration of these elements in the industrial wastewater of interest for AnMBR, the applied high loading rates, and the chemistry of carbon dioxide equilibrium (Stuckey, 2010). Struvite was reported as one of the most important precipitates affecting the filtration performance of inorganic membranes (Choo and Lee, 1998; Choo et al., 2000; Kang et al., 2002). Inorganic fouling should not be underestimated when treating complex wastewaters such as industrial effluents with high concentrations of nitrogen and phosphorus. Inorganic species can interact with soluble microbial products in the reactor and enhance the mechanical stability of the fouling layer (Lin et al., 2009). Furthermore, the pH increase in permeate pipes due to release of carbon dioxide under atmospheric pressure can also cause scaling problems in the permeate lines.

Since particles with the smallest size determine filterability in membrane processes, continuous particle size reduction due to applied shear rate decreases the attainable flux in AnMBRs in long term operation. Torres et al. (2011) suggested that modification of sludge properties should be considered to obtain high fluxes in AnMBRs. For that purpose, additives such as PAC, coagulants/flocculants can be used. Choo et al. (2000) assessed that PAC addition increases the mean particle size resulting in a lower specific cake resistance. Moreover, PAC could also reduce fouling by sorbing and/or coagulating colloidal matter in the reactor. Park et al. (1999) obtained an increased flux at high cross-flow velocities by addition of PAC and attributed this result both to the scouring effect of PAC and removal of colloidal matter by adsorption. Further research is needed to determine the effects of additives on improvement of filterability characteristics for AnMBRs.

The complete retention of slow growing methanogenic biomass in the reactor by membrane filtration may enable a faster start up of AnMBRs compared to conventional high rate anaerobic reactors. However, AnMBRs still need an appropriate start-up strategy in order to achieve a good filtration performance for long term. Moreover, selection of a suitable inoculum free of fibrous material and inert solids may be required to obtain good filtration performance. The fibrous material in the inoculum

may also clog the pumps and pipes before the membrane module entrance and inert material like silt can be abrasive for the membranes during long term operation. Trzcinski and Stuckey (2009) reported that in order to achieve a successful start-up period for AnMBRs, a low initial OLR, low shear rate, and long acclimation periods were needed, which are anyway shorter compared to other anaerobic reactor types. The sludge development in agreement with the influent composition would automatically occur in due time. The substrate composition determines the microbial diversity and it can play a role on both biological and long term filtration performance of AnMBRs.

Full scale implementation of AnMBRs will be largely dependent on the flux levels that can be achieved, on a long term basis. Membrane filtration was originally combined with anaerobic treatment by using side-stream configurations, where high levels of shear rate are provided at the expense of high energy requirements. Main disadvantages of high cross-flow side-stream configuration are the high energy demands, combined with the concern about a potential negative effect of a high shear rate over biomass activity (Liao et al., 2006). The down side of submerged full-scale configurations is, similar to aerobic MBRs, higher costs for maintenance procedures. Nonetheless, although lab-scale applications of AnMBR technology are generally carried out in side-stream configuration, interestingly almost all of the full scale installations are operated in submerged configuration. Apparently, full-scale experiences with submerged AnMBRs are thus far sufficiently satisfactory. However, according to Martin et al. (2011) energy efficiency of submerged AnMBRs are not much different than side-stream cross-flow AnMBRs as it is not the case for aerobic MBRs. This is attributed to the required high biogas flows for scouring the membrane in submerged AnMBRs. Apparently, biogas sparging for fouling control is an important cost factor and should be optimized to improve the feasibility of submerged AnMBRs.

Even though membrane separation represents a highly effective way for biomass retention, it inevitably involves higher operational and investment costs, compared to granular sludge or biofilm based technologies. However, a recent feasibility study revealed that AnMBRs are more suitable for wastewaters with COD concentrations exceeding $4\text{-}5\text{ g}\cdot\text{L}^{-1}$ (Martin et al., 2011). Then, MBR feasibility under anaerobic conditions will be determined by the balance between the techno-economic benefits

that the membrane enhanced retention can provide and the increase in treatment costs that comes from the application of membrane filtration processes. Capital and operational costs of MBR systems are directly related with the applied surface membrane area, which is proportional to operational flux. It is clear then that a high operational flux is a required condition for the economic feasibility of AnMBR full scale application for moderate to low strength wastewaters. However, for highly concentrated industrial wastewaters the operational flux and thus membrane costs may not be a limiting factor due to the low volumes of wastewater to be treated by the system. Therefore, the share of membrane costs in the whole investment may not be considerably high and the system may still be feasible considering the superior effluent quality and the reuse options.

2.5 Conclusions

In conclusion, AnMBR systems can be considered as an important potential technology that can fill in the gap between high rate anaerobic treatment systems and conventional slurry digesters. However, further investigations are still needed to find reasonable solutions to commonly encountered problems such as fouling and low flux that limits the widespread application and acceptance of AnMBRs for industrial wastewater treatment. There is still a large knowledge gap for the design and operation of full scale AnMBRs. To meet this deficit, information and experience gained from pilot scale systems are certainly helpful.

3. EFFECT OF LIPIDS ON BIOLOGICAL PERFORMANCE ²

3.1 Introduction

The wastewaters from food processing industries such as dairies, fish canning factories, olive mills, ice cream manufacturing and slaughterhouses are characterized by a high lipid content. In addition to these industrial processes, bioethanol production from corn which has increased rapidly in the last decade and became popular especially in United States, also generates a lipid rich stream called thin stillage. In corn-to-ethanol distilleries a part of thin stillage is recycled in the process, whereas the rest is generally evaporated to reduce its volume. The evaporator syrup, called distiller's solubles, is then mixed with other waste streams to be sold as animal fodder. Corn-to-ethanol thin stillage is a complex wastewater containing high concentrations of carbohydrates (glucan, xylose, xylan, arabinose, arabinan), proteins, lipids, glycerol and lactic acid (Kim et al., 2008). Corn typically contains 4% oil on dry basis and depending on the corn kernel breaking method applied in dry-milling process, around 50% of the oil in the raw material ends up in thin stillage (Wang et al., 2008). Moreover, the amount of oil passing to the thin stillage fraction is closely correlated with the dry matter content of thin stillage, which suggests that a significant fraction of oil is bound to particles (Wang et al., 2008). Moreau et al. (2011) determined that thin stillage contained 1.64-2.1% (w:w wet sample) crude oil and of that 9-10% was free long chain fatty acids (LCFA). Similar results on free LCFA content were also reported by other researchers (Noureddini et al., 2009; Wang et al., 2008).

Although lipids are potentially ideal substrates for anaerobic digestion, with high degradability and methane yield, anaerobic treatment of lipid rich wastewaters is often regarded as challenging due to the problems with biomass adsorption, deteriorating sludge retention, sludge activity and inhibition. LCFAs, lipid hydrolysis intermediates,

² This chapter was published as:

Dereli, R.K., van der Zee, F.P., Heffernan, B., Grelot, A., van Lier, J.B. (2014) Effect of sludge retention time on the biological performance of anaerobic membrane bioreactors treating corn-to-ethanol thin stillage with high lipid content. *Water Research* 49, 453-464.

exert a surface proportional toxicity to anaerobic biomass at millimolar concentrations (Hwu and Lettinga, 1997). They exhibit a similar type of toxicity as surfactants on cell membranes of methanogens, which can lead to cell lysis (Hwu and Lettinga, 1997; Rinzema et al., 1994). In addition to their bactericidal effect, LCFAs are also known to suppress the sludge activity by adsorbing on to the anaerobic biomass, thereby limiting access to substrate and nutrients. However, the biomass activity can be restored by the degradation of biomass associated LCFA in a prolonged time. The reversibility of LCFA inhibition indicates that it is largely related to mass transfer limitations than to the loss of metabolic functions and cell lysis (Cirne et al., 2007). Aside from suppressing biological activity, LCFAs have also been reported to impair granule formation (Hawkes et al., 1995) and to cause granular sludge flotation and washout in granular sludge bed reactors treating lipid rich wastewaters (Rinzema et al., 1993; Hwu et al., 1998a). Although granular sludge was found to be more resistant to LCFA inhibition than flocculent sludge due to its lower specific surface area (Hwu et al., 1996), Hwu et al. (1998a) reported that the sludge flotation threshold concentration was even lower than the inhibition concentration. Hence, efficient biomass retention is required for the success of high rate granular sludge bed reactors for the treatment of lipid rich wastewaters. To mitigate the sludge flotation and washout problems, Alves et al. (2007) developed an (inverted) anaerobic sludge blanket reactor fed from the top and equipped with a separation step at the bottom. Moreover, Kleyböcker et al. (2012) proposed to use divalent cations such as calcium and magnesium, which form less soluble precipitates with LCFAs, to control free LCFA concentrations and lower their toxicity.

Anaerobic membrane bioreactors (AnMBRs) have drawn an ever growing scientific interest since 2006 (Chapter 2). In AnMBRs, biomass is retained in the reactor, independent from its settling properties, with the aid of membranes. AnMBRs enable reactor operation at high biomass concentration, better control of sludge retention time (SRT), and therefore high organic loading rates (Jeison, 2007). Thus, AnMBRs can provide a possible alternative for the treatment of lipid rich wastewaters where biomass aggregation cannot be ensured and sludge flotation/washout is inevitable. Pereira et al. (2002) reported clear LCFA inhibition in a lab-scale expanded granular sludge bed reactor (EGSB) treating oleate at an organic loading rate (OLR) of $8 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. They concluded that suspended sludge, provided that it can be efficiently retained

inside the reactor, is advantageous over granular sludge due to its higher LCFA adsorption and its surface related degradation capacity. Diez et al. (2012) could treat snacks factory wastewater with high lipid content up to $6000 \text{ mg}\cdot\text{L}^{-1}$ in an AnMBR system consisting of a UASB reactor and hollow fiber microfiltration (MF) membranes. They reported high COD removal efficiency (97%) at moderate OLRs, i.e. $5.1 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Similarly, Dereli et al. (2012b) reported that a pilot-scale AnMBR could treat corn thin stillage at OLRs ranging between 4.5 and $7 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. However, these papers focused more on membrane filtration than on lipid biodegradation.

So far, most of the research on LCFA inhibition was conducted with synthetic wastewaters containing either a single type or a mixture of LCFAs. However, real industrial wastewaters are complex matrices of various organic and inorganic compounds. Therefore, there is a necessity to investigate the anaerobic treatment of real industrial wastewaters with high lipid content in continuously fed reactors in order to understand the process dynamics, stability and the interaction or effect of other compounds present in real wastewaters. The high suspended solids and lipid content of corn-to-ethanol thin stillage makes it a promising and challenging substrate for conventional high rate anaerobic treatment. Although the effects of lipids on anaerobic treatment and especially granular sludge bed systems are well documented in literature, their impact on the performance of AnMBR systems has not been investigated so far. Moreover, the SRT, one of the main parameters for bioreactor operation, has never been taken into consideration in AnMBR research as an important factor for LCFA degradation and inhibition. The aim of this chapter is to investigate the effects of high lipid concentration in corn-to-ethanol stillage on the biological performance of AnMBRs operated at different SRTs. Moreover, the importance of LCFA precipitation with divalent cations and adsorption on the sludge for LCFA removal and inhibition was evaluated.

3.2 Materials and Methods

3.2.1 Wastewater characterization

The feed was periodically prepared by diluting the evaporator syrup received from a full scale corn based bio-ethanol plant to simulate thin stillage. The syrup and diluted feed were stored in the fridge at 4°C until they were used. The characterization of the

feed used in the study is given in Table 3.1. As can be seen from the table, the most remarkable characteristic of the feed is its very high lipid concentration and around 45% of the COD was calculated as originating from lipid (assuming 2.87 g COD·g⁻¹ lipid).

Table 3.1 : Wastewater characterization (mean±standard deviation).

Parameter	Unit	Value
COD	g·L ⁻¹	72.2 ± 8.6
COD _{soluble}	g·L ⁻¹	34.7 ± 6.2
Lipid	g·L ⁻¹	11.3±0.5
TS	g·L ⁻¹	41.8 ± 6.2
VS	g·L ⁻¹	37.1 ± 5.6
SS	g·L ⁻¹	19.2 ± 2.7
VSS	g·L ⁻¹	19.2 ± 2.8
TN	mg·L ⁻¹	1193 ± 263
TP	mg·L ⁻¹	909 ± 85
PO ₄ ³⁻ -P	mg·L ⁻¹	654 ± 133
SO ₄ ²⁻	mg·L ⁻¹	948 ± 165
pH	-	3.89
Ca ²⁺	mg·L ⁻¹	89 ± 11
Mg ²⁺	mg·L ⁻¹	307 ± 31
K ⁺	mg·L ⁻¹	1453 ± 250

3.2.2 Reactor configuration and operation

The lab-scale AnMBR system consists of a continuously mixed 10 L anaerobic digester and a tubular cross-flow ultrafiltration membrane with a surface area of 0.0115 m² (Figure 3.1). The membrane was made of PVDF (Pentair X-Flow) with a mean pore size of 0.03 µm. The membrane was operated in cyclic mode, i.e., 5 minutes filtration and 30 seconds backwash, at a cross-flow velocity of 0.5 m·s⁻¹. The gross flux was between 10-14 L·m⁻²·h⁻¹. The reactor was gently stirred at 35 rpm by a top-entry mechanical mixer and by sludge recirculation through the membrane loop. The biogas production and pH were recorded on-line. The reactors were fed semi-continuously, at 60-min intervals. The feed and permeate flow rates were manually checked.

Two AnMBRs were operated in parallel at different SRTs, i.e. 20 and 30 days, for 3 months. The SRT was then increased from 30 days to 50 days in the second reactor for another 3 months. The reactors are named according to their SRTs, i.e. R-20, R-30, R-50, respectively. All reactors were operated at similar HRTs of 10-12 days. The reactors were inoculated with crushed and sieved (600 µm) granular sludge from a full scale EGSB reactor and they were operated under mesophilic conditions at 37±1 °C.

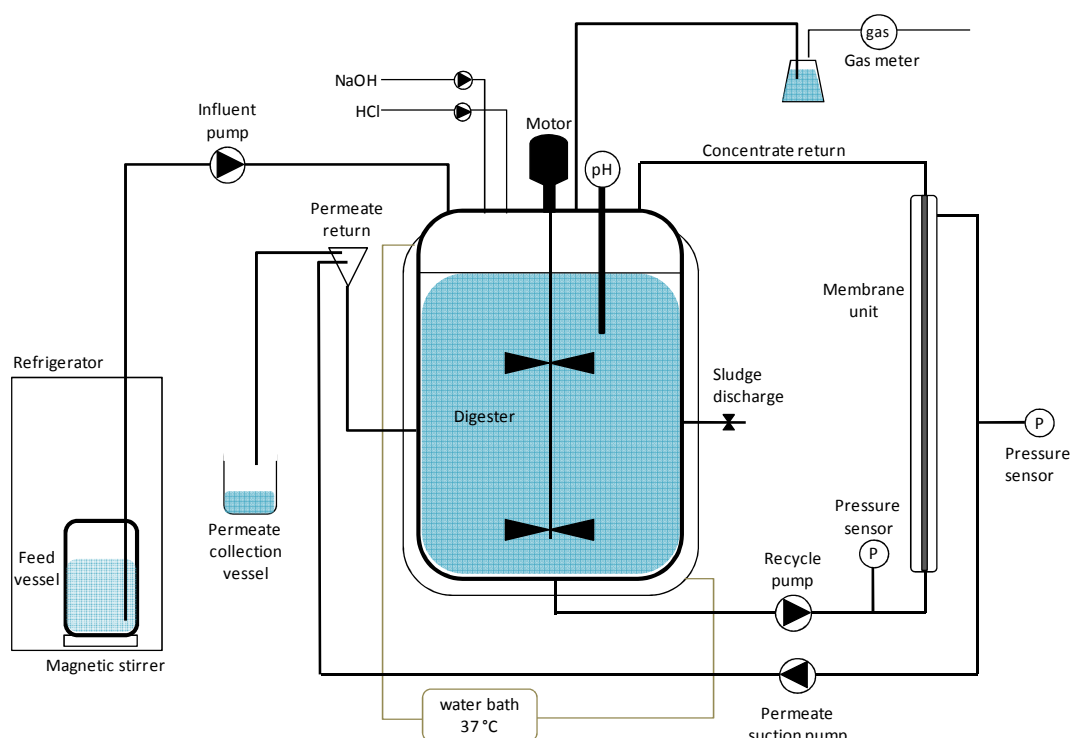


Figure 3.1 : Lab-scale cross-flow AnMBR set-up.

3.2.3 Experimental methods

Frequent analyses were performed to check the characteristics of the feed, permeate 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 ($\text{PO}_4^{3-}\text{-P}$), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were measured with Hach-Lange test kits. The VFAs were analyzed by a gas chromatograph (GC, 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.

Lipid contents of feed and sludge were determined by ether extraction according to Netherlands Norm (NEN) 6671. The sample was first acidified with 12N HCl and then filtered through a diatom covered filter paper. The lipids captured by the filter was extracted in a standard soxlet distillation unit with ether as the solvent. Individual LCFA composition of sludge and LCFA precipitates were measured according to Neves et al. (2009a). The samples were lyophilized and a defined amount was transferred to the glass vials previously dried at 75°C. The solutions of internal standard (pentadecanoic acid, 1.5 mL), HCl:1-Propanol (1.5 mL), dichloromethane (2

mL) and ultra-pure water (2 mL) were further added to the vials. The mixture was vortex-mixed and digested at 100 °C for 3.5 hours. Then, the content of the vial was transferred with 2 mL of ultra-pure water to a different vial. These new vials were kept in inverted position for 30 minutes, after which 2 mL of the organic phase was collected. 1mL of this sub sample was analyzed by GC (CP-9001 Chrompack) equipped with a flame ionization detector (FID). Elemental composition of LCFA precipitates was analyzed with inductively coupled plasma optical emission spectrometry (Perkin Elmer 5300DV).

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 tests were carried out at an initial food to mass ratio (F/M) of 1 g COD·g⁻¹ VSS. Total liquid volume in the bottles was 50 mL and the biomass concentration was 2.0 g VSS·L⁻¹. The bottles were sealed with butyl rubber stoppers and aluminium caps. The anaerobic medium was prepared by dissolving 3.5 g·L⁻¹ NaHCO₃ in tap water without the addition of extra nutrients. The head space was flushed with N₂:CO₂ (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.

The biochemical methane potential of LCFA precipitates were measured in 1070-mL serum bottles by applying the same pressure measurement technique. The liquid volume in the bottles was 0.25 L and the F/M ratio was 1.5 g COD·g⁻¹ VS. The LCFA precipitates were first grinded with a kitchen blender and then sieves with different mesh sizes were used to fractionate them in to different particle sizes. Crushed granular sludge was used as the inoculum. The sludge was washed twice with anaerobic medium (5 g·L⁻¹ NaHCO₃) by centrifuging and decanting the supernatant in order to reduce calcium and magnesium and to prevent further precipitation of LCFA with divalent cations present in the inoculum. In order to evaluate the reversibility of LCFA inhibition, two sets of batch experiments were carried out at the end of the study:

1. The biomass was kept in the reactor at 37 °C without feeding for 1 week and a batch activity test was carried out.

2. The sludge was put in to serum bottles and kept in 37 °C without substrate addition in order to degrade the accumulated LCFA on the biomass. When the biogas production reached to a plateau after 2.5 weeks, substrate was added into the bottles to test the activities on different VFAs.

3.3 Results

3.3.1 Reactor Performance

Figure 3.2 shows the permeate COD and COD removal efficiencies of the reactors together with the applied OLR. Higher loading rates could be applied to R-20 and R-30 than to R-50 and the average OLR for R-20, R-30 and R-50 were 8.3, 7.8, 6.1 kg COD·m⁻³·d⁻¹, respectively. The lab-scale AnMBRs showed a good and reliable permeate quality during continuous operation.

The COD removal efficiencies of R-20 and R-30 based on permeate quality was higher than 99% during the long term study, however, as a consequence of instabilities the performance of R-50 occasionally dropped down to 96%, which is still a very high removal efficiency thanks to the membrane filtration. As a result of VFA accumulation, the permeate COD of R-50 was always higher than that of other two reactors. R-50 reactor was only stable during the last period of the study at an OLR of 5.1 kg COD·m⁻³·d⁻¹ which yielded no VFA accumulation. The permeate COD at stable operating conditions in R-20, R-30 and R-50 was 470±60, 570±60, 1070±30 mg·L⁻¹, respectively, reflecting an obvious increase with increasing SRT.

The biological performances of the reactors operated at different SRTs are summarized in Table 3.2. In terms of applicable OLR, R-50 showed the worst performance and LCFA inhibition led to instable performance. Considering the fact that R-50 was first operated as R-30, long term LCFA accumulation on biomass may be a reason for instable performance in this reactor. However, we did not observe any instability such as VFA accumulation or biomass activity loss as an indicator of LCFA inhibition during long term operation of R-30. In general, higher OLRs could be applied to R-20 and R-30 without any instability in the process performance. This may be due to the dominant mechanism for LCFA removal, such as precipitation with cations or adsorption onto biomass.

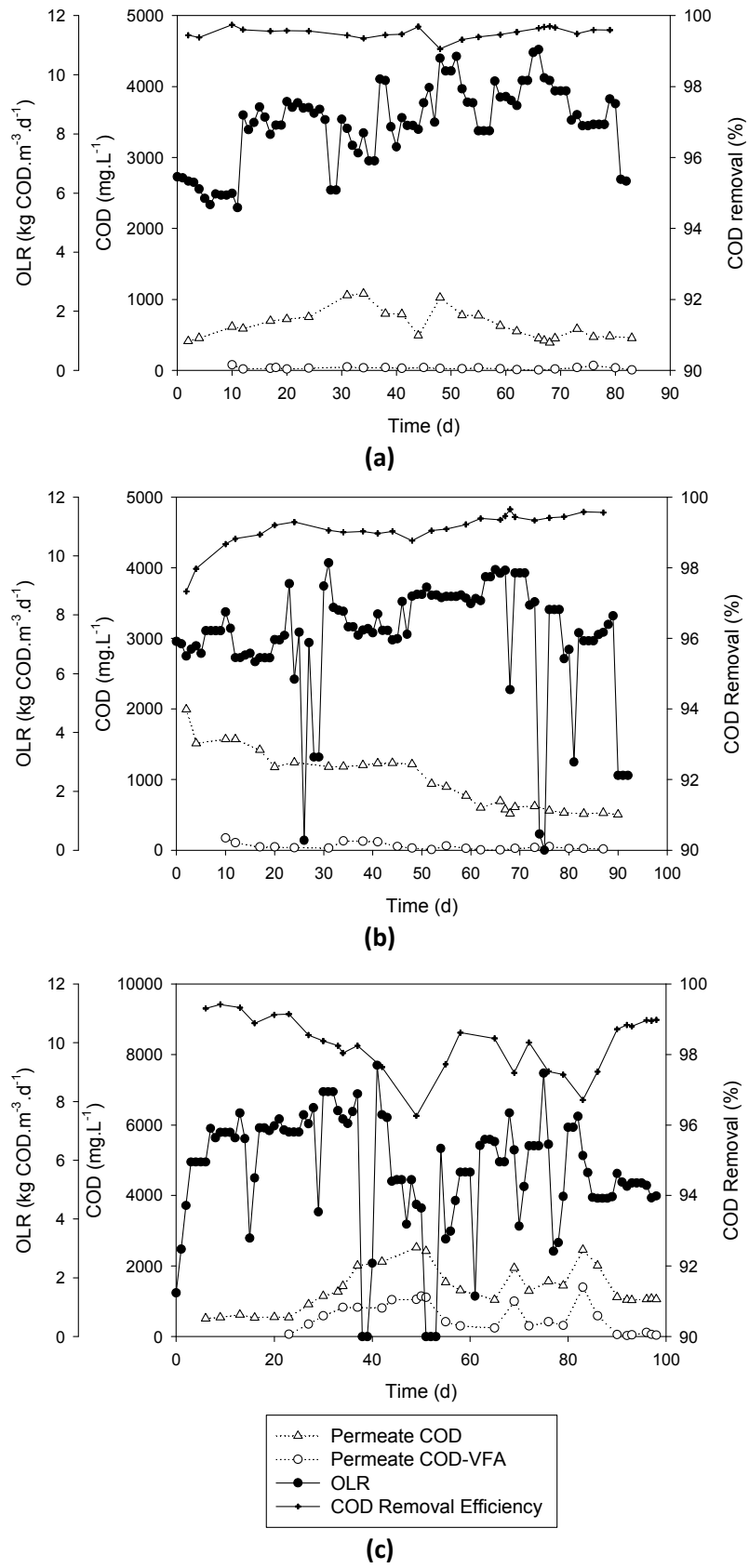


Figure 3.2 : OLR, permeate COD COD_{VFA} and COD removal efficiency at different SRTs (a: R-20, b: R-30, c: R-50).

As a matter of fact, since a high amount of COD originating from lipids accumulated as very large LCFA precipitates at short retention times, becoming less available for biodegradation, the actual OLR applied to R-20 and R-30 reactors, in terms of bio-convertible COD, will have been less in practice. A similar phenomenon was observed by other researchers (Alves et al., 2001a; Pereira et al., 2002; Cavaleiro et al., 2001). They reported a reduction in the effective organic loading rate due to the precipitation of free LCFA with Ca^{2+} and Mg^{2+} cations present in the feed. Stoichiometrically, each mole of Ca^{2+} and Mg^{2+} can precipitate two moles of free LCFA (Alves et al., 2001a; Pereira et al., 2005). Table 3.3 shows the LCFA-precipitation potential of the corn-based thin stillage investigated, assuming LCFA-precipitation as sole mechanism of calcium and magnesium removal.

Table 3.2 : Comparison of the steady state performance of reactors at different SRTs (mean±standard deviation).

Parameter	Unit	R-20	R-30	R-50
OLR	kg COD·m ⁻³ ·d ⁻¹	8.3±1.3	7.8±0.9	6.1±1.4
TSS	g·L ⁻¹	16.5±0.8	17.2±1.8	28.3±1.1
VSS	g·L ⁻¹	15.2±0.6	15.3±1.4	24.9±1.2
F/M ratio	kg COD·kg VSS ⁻¹ ·d ⁻¹	0.53±0.1	0.47±0.12	0.30±0.09
Lipid/Mass ratio	kg Lipid·kg VSS ⁻¹ ·d ⁻¹	0.10±0.01	0.08±0.02	0.04±0.01
Permeate COD	mg·L ⁻¹	470±60	570±60	1070±30
COD removal efficiency based on permeate quality	%	99±0.2	99±0.5	98±0.8
Methane production ¹	L·d ⁻¹	21±3.1	22.6±2.9	16.9±1.7
Digestion efficiency ²	%	73±4	80±6	83±10
Methane yield	Nm ³ CH ₄ ·kg ⁻¹ COD _{removed}	0.26±0.01	0.28±0.02	0.29±0.03

¹ At standard temperature and pressure (0 °C, 1 atm)

² Daily methane production (as g COD) divided by daily total load (as g COD)

Table 3.3 presents that calcium and magnesium were removed to a large extent in all three reactors. However, it is difficult to estimate the amount of calcium and magnesium used for LCFA precipitation, since it is dependent on pH and precipitation of other species such as struvite, magnesium phosphate, calcium carbonate, etc. It is expected that precipitation with cations can decrease the toxic concentrations of LCFA and enables more stable reactor operation. On the other hand, LCFA precipitation will decrease the accessibility of LCFA for methanogenic conversion and will thus lead to solid phase LCFA accumulation. Remarkably, we observed a severe toxicity and sludge activity loss in R-50 reactor. We hypothesize that the applied long SRT, brought about by an increased biomass concentration in the reactor, led to a lower Lipid/Mass ratio and, very likely, higher degree of LCFA adsorption to the methanogenic sludge. The latter, likely resulting in increased inhibition and/or mass transfer limitation. Interestingly, in R-20 and R-30 reactors, operated at shorter SRTs and thus higher Lipid/Mass ratio, we observed the formation of LCFA clumps, possibly leading to a

lower adsorption of LCFA to the sludge and thus lower inhibition. Additionally, at lower SRTs a significant fraction of free or solid-phase LCFA, might be discarded from the reactors with the sludge discharge.

Table 3.3 : LCFA fed to the reactors and removal efficiency of calcium and magnesium (mean±standard deviation).

Reactor	Lipids in feed ¹	LCFA ²	Ca removal		Mg removal		LCFA precipitated ³	
	g·d ⁻¹		%	mmol·d ⁻¹	%	mmol·d ⁻¹	mmol·d ⁻¹	%
R-20	14.2±1.2	52.8±4.4	88±10	2.5±0.3	62±11	9.2±1.3	23.1±3.4	44
R-30	12.6±1.6	46.9±6.0	93±7	2.3±0.3	82±6	11.7±2.1	27.7±4.1	59
R-50	8.6±1.8	32.0±6.8	73±15	1.3±0.3	88±5	8.8±1.3	19.3±3.2	60

¹Calculated as 45% of feed COD is originating from lipids.

²LCFA equivalent of lipids present in the feed was calculated assuming 1 mole of lipid (triglyceride) is producing 3 moles of LCFA (palmitate) and 1 mole of glycerol during hydrolysis process.

³Assuming calcium and magnesium precipitated with only LCFA, the molar amounts were multiplied with stoichiometric ratio: 2.

3.3.2 COD mass balance

It is well known that COD removal efficiency does not always correspond to COD biodegradation when treating lipid rich wastewaters (Hwu et al., 1998a). LCFA can absorb on to the sludge and/or precipitate with cations and accumulate in the reactor without being converted to methane. Hwu et al. (1998b) reported significantly lower methane conversion efficiency compared to the COD removal efficiency of an EGSB reactor treating LCFA rich synthetic wastewater at mesophilic conditions. This indicates that physicochemical processes, i.e. biosorption and precipitation, significantly contribute to LCFA removal and, thus, a high COD removal efficiency does not necessarily mean that it is biodegraded efficiently. Sayed et al. (1987) also reported a large difference between the methane production efficiency and COD removal efficiency in UASB reactors treating slaughterhouse wastewater. Therefore, permeate quality should be evaluated together with COD conversion efficiency to methane to get more realistic performance comparison. The average methane conversion efficiencies in R-20, R-30 and R-50 were 73%, 80%, 83%, respectively. In general, the conversion efficiency improved with increasing SRT. The improved digestion efficiency may be attributed to a lower Lipid/Mass ratio and thus, better bioconversion of lipids to methane at higher SRTs (Table 3.2).

Table 3.4 summarizes the results of the COD mass balance calculations obtained for each reactor. Significant discrepancies in the COD mass balance were noted for R-20

and R-30. The missing fraction of COD in the mass balance decreased at higher SRTs. This can be explained by the occurrence of floating LCFA precipitates in the form of clumps, at the upper part of the reactors. These LCFA clumps grew out to very large “fat balls” with diameters up to several centimeters. The floating fat balls were not extracted during regular sampling since the sampling port is located in the middle of the reactor and moreover, they were far too big to pass through the sampling port. Therefore, a significant fraction of COD entrapped in LCFA precipitates could not be measured as wasted sludge COD, thus creating a significant gap in COD mass balance.

Table 3.4 : COD mass balance (mean±standard deviation).

Stream	R-20		R-30		R-50	
	g COD·d ⁻¹	%	g COD·d ⁻¹	%	g COD·d ⁻¹	%
Influent	88.2±7.6	100	80.6±7.1	100	55.5±5.7	100
Permeate	0.4±0.1	0.5±0.1	0.6±0.2	0.7±0.3	1.1±0.4	1.9±0.6
Wasted sludge	14.2±1.3	16.1±1.3	8.9±0.4	11.1±1.3	5.7±2.6	10.7±5.7
Biogas	59.9±5.9	73.1±3.6	64.5±6.7	80±6	45.8±3.2	83.1±9.9
Missing	8.5±3.8	9.7±4.1	6.8±4.9	8.3±5.9	5.2±4.6	4.2±14.4

In R-50, LCFA precipitates as large as the ones in R-20 and R-30 reactors did not occur. This may be attributed to the larger biomass surface availability for adsorption and the higher degree of lipid degradation at higher SRTs due to the higher concentration of bio-catalysts for LCFA conversion to methane. However, consequently, this resulted in LCFA accumulation on the biomass and impairment of biomass activity due to mass transfer limitations caused by LCFA layer covering the biomass. It is noted that the missing COD fraction and methane generation had a very large variation for the R-50 reactor, likely attributable to the accumulation of non-degraded substrate. The accumulated substrate was converted into biogas when the feed flow was decreased or even stopped due to high VFA concentrations. Therefore, the reactor continued to produce significantly more biogas than the theoretically expected amount at the actual loaded conditions due to the digestion of accumulated substrate. This situation caused an imbalance in COD mass balance. Hwu et al. (1998a) observed that biogas production still continued even after the depletion of oleate from the bulk liquid which indicates that the absorbed LCFA was slowly converted to methane. Cavaleiro et al. (2008) also observed higher methane production than the theoretical expectation due to the degradation of previously accumulated LCFA in pulse fed reactors.

3.3.3 LCFA precipitation with calcium and magnesium

LCFAs are known to precipitate with calcium and magnesium present in the feed and this can make them become less toxic for biomass and less bioavailable for conversion into methane (Alves et al., 2001a; Pereira et al., 2005). In our study, LCFA precipitates agglomerated and formed fat balls of various sizes up to 2-3 cm in diameter (Figure 3.3). Several researchers have reported LCFA precipitation in different types of reactors (Rinzema et al., 1993; Hwu et al., 1998b; Pereira et al., 2005) but not in the extent as observed in our study. The gentle mixing conditions in our reactors may promote the aggregation of these small precipitates through hydrophobic surface interactions. The fat balls caused several operation problems by clogging the tubes and cross-flow pump inlet and the reactor sludge had to be sieved several times to discard the LCFA precipitates from the reactor. Likewise, Hwu et al. (1998b) observed fat-like materials floating on top of EGSB reactors which required daily removal for continuous reactor operation. Moreover, they observed that the color of the granules changed from black into white due to multilayer LCFA adsorption on granules. Also in our reactors, a gradual color change of the sludge was observed.

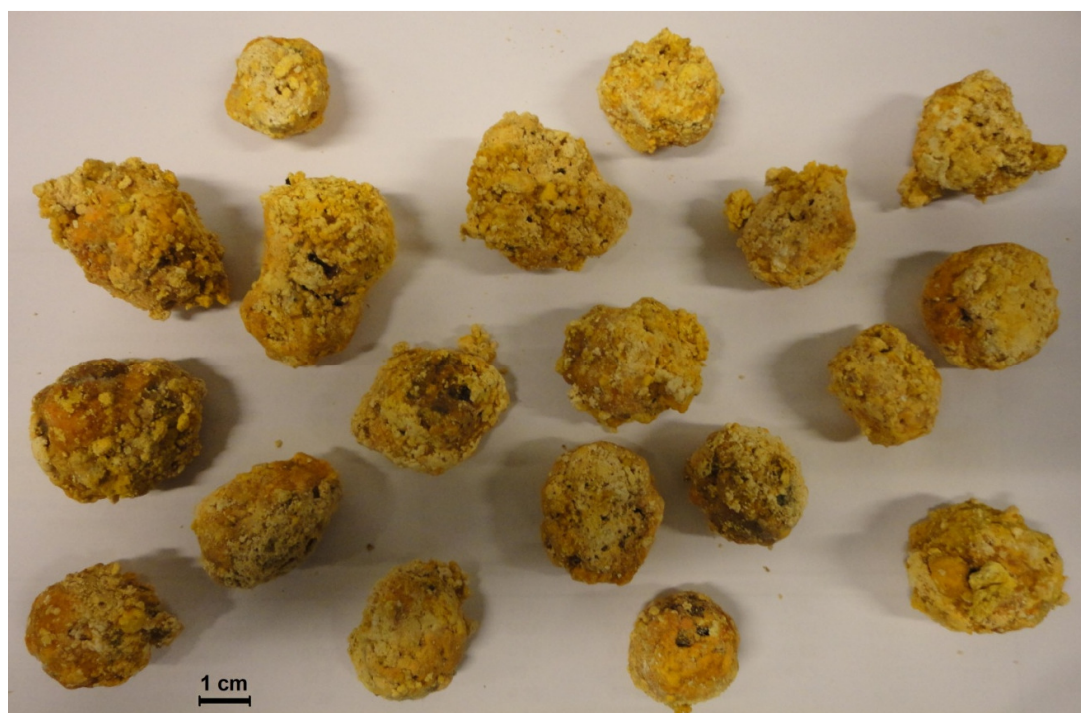


Figure 3.3: LCFA precipitates

Elemental analysis revealed that the calcium and magnesium were the most abundant elements in the precipitates (Table 3.5). Similarly, Roy et al. (1985) reported that the

precipitates formed during LCFA digestion were mainly composed of LCFA, calcium and phosphorus. This clearly indicates that these ions form LCFA salts. Phosphorus, nitrogen and potassium were the other important elements found in the precipitates. The high nitrogen content of the precipitates might be due to LCFA protein complexes or entrapment of bacteria inside the precipitates. The dry matter and lipid content of the precipitates were measured as 34% and 380 g/kg dry solids, respectively.

Table 3.5 : Elemental composition of LCFA precipitates from R-20.

Elements	Concentration (mg·kg⁻¹ TS)	Concentration (mmol·kg⁻¹ TS)
Aluminium (Al)	38	1.4
Calcium (Ca)	20900	521
Cobalt (Co)	3.0	0.05
Copper (Cu)	4.2	0.07
Iron (Fe)	557	10
Magnesium (Mg)	18400	757
Manganese (Mn)	345	6
Molybdenum (Mo)	0.9	0.01
Nickel (Ni)	3.9	0.07
Nitrogen (N)	7100	507
Phosphorus (P)	1250	40
Potassium (K)	1153	29
Selenium (Se)	< 0.05	-
Sodium (Na)	1130	49
Sulfur (S)	630	20
Zinc (Zn)	5	0.07

Table 3.6 shows the results of detailed LCFA composition of precipitates. It appears that the fat deposits mainly consist of palmitic acid (C16:0), which is one of the most common LCFA intermediates when digesting wastewaters containing high oleate concentrations. Analogously, Pereira et al. (2005) reported that LCFA precipitates formed during the treatment of oleate based wastewater were mainly composed of palmitate. The LCFA/([Ca²⁺]+[Mg²⁺]) ratio in the precipitates was 1.8, which is close to the stoichiometric value for LCFA and divalent cations precipitation. Based on their solubility product constants, saturated LCFA-Ca salts were reported to be less soluble compared to unsaturated LCFA-Ca salts (Irani and Callis, 1960; Roy et al., 1985). Therefore, the bioavailability of LCFA is restricted by the formation of LCFA-Ca salts with low solubility (Rinzema et al., 1993; Pereira et al., 2002). Moreover, the particle size of LCFA precipitates also affects their bioavailability. This is shown in Figure 3.4, from which it is obvious that the methane production rates decrease with particle

size, indicating problems for the biomass to access the conglomerated substrate. The figure also shows very low degradation rates with (non-particular) corn oil, probably as a result of the expected higher toxicity of free LCFA in contrast to the lower toxicity of precipitated LCFA.

Table 3.6 : LCFA composition of fat deposits from R-20 (mean±standard deviation).

LCFA	Concentration (mg LCFA·g ⁻¹ TS)
Lauric (C12:0)	< 1
Myristic (C14:0)	43.4 ± 1.1
Palmitic (C16:0)	475.2 ± 7.8
Palmitoleic (C16:1)	4.8 ± 0.7
Stearic (C18:0)	27.5 ± 1.1
Oleic (C18:1)	15.6 ± 1.0
Linoleic (C18:2)	3.4 ± 0.78

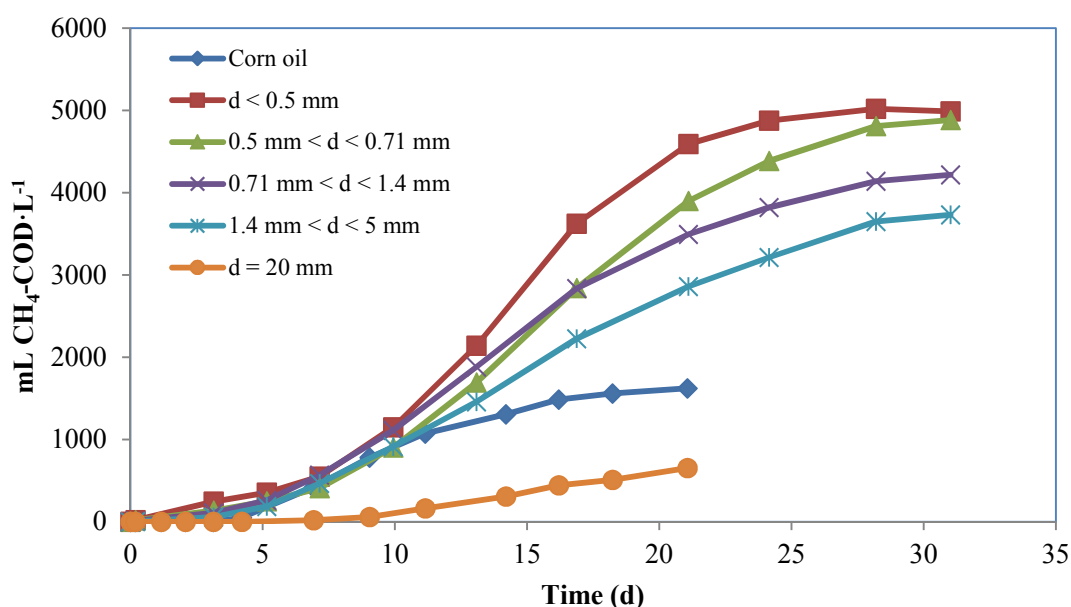


Figure 3.4 : Methane production during incubation of LCFA precipitates with different particle sizes, and corn oil with an initial concentration of about 10 g COD·L⁻¹. Inoculum concentration was about 6.6 g VSS·L⁻¹. The methane production data are corrected for the methane production in the substrate-free controls. All values are expressed in mg COD per liter liquid phase.

3.3.4 LCFA accumulation

At the end of the study, lipid analyses were performed on bulk sludge samples from each reactor (Figure 3.5) and the highest lipid concentration was measured in R-20. This may be attributed to insufficient retention time of either lipids or biomass to convert the LCFA into methane. Moreover, hydrolysis of lipids may be rate limiting at short SRTs (Miron et al., 2000). On the other hand, the accumulated lipids

concentration had gradually increased in R-50 during operation and at the end of the study it was higher than R-30. Since the SRT was significantly higher in this reactor the accumulated lipids stayed in the reactor for a longer time.

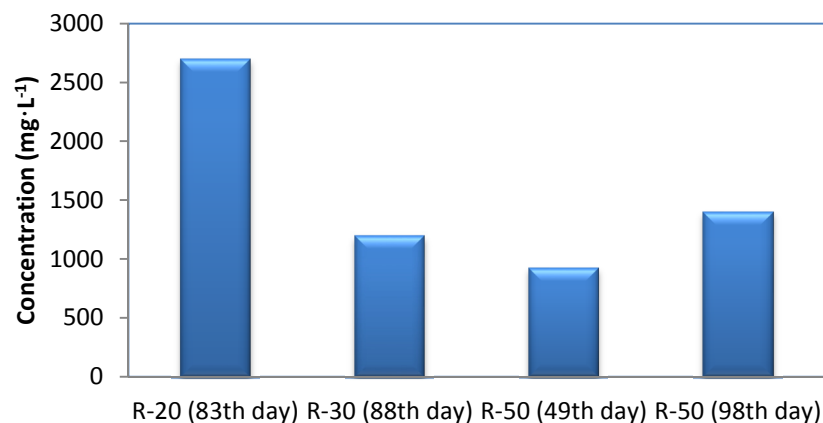


Figure 3.5 : Lipid concentrations in each reactor.

Direct measurement of LCFAs accumulated in anaerobic reactors can provide a better insight on LCFA inhibition. The detailed LCFA composition accumulated in different reactors is presented in Table 3.7. Although, linoleic and oleic acids were reported as the most dominant LCFA types (55% and 28% of total LCFA, respectively) in corn-to-ethanol thin stillage (Winkler-Moser and Breyer, 2011), the palmitic acid was the most abundant LCFA found in all reactors. In fact, palmitic and myristic acids were reported as the most important intermediate LCFAs during the oleic acid and linoleic acid degradation (Lalman and Bagley, 2000; Lalman and Bagley, 2001). Similar results were reported by several researchers (Gonçalves et al., 2012; Pereira et al. 2005; Neves et al., 2009b; Palatsi et al., 2012) for the degradation of oleate. Neves et al. (2009b) reported palmitic acid as the main accumulated LCFA (72%), while treating oily waste from fish processing industry with a similar LCFA content (C18:1 (43%), C18:2 (38%), C16:0 (13%)) to corn oil.

The total accumulated LCFA concentration in R-20, R-30 and R-50 were 62, 48, 61 mg LCFA-COD·g⁻¹ TS, respectively. It is interesting to note that we observed no methanogenic activity loss due to LCFA inhibition in R-20 and R-30, whereas in R-50, although a similar amount of LCFA was accumulated as in R-20 (Table 3.7), severe LCFA inhibition was observed. The reason for that may be the different mechanisms of LCFA accumulation in the reactors. In R-20 and R-30, the LCFA mainly accumulated as large LCFA precipitates (clumps) which were not degraded. In

contrast, in R-50 the LCFA-precipitates were smaller and LCFA associated with biomass, presumably causing mass transfer limitation and thus hindering sludge activity.

Table 3.7 : LCFA concentrations in different reactors at the end of operation (mean±standard deviation).

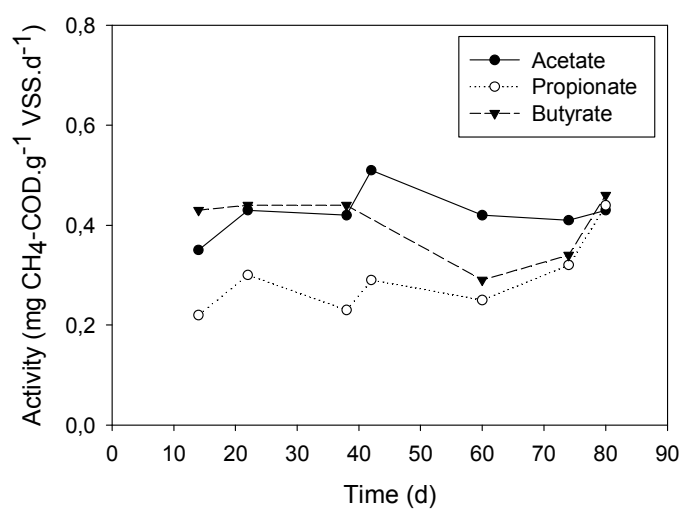
LCFA	R-20 (83 rd day)		R-30 (83 rd day)		R-50 (98 th day)		R-50 (104 th day*)	
	mg LCFA·g ⁻¹ TS	mg·L ⁻¹	mg LCFA·g ⁻¹ TS	mg·L ⁻¹	mg LCFA·g ⁻¹ TS	mg·L ⁻¹	mg LCFA·g ⁻¹ TS	mg·L ⁻¹
Lauric (C12:0)	<1	-	<1	-	<1	-	<1	-
Myristic (C14:0)	3.47 ± 0.94	70	<1	-	1.29 ± 0.52	45	<1	-
Palmitic (C16:0)	10.70 ± 2.48	217	8.75 ± 0.56	193	14.50 ± 6.10	508	3.67 ± 0.93	108
Palmitoleic (C16:1)	1.52 ± 0.01	31	1.33 ± 0.04	29	1.85 ± 0.54	65	1.28 ± 0.06	38
Stearic (C18:0)	2.31 ± 0.52	47	1.21 ± 0.07	27	< 1	-	< 1	-
Oleic (C18:1)	1.66 ± 0.21	34	3.12 ± 0.19	69	1.43 ± 0.05	50	1.61 ± 0.28	47
Linoleic (C18:2)	2.13 ± 0.27	43	2.21 ± 0.18	49	2.14 ± 0.25	75	1.78 ± 0.33	52
Total (mg LCFA-COD·g ⁻¹ TS)	62		48		61		24	
Total (mg LCFA-COD·L ⁻¹)	1270		1050		2130		570	

*1 week after shutdown without any feed

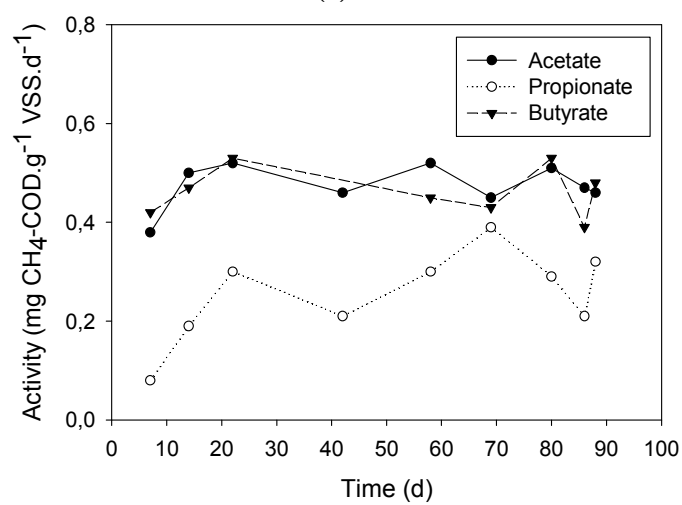
The palmitate content of R-50 decreased significantly after 1 week without feeding, indicating conversion to methane. This result confirms that the pulsed feeding regime suggested by Cavaleiro et al. (2008) can be applied to improve lipid digestion efficiency and to avoid possible LCFA inhibition problems. Pereira et al. (2002) and Alves et al. (2001b) observed that under the presence of biomass associated LCFA, continuous feeding of LCFA can totally inhibit the process or significantly delay methane production. Therefore, interruption of feeding to allow mineralization of biomass associated LCFA can restore the process. Cavaleiro et al. (2008) observed very long lag-phases in the further conversion of added LCFA under the presence of biomass associated LCFA up to 5200 mg COD·g⁻¹ VSS. However, an unfed resting phase to allow the sludge to degrade the biomass associated LCFA significantly decreased the lag-phase.

3.3.5 Sludge activity loss and restoration

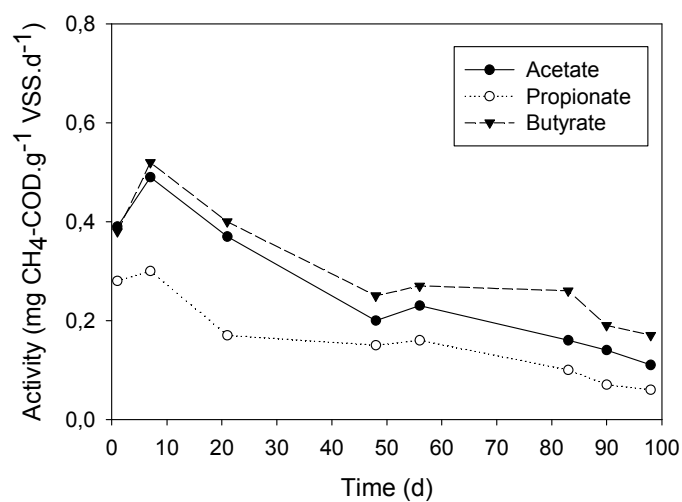
One of the most important concerns about the cross-flow AnMBRs is the loss of biomass activity due to the disruption of syntrophic associations between propionate degraders and hydrogen utilizers by the applied shear rate (Brockmann and Seyfried, 1997). The activities of biomass on specific VFAs in different reactors were monitored throughout the operational period and the results are presented in Figure 3.6.



(a)



(b)



(c)

Figure 3.6 : Methanogenic activity on individual VFAs at different reactors (a: R-20, b: R-30, c: R-50).

There was no apparent loss of sludge activity in R-20 and R-30, meaning that the cross-flow velocity of $0.5 \text{ m}\cdot\text{s}^{-1}$ and the gentle mixing intensity applied in this study were appropriate for sustaining the syntrophic consortia. In other AnMBR studies described in literature much higher cross flow velocities are applied, i.e. $1\text{-}5 \text{ m}\cdot\text{s}^{-1}$ (Chapter 2). However, a clear trend of methanogenic activity loss for all VFAs, was observed with sludge from R-50. This situation clearly explains why R-50 could not be stably operated at OLRs as high as the other two reactors with shorter SRTs. The continuous reduction in sludge activity led to VFA accumulation at OLRs higher than $6 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. The most plausible reason of activity loss is the mass transfer limitations caused by the accumulation of biomass associated LCFA at high SRT.

At the end of operation, methanogenic activities on acetate, propionate and butyrate decreased 78%, 80% and 67%, respectively, compared to the initial activities in R-50 (Figure 3.6). Considering the fact that acetotrophic methanogens and syntrophic acetogens are the most sensitive groups of bacteria to LCFA inhibition, these results are in accordance with the literature. Lalman and Bagley (2001) reported that the inhibitory effect of oleic acid was much more pronounced for acetotrophic methanogenesis than for hydrogenotrophic methanogens. Alves et al. (2001a) observed a significant decrease in acetotrophic activity when treating oleate based wastewater. Cavaleiro et al. (2008) indicated that the acetate conversion activity of the sludge decreased significantly when it was exposed to high LCFA concentrations. However, this phenomenon was reversible and the activity could be restored after mineralization of adsorbed LCFA.

As it can be seen in Figure 3.7, the acetate and butyrate degradation activity improved significantly when the feeding was stopped (1st batch experiment) for 1 week and it could be almost completely recovered after 2.5 weeks of lipid degradation (2nd batch experiment). However, activity on propionate was somewhat lower (30%) compared to its initial value at the start up, but it was still 3.5 fold higher than the activity at reactor shutdown. Cavaleiro et al. (2001) observed that the methanogenic activities on propionate and butyrate could not recover to their initial values even after 90 days from an oleic acid overload. In contrast, acetoclastic activity even exceeded the initial value. This might indicate a higher abundance of acetotrophic methanogens compared to the other groups, meaning that acetate conversion is more rapidly restored when the LCFA blockage is lifted. In addition, within LCFA conversion, a high molar ratio of acetate

is produced, providing ample substrate for rapid growth. Pereira et al. (2005) also reported a low degree of restoration of the propionate-degrading activity after the mineralization of biomass associated LCFA. It can also be speculated that the acetotrophic methanogens and syntrophic butyrate degrading bacteria might be enriched during the conversion of biomass associated LCFA, since acetate and butyrate are the products of β -oxidation of LCFA with even number of carbon atoms (palmitate, C:16). However, propionate degraders might not directly benefit from this process (Pereira et al., 2005; Silvestre et al., 2011). Nevertheless, the results indicate that the activity can be restored after the LCFA layer limiting the transfer of substrate and nutrients to the biomass is degraded. Therefore, by allowing the mineralization of the biomass-associated LCFA, the inhibitory/transport limitation effects can be eliminated.

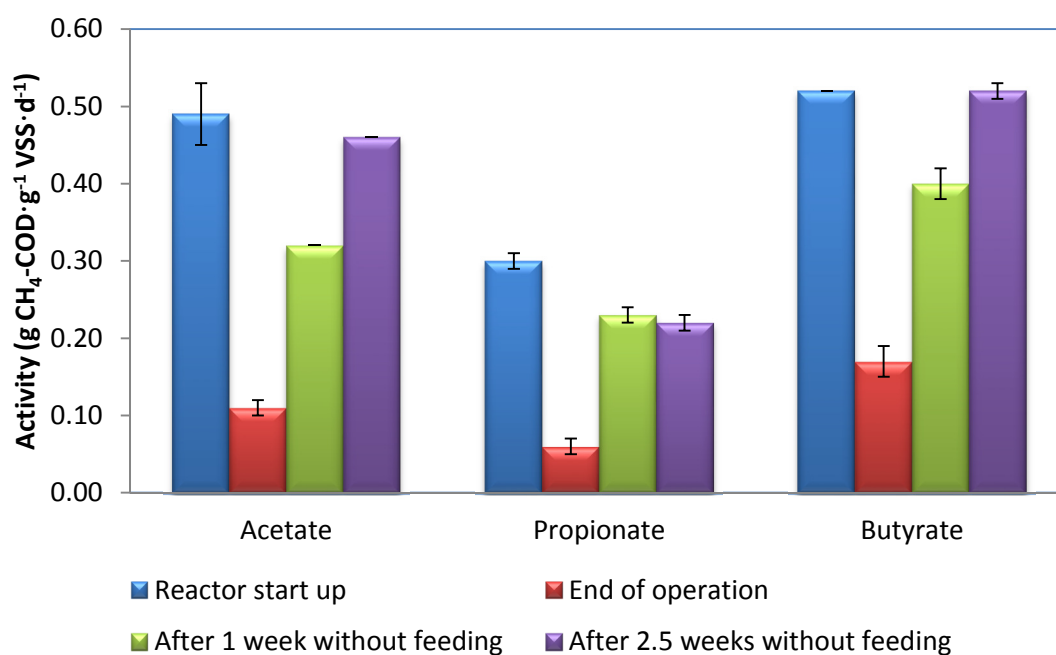


Figure 3.7 : Activity of the sludge from R-50 reactor.

3.4 Discussion

LCFA precipitation with calcium and magnesium reduces the free LCFA concentrations, thus ameliorating LCFA inhibition. In fact, using Ca and Mg to reduce LCFA inhibitory effects in anaerobic reactors was proposed by several other researchers previously (Hanaki et al., 1981; Roy et al., 1985; Alves et al., 2001a; Ahn et al., 2006). Ahn et al. (2006) observed better lipid removal efficiency by increasing

the Ca concentration up to $3 \text{ g}\cdot\text{L}^{-1}$. Recently, Kleyböcker et al. (2012) proposed to use the calcium concentration as an early warning indicator against overloading in anaerobic reactors treating lipid rich wastewaters. In our study, although a significant fraction of LCFA would be precipitated by Ca and Mg present in the feed, this did not prevent long term LCFA to biomass adsorption, enhancing mass transfer limitation at high SRTs.

Corn-to-ethanol contains a high amount of un-saturated LCFAs such as linoleic and oleic acids (~82% of total LCFA). It is believed that unsaturated LCFAs must be hydrogenated first in order to proceed with β -oxidation (Lalman and Bagley, 2001). However, the absence of stearic acid as an intermediate product in oleic and linoleic acid degradation contradicts this hypothesis (Lalman and Bagley, 2000; Lalman and Bagley, 2001). In our study, we measured a significant amount of stearic acid both in LCFA precipitates and in bulk sludge of R-20 operated at short SRT. This supports the hypothesis that the unsaturated LCFA has to be saturated first to stearic acid. Nevertheless, saturation of unsaturated acids and the first step of β -oxidation from stearate to palmitate seemed to be not the rate limiting step in LCFA degradation (Lalman and Bagley, 2000; Lalman and Bagley, 2001; Pereira et al., 2005). However, the β -oxidation of palmitate is retarded due to its accumulation on the biomass and it proceeds very slowly because of the mass transfer limitations of the substrate and products (Pereira et al., 2005). Evidently, in our study, palmitate was measured as the most abundant LCFA present accumulated in precipitates and bulk sludge in all reactors.

We measured significantly lower concentrations of accumulated LCFA in our reactors compared to literature. However, it was enough to hinder biomass activity at high SRTs. Pereira et al. (2004) applied an inhibition model and calculated a value around $1000 \text{ mg LCFA}\cdot\text{COD}\cdot\text{g}^{-1} \text{ VSS}$ for the optimal sludge LCFA content that allowed the maximum mineralization rate for biomass associated LCFA. However, this value is rather a rough estimation since LCFA inhibition mechanism is a very complex process involving several intermediate substrates, different trophic bacteria species, substrate and product diffusion limitations. Moreover, some other researchers reported much lower threshold concentrations for LCFA accumulation when LCFAs were directly measured. Gonçalves et al. (2012) measured significantly lower concentrations of biomass associated LCFA, i.e. 180 and $410 \text{ mg LCFA}\cdot\text{COD}\cdot\text{g}^{-1} \text{ VS}$, when treating

lipid rich olive mill wastewater. Cirne et al. (2007) observed inhibition of methanogenesis at 145-330 mg COD·g⁻¹ TS concentrations of palmitate. Neves et al. (2009b) demonstrated that threshold values for LCFA and palmitate accumulation onto the solid matrix, of about 180–220 mg LCFA-COD·g⁻¹ TS and 120–150 mg palmitate-COD·g⁻¹ TS, should not be surpassed in order to prevent persistent reactor failure. Hwu et al. (1996) showed that the susceptibility to LCFA toxicity is dependent on the sludge specific surface area. Thus, the granular sludge is less sensitive to oleate inhibition compared to flocculent sludge due to its larger particle size and lower surface area. In this context, considering the very small sludge particle size in AnMBRs, it is plausible that these reactors can suffer from LCFA toxicity at lower concentrations. But on the other hand, by using the suspended sludge in AnMBR systems, a much larger biomass surface is available for adsorption. Since LCFA conversion is very likely a surface proportional conversion, suspended sludge in AnMBR offers interesting perspectives for high-rate LCFA treatment, provided the free LCFA liquid concentration is kept below toxicity level. Also the formation of non-biomass associated LCFA clumps, the so-called fat balls, offers perspectives to further research. Deliberately overloading the system results in formation of such clumps protecting the methanogenic biomass from LCFA adsorption. A substantial fraction of the COD is then removed as solid phase LCFA with a possible other outlet than only methanation.

3.5 Conclusions

The corn-based thin stillage could be treated with COD removal efficiencies over 99% and digestion efficiencies from 73% to 83% at OLRs up to 8 kg COD·m⁻³·d⁻¹ in AnMBR systems operated at different SRTs. The major drawbacks, i.e. sludge flotation, insufficient granulation, biomass washout, of granular sludge bed systems when treating high lipid containing wastewaters, can all be addressed at once by membrane assisted biomass retention in AnMBRs. However, this study underlines that although biomass retention is guaranteed by the membrane, the AnMBR process still suffers from reversible LCFA inhibition, mainly caused by LCFA adsorption, and requires an appropriate adaptation/operation strategy to obtain better biodegradation performance for the treatment of lipid rich wastewaters. Operating the reactor at short SRTs may result in the washout of free and adsorbed LCFA from the reactor, therefore

reducing the effective concentration of LCFA causing inhibition or transport limitation. However, a significant fraction of LCFA would remain undegraded, reducing the methane recovery potential which is important for the feasibility of the process unless another outlet for the precipitated LCFA is found. On the other hand, at high SRTs more LCFA remains in the reactor and this may increase the amount of LCFA adsorbed on to the biomass and hinder process stability. In order to sustainably treat all LCFA only very low Lipid/Mass ratios can be applied.

4. EFFECT OF SRT AND LIPIDS ON FILTRATION PERFORMANCE ³

4.1 Introduction

The success and widespread application of aerobic membrane bioreactors (MBRs) stimulated the combination of membrane filtration and anaerobic processes for municipal and industrial wastewater treatment (Dereli et al., 2012a; Ozgun et al., 2013). Likewise their aerobic counterparts, AnMBRs take advantage of membrane filtration for the separation of the biomass from effluent and provide complete sludge retention. In fact, AnMBR is an emerging treatment technology for industrial wastewaters/slurries with extreme properties, which often cause biomass retention problems in conventional anaerobic granular sludge bed reactors. Thus, AnMBRs can provide an alternative reactor technology with a high treatment performance and permeate quality that enables water reuse for industrial wastewaters. Moreover, anaerobic digestions is a low sludge producing and net energy yielding process, which can help to reduce carbon emissions related to fossil fuel consumption (van Lier, 2008).

Despite their many advantages, anaerobic membrane bioreactors (AnMBRs) obviously inherit some constraints related to the membrane filtration process such as membrane fouling, high investment and operation costs, and process complexity with increased automation needs due to additional pumps and pressure sensors used on membrane modules. Among these constraints, membrane fouling seems to be most important, limiting the wide-spread application of AnMBRs. Membrane fouling is a very complex and multi-variable phenomenon dependent on sludge characteristics as well as on the filtration process itself (Le-Clech et al., 2006). Thus, parameters related

³ The content of this chapter was published as:

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

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

to mixed liquor characteristics such as biomass concentration, extracellular polymeric substances (EPS), soluble microbial products (SMP), particle size distribution (PSD), hydrophobicity and surface charge, seem to have an influence on fouling (Meng et al., 2009). In fact, all these parameters are related to substrate characteristics, bioreactor operation, i.e. SRT, organic and sludge loading rate (OLR and F:M ratio), and membrane operation, i.e. shear rate, and the filtration-backwash-relaxation cycles. It is therefore, considered imperative to better understand the relation of these factors with each other, enabling possible optimization for each specific case to improve sludge filterability and to mitigate fouling.

The solids retention time (SRT) is often considered as the most fundamental parameter that controls the process performance in biological treatment systems. The SRT in AnMBRs, can be controlled much easier than in other types of anaerobic reactors and it is completely independent from the hydraulic retention time (Liao et al., 2006). Theoretically, the operation of AnMBRs at infinite SRT, i.e. without any sludge withdrawal, is possible. In literature, AnMBRs were reported to operate at various SRTs in the range of 30-350 days (Chapter 2). Generally, high SRTs correspond to more biogas production due to the improved stabilization of organic matter by overcoming hydrolysis limitations, less sludge production and higher biomass concentrations in the reactor. However, increased SRTs also yields to sludge stabilization, which means less active biomass and accumulation of inert organic and inorganic matter, such as biomass decay products and inorganic precipitates, in the reactor (Lee et al, 2003; Liao et al., 2006). The SRT is considered as the most important parameter which determines the fouling propensity of the mixed liquor cultivated in MBRs (Le-Clech et al., 2006). However, SRT cannot be regarded as an isolated parameter. In fact, it is very difficult to assess and evaluate the impact of SRT on membrane fouling since many parameters, i.e. biomass concentration, sludge viscosity, F:M ratio and EPS concentrations, which are also related to fouling, inevitably change when the SRT is altered. Therefore, contradictory results appear in the literature on this subject (Meng et al., 2009). Thus far, the exact role of SRT on biological and filtration performance of AnMBRs has not yet been elucidated, requiring further investigations.

Lipids and their hydrolysis products, i.e. long chain fatty acids (LCFAs), are potentially very suitable substrates for anaerobic digestion with high degradability and

methane yields. However, they are known to cause sludge flotation, biomass washout and toxicity problems in anaerobic granular sludge bed reactors (Hwu et al., 1996; Hwu et al., 1998b). Besides, LCFAs can accumulate and form a layer on the biomass, which hinders the mass transfer of substrate, nutrients and biogas (Pereira et al., 2005). Fortunately, biomass activity loss due to LCFA accumulation can be restored when the biomass is given sufficient time to degrade the accumulated LCFA layer with a discontinuous feeding regime (Cavaleiro et al., 2008; Chapter 3). Generally, lipids are also regarded as problematic due to their hydrophobic nature and tendency to accumulate and cause fouling on polymeric membranes in aerobic MBRs through hydrophobic interactions. Therefore, as a common precaution the lipids are generally removed from the wastewater by pretreatment units, such as dissolved air flotation, before the MBRs.

The influence of lipids on fouling in aerobic MBRs might be related to hydrophobic interactions with the membrane and/or indirect effect on biology and sludge characteristics. Only a few studies have reported the treatment of wastewaters with a high lipid content in aerobic MBRs and AnMBRs. Yang et al. (2012a) observed reduced membrane fouling in an aerobic MBR treating wastewater with high oil and detergent content than the control MBR. Diez et al. (2012) could treat high lipid containing industrial wastewater in an AnMBR without any problems in bioreactor stability and membrane filtration. In Chapter 3, it was shown that biomass retention problems can be solved by the membranes in AnMBRs, but the process still suffered from long term LCFA accumulation and biomass activity loss, thus it requires an appropriate operation strategy. Al-Halbouni et al. (2009) showed that LCFAs originating from bacteria and incoming wastewater can adhere to ultrafiltration membranes and therefore impact fouling layer formation in MBRs. Ramos et al. (2014) detected higher lipid concentrations in the fouling layer compared to the bulk sludge of an AnMBR treating lipid rich wastewater, suggesting site-specific accumulation. Apparently the exact role of LCFAs in AnMBR has not yet been elucidated and more research should be conducted to further study the effects of lipids on sludge characteristics and membrane fouling.

AnMBR is a promising technology for the treatment of industrial wastewaters with high lipid content due to the guaranteed biomass retention. However, the influence of high lipid concentrations on membrane fouling is still unclear. This study was

undertaken to investigate the effect of high lipid containing wastewater on the filtration performance of AnMBRs operated at different SRTs. Membrane fouling characteristics in respect to wastewater composition and operating conditions were evaluated. Moreover, long term evolution of the sludge physicochemical characteristics has been monitored by using standardized tests to establish a link between sludge filterability characteristics and long term membrane filtration performance.

4.2 Materials and Methods

4.2.1 Reactor set-up and operation

Three AnMBRs were operated, each having 10 L effective volume and equipped with cross-flow tubular membranes. Each reactor was mixed by a mechanical stirrer, operated at 35 rpm and by a cross-flow pump (~3.5 volume turnovers per hour). Mesophilic conditions (37 °C) were maintained by recirculating hot water through the double-walled water jacket around reactors. SRT was maintained by discarding different amounts of sludge, corresponding to 20, 30, and 50 days SRT from the reactors each day. The reactors were named after their respective SRT, i.e. R-20, R-30 and R-50. A similar hydraulic retention time of 10-12 days was kept in all reactors by adjusting the feed concentration. The reactors were controlled and operated by a programmable logic controller (PLC) unit and online data acquisition was performed for recording the transmembrane pressure (TMP) by a standard computer. A detailed description and schematic representation of the lab-scale reactors can be found in Chapter 3.

The tubular ultrafiltration membrane was supplied by Pentair X-flow. The properties of the membranes used in the study are given in Table 4.1. The membranes were de-conditioned by soaking them in 200 ppm NaClO solution for 1 hour and 10-20% ethanol solution for half an hour. After de-conditioning, the initial clean water permeability of the membranes was determined with tap water at 37 °C.

In the first part of the study, two reactors were operated in parallel at different SRTs, i.e. 20 and 30 days, for 3 months. Subsequently, the SRT was increased to 50 days in the R-30 reactor and operation was continued for another 3 months. In order to shorten the start-up period, R-20 and R-30 were inoculated with crushed granular sludge,

characterized by a high specific methanogenic activity (SMA), i.e. $0.5 \text{ g CH}_4\text{-COD}\cdot\text{g}^{-1} \text{ VSS}\cdot\text{d}^{-1}$. The membrane was operated at a cross-flow velocity of $0.5 \text{ m}\cdot\text{s}^{-1}$ and to limit fouling, cyclic membrane operation was carried out, imposing 300 seconds filtration and 30 seconds backwash. Backwash was done by simply reversing the pump flow. Only in the last 3 weeks of the R-20 experiment, the membrane was operated in continuous filtration mode and backwash was done once in every 5-6 days. The membranes of R-20, R-30 and R-50 were operated at a gross flux of 12, 10 and $14 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, respectively. No chemical cleaning was conducted during the entire operation.

Table 4.1 : Membrane and module properties.

Parameter	Unit	Membrane (F 4385)
Mean pore size	nm	30
Material	-	Polyvinylidene fluoride (PVDF)
Carrier material	-	Polyester woven/non-woven
Structure	-	Asymmetric
Hydrophobicity	-	Hydrophilic
Geometry	-	Tubular
Diameter	mm	5.2
Length	cm	70
Membrane area	m^2	0.0114

4.2.2 Experimental methods

4.2.2.1 Analytical methods

The feed wastewater characterization and reactor performance was monitored by wet analyses. Standard methods (APHA, 1998) were applied for suspended solids (TSS) parameter. Chemical oxygen demand (COD) was determined with Hach-Lange kits. Soluble COD samples were prepared by centrifuging sludge at $17,500 \text{ g}$ for 10 minutes, and then by filtering the supernatant through $0.45 \mu\text{m}$ membrane filter. VFAs were analyzed by a gas chromatograph with the properties given in Chapter 3. Extracellular polymeric substances (EPS) were extracted by heat treatment at 100°C for 1 hour (Chang and Lee, 1998). After heat extraction, the sample was centrifuged at $17,500 \text{ g}$ for 10 minutes and then filtered through $0.45 \mu\text{m}$ filter. The same procedure was also applied for soluble microbial products (SMP) samples without heat treatment. Total EPS and SMP were determined by measuring proteins (BCA Protein Assay Kit, Sigma Aldrich) and polysaccharides (Dubois et al., 1956). Bovine serum albumin and d-glucose were used respectively as standards in protein and polysaccharide assays.

Each sample was measured in duplicates. Bound EPS concentrations were calculated according to Le-Clech et al. (2006). Besides direct measurement techniques, SMP concentrations were also calculated by subtracting VFA (as COD) from soluble COD measured in reactor bulk liquid (Hu and Stuckey, 2007; Akram and Stuckey, 2008b).

4.2.2.2 Sludge filtration characteristics

Sludge filtration characteristics were determined by a number of standard analyses performed at room temperature (20-22 °C). CST of the sludge was measured with Triton Capillary Suction Timer (304M) and standard filter paper (Whatman No.17) supplied from Triton Electronics. Each sample was measured in duplicates. The CST was normalized by dividing the results by the TSS concentration of the sludge. SRF was measured in a dead-end filtration cell (Millipore 8050). The sludge was first diluted to 10 g·L⁻¹ TSS concentration with permeate. Then, the diluted sample was filtrated under a pressure of 0.5 bar at 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 SRF was calculated according to the following formula (4.1) (Police et al., 2008):

$$SRF = \frac{2 \cdot \Delta P \cdot A^2 \cdot b}{\mu \cdot C} \quad (4.1)$$

ΔP : Pressure (Pa), A : Effective filtration area (m²), b : Slope (s·L⁻²), μ : Viscosity (Pa·s), C : TSS concentration (kg·m⁻³)

Supernatant filterability was determined by a method adapted from Rosenberger et al. (2006). The method was adapted because the sludge supernatant of the anaerobic sludge was much more difficult to filtrate than the one of an aerobic sludge. The supernatant was prepared by centrifuging (Hermle Z383 K) the sludge at 17,500 g for 10 minutes. The supernatant filterability was measured 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 during the test in order to prevent extensive accumulation of soluble macromolecules and immediate blocking of the membrane pores. The 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.

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. Each sample was measured in triplicates. Critical flux measurements were done according to the flux step method of Le Clech et al. (2003) with $2 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ step height and 15 minute step length. Critical flux was determined according to its week definition and a slope of $dP/dt \geq 1 \text{ mbar} \cdot \text{min}^{-1}$ was arbitrary chosen to decide whether the critical flux was reached.

4.2.2.3 Sludge relative hydrophobicity

Sludge relative hydrophobicity (RH) was measured according to microbial adhesion to hydrocarbons (MATH) test (Rosenberg et al., 1980). Dodecane was used as the hydrocarbon phase. The sludge sample was diluted with permeate to achieve $1 \text{ g} \cdot \text{L}^{-1}$ TSS concentration. The diluted sample was measured as the initial value at 600 nm (Abs_i) in a UV spectrophotometer (Jenway 7315, Bibby Scientific, UK) with permeate as blank. After hydrocarbon treatment the absorbance of the aqueous phase of the sample was measured (Abs_f) and RH was calculated as follows (4.2):

$$RH (\%) = \left(1 - \frac{Abs_f}{Abs_i} \right) \cdot 100 \quad (4.2)$$

4.2.2.4 Membrane cleaning

At the end of the operational period, the membranes were cleaned with a sequential cleaning procedure in order to evaluate the cleaning efficiency and calculate different fouling resistances. The membranes were first physically cleaned by flushing with tap water in order to remove cake layer and then chemically cleaned by soaking in 200 ppm NaClO and 1% (w/v) citric acid solution sequentially for one hour. After each step the clean water permeability of the membranes were measured to determine the permeability recovery. Total resistance in a membrane filtration process can be formulized with the below given equation (4.3) (Meng et al., 2009):

$$R_{total} = \frac{\Delta P_T}{\eta \cdot J} = R_{intrinsic} + R_{removable} + R_{irreversible} + R_{irrecoverable} \quad (4.3)$$

R_{total} : Total filtration resistance (m^{-1})

$R_{intrinsic}$: Intrinsic membrane resistance (m^{-1})

$R_{removable}$: Cake layer resistance which can be physically removed by a water jet (m^{-1})

$R_{irreversible}$: Resistance caused by organic and inorganic foulants, which can be removed by chemical cleaning (m^{-1})

$R_{irrecoverable}$: Resistance caused by foulants that cannot be removed by neither physical nor chemical cleaning (m^{-1})

J : Flux ($m^3 \cdot m^{-2} \cdot h^{-1}$)

ΔP_T : Transmembrane pressure (TMP) (Pa)

η : Dynamic viscosity of water (Pa·s)

The membrane permeability P ($L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$) with temperature correction (4.4) and cleaning efficiency (E) (4.5) was calculated as;

$$P_{20C} = \frac{J_T [L \cdot m^{-2} \cdot h^{-1}] \cdot e^{-0.0239 \cdot (T-20)}}{TMP [bar]} \quad (4.4)$$

$$E(\%) = \frac{J_{clean}}{J_{initial}} \cdot 100 \quad (4.5)$$

4.2.2.5 Membrane autopsy

At the end of the study, a membrane autopsy was performed. Several pieces of membrane were cut from the middle of the membrane module. The samples were fixed with 3.0% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. They were dehydrated with ethanol solutions with increasing concentrations (50, 70, 80, 90, 95%) and then observed using scanning electron microscope (Jeol JSM 5600 LV). Elemental composition of foulants on the membrane was measured by EDX analysis (PV 7756/78 ME). The major functional groups of biopolymers accumulated on membrane were characterized with a Fourier Transform Infrared Spectrometer (Perkin Elmer, Spectrum 100 FT-IR) in a spectrum ranged between 550 and 4000 cm^{-1} .

4.2.3 Wastewater characterization

Stillage received from a full scale corn based bio-ethanol plant was used as the substrate. The average total COD, soluble COD and TSS concentrations were 72.2 ± 8.6 , 34.7 ± 6.2 and 19.2 ± 2.7 $\text{g} \cdot \text{L}^{-1}$, respectively. The most prominent characteristic of the feed was its very high lipid content, with about 45% of the feed COD originating from lipids. The wastewater also contained high concentrations of inorganic compounds such as nitrogen, phosphorus, calcium, magnesium and potassium. A detailed characterization of the feed can be found in Chapter 3.

4.2.4 Statistical analysis

The data was statistically analyzed with the help of Sigma Plot 12.0 (Systat Software, Inc.) software. The Pearson product momentum correlation test was used to determine linear relationships. The Pearson correlation coefficient varies between -1 and +1, where -1 means a perfect negative correlation and +1 is a perfect positive correlation while 0 means absence of correlation. Correlations are considered statistically significant at 95% confidence level ($P < 0.05$). In order to estimate whether the difference in supernatant COD measured in the reactors was statistically significant, ANOVA tests were conducted at a confidence interval of 95%.

4.3 Results

4.3.1 Treatment performance

The operating conditions and performance of the reactors are summarized in Table 4.2. The average OLRs applied to R-20, R-30 and R-50 were 8.3, 7.8, 6.1 $\text{kg COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, respectively. Although higher OLRs could be applied at shorter SRTs, substrate bioconversion efficiency increased at higher SRTs. This may be due to longer contact time of slowly degradable substrate such as LCFA and particulate organic matter with biomass at higher SRTs (Chapter 3). On the other hand, severe LCFA inhibition observed in R-50 caused instable reactor performance, thus lower organic loadings could be applied. The adsorption of LCFA on bacterial flocs led to a decrease in biomass activity and volatile fatty acids accumulation. At the end of operation, the total LCFA concentration measured in R-20, R-30 and R-50 was 440, 370 and 740 $\text{mg} \cdot \text{L}^{-1}$, respectively. The most dominant LCFA that accumulated in the reactors was palmitate. The effect of high lipid concentrations on the biological performance of the

reactors was comprehensively discussed in Chapter 3. At stable operating conditions, all reactors provided a high COD removal efficiency up to 99% but the permeate quality became worse with increased SRT. This may be due to the cell lysis and release of soluble microbial products at prolonged SRTs, possibly aggravated by LCFA inhibition.

Table 4.2 : Operating parameters and treatment performance of each reactor.

Parameter	Unit	R-20	R-30	R-50
OLR	kg COD·m ⁻³ ·d ⁻¹	8.3 ± 1.3	7.8 ± 0.9	6.1 ± 1.4
Permeate COD	mg·L ⁻¹	470 ± 60	570 ± 60	1070 ± 30
COD removal efficiency	%	99 ± 0.2	99 ± 0.5	98 ± 0.8
Substrate conversion efficiency to methane	%	73 ± 4	80 ± 6	83 ± 10
Methane yield	Nm ³ CH ₄ kg ⁻¹ COD _{removed}	0.26 ± 0.01	0.28 ± 0.02	0.29 ± 0.03

4.3.2 Long term filtration performance

The filtration performances of the reactors are given in Figure 4.1. In general, good filtration performance could be obtained in all reactors without any significant fouling problems. This is remarkable, considering the relatively low cross-flow velocity applied in the study compared to those (1-3 m·s⁻¹) reported in literature (Chapter 2; Ozgun et al., 2013). Probably, the good filtration performance can be attributed to the cyclic membrane operation and/or operation below the critical flux, which limits the irreversible fouling and cake compaction (Jeison and van Lier, 2006a; Jeison and van Lier, 2007b; Yigit et al., 2009). At steady state conditions, reactors R-20, R-30 and R-50 reactors were operated at a gross flux of 12, 10 and 14 L·m⁻²·h⁻¹, respectively. These operational fluxes were well below the critical fluxes measured in the reactors (Figure 4.1).

R-20 was operated at filtration and backwash mode for 15 days between 45th and 60th days. The fouling rate in this period of operation was calculated as 1.15 mbar·d⁻¹. In order to compare the effect of long term continuous filtration on membrane fouling with the cyclic membrane operation, the membrane was operated at continuous filtration mode in the last 3 weeks of operation. During this period backwash was done once in 5-6 days for 1 min.

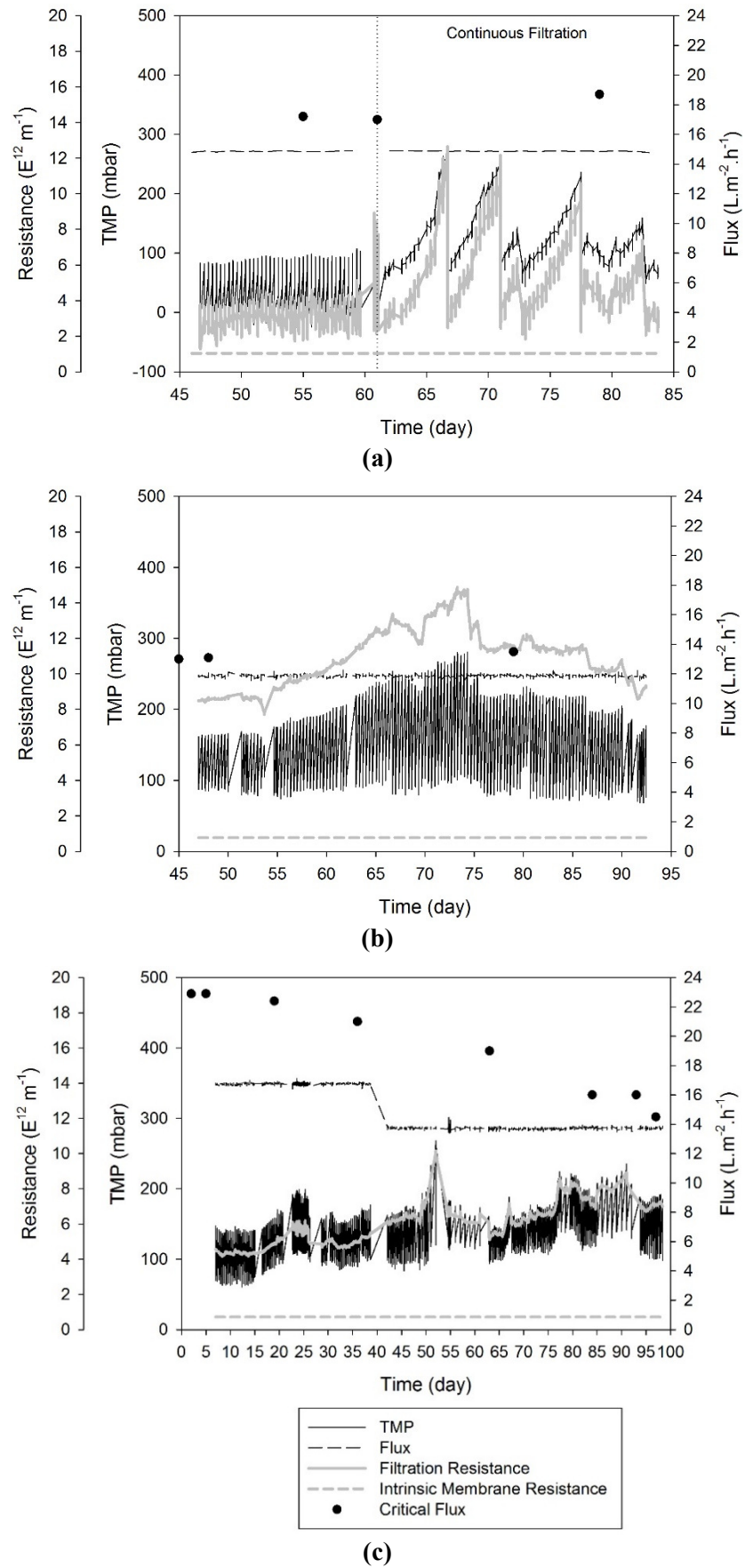


Figure 4.1 : Filtration performance of the reactors (a: R-20, b: R-30, c: R-50).

As it can be seen in Figure 4.1, the TMP and membrane resistance continuously increased during the long filtration cycles of 5-6 days, but the initial TMP at the beginning of the filtration could be almost completely recovered after backwash. The average TMP increase rate of this period was calculated as $37 \text{ mbar} \cdot \text{d}^{-1}$ which was much higher compared to the cyclic membrane operation period. Similar results were presented by Yigit et al. (2009), who investigated the effects of various backwash scenarios on membrane fouling in an aerobic MBR. They reported decreasing fouling rates and improved fouling control with more frequent back-wash, i.e., 15 s in each 5 minutes of filtration. The continuous increase in membrane resistance during the long filtration cycles seems to be linked to the cake layer build up, due to insufficient back transport of the particles with the applied shear. As backwash could completely remove the cake layer resistance, the cake layer appeared to be loosely attached to the membrane and cake compaction was apparently of minor importance.

Relatively low fluxes were obtained in R-30 compared to the other two reactors. During operation, the TMP and membrane resistance gradually increased and stabilized at about 200 mbar and $10 \text{ E}^{15} \cdot \text{m}^{-1}$, respectively. Moreover, the critical fluxes measured in this reactor were also significantly lower than the other reactors. R-50 was initially started with a moderate flux of $16 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ but the flux was gradually decreased to $14 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ in accordance to the decreasing critical flux (Figure 4.1). Reactor R-50's filtration resistance has gradually increased during the study, but it was still comparable with R-20 and R-30, considering the fact that this reactor was operated at a higher flux and TSS concentrations. The average TSS concentrations in R-20, R-30 and R-50 at stable operating conditions were 16.5 ± 0.8 , 17.2 ± 1.8 , $28.3 \pm 1.1 \text{ g} \cdot \text{L}^{-1}$, respectively.

Critical fluxes measured in R-20, R-30 and R-50 under stable operation conditions were 18, 13 and $14 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$, respectively (Figure 4.1). The critical flux of R-20 was significantly higher compared to the other reactors. The reason for this might be attributed to the removal of the small sized particles, which play a significant role in membrane fouling and cake compaction, with the discharged sludge. Following this explanation, wasting more sludge in membrane bioreactors may help to prevent the excessive accumulation of fine particles. The critical flux of R-30 did not change significantly whereas it gradually decreased in R-50 during the operation. The decrease in critical flux in R-50 may be due to an increased TSS concentration during the

experimental period. Thus, a significant negative correlation was observed between the TSS concentration and the critical flux of the R-50 reactor (Pearson correlation coefficient: -0.926 and P value: 0.0027). A clear relationship between TSS concentrations and critical fluxes of R-20 and R-30 could not be established.

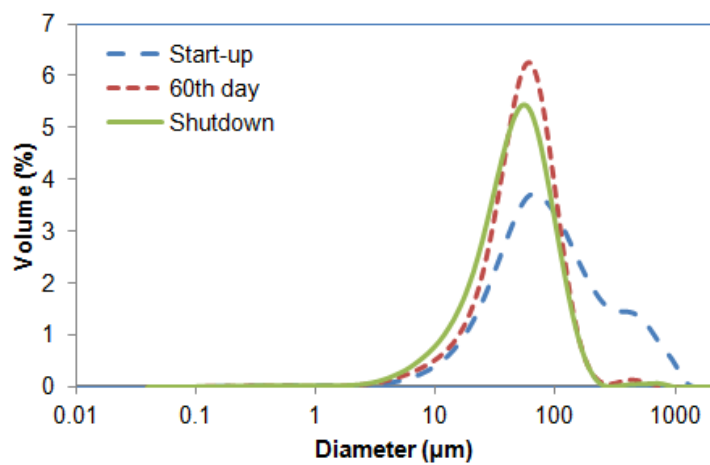
4.3.3 Sludge characteristics

In order to investigate the effect of SRT on sludge filtration characteristics, supplementary indicators such as sludge particle size distribution, CST, SRF and supernatant filterability were monitored throughout the study. Although these parameters do not provide direct information on the real membrane filtration process taking place in the reactors, they are valuable tools to compare the sludge filterability at different reactors under standardized conditions.

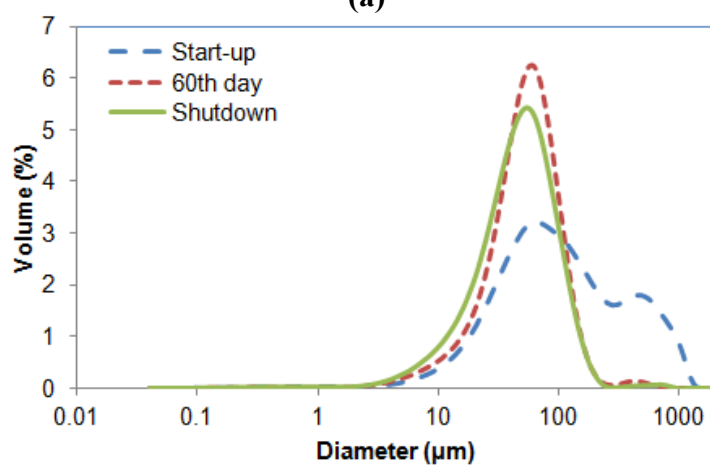
4.3.3.1 Particle size distribution

During long term operation, the particle size decreased in all reactors (Figure 4.2). However, after a remarkable initial decrease in particle size, the PSD remained stable for the rest of the operation period in R-20 and R-30. The significant decrease in the median particle size at the initial stages may be attributed to the disruption of sludge flocs by the cross-flow pump. Several researchers reported that the shear stress applied for scouring the particles from the membrane surface results in a decrease in particle size in AnMBRs (Jeison et al., 2009b; Ho and Sung, 2009). The initial median particle size in R-50 was already low, since this reactor was a continuation of R-30 at an increased SRT. On the other hand, the median particle size in R-50 did not stabilize, as we observed in the other reactors, but continued to shift to lower sizes. Considering the fact that all reactors were operated at the same cross-flow velocity and shear rate, the difference in particle size can be attributed to the accumulation of small size particles in R-50 due to increased SRT. Another plausible reason for decreasing particle size may be LCFAs which may cause toxicity on biomass (Hwu et al., 1996). It is reported that the instabilities such as sudden organic load, temperature and pH shocks, may cause floc breakage, resulting in a decrease in particle size in AnMBRs (Akram and Stuckey, 2008b; Gao et al., 2010a; Gao et al., 2011a). It is also reported that LCFAs can cause degranulation in granular bed reactors due to their surfactant characteristics at neutral pH (Sam-Soon et al., 1991; Amaral et al., 2004). Very likely,

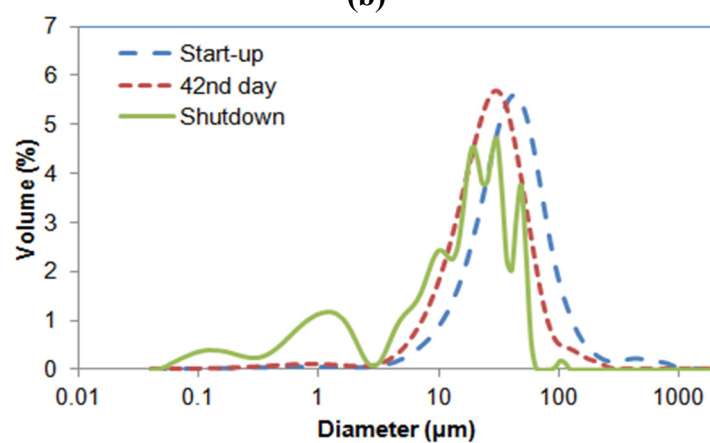
the observed continuous particle size reduction in R-50, might be very well attributed to LCFA inhibition (Chapter 3).



(a)



(b)



(c)

Figure 4.2 : Sludge particle size distribution in the reactors, a: R-20, b: R-30, c: R-50.

4.3.3.2 EPS and SMP

EPS and SMP measured as protein and carbohydrate, are often linked to observed membrane fouling (Meng et al., 2009). The SMP and bound EPS concentrations measured at the end of reactor operation are presented in Table 4.3. Both SMP and bound EPS concentrations increased at higher SRTs. The increase in SMP concentrations may be attributed to the accumulation of these compounds in the reactor due to decreased sludge discharge at elevated SRTs. Moreover, it is reported that LCFAs may exhibit a bactericidal effect on anaerobic biomass (Rinzema et al., 1994; Hwu et al., 1996). Therefore, the previously described LCFA inhibition observed in R-50 (Chapter 3) may result in cell lysis, releasing bacterial decay products to the bulk liquid. It is also reported that the bacteria might secrete more SMP during instable operating conditions (Le-Clech et al., 2006).

The better membrane permeability observed in R-20 reactor may be due to lower SMP and bound EPS concentrations compared to the other reactors. It is also noted that the protein – carbohydrate (P:C) ratio of bound EPS tended to decrease at higher SRTs. Since the protein fraction of EPS plays a major role in sludge aggregation and bioflocculation, a decrease in P:C ratio is often correlated with a decrease in sludge particle size (Liao et al., 2001; Massé et al., 2006). The low P:C ratio agreed well with the small median particle size observed in R-50.

Table 4.3 : SMP and bound EPS concentrations at the end of operation.

Parameter	Unit	R-20	R-30	R-50
Soluble protein	mg·L ⁻¹	440	580	1160
Soluble polysaccharide	mg·L ⁻¹	58	115	180
P:C ratio of SMP	-	7.6	5.0	6.4
Bound protein	mg·g ⁻¹ VSS	82	95	91
Bound polysaccharide	mg·g ⁻¹ VSS	11	17	23
P:C ratio of bound EPS	-	7.5	5.6	4.0

4.3.3.3 Relative hydrophobicity

The RH of the sludges from R-20, R-30 and R-50 was measured as 40%, 34% and 58%, respectively. Cell surface hydrophobicity is dependent on many factors such as substrate, EPS and bacterial culture composition. For instance, it is reported that acetogens that degrade LCFAs are hydrophobic (Daffonchio et al., 1995; Nielsen et al., 2002). Besides, LCFAs can absorb on to the biomass forming a hydrophobic layer around bacterial membranes (Alves et al., 2009). This situation can strongly alter the

RH and surface characteristics of the sludge. The R-50 reactor, which suffered from LCFA inhibition due to the accumulation of LCFA on the biomass (Chapter 3), exhibited the most hydrophobic sludge characteristics. Moreover, R-20 sludge was slightly more hydrophobic compared to R-30. This may be due to incomplete LCFA degradation indicated by the lowest substrate biodegradation efficiency in this reactor. Therefore, the non-degraded LCFA might accumulate in the sludge. Another important factor that influences sludge hydrophobicity is the EPS composition, for example, bound proteins are often correlated with sludge hydrophobicity (Mikkelsen, and Keiding, 2002; Wilen et al., 2003). In this study, similar bound protein concentrations were measured in R-30 and R-50 (Table 4.3). Therefore, the significant difference observed between the RH of the sludges can be mainly attributed to the effect of LCFA adsorption on the biomass surface.

4.3.4 Sludge filterability

4.3.4.1 Specific resistance to filtration

Cake layer formation is generally regarded as the most important fouling mechanism in AnMBRs (Jeison and van Lier, 2007b; Charfi et al., 2012). Therefore, the parameter SRF, indicating the sludge filterability, may also indicate the quality of cake accumulating on the membrane surface. Thus a high SRF means a compact and less porous cake layer formed with small-sized particles, whereas a low SRF indicates a cake layer with high porosity. The SRF of the sludge in R-20 and R-30 showed an increasing trend in the initial stages of the study and then stabilized during the long term operation (Figure 4.3). The reason for that might be the initial disruption of the flocs due to applied shear stress which may yield a more compact cake layer. R-50 was inoculated with the sludge of R-30; therefore, particle size in this reactor was already low from the start. As a result of that, the SRF did not change so much in this reactor. However, instabilities in biological performance due to LCFA inhibition between 20 and 60 days (Chapter 3), led to a remarkable increase in SRF.

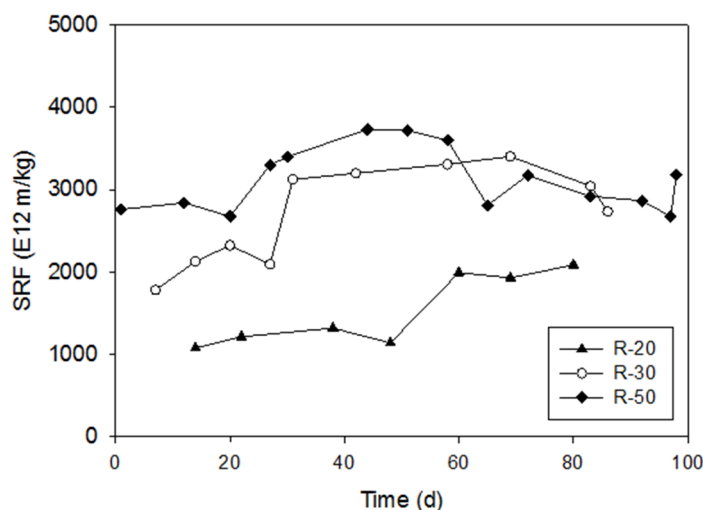


Figure 4.3 : Evolution of SRF of the sludge during reactor operation.

The average SRF of R-20, R-30 and R-50 at stable conditions were calculated as 2001 ± 78 , 3137 ± 262 and $2963 \pm 218 \text{ E}^{12} \text{ m} \cdot \text{kg}^{-1}$, respectively. Recently, Ersahin et al. (2013) reported similar values for dynamic AnMBRs. The SRF of the sludge in our study was about 1-3 orders of magnitude higher than the values reported for aerobic MBRs (Pollice et al., 2008, Guglielmi et al., 2010). This may be due to different floc size and structure of aerobic and anaerobic sludges. Chang and Kim (2005) stated that the SRF is directly dependent on sludge particle size and cake porosity. Since R-20 had a higher particle size distribution, it is not surprising that the SRF measured in this reactor was lower compared to that of R-50. Interestingly, it is noted that although R-30 sludge had a similar PSD with R-20, its SRF was as high as R-50 sludge which had a significantly higher fraction of small size particles. Therefore, several other parameters such as EPS, shear sensitivity and degree of dispersion which may have an influence on the parameter SRF, should also be taken into consideration (Liu et al., 2012a; Mikkelsen et al., 2002). SMP, colloidal fraction of biopolymer clusters, and humic substances were also reported to have a positive correlation with SRF (Guglielmi et al., 2010; Buntner et al., 2014).

4.3.4.2 Capillary suction time

CST, which is a common parameter used for sludge dewaterability, followed the same trend and showed a good correlation with TSS concentration in R-50. However, in R-20 and R-30 reactors it tended to increase regardless of the decreasing TSS concentration and then it stabilized as the reactor TSS concentration reached to a

steady state (Figure 4.4). The average CSTs measured in R-20, R-30 and R-50 reactors were 951 ± 128 , 1743 ± 187 and 2414 ± 145 seconds, respectively.

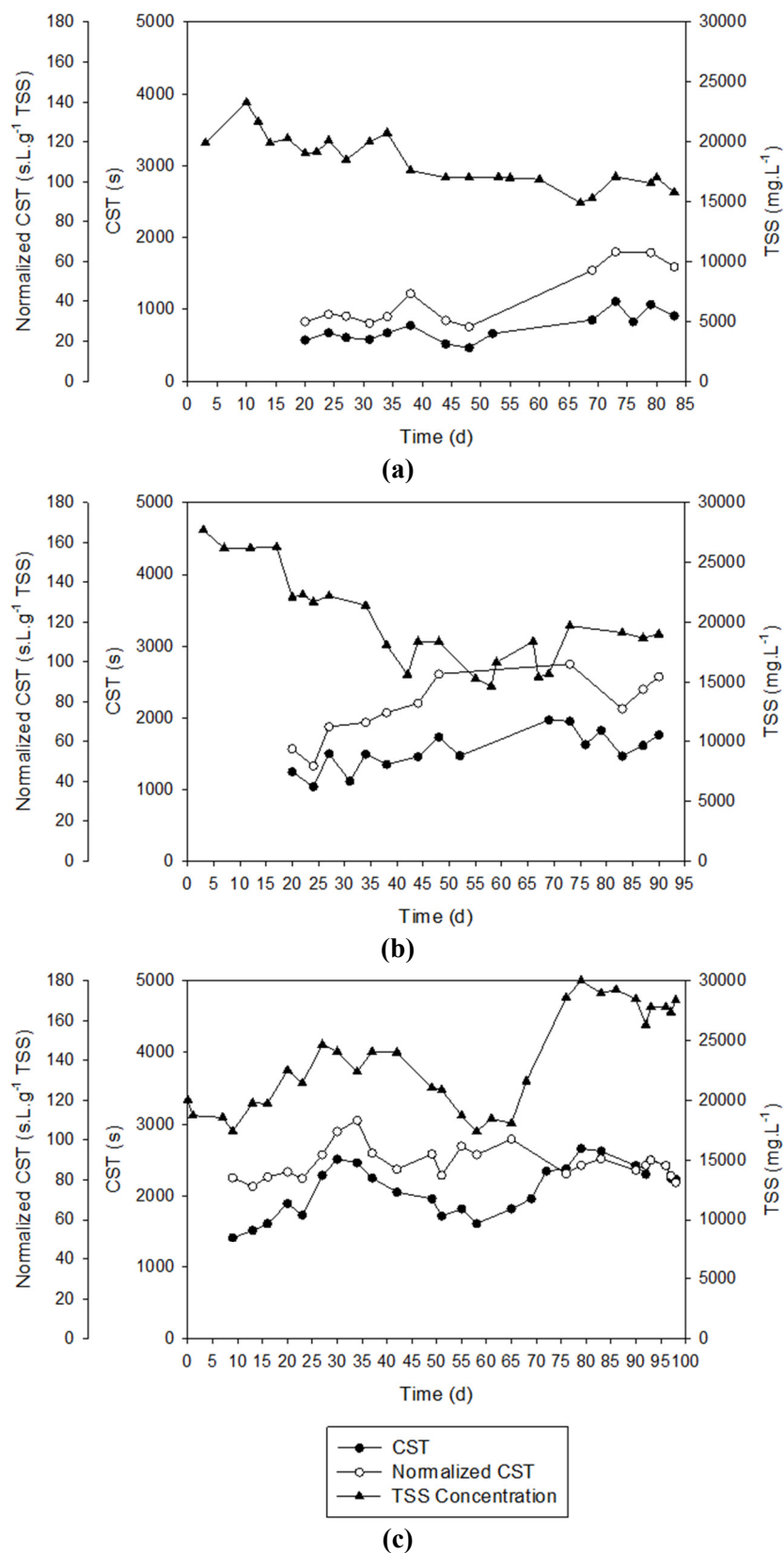


Figure 4.4 : Evolution of CST together with TSS concentration (a: R-20, b: R-30, c: R-50).

The normalized CST results for R-20, R-30 and R-50 reactors were 61 ± 5 , 90 ± 12 , 86 ± 4 $\text{s} \cdot \text{L} \cdot \text{g}^{-1}$ TSS, respectively. The normalized CST in R-30 and R-50 reactors were similar, but significantly higher than R-20. These results were much higher compared to the CST of sludge from aerobic MBRs, which was reported to be less than 120 seconds (Wu et al., 2007; Police et al., 2008). Moreover, they were also higher than the normalized CST values of anaerobically digested waste activated sludge, which was reported to be in the range of 40-50 $\text{s} \cdot \text{L} \cdot \text{g}^{-1}$ TSS (Xu et al., 2011).

4.3.4.3 Supernatant filterability

The supernatant filterability provides information about the fouling propensity of fine particles and solutes such as SMP and colloids in the sludge supernatant, which yields to pore blocking in the membranes (Le-Clech et al., 2006; Meng et al., 2009; Tian et al., 2011). The supernatant filterability did not change significantly throughout the operation period in all reactors (Figure 4.5). The supernatant filterability of R-30 and R-50 reactors was similar but it was significantly lower than that of R-20 reactor. Lee et al. (2003) stated that the filtration resistances of the supernatants from aerobic MBRs operated at different SRTs, i.e. 20, 40, 60 days, were similar regardless of the SRT. However, one could expect that the supernatant filterability increases at shorter SRTs due to the washout of solutes and fine particles with sludge wasting. Therefore, the better filterability of R-20 supernatant may be due to this phenomenon, coinciding with the higher critical fluxes measured in this reactor.

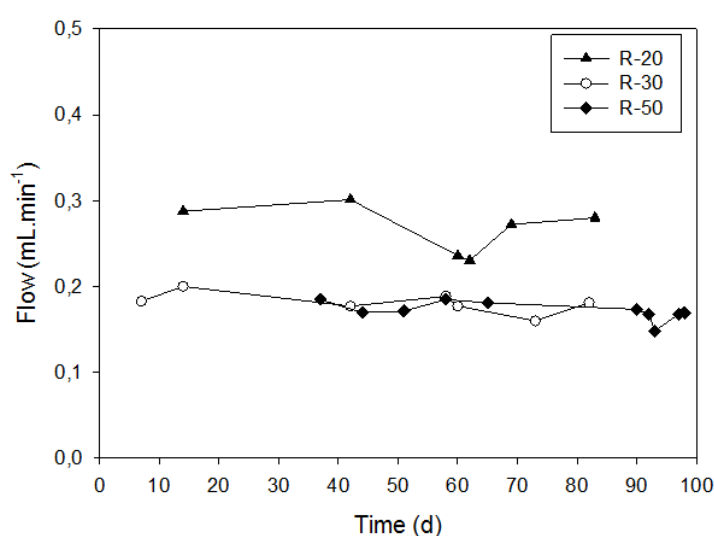


Figure 4.5 : Supernatant filterability at different SRTs.

Figure 4.6 shows the COD fractions of the supernatant from the reactors. A one way ANOVA test ($\alpha = 0.05$) revealed that the mean COD concentrations of supernatant in reactors are significantly different. It is clear that the supernatant COD, concentration of solutes and fine particles increased at elevated SRTs. Therefore, the parameter SRT may help to control the accumulation of solutes and fine particles in AnMBRs. A pair wise comparison with Holm-Sidak method ($\alpha = 0.05$), applied as a complementary test, indicated that soluble and fine particle COD fractions in R-30 and R-50 were not statistically different. This result coincides with the similar supernatant filterability measured in these reactors.

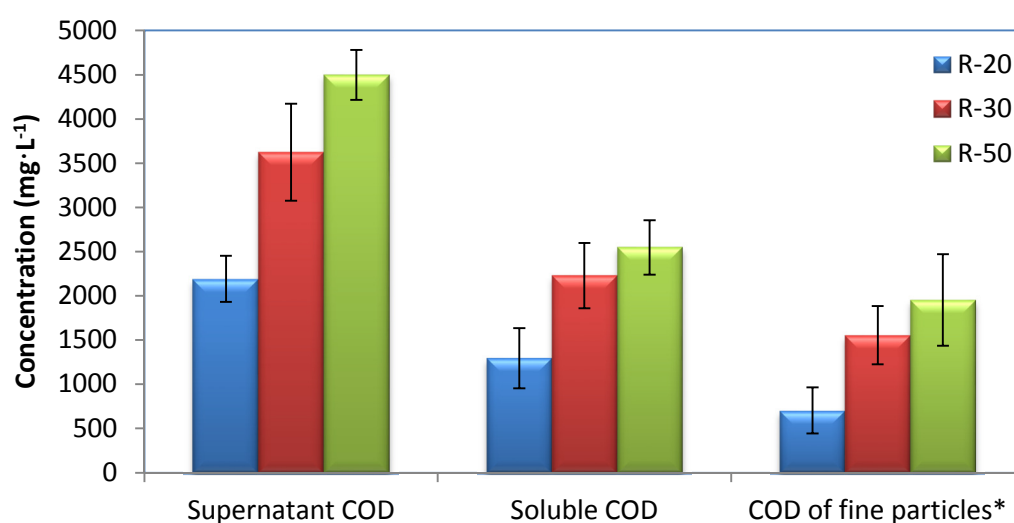


Figure 4.6 : COD fractions of supernatant from the reactors.

In order to get more insight about the supernatant filterability, the supernatant was fractionated with 2 different sized membranes (1.6 and 0.7 μm) and filterability tests were carried out with the filtrates. According to the results (Figure 4.7), the filterability of the supernatant of both reactors gradually improved after selective removal of foulants with different pore sized membranes. Moreover, it is noted that the improvement of the filterability in R-20 after 0.7 μm membrane filtration was more remarkable compared to R-30. This means that the amount of foulants smaller than 0.7 μm present in R-20 was much less compared to R-30.

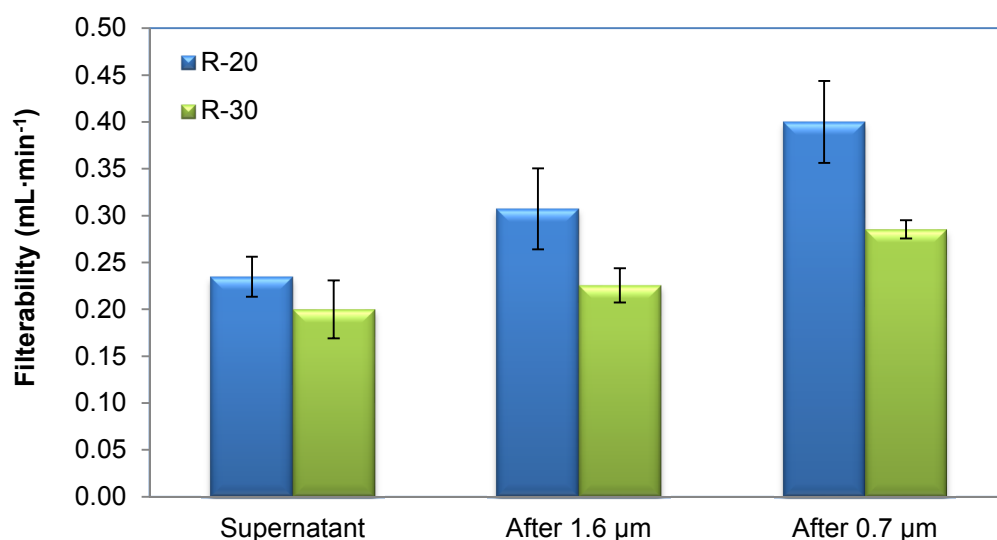


Figure 4.7 : Filterability of different supernatant fractions of R-20 and R-30.

4.3.5 Relationship between sludge characteristics and real membrane performance

A statistically significant correlation between sludge filtration characteristics and real membrane filtration resistance during operation could not be observed for R-20 and R30. However, the results of Pearson Correlation Test ($p < 0.05$) indicated that there is a significant correlation between some filterability parameters and membrane filtration resistance for R-50 (Table 4.4). The results indicated that there is a positive correlation between TSS, protein, carbohydrate and COD_{SMP} concentrations, CST and membrane filtration resistance. Wu et al. (2007) reported that CST, SCOD, soluble carbohydrate and protein are inter-correlated to each other. These parameters were also found to be influencing membrane filtration resistance. Similar results were also reported by several other researchers (Pan et al., 2010; Guglielmi et al., 2010). Controversially, Khongnakorn et al. (2007) stated that the CST displayed a good correlation with TSS concentrations, however, neither the sludge physical characteristics, i.e. TSS concentration, CST and SRF, nor SMP could be linked to the TMP evolution of an aerobic MBR. Some researchers also could not find any correlation between TSS concentrations and the CST parameter (Laera et al. 2009; Wu et al., 2007; Guglielmi et al., 2010).

Unsurprisingly, the supernatant filterability was negatively correlated with filtration resistance, which means that an increase in the supernatant filterability yields to less membrane resistance during operation (Table 4.4). This is reasonable, since the

supernatant fraction of the sludge mainly contains the solutes and fine particles which were reported to play an important role in membrane fouling (Gao et al., 2013). Several researchers reported that the contribution of the supernatant to the total filtration resistance was between 17% and 81% (Wisniewski and Grasmick 1998; Defrance et al., 2000; Bouhabila et al., 2001; Lee et al., 2003, Le-Clech et al., 2006). The wide variation of the results reported on the influence of supernatant on fouling may be attributed to the differences in the methods used for fractionating sludge, bioreactor and membrane operating conditions.

By definition, the parameter SRF is independent from TSS concentration, however it was found inversely correlated with the sludge TSS concentration in our study. Although the correlation was significant for only R-20 and R-30, the trend was the same for all reactors which indicated that an increase in TSS concentration led to higher SRF of the sludge (Figure 4.8). A similar trend between SRF and TSS concentration was also reported by Chang and Kim (2005). They suggested that the SRF should not be a proper criterion for estimating the degree of cake fouling especially at low TSS concentrations, i.e. below $3.7 \text{ g} \cdot \text{L}^{-1}$. Interestingly, Lousada-Ferreira et al. (2015) found an increased sludge filterability with increasing TSS concentration in an aerobic MBR, until a maximum was reached when the sludge concentration amounted $10\text{-}11 \text{ g TSS} \cdot \text{L}^{-1}$. At higher sludge concentrations the filterability dropped again. They postulated that TSS may act as scavenger for colloids, SMP, EPS, enhancing the filterability. Although their sludge concentrations in the aerobic MBR were much lower, a similar principle may explain our present results.

Table 4.4 : Correlations between selected filterability parameters for R-50.

Parameter	Soluble protein	Soluble carbohydrate	COD _{SMP} ¹	CST	Supernatant Filterability	SRF	Filtration resistance
TSS concentration	0.505 ²	0.751*	0.599*	0.874*	-0.594	-0.383	0.7*
Soluble protein	-	0.695*	0.858*	0.173	-0.384	0.0192	0.911*
Soluble carbohydrate	-	-	0.645*	0.504	-0.394	-0.544	0.646*
Non VFA soluble	-	-	-	0.417*	-0.711*	-0.353	0.731*
COD	-	-	-	-	-0.702*	-0.208	0.536*
CST	-	-	-	-	-	0.192	-0.832*
Supernatant filterability	-	-	-	-	-	-	-0.103
SRF	-	-	-	-	-	-	-0.103

¹ Calculated by subtracting COD_{VFA} from soluble COD measured in the reactor

² Pearson correlation coefficient (“-” indicates negative correlation)

* Indicates significant correlation (P<0.05)

The SRF was also found to be correlated with the normalized CST parameter in all reactors (Figure 4.9). Guglielmi et al. (2010) reported that CST displayed a good

correlation with the sludge cake resistance. Sawalha and Scholz (2010) modeled the relationship between CST and SRF with a non-linear equation, based on normalized CST and temperature. Apart from the interrelation between SRF and CST, interestingly, the parameter SRF was found uncorrelated with membrane resistance in R-50 (Table 4.4). The hydrodynamic differences between the membrane operation and SRF test conditions may explain the lack of correlation. In general, it can be stated that the cake layers formed in cross-flow filtration are less porous and have a higher resistance than the ones in dead end filtration tests (Le-Clech et al., 2006).

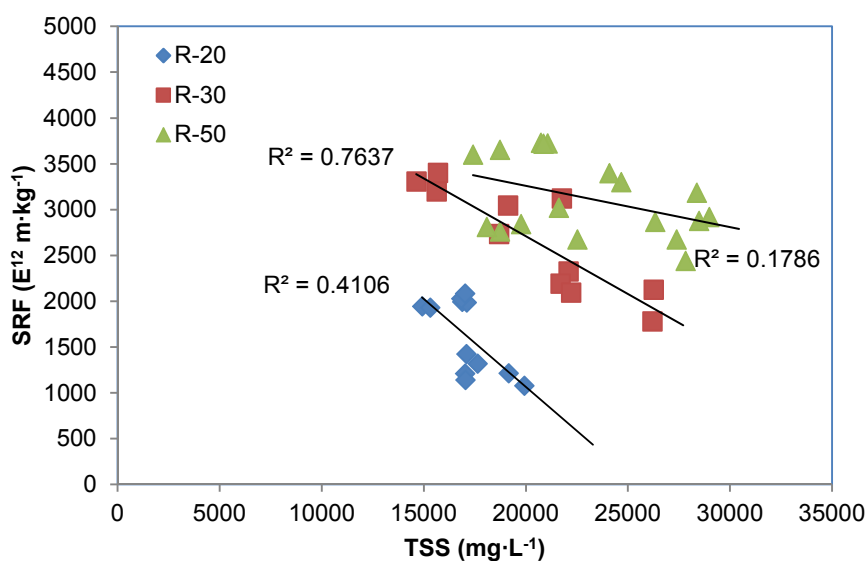


Figure 4.8 : Relationship between TSS concentration and SRF parameter.

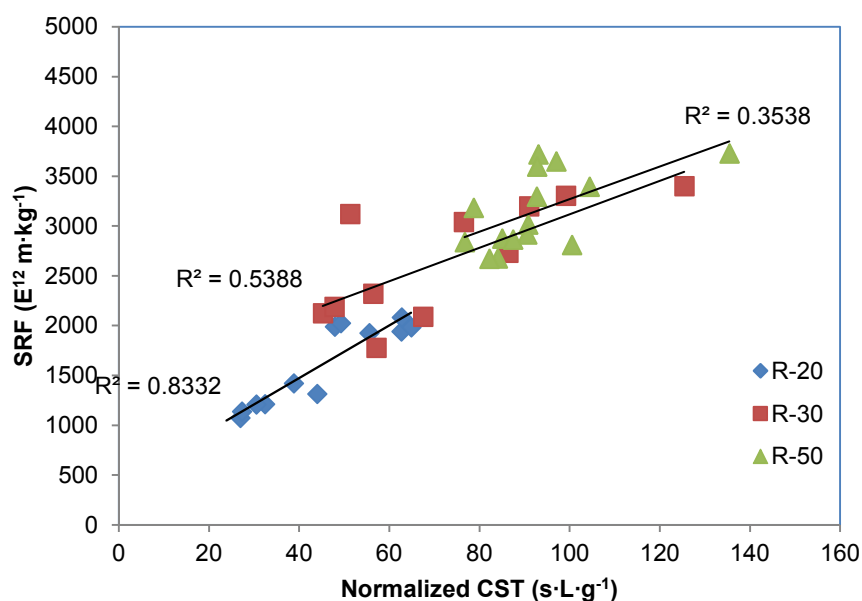


Figure 4.9 : Relationship between CST/TSS and SRF parameter.

4.3.6 Membrane cleaning

Physical cleaning by flushing the membrane with tap water could significantly restore the membrane permeability and decreased the resistance in R-20 and R-50 reactors (Table 4.5). This indicated that a loosely attached cake layer was present on these membranes. However, physical cleaning was not that effective for R-30 reactor indicating a higher degree of irreversible fouling.

Table 4.5 : Permeability and filtration resistance of reactors R-20 (at day 60 and day 83) and R-30, measured after physical cleaning of the membranes.

Procedure	Parameter*	Unit	R-20	R-30
Virgin membrane	Permeability	$L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	339	507
	Resistance	$10^{12} m^{-1}$	1.04	0.77
			60th day (End of cyclic operation)	83rd day (End of operation)
Before cleaning	Permeability	$L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	85	54
	Resistance	$10^{12} m^{-1}$	4.05	6.29
After physical cleaning	Permeability	$L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	207	260
	Resistance	$10^{12} m^{-1}$	1.72	1.38
				86th day

*Permeability and resistance were calculated for 20 °C.

Figure 4.10 shows the proportions of different fouling resistances in total filtration resistance of R-20 and R-30 reactors. In general, cake layer formation constituted the highest portion of total filtration resistance, which confirmed that the main fouling mechanism in the AnMBRs was the cake layer accumulation (Jeison et al., 2007; Charfi et al., 2012). However, a significantly higher degree of irreversible fouling was observed for the R-30 reactor.

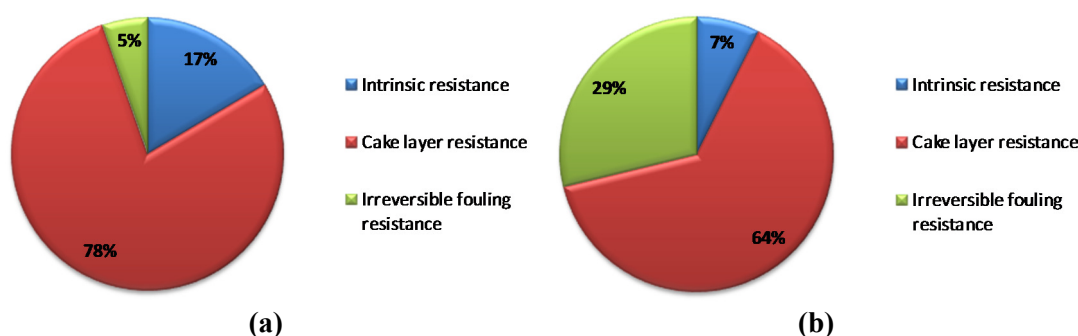


Figure 4.10 : The proportions of different fouling resistances in total filtration resistance at the end of operation of reactors (a): R-20 and (b): R-30.

A detailed cleaning procedure was applied for the membrane of R-50 and the results are presented in Figure 4.11. The results indicated that removal of the cake layer by physical cleaning could significantly improve the membrane permeability.

Surprisingly, a negligible degree of irreversible organic fouling was observed in this reactor based on the cleaning results with NaClO. Since significantly higher SMP concentrations were measured in R-50, a higher degree of irreversible organic fouling was expected to occur on and in this membrane. Possibly, not only the quantity but also the quality of the SMP such as molecular size, hydrophobicity and charge is important for membrane fouling (Liang et al., 2007). On the other hand, the R-50 membrane was found remarkably fouled by inorganic foulants (Figure 4.11). Fortunately, chemical cleaning with citric acid could completely restore the membrane permeability.

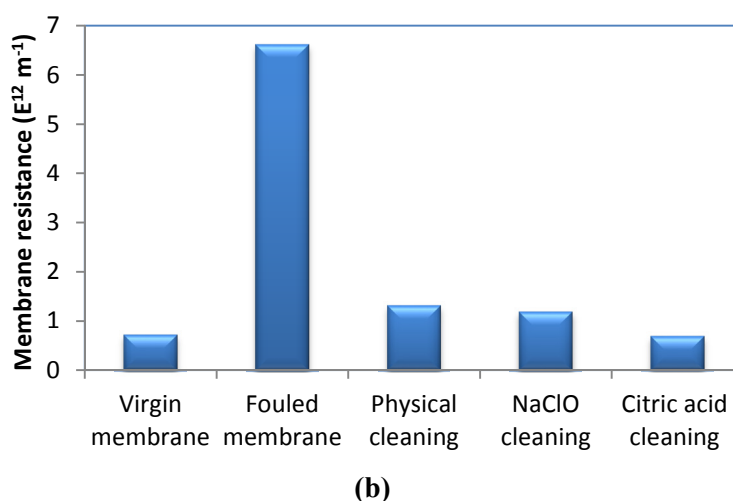
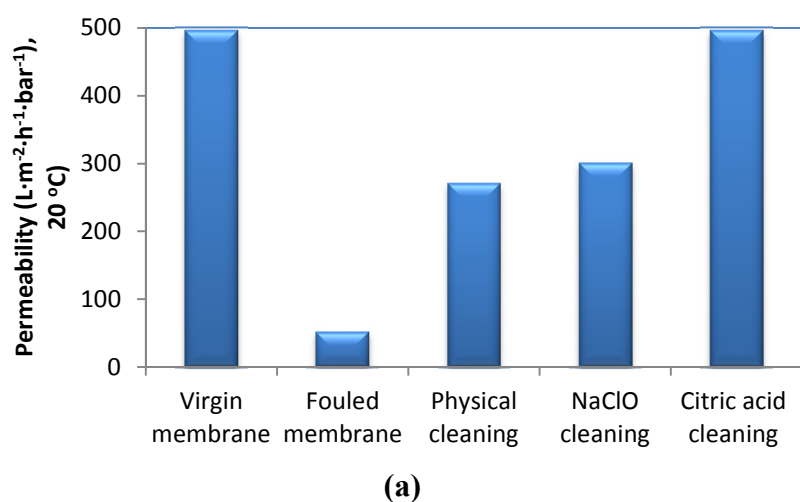


Figure 4.11 : Efficiency of cleaning on permeability and filtration resistance recovery for the R-50 membrane (a) Permeability after cleaning, (b) Resistance after cleaning.

4.3.7 Membrane autopsy

4.3.7.1 FT-IR analysis of membrane foulants

Figure 4.12 presents the FT-IR spectra of virgin, fouled and physically cleaned membranes. All spectra show a broad region of absorption at around 3300 cm^{-1} , which is due to stretching of the O–H bond in hydroxyl functional groups, and a peak at around 2930 cm^{-1} , which is due to stretching of C–H bonds of alkane class (Gao et al., 2011b). Martinez et al. (2012) attributed the peak around 2930 cm^{-1} to the stretching of C–H bonds in LCFAs. The peaks in the vicinity of 1640 cm^{-1} (stretching vibration of C=O and C–N) and 1540 cm^{-1} (N–H deformation and C=N stretching) are typical for secondary protein structures, namely amide I and II (Gao et al., 2011b). The peaks at 1240 and 1400 cm^{-1} imply the presence of amides III (Lin et al., 2009). These results indicate the presence of proteins in the cake layer.

The broad peak of around 1040 cm^{-1} typical for C–O stretching demonstrates the presence of polysaccharides or polysaccharide-like substances (Meng et al., 2006). C–H rocking vibrations are present in the fingerprint regions between $635\text{--}855\text{ cm}^{-1}$ of the spectra and the doublet at 883 cm^{-1} are due to C–C stretching (Tian et al., 2011). Interestingly, although the spectra of R-30 and R-50 seem to be similar, some foulants such as polysaccharides and amide III associated to the wave numbers between $1000\text{--}1400\text{ cm}^{-1}$ could not be detected on R-20 membrane indicating a lesser degree of fouling. Moreover, physical cleaning could almost completely remove the foulants from the membranes of R-20 and R-50 but was less effective on the R-30 membrane.

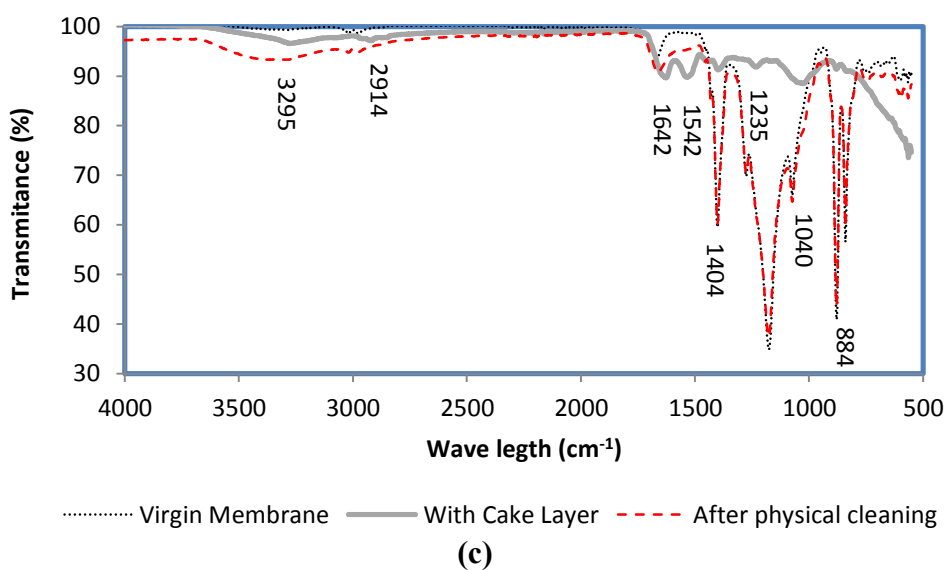
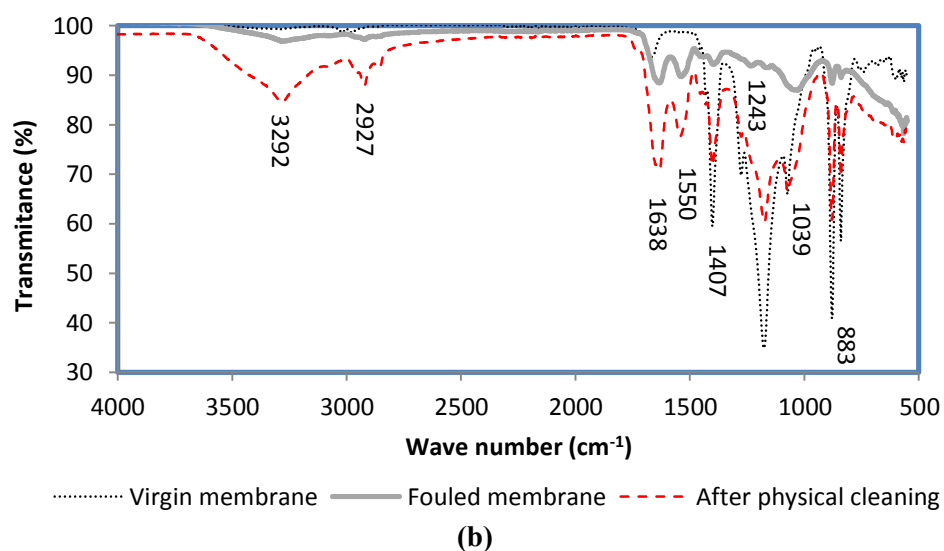
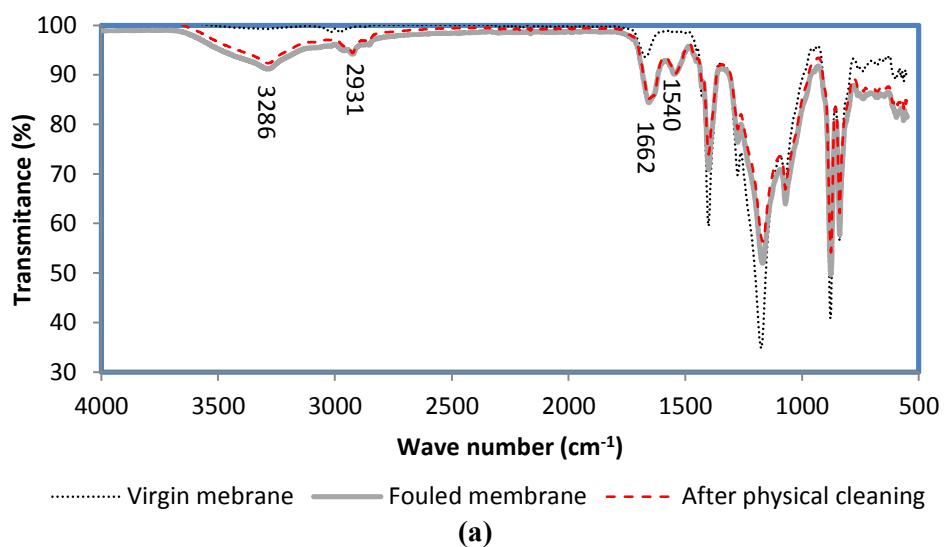


Figure 4.12 : FT-IR spectra of the membranes from reactors (a): R-20, (b): R-30, and (c): R-50.

4.3.7.2 SEM-EDX

As it is shown in Figure 4.13, the cake layer on the membranes from R-20 and R-50 reactors have remarkable similarities. Both cake layers seem to be composed of rod and filamentous shaped microorganisms and the cake layers almost could be totally removed by physical cleaning, i.e. flushing with tap water. Alves et al. (2009) and Hatamoto et al. (2007) reported that rod shaped microorganisms dominate the reactors treating LCFA containing wastewaters under mesophilic and thermophilic conditions. Thus, it is not surprising to see this type of bacteria in the cake layers of the AnMBRs treating corn-to-ethanol stillage with high lipid content. Interestingly, the cake layer on the membrane from R-30 was significantly different compared to the others. The cake layer of R-30 membrane had a fluffy structure which was probably composed of EPS and cells. Moreover, it could not be totally removed by physical cleaning. After flushing with water, the fluffy layer had been removed revealing a similar structure to R-20 and R-50 cake layers. However, it seems that this layer attached more tightly to the membrane. The reason for this may be related to the differences in the hydrophobicity of the sludge in the reactors.

The EDX analysis revealed that inorganic precipitates consisting of e.g. phosphorus, potassium and sulphur were incorporated within the cake layer (Table 4.6). Moreover, some heavy metals such as iron and copper which may form metal-sulphur precipitates were also found on the membrane of R-50 reactor. Struvite was reported as one of the main reasons of inorganic membrane fouling in AnMBRs (Kang et al., 2002; Meabe et al., 2013). During long term operation, we observed a lot of precipitation in the reactors. An elemental analysis of these precipitates obtained from the bottom of the reactors revealed that the most abundant elements were Mg and P with concentrations of 7.7 and 7.4 mol·kg⁻¹ TS, respectively. Therefore, it is likely that there was also some precipitation on the membrane. EDX analysis of precipitates found on the membrane surface indeed indicated that they contain magnesium, phosphorus and potassium (Figure 4.14).

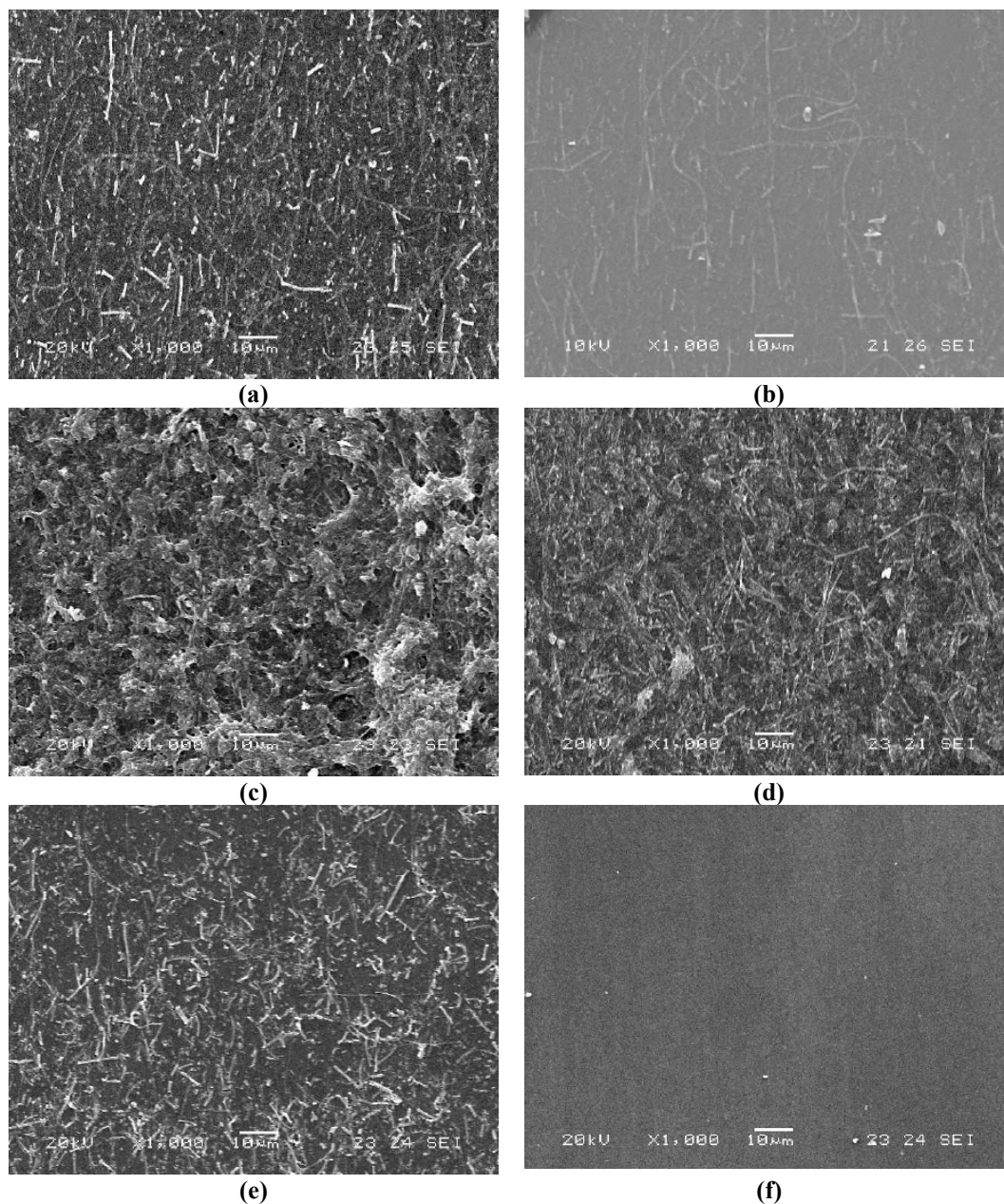


Figure 4.13 : SEM photographs of the membranes from the reactors before and after physical cleaning, (a): R-20 before cleaning, (b): R-20 after cleaning, (c): R-30 before cleaning, (d): R-30 after cleaning, (e): R-50 before cleaning, and (f): R-50 after cleaning.

Table 4.6 : The results of EDX analysis on fouled and cleaned membranes.

Parameter	Elements (%)									
	C	N	O	F	Na	S	P	Fe	K	Cu
Virgin membrane	68	6	3	23	-	-	-	-	-	-
R-20 fouled membrane	32	8	6	51	1	-	1	-	1	-
R-20 after physical cleaning	41	-	8	45	1	1	2	-	2	-
R-30 fouled membrane	46	23	24	-	1	1	3	-	3	-
R-30 after physical cleaning	44	14	15	25	-	1	1	-	1	-
R50 fouled membrane	32	15	-	49	-	1	1	-	-	2
R-50 after physical cleaning	39	-	-	57	-	1	-	1	-	2
R-50 cleaned with NaOCl	35	-	5	58	-	-	-	1	-	-
R-50 cleaned with NaOCl and citric acid	29	17	-	53	-	-	1	-	-	-

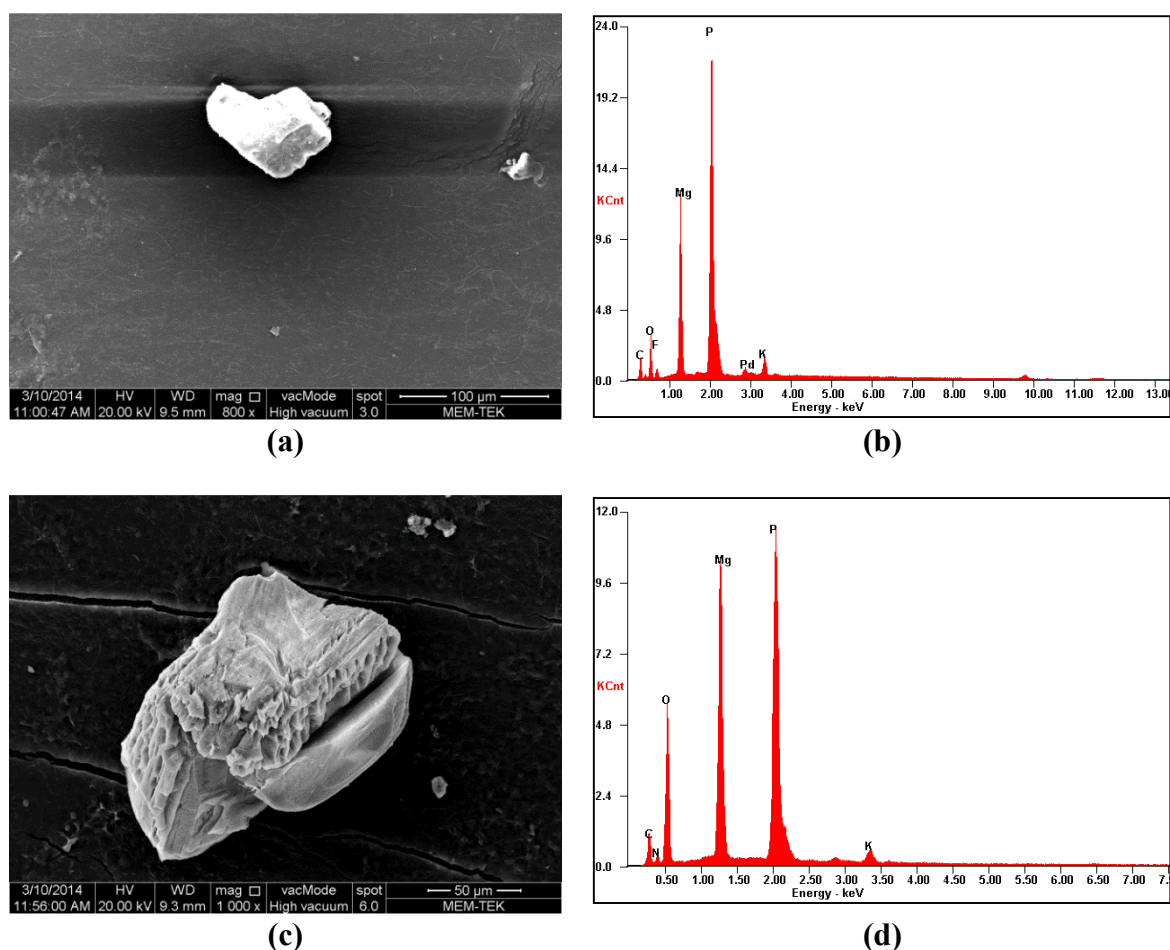


Figure 4.14 : SEM-EDX analysis of precipitates found on membrane surface (a-c: SEM photographs, b-d: EDX analysis).

4.4 Discussion

The adsorption of LCFA on biomass, as discussed in the previous chapter, not only affected the bioreactor stability and biomass activity but also strongly altered the sludge RH. Thus, the substrate composition together with reactor operating conditions indirectly affected the sludge's fouling propensity. Although LCFA inhibition may be the reason for decreased particle size and deteriorating sludge filterability, we observed a lesser degree of fouling at increased sludge hydrophobicity. Van den Broeck et al. (2011) stated that the hydrophobic flocs interacted less with the hydrophilic membrane and tended to foul the hydrophilic membrane surface less. Therefore, the lower sludge RH may be the reason for poor membrane performance and higher degree of fouling observed in R-30. Generally, low hydrophobicity of flocs is assumed to cause higher fouling due to floc deterioration and stronger interactions with the typically hydrophilic membrane (Le-Clech, 2006; Drews, 2010). Qin et al. (2012) reported a significant flux enhancement due to the increase in sludge hydrophobicity in an aerobic

MBR treating frying oil and rhamnolipids as surfactant. Yang et al. (2012a) observed a lower membrane fouling rate when treating simulated restaurant wastewater composed of oil and detergents. They suggested that oil and detergents modify the sludge and membrane surface properties. Although there are reports on the positive effect of lipids on sludge filterability, Ramos et al. (2014) reported that a high amount of lipids were present in the organic foulants removed by chemical cleaning of membranes from an AnMBR treating lipid rich wastewater.

The chosen operational conditions, i.e. short filtration/backwash cycles and applying a membrane flux below the critical flux, was found effective for preventing the extensive deposition of particles and cake layer consolidation. However, even at very low fluxes, fouling can still progress through the adsorption of solutes on to the membrane (Le-Clech et al., 2003). Thus, hydrophobic interactions, electrostatic repulsion and size exclusion can be the governing mechanisms of fouling on the membranes, when operated below the critical flux (Shen et al., 2010; Xiao et al., 2011). The absorption process can significantly alter the original membrane surface properties and the affinity of hydrophobic proteins to accumulate on the membrane surface or inside the pores can play a major role in organic fouling such as pore blocking, gel layer formation and subsequent cake layer development (Le-Clech et al., 2006). Recent studies on the spatial distribution of biopolymers in fouling layers accumulating on the membrane revealed that the bottom part of the cake layer in the vicinity of the membrane is rich with proteins (Metzger et al., 2007; Gao et al., 2011b). This clearly confirms that proteins can form a sticky layer on the membrane surface which accelerates the following fouling process. The detailed cleaning studies carried on the fouled membrane of R-50 showed that cake layer formation and inorganic fouling were the major contributors of filtration resistance, whereas organic fouling apparently had a minor effect. Although the concentrations of SMP as proteins and carbohydrates were much higher in this reactor, it seems that SMP had a minor effect on the fouling. Liang et al. (2007) observed that although the concentration of hydrophobic SMP increased at elevated SRTs, its fouling potential was relatively low at long SRTs. Shen et al. (2010) found that the hydrophilic fraction of SMP was the dominant foulant for flux deterioration in a hydrophilic PVDF membrane. Moreover, flux decline caused by hydrophilic foulants was much less recoverable by hydraulic cleaning such as backwash. Thus, not only SMP concentrations but also the

physicochemical properties of the SMP, such as hydrophobicity and surface charge, must be taken in to consideration when evaluating membrane fouling.

Better sludge filtration characteristics were observed in R-20 operated at a shorter SRT compared to the other two reactors. This may be due to washout of fine particles and SMP, which have been reported as the main membrane foulants, with the wasted sludge. The better supernatant filterability, the higher particle size of the sludge and the lower supernatant COD concentrations, were all in line with the better sludge filterability and higher operating fluxes observed in this reactor. The sludge filterability tended to decrease, when the SRT was increased from 20 to 30 days, but a further increase to 50 days did not change the filterability significantly. Pollice et al. (2008) observed that the optimum SRT, which provides the best filterability in terms of low CST and SRF values, was between 40 and 80 days in aerobic MBRs. Grelier et al. (2006) reported better sludge filterability and less fouling when the SRT was increased from 8 to 40 days in aerobic MBRs. Meng et al. (2009) reported that there is an optimum SRT for each specific case in MBRs, regarding the differences in substrate composition, reactor configuration and membrane operation. The optimum SRT which provides the best sludge filterability does not necessarily have to be the optimum SRT for biological parameters and system feasibility (Berube et al., 2006). As presented in Chapter 3, reactor R-20 was characterized with the best sludge filterability, but offered the worst substrate conversion efficiency to methane (Chapter 3). Therefore, a compromise between membrane and biological performances must be found. Moreover, it should also be mentioned that sludge filterability can be improved by other methods such as addition of adsorbents and coagulants (Vyrides and Stuckey, 2009; Wu et al., 2009). It was proven that especially powdered activated carbon removes soluble and colloidal foulants in AnMBRs (Trzcinski and Stuckey, 2010). Therefore, it helps to improve attainable flux and reduce membrane fouling.

Interestingly, sludge filterability indicators such as TSS concentration, SRF and CST did not correlate well with the long term membrane filtration resistance observed in R-20 and R-30, whereas most of the parameters correlated well for R-50. The reasons for that may be various. In our study, the membranes were operated below the critical flux and were frequently backwashed. This kind of operation may help to limit cake compaction and thus rapid buildup of filtration resistance. Thus, the link between sludge filterability characteristics and membrane filtration resistance was weak in R-

20 and R-30. Moreover, hydrodynamic conditions for some filterability tests such as SRF may be completely different than real membrane filtration. The cake layer formed under cross flow filtration was reported to have a higher resistance in comparison to dead end filtration, due to the selective deposition of fine particles on the membrane surface (Le-Clech et al., 2006; Delrue et al., 2011). CST correlated well with actual membrane performance in R-50. In several studies, CST was found to be a simple and comprehensive parameter that could represent the overall effect of different parameters to indicate the sludge filterability and membrane fouling (Wang et al., 2006; Wu et al., 2007; Pan et al., 2010). However, there are also contradictory results. For instance, after two years of extensive monitoring and data evaluation, Lyko et al. (2008) could not find a correlation between CST and sludge filterability in a full scale MBR. On the other hand, in a full-scale plant, it could be more difficult to find direct correlations because the membranes are probably operated at a safe flux, whereas the operating parameters are usually fixed. Moreover, it is also difficult to evaluate the influence of the membrane history.

In literature, several attempts have been made to correlate sludge filtration characteristics to membrane filtration resistance and fouling (Khongnakorn et al., 2007; Lyko et al., 2008; Delrue et al., 2011; Liang et al., 2013). Although the relationship between sludge dewaterability parameters and physicochemical characteristics were confirmed by several authors, the link between these parameters and the actual filtration process in MBRs was contradictory. For instance, Lyko et al. (2008) observed that dissolved macromolecular compounds in the sludge supernatant correlated well with CST but their correlation with sludge filterability was weak. Wang et al. (2006) and Pollice et al. (2008) proposed CST as a representative parameter for sludge filterability and membrane fouling. Moreover, Delrue et al. (2011) reported that, although mixed liquor properties had no impact on filtration performance of a specific full scale plant, in another full scale plant they found a significant relationship between them. These results suggest that bulk suspension characteristics do not provide enough information to identify the membrane fouling phenomenon and some complementary parameters should be evaluated. Van den Broeck et al. (2011) stated that no single parameter can represent sludge filterability alone, thus a combination of parameters is more reliable to predict filterability and fouling propensity. Therefore, a set of parameters has to be evaluated to gain deeper insight about membrane filtration

and fouling. They suggested that a combination of sludge morphology, obtained from microscopic image analysis, and relative hydrophobicity allow for a clear classification of activated sludge in terms of filterability. It is also worth to note that some parameters are not totally independent. Moreover, the observed lack of correlations does not necessarily mean that there exists no relationship between these parameters, thus, the relationship may be more complex and non-linear.

It is also worth to underline that the filterability and dewaterability of AnMBR sludge seem to be worse than aerobic MBR sludge (Table 4.7). This may be due to the different floc and EPS structure under aerobic and anaerobic conditions. Moreover, it can also be attributed to different wastewater characteristics and reactor operating conditions such as sludge loading rates, TSS concentrations, temperature and shear rate. Understanding the differences in sludge filterability with the help of standard tools would certainly help to optimize the operating conditions and to mitigate membrane fouling in AnMBRs.

Table 4.7 : Comparison of sludge characteristics between AnMBRs and MBRs.

Reference	Reactor type	Wastewater type	SRT (d)	TSS (g·m ⁻³)	CST (s)	SRF (E ¹⁵ kg·m ⁻¹)	Median floc size (μm)
This study	Cross-flow AnMBR	Corn-based ethanol thin stillage	20	16.5	951	2001	50
This study	Cross-flow AnMBR	Corn-based ethanol thin stillage	30	17.2	1743	3137	41
This study	Cross-flow AnMBR	Corn-based ethanol thin stillage	50	28.3	2414	2963	16
Ersahin et al. (2013)	Submerged anaerobic dynamic MBR	High strength synthetic wastewater	20	14-16	210 ¹	770	45
Ersahin et al. (2013)	Submerged anaerobic dynamic MBR	High strength synthetic wastewater	40	14-16	525 ¹	1410	37
Guglielmi et al. (2010)	Submerged MBR	Pre-screened (2 mm) municipal wastewater	20-35	6.8	15-100	150 ¹	-
Pan et al. (2010)	Submerged MBR	Mainly soluble synthetic wastewater	10	3	16	-	164 ²
Grelot et al. (2009)	Submerged MBR	Pre-screened (1 mm) municipal wastewater	25	9.1	10	1.4	28.2
Pollice et al. (2008)	Submerged MBR	Pre-settled and screened (1 mm) municipal wastewater	20-Infinite	3.7-22.9	6-18	0.04-0.2	-
Lyko et al. (2008)	Submerged MBR	Municipal wastewater	28.6	10-14	20-60	-	-
Wang et al. (2006)	Submerged MBR	Pre-screened (0.9 mm) municipal wastewater	10-40	4.6-22.7	10-100	-	-

¹ Calculated from reported data

² Reported as mean value

4.5 Conclusions

The substrate characteristics and operating conditions had a strong effect on sludge filterability and membrane fouling. LCFA inhibition at high SRTs accelerated the floc breakage and SMP release. On the other hand, the accumulated LCFA on sludge flocs modified their hydrophobicity and fouling propensity. A lesser degree of fouling was observed at higher sludge RH. Apparently, foulants interacted less with the hydrophilic PVDF membrane while the AnMBR was operated below the critical flux. Cake layer formation was the dominant fouling mechanisms in all reactors. A low degree of organic fouling was observed at the end of the study, whereas the inorganic fouling, which was due to precipitation of phosphate salts, resulted in a significant permeability loss during long term operation. When treating corn-to-ethanol thin stillage, it is suggested to perform regular cleaning with acidic solutions in order to prevent permeability loss due to inorganic fouling.

Reactor results showed a good filtration performance and net permeate fluxes between 9 and 13 L·m⁻²·h⁻¹ were obtained, operating the membranes at a cross-flow velocity of 0.5 m·s⁻¹. Cyclic membrane operation with frequent backwash was found to effectively control rapid cake consolidation and fouling. The results showed that SRT is one of the most important parameters that effect sludge filterability. Thus, short SRTs may help to obtain better sludge filterability due to the washout of solutes and fine particles with the discharged sludge. The use of standard methods such as CST and SRF allowed comparing the effect of SRT on sludge filterability. Sludge filterability tended to become worse, when the SRT was increased from 20 to 30 days. In our study, a further increase in SRT to 50 days did not change the sludge filtration characteristics significantly. The sludge physicochemical characteristics such as particle size distribution, TSS and SMP concentrations, correlated well with sludge filterability parameters, i.e., CST and supernatant filterability.

5. EFFECT OF SUBSTRATE ACIDIFICATION DEGREE ⁴

5.1 Introduction

The anaerobic digestion process can be regarded as an intertwined collaboration of different microorganisms with specific functionalities. So far, high rate anaerobic reactors that rely on granulation for biomass retention have been successfully applied for the treatment of various types of wastewaters (van Lier, 2008). Short hydraulic retention times applied in these reactors exert a selection mechanism which promotes microbial aggregation, eventually resulting in anaerobic sludge granules, and the wash out of microorganisms incapable of forming these granules. However, some wastewater types are known as notorious for treatment in granular sludge bed reactors due to the problems with granule formation and biomass retention (Chapter 2). Membranes, as a physical barrier for particulate matter, present an ultimate solution for biomass retention problems in anaerobic reactors. Thus, anaerobic membrane bioreactors (AnMBRs) have received an ever growing interest, especially for the treatment of wastewaters with extreme characteristics. AnMBRs ensure the retention of slow growing anaerobic bacteria inside the reactor and provide excellent effluent quality free of suspended solids. However, membrane fouling, indicated by the reduction in permeate flux or increase in filtration resistance, due to e.g. clogging of the membrane pores, limits the wide spread application of AnMBRs (Liao et al., 2006). Membrane fouling is a very complex process, which depends on multiple factors such as reactor and membrane operating conditions, substrate and sludge characteristics and membrane properties. Although extensive research has been carried out on these parameters, the influence of substrate characteristics has generally been overlooked and rarely investigated (Gao et al., 2013). The substrate characteristics can indirectly influence the biomass filterability, organic and inorganic fouling. Substrate

⁴ This chapter was published as:

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

composition not only alters the dominant microorganisms in the reactors, but also affects the concentration and composition of extracellular polymeric substances (EPS) and soluble microbial products (SMP), which were associated to membrane fouling (Le-Clech et al., 2006). McAdam et al. (2007) showed that the substrate composition had an effect on floc mechanical strength and thus, influenced the sludge filterability in MBRs fed with acetate and ethanol as the sole carbon source. Gao et al. (2013) reported significantly different filtration resistances in MBRs treating different types of industrial wastewater under aerobic and anaerobic operating conditions. They concluded that the colloidal fraction and particle size distribution (PSD) of the incoming wastewater can partially influence the PSD of bulk and cake sludge in MBR systems, thus inevitably affecting filtration performance. Arabi and Nakhla (2008) systematically investigated the effect of the protein to carbohydrate ratio in the feed wastewater on the filtration performance of MBRs. High protein content in the feed stimulated the excretion of both the proteaceous and carbohydrate type SMP. They observed an increased rate of fouling and attributed this to SMP accumulation and occurrence of small-sized flocs at higher protein to carbohydrate ratio in the feed.

Only a few studies reported about the influence of substrate characteristics on the filtration performance and fouling in AnMBRs. In an attempt to investigate the effect of wastewater acidification degree on sludge filterability, Jeison and van Lier (2007a) operated two identical AnMBRs fed with a volatile fatty acids mixture (VFA) and a VFA/glucose mixture. They observed a higher degree of fouling in VFA/glucose AnMBR and attributed this to extensive growth of acidogenic biomass as individual cells on glucose. Therefore, they asserted that the acidification degree of substrate can strongly influence the physical properties of the sludge and determine the applicable flux and fouling rate. Jeison et al. (2009a) further confirmed that the single cells present in the supernatant of an AnMBR treating non-acidified substrate were mainly acidogenic biomass. Thus, they concluded that a small fraction of sludge can determine the rheology, particle size distribution and filtration behavior of the whole sludge. On the other hand, although Torres et al. (2011) reaffirmed that the supernatant fraction of the sludge played an important role in filterability, they could not validate selective enrichment of single cell acidogenic bacteria in the supernatant of AnMBR sludge. Thus, the impact of acidogenic biomass on sludge filterability remains unclear and needs further investigations. Moreover, recent studies showed that the microbial

diversity of the bulk sludge and cake layer depositing on the membrane can be significantly different (Calderon et al., 2011; Lin et al., 2011). This indicates that some species have a higher affinity to attach and colonize on the membrane surface, and may play an important role in membrane fouling.

Based on the studies cited above, this chapter focuses on the influence of acidogenic biomass on sludge filterability. It is aimed to bring a systematic insight to the influence of substrate acidification degree on sludge filtration characteristics by presenting results obtained from anaerobic reactors fed with acidified and non-acidified cheese whey.

5.2 Materials and Methods

5.2.1 Reactor setup and operation

Four lab-scale reactors, each consisting of 2 L effective volume, were operated (Figure 5.1). In order to maintain mesophilic conditions (37 ± 1 °C), warm water was recirculated through double wall water jacket of the reactors. pH was controlled with stand alone pH controllers and dosing pumps for acid or caustic. The reactors were continuously mixed with a stirrer motor. To simulate the shear effect in AnMBRs the sludge was continuously recirculated with a peristaltic pump at a flow rate of $12\text{ L}\cdot\text{h}^{-1}$ (~6 volume turnovers per hour). The biogas was measured with wet tipping gas meters and daily biogas production was recorded online. Methane composition of the biogas was checked daily by washing 10 mL of biogas in a graduated glass column filled with 1 M NaOH solution.

Three reactors (R-2, R-3 and R-4) were operated under methanogenic conditions by controlling the pH between 7 and 8, and one reactor (R-1) was operated under acidogenic conditions at pH 5.5. All reactors, except R-1 were operated as continuous stirred tank reactors (CSTRs). R-1 was operated as a timer controlled sequencing batch reactor (SBR) with 19 h continuous ‘feed & react’, 4 h settle and 1 h decant phases. All reactors except R-1 were inoculated with mechanically disintegrated granular sludge from a full scale expanded granular sludge bed (EGSB) reactor treating lactose based industrial wastewater. R-1 was started up without any seed and acidogenic biomass grew rapidly within a few days. Solids retention time (SRT) was controlled by daily extracting a certain volume of sludge from the reactors.

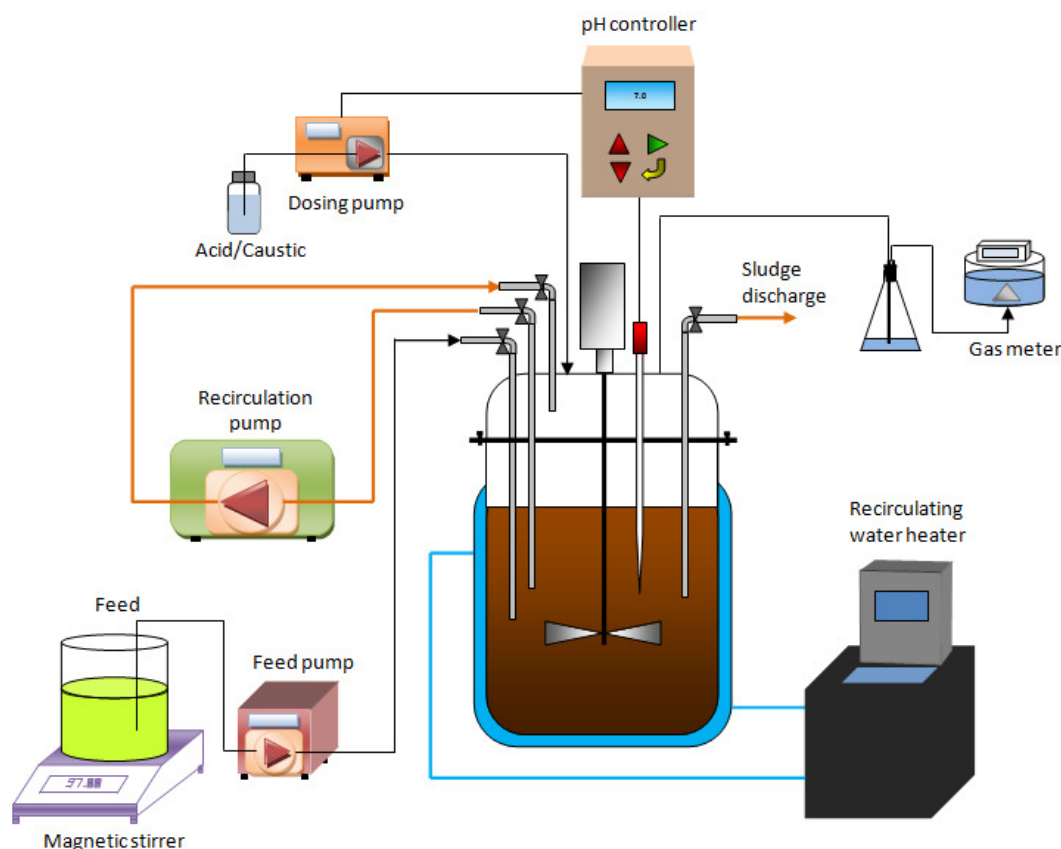


Figure 5.1 : Schematic representation of reactors.

5.2.2 Experimental methods

5.2.2.1 Analytical methods

Analyses were performed on the wasted sludge to check the biological performance of the reactors. Chemical oxygen demand (COD), soluble COD, total phosphorus (TP) and phosphate were measured with Hach-Lange kits. Standards Methods (APHA, 1998) were used for the determination of total solids (TS), total suspended solids (TSS), total Kjeldahl nitrogen (TKN) and ammonium. In order to measure soluble compounds, sludge samples were centrifuged at 17,500 g for 10 minutes and the supernatant was filtered through 0.22 μm disposable filters. A gas chromatograph (GC, Varian 3900) with the specifications given in Chapter 3 was used to measure VFAs. Sludge relative hydrophobicity (RH) was determined according to microbial adhesion to hydrocarbons (MATH) test (Rosenberg et al., 1980) as described in Chapter 4. Each sample was measured in triplicates.

Two different methods were used for the determination of SMP concentrations. SMP was calculated indirectly by subtracting COD equivalent of VFA from soluble COD measured in the reactors (Akram and Stuckey, 2008b; Jeison et al., 2009a). SMP was

also determined by directly measuring proteins and polysaccharides as it is commonly applied in MBR studies (Le-Clech et al., 2006). Protein and polysaccharides were measured according to bicinchoninic acid (Sigma Aldrich BCA) and phenol-sulfuric acid methods (Dubois et al., 1956), respectively. Each sample was measured in duplicates. Sludge activity on different VFAs was measured in sealed serum bottles by monitoring pressure increase with a pressure transducer. The bottles were incubated at 37 °C and the tests were carried out in duplicates.

5.2.2.2 Sludge filterability

Standard filterability analysis such as capillary suction time (CST), specific resistance to filtration (SRF) and supernatant filterability was measured according to the methods described in Chapter 4. For supernatant filterability test, the sludge was centrifuged at 1000 rpm for 10 minutes to obtain supernatant fraction. PSD of the sludge between 0.4 and 2000 μm was determined with laser diffraction analysis (Beckman Coulter LS230). Critical flux tests were performed in a cross-flow AnMBR setup equipped with an inside-out tubular ultrafiltration membrane (Pentair X-Flow). The membrane had a nominal pore size of 0.3 μm and total membrane area was 0.0114 m^2 . Critical flux measurements were performed according to the flux step method (Le-Clech et al., 2003) at two different cross flow velocities of 0.5 and 1.0 $\text{m}\cdot\text{s}^{-1}$. Step height and duration were 2 $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ and 15 minutes, respectively. Weak definition of critical flux was used in data analysis (Le-Clech et al., 2003) and a slope of $\text{dP}/\text{dt} \geq 1 \text{ mbar}\cdot\text{min}^{-1}$ was arbitrarily chosen to decide critical flux was reached.

5.2.3 Substrate characteristics

Concentrated whey permeate obtained from a cheese production plant was used as the main substrate in the study. Whey is known as the remaining part of milk after precipitation and removal of proteins and fats during cheese making process. It is a highly biodegradable (~99%) effluent and the main portion of the COD can be attributed to lactose content inherited from the milk (Siso, 1996). Cheese whey rapidly acidifies at room temperature, thus in order to keep its composition stable it was refrigerated at 4 °C until it was used. Concentrated whey was diluted with tap water to the desired COD concentration and supplemented with urea as additional nitrogen source for biomass growth. R-1 and R-4 was fed with raw whey as substrate (Figure 5.2). The effluent of R-1, basically acidified whey, was directly fed to R-2. Therefore,

the substrate of R-2 was mainly a VFA mixture and the acidifiers from R-1. A portion of R-1 effluent was centrifuged and filtered in order to remove the acidogenic biomass. The filtrate without any suspended solids was fed to R-3. The operation of R-2 and R-3 was divided in to two stages. In the second stage of the study (after 78th day), higher organic loads were applied to the reactors by increasing the feed concentration. R-2 feed was concentrated by adding the pellets of centrifuged R-1 effluent. Consequently, this led to an increase in TSS and acidogenic biomass concentration fed to R-2. Similarly, the feed of R-3 was boosted with additional VFA, mainly acetate and butyrate. Table 5.1 presents the substrate composition fed to each reactor.

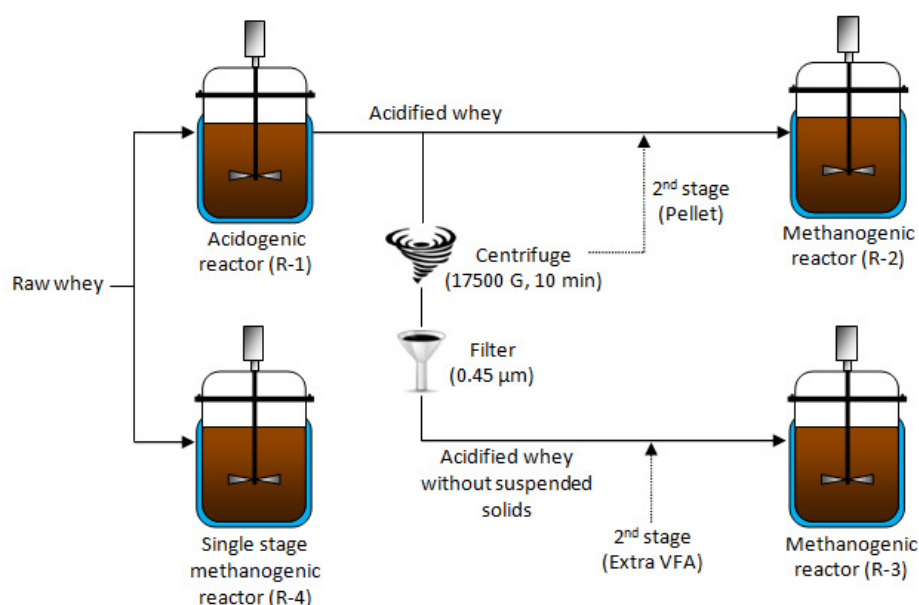


Figure 5.2 : Substrate fed to each reactor.

Table 5.1 : Substrate composition fed to the reactors (mean $\pm$ standard deviation).

Parameter	Unit	R-1	R-2		R-3		R-4
			1 st stage	2 nd stage	1 st stage	2 nd stage	
Total COD	$\text{g}\cdot\text{L}^{-1}$	62.8 ± 4.7	57.2 ± 3.1	70.5 ± 7.2	55.1 ± 2.9	96.3 ± 3.2	96.0 ± 0.8
Soluble COD	$\text{g}\cdot\text{L}^{-1}$	62.5 ± 4.5	55.1 ± 2.9	57.0	55.1 ± 2.9	96.3 ± 3.2	95.4 ± 1.2
Particulate COD	$\text{g}\cdot\text{L}^{-1}$	0.3 ± 0.2	2.5 ± 0.7	13.5 ± 7.2	n.a. ^b	n.a.	0.6 ± 0.4
COD _{VFA}	$\text{g}\cdot\text{L}^{-1}$	0.6 ± 0.1	34.2 ± 3.1	30.0	34.0 ± 3.1	78.2 ± 0.7	1.0 ± 0.8
TSS	$\text{g}\cdot\text{L}^{-1}$	1.3 ± 0.2	3.3 ± 1.2	11.0 ± 5.7	n.a.	n.a.	n.m.
VSS	$\text{g}\cdot\text{L}^{-1}$	1.2 ± 0.3	3.2 ± 1.2	10.9 ± 5.7	n.a.	n.a.	n.m.
TKN	$\text{mg}\cdot\text{L}^{-1}$	620 ± 30	n.m. ^a	n.m.	n.m.	n.m.	685 ± 31
NH ₄ ⁺ -N	$\text{mg}\cdot\text{L}^{-1}$	65 ± 40	n.m.	n.m.	n.m.	n.m.	n.m.
TP	$\text{mg}\cdot\text{L}^{-1}$	330 ± 45	n.m.	n.m.	n.m.	n.m.	600 ± 90
PO ₄ ³⁻ -P	$\text{mg}\cdot\text{L}^{-1}$	250 ± 105	n.m.	n.m.	n.m.	n.m.	420 ± 30

^a Not monitored.

^b Not available.

5.3 Results and discussion

5.3.1 Biological performance

Table 5.2 summarizes operating conditions and biological performance of the reactors. R-1, used as a pre-fermenter, achieved a high acidification performance and around 67% of the effluent COD was VFAs. Butyrate was the most dominant VFA (85%), while propionate and acetate concentrations were quite low. This indicates that lactose in the substrate was converted mostly to butyrate under the operating conditions of R-1. Similar results were also reported in several studies about the fermentation of lactose (Yu and Pinder, 1993; Yang et al., 2003). In general, all methanogenic reactors achieved high COD removal efficiencies. As a result of the increased organic load at the 2nd stage of the study, the F:M ratio in R-2 and R-3 also increased. The increase in organic loading rate (OLR) did not cause any stability problems and the VFA concentrations remained low after an initial peak.

Table 5.2 : Operating conditions and biological performance (mean $\pm$ standard deviation).

Parameter	Unit	R-1	R-2		R-3		R-4
			1 st stage	2 nd stage	1 st stage	2 nd stage	
HRT	d	2.2 $\pm$ 0.4	29 $\pm$ 3		27 $\pm$ 4		31 $\pm$ 3
OLR	kg COD·m ⁻³ ·d ⁻¹	30.8 $\pm$ 4.9	1.9 $\pm$ 0.2	2.4 $\pm$ 0.3	2.1 $\pm$ 0.2	3.7 $\pm$ 0.6	3.1 $\pm$ 0.3
F:M	kg COD·g ⁻¹ VSS·d ⁻¹	5.7 $\pm$ 1.4	0.32 $\pm$ 0.06	0.57 $\pm$ 0.07	0.40 $\pm$ 0.09	0.91 $\pm$ 0.15	0.37 $\pm$ 0.02
TSS	g·L ⁻¹	6.3 $\pm$ 0.9	6.1 $\pm$ 0.8	5.4 $\pm$ 1.0	5.4 $\pm$ 0.8	5.3 $\pm$ 0.6	10 $\pm$ 0.5
VSS	g·L ⁻¹	6.1 $\pm$ 0.8	5.3 $\pm$ 0.9	4.4 $\pm$ 0.5	5.0 $\pm$ 0.6	4.1 $\pm$ 0.4	8.5 $\pm$ 0.3
pH	-	5.5 $\pm$ 0.1	7.5 $\pm$ 0.1	7.6 $\pm$ 0.1	7.0 $\pm$ 0.1		
Sludge yield	g VSS·g ⁻¹ COD _{removed}	0.26 $\pm$ 0.05	0.06 $\pm$ 0.01		0.04 $\pm$ 0.01		0.08 $\pm$ 0.02
Specific CH ₄ production ¹	L·g ⁻¹ COD _{removed}	-	0.31 $\pm$ 0.04		0.29 $\pm$ 0.4		n.m. ²
COD removal efficiency	%	17 $\pm$ 3	88 $\pm$ 3		90 $\pm$ 4		86 $\pm$ 2
Effluent COD	mg·L ⁻¹	52400 $\pm$ 3000	7.3 $\pm$ 2.0		6.5 $\pm$ 0.9		13.0 $\pm$ 1.6
Effluent COD _{VFA}	mg·L ⁻¹	35000 $\pm$ 5000	31 $\pm$ 30		50 $\pm$ 47		14 $\pm$ 10

¹ At standard temperature and pressure (0 °C, 1 atm).

² Not measured.

Acidogenic bacteria were reported to have a high growth rate and biomass yield compared to methanogens (Batstone et al., 2002). Therefore, a much higher sludge yield was observed in R-1 compared to the methanogenic reactors. As expected, the sludge yield of R-4 was the highest among the methanogenic reactors due to the proliferation of acidogens in this reactor, which was fed with raw whey. Activity measurements on different VFAs indicated that no acetotrophic methanogenic activity

was present in R-1 sludge. Test results clearly showed that a microbial culture composed of mainly acidogens was developed in R-1, which was started up without any inoculum. Table 5.3 presents the results of the activity tests on the sludge and the inoculum of the methanogenic reactors. It is clear that propionate oxidizers could not manifest in R-2 and R-3, likely due to the low concentrations of this VFA in the pre-fermented whey. Supplementation of extra butyrate to the feed of R-3 promoted the enrichment of butyrate oxidizers at the 2nd stage of the study. Addition of more acidogenic biomass in to R-2 feed induced a decrease in the specific methanogenic activity (SMA) on acetate and butyrate. Moreover, the extensive growth of acidogenic biomass in R-4, which was operated as a single stage methanogenic reactor fed with non-acidified substrate, resulted in a low acetotrophic methanogenic activity. Jeison et al. (2009a) stated that the growth of acidogenic bacteria “dilutes” methanogenic and acetogenic microorganisms in a mixed culture and this may lead to a decrease in SMA. On the other hand, the SMAs measured in R-2 were much higher compared to R-4. This striking difference was unexpected since in principle both R-2 and R-4 sludge was a mixture of acidogens and methanogens, with the only difference that the trophic groups have been cultivated separately in R-2 and simultaneously in R-4. However, applying a two-stage and a single stage reactor configuration may cause a difference in the growth conditions of acidogens. Thus, consumption of rapidly fermentable substrate, i.e. lactose, in the first stage R-1 reactor may cause substrate limitation for acidogens in R-2. This may restrict the growth of acidogens, even leading to net decay. Thus, the fraction of acidogenic biomass in mixed sludge may be different in R-2 and R-4 reactors.

Table 5.3 : Biomass activity in g CH₄-COD·g VSS⁻¹·d⁻¹ on different VFAs.

VFA	Methanogenic inoculum	R-2		R-3		R-4
		1 st stage	2 nd stage	1 st stage	2 nd stage	
Acetate	0.67	0.59	0.40	0.46	0.47	0.27
Propionate	0.20	0.24	0.09	0.27	0.09	0.17
Butyrate	0.36	0.71	0.47	0.56	0.74	0.14

Whey permeate contains mainly lactose, a disaccharide, since proteins and fats are mostly removed during cheese production. *Clostridia* species, such as *Clostridium butyricum*, *Clostridium propionicum* and *Clostridium sticklandii*, are known as the most common acidogens present in anaerobic reactors treating whey and dairy wastewaters (Kim et al., 2011). These bacteria degrade lactose to various intermediates

such as lactate, ethanol, propionate, butyrate, acetate and hydrogen, depending on the operating conditions such as SRT, pH and temperature. The distribution of metabolites depends mainly on the thermodynamics of metabolic pathways. Thus, environmental conditions such as hydrogen partial pressure and pH are very important on the shifts of intermediates. It is reported that high hydrogen partial pressure favors the butyrate and acetate production from lactose, whereas in case of low hydrogen concentration, such as methanogenic conditions, propionate pathway becomes more dominant (Yu and Pinder, 1993). Therefore, separating the acidogens from methanogens may result in alterations in the intermediary metabolite routes and thus, the dominant species. We observed that the substrate was mainly converted to butyrate in R-1 operated at a pH of 5.5. On the other hand, non-acidified whey was directly fed to R-4 operated under methanogenic conditions. Therefore, the dominant acidogenic species in R-1, and consequently in R-2, may distinctly differ from those in the methanogenic reactor R-4, due to different operating conditions such as SRT, pH and hydrogen partial pressure. This may also influence the fouling propensity of acidogens in single stage and two-stage anaerobic reactors.

5.3.2 Sludge characteristics

5.3.2.1 Particle size distribution

PSD of the sludges from each reactor at the end of operation are presented in Figure 5.3 and Table 5.4. Interestingly, acidogenic sludge from R-1 and methanogenic sludge from R-3 showed bimodal size distribution whereas, R-2 and R-4 reactors in which the acidogenic biomass was present together with methanogens showed a similar unimodal PSD. Acidogenic sludge of R-1 had the smallest median particle size but it also contained some large flocs indicated by the wide particle size range. The SBR regime of R-1 may present a selection mechanism for the biomass that forms flocs and settles. Thus, it may have an influence on the overall PSD of R-1 sludge. R-3, receiving mainly acidified whey, had a significantly different PSD and a larger mean particle size compared to R-2 and R-4. These results confirm that the presence of acidogenic biomass has an impact on the sludge PSD. Similarly, Jeison and van Lier (2007a) reported a decrease in sludge particle size when the AnMBRs were fed with glucose as a non-acidified substrate.

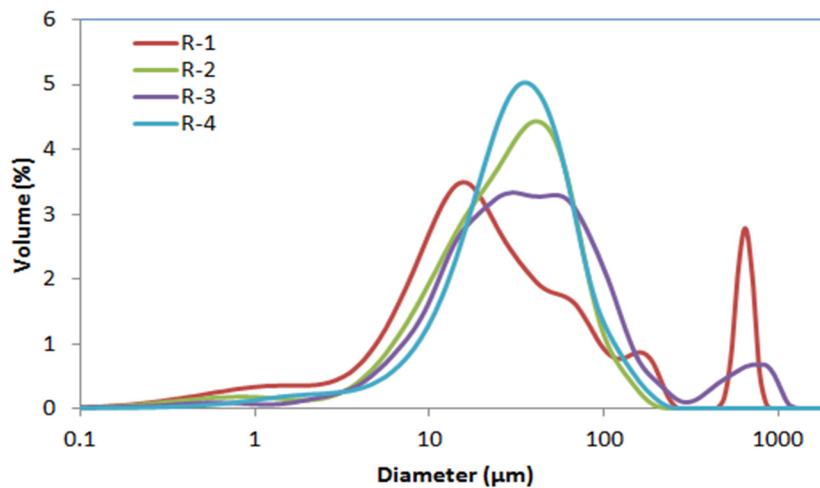


Figure 5.3 : Volume based particle size distribution of the sludges.

Table 5.4 : Granulometry of the sludge from each reactor.

Reactor	Mean (μm)	d10 (μm) ¹	Median – d50 (μm) ²	d90 (μm) ³
Methanogenic inoculum	362	18	202	958
R-1	91	4	21	198
R-2	37	7	30	76
R-3	88	9	37	144
R-4	41	10	33	81

¹ Size that 10% of particles are smaller

² Size that 50% of particles are smaller

³ Size that 90% of particles are smaller

Jeison et al. (2009a) fractionated the AnMBR sludge fed with glucose/VFA mixture with a centrifuge, and reported the median particle size of the supernatant, pellet and bulk sludge as 1, 20 and 10 μm, respectively. According to FISH analysis and specific acidogenic activity tests, they inferred that the supernatant mainly contains acidogenic biomass. In our study, it is noted that the median particle size of sludge in R-1 (21 μm) was similar to that of pellet with a good filterability in Jeison et al.'s (2009a) study. This may be a result of SBR regime applied in R-1 reactor, which inevitably removes the non-settling fine particles with the effluent. On the other hand, the median particle size of R-3, receiving acidified substrate without acidogens, was higher compared to the others. This may be due to methanogenic conditions that promote floc formation instead of dispersed growth to enable interspecies hydrogen transfer between acetogens and methanoges (Thiele et al., 1988). Moreover, considering the low growth yield in reactor R-3, it is postulated that the large flocs present in R-3 may also derive from the original seed sludge.

5.3.2.2 Soluble microbial products

The concentrations of SMP were regularly monitored in reactors R-2 and R-3 (Figure 5.4). According to the unified EPS theory proposed by Laspidou and Rittman (2002), SMP can be divided into two categories, i.e. metabolically associated products produced during substrate consumption, and biomass associated products, generated during biomass decay. A gradual increase in SMP concentrations was observed in both reactors at the 2nd stage of operation. However, the reason for SMP accumulation may be different in each reactor. The organic load of R-2 was increased by supplementing more acidogenic biomass harvested from R-1 to the feed. Thus, it can be expected that a significant fraction of acidogenic biomass will undergo bacterial decay releasing of decay products. In contrast, the organic load of R-3 was increased by adding extra butyrate and acetate to the acidified whey. This would have stimulated the growth of butyrate utilizing acetogens and acetotrophic methanogens and the release of growth associated products. In both cases, an increase in the F:M ratio would have induced the release of SMP to the bulk liquid.

5.3.2.3 Relative hydrophobicity

The relative hydrophobicity of the sludge from R-1, R-2, R-3 and R4 reactors was 3, 22, 57 and 32% respectively. The acidogenic sludge was found to be highly hydrophilic whereas the sludge receiving mainly acidified substrate demonstrated a high hydrophobicity. The sludge from R-2 and R-4 was moderately hydrophilic. Daffonchio et al. (1995) stated that the hydrophobicity of a sludge mixture was determined by mixing ratio of hydrophilic and hydrophobic cells. They reported that most acidogens are hydrophilic but most of the acetogens and methanogens are hydrophobic. Moreover, they showed that long term enrichment of sludge with sugars selected for hydrophilic acidogens.

Hydrophobic interaction between the bacterial cells and miscellaneous interfaces is one of the most important factors which govern the mechanism of adherence (van Loosdrecht et al., 1987). Analogously, in membrane processes, hydrophobic interactions between bacterial flocs, solutes and membrane surface play an important role in membrane fouling. Recent studies showed that the microbial species distribution of cake layer and bulk sludge differs, thus suggesting that some bacteria have a higher tendency to adhere and colonize on the membrane surface (Calderon et

al., 2011; Lin et al., 2011). Hydrophilic membranes are generally preferred in MBR applications since they are less prone to organic fouling (Le-Clech et al., 2006). Our results showed that acidogens increased the hydrophilicity of the anaerobic sludge. Therefore, this may increase membrane fouling propensity. Several studies reported that membrane fouling decreased with increased sludge hydrophobicity (van den Broeck et al., 2011; Qin et al., 2012). They attributed this to less interaction of hydrophobic sludge flocs with typically hydrophilic membrane. Therefore, the high hydrophilicity of acidogens will likely result in increased adherence to the hydrophilic membranes.

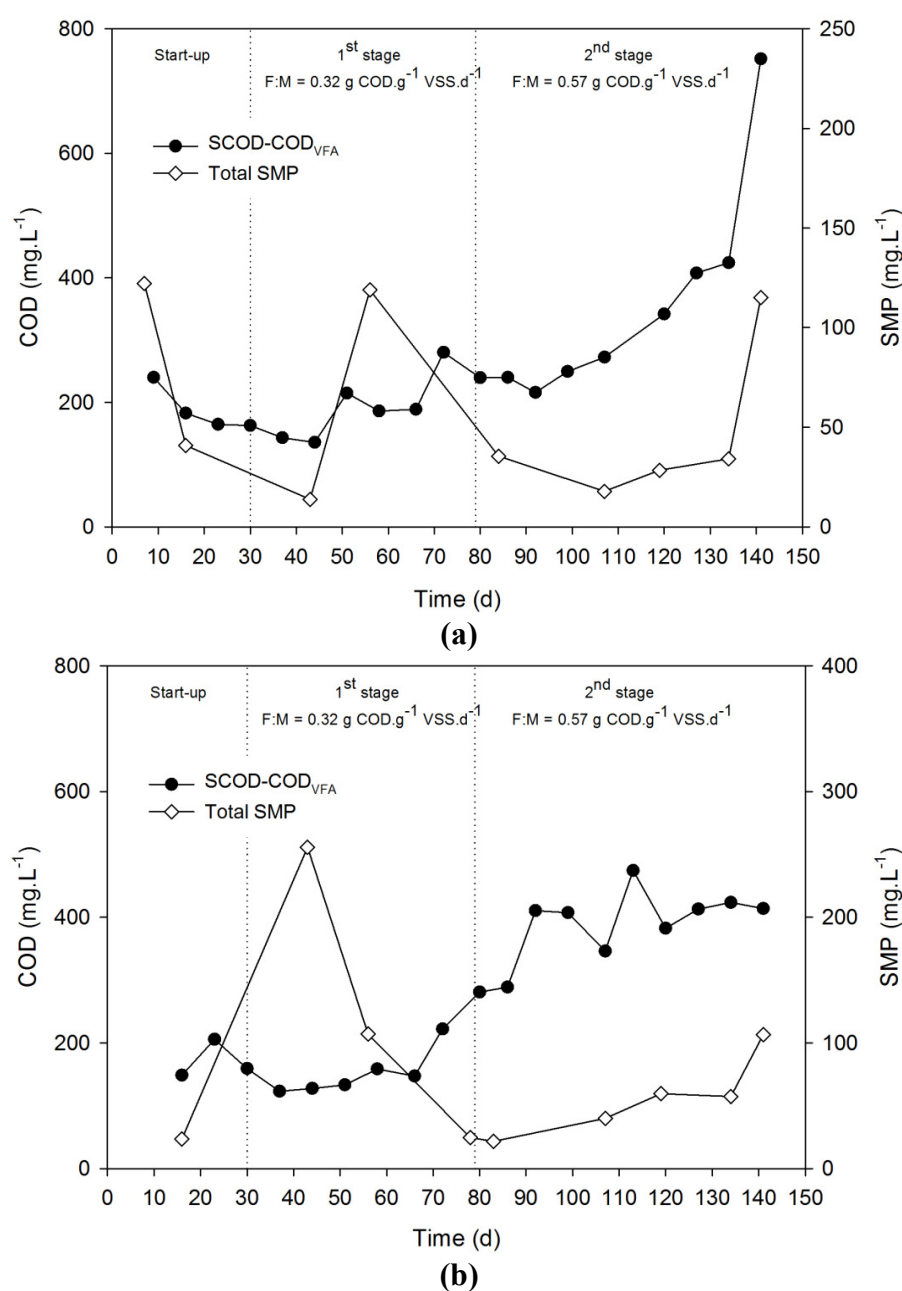


Figure 5.4 : Evolution of SMP in reactors (a: R-2, b: R-3).

5.3.3 Sludge filterability

5.3.3.1 CST and SRF

CST and SRF parameters are often used as indicators for sludge filterability in MBR studies (e.g. Pollice et al., 2008; Chapter 4). Both parameters showed significant positive correlation, based on a Pearson Correlation test ($p < 0.05$), in all reactors except R-1 (Figure 5.5). The lack of correlation in R-1 can be attributed to limited amount of data pairs. Interestingly, at stable operation the average SRF of acidogenic sludge in R-1 ($480 \text{ m} \cdot \text{kg}^{-1}$) was slightly lower than that of R-2 ($600 \text{ m} \cdot \text{kg}^{-1}$). It is clear from the results that, the filterability of R-3 in the first stage of the study was significantly better than all reactors. The filterability of R-4 rapidly deteriorated in a very short time. This may be due to the fast growth of acidogenic biomass in R-4.

SRF and CST showed a similar increasing trend in both reactors R-2 and R-3 at the 2nd stage of the study. It seems that an increase in acidogenic biomass in the feed of R-2 stimulated the increase in SRF and CST. Since the growth of acidogenic biomass was restricted due to limited availability of rapidly fermentable substrate in R-2, the reason for worsening sludge filterability may be the release of bacterial decay products of acidogens. The increase in OLR and F:M ratio also caused a gradual decrease in sludge filterability of R-3, receiving acidified substrate consisting of mainly butyrate and acetate. This may be due to the increased secretion of growth related SMP, which are often related to poor filterability. It was reported that the microbial products produced at the different physiological states of the biomass, such as exponential growth or decay, may result in different characteristics and fouling propensity (Chang and Lee, 1998). These results clearly show that not only the presence of acidogens but also the F:M ratio play a very important role on filterability.

In order to further elucidate the effect of acidogens on the SRF parameter, R-1 sludge was mixed with sludge from R-2 and R-3 at a 1:1 volume ratio and tests were carried out. The average SRF of R-2 and R-3 was calculated as 450 ± 110 and $250 \pm 60 \text{ m} \cdot \text{kg}^{-1}$, respectively. When R-1 sludge was mixed with R-2 and R-3 sludge, the SRF of the mixtures remarkably increased to 840 ± 80 and $820 \pm 150 \text{ m} \cdot \text{kg}^{-1}$, respectively. This result clearly demonstrated that the presence of acidogens significantly increased the SRF. The SRF parameter strongly depends on the particle size (Endo and Alonso, 2001). Thus, the small particle size of acidogenic sludge may increase the SRF values.

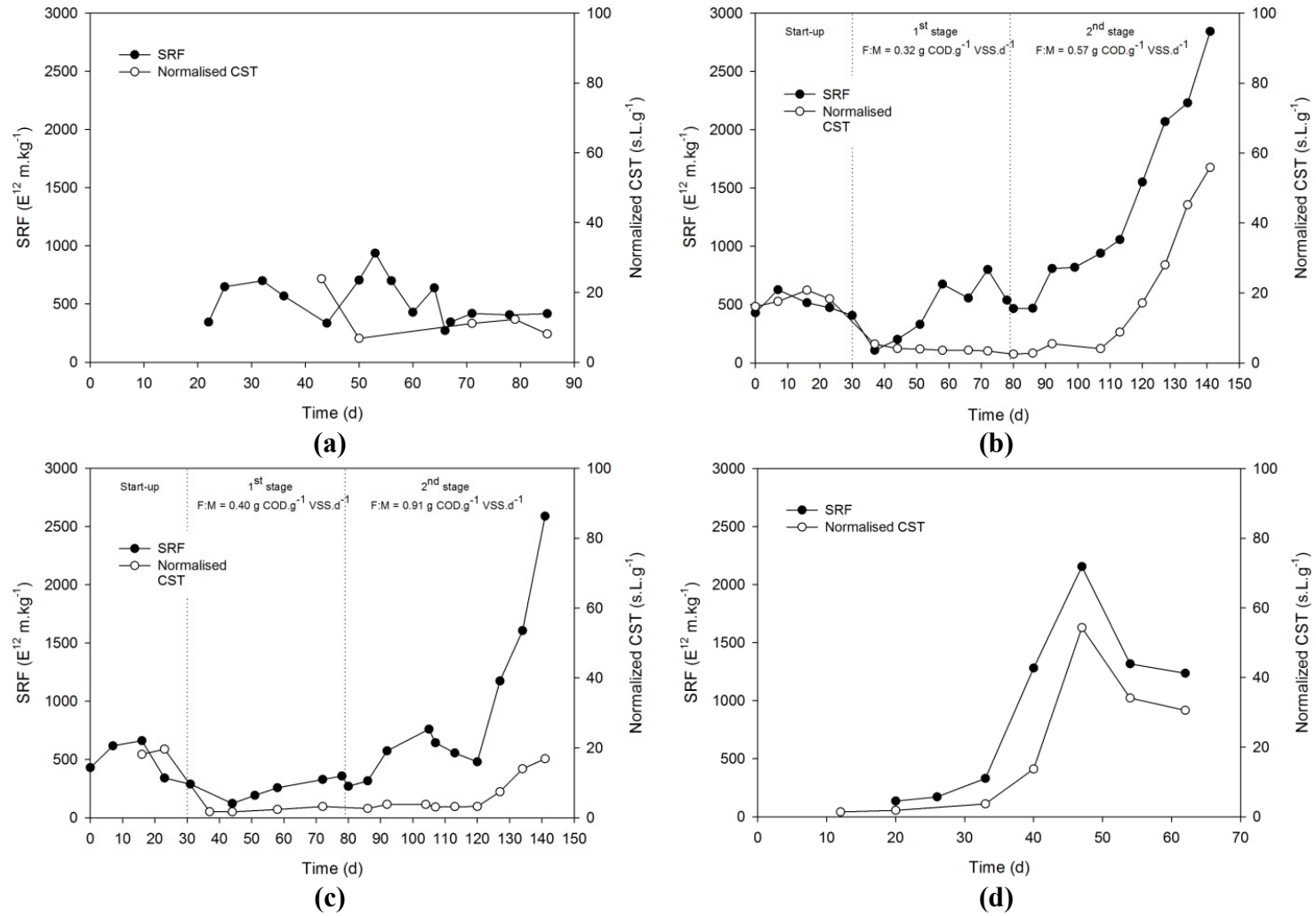


Figure 5.5 : The evolution of SRF and CST in reactors R-1 (a), R-2 (b), R-3 (c), and R-4 (d).

5.3.3.2 Supernatant filterability

Jeison et al. (2009a) reported that the supernatant fraction of the sludge determined the filtration behavior of the whole sludge. They attributed this to the enrichment of acidogens, growing as single cells, in the supernatant. It must be noted that not only the fine particles but also solutes, such as macromolecules and SMP, may play a significant role on the filterability of the supernatant fraction in MBRs (Le-Clech et al., 2006). Our results showed that the supernatant filterability of R-1 ($0.37 \pm 0.11 \text{ mL} \cdot \text{min}^{-1}$) was as high as the other reactors. The filterability of R-2 and R-3 in the 1st stage was measured as 0.3 ± 0.01 and $0.28 \pm 0.02 \text{ mL} \cdot \text{min}^{-1}$, respectively. However, their filterability respectively decreased to 0.16 ± 0.01 and $0.20 \pm 0.01 \text{ mL} \cdot \text{min}^{-1}$, when the F:M ratio was increased. This result correlates well with the increasing SMP concentrations in the 2nd stage of operation. Moreover, when the filterability of R-2 in the 1st stage and R-4 ($0.21 \pm 0.02 \text{ mL} \cdot \text{min}^{-1}$) were compared at similar F:M ratios, it was clear that R-4 exhibited a poorer filterability. The supernatant filterability of R-3 at the 1st stage of operation was also significantly higher than R-4.

Although Jeison et al. (2009a) attributed the poor filterability to the presence of acidogens in the supernatant, Torres et al. (2011) could not find any difference between the acidogenic activity of sludge supernatant and pellet. This may be a result of different G forces applied for fractionating the sludge in these studies. According to our results, although acidogenic sludge seemed to be involved in poor sludge filterability, the F:M ratio was another important parameter in filterability. SMP accumulation was stimulated at increased F:M ratios in both R-2 and R-3 reactors, which led to a dramatic decrease in supernatant filterability. Liu et al. (2012b) systematically evaluated the impact of F:M ratio on membrane fouling in AnMBRs. They concluded that F:M ratio greatly influenced the concentration and properties of the SMP and EPS, as well as the PSD of the bulk sludge. They observed a higher degree of membrane fouling at elevated F:M ratios.

5.3.3.3 Critical flux

The results of the critical flux tests of different sludges, performed at 0.5 and $1 \text{ m} \cdot \text{s}^{-1}$ cross flow velocities, are presented in Table 5.5. Expectedly, increasing the cross-flow velocity resulted in higher critical fluxes for all sludges due to improved shear force over the membrane surface. Interestingly, the critical flux of R-1 sludge was as high

as the sludge from other reactors. Obviously, increasing the F:M ratio in R-2 and R-3 led to a poorer filterability compared to the 1st stage of operation. This result was in accordance with the other filterability indicators, such as SRF and CST, and further confirmed that F:M ratio was one of the most important parameters for MBR filtration performance. Our present results suggest that short term filterability of acidogenic sludge alone (R-1) was not remarkably different than methanogenic sludge (R-3) at 1st stage of operation. Moreover, the critical flux of R-2 and R-4 at similar F:M ratio was also alike. However, when the amount of acidogenic sludge fed to R-2 was increased at the 2nd stage of the study, the CF of R-2 substantially decreased compared to R-4 where substrate acidification and methanogenesis took place in the same vessel.

Table 5.5 : Critical flux ($L \cdot m^{-2} \cdot h^{-1}$) of different sludges.

Cross-flow velocity ($m \cdot s^{-1}$)	Methanogenic inoculum	R-1	R-2		R-3		R-4
			1 st stage	2 nd stage	1 st stage	2 nd stage	
0.5	17	15	17	11	17	11	16
1.0	36	30	24	18	26	21	20

Interestingly, critical flux of acidogenic sludge in R-1 was as high as the others, but this may be a consequence of SBR regime as discussed above. The short term critical filtration tests showed that the presence of acidogens did not significantly affect the critical flux. This seems contradictory to what was reported in literature (Jeison et al., 2009a; Torres et al., 2011). However, it should be kept in mind that in our study the critical flux test were applied to whole sludge whereas in other studies the sludge was fractioned in to supernatant and pellet. The fractionation procedure inevitably selects for fine particles, which may be acidogens, to be in the supernatant fraction. Thus, it is reasonable that the supernatant had a lower critical flux compared to the pellet. While in a poly-dispersed solution such as mixed sludge, particle to particle interactions may play a role on the deposition and formation of cake layer on the membrane effecting the attainable flux. Therefore, the effect of acidogens on long term cake layer compaction and membrane fouling should be further investigated in continuous filtration tests.

5.3.4 Single stage versus two-stage AnMBRs

There are only a few studies discussing the performance of two staged AnMBRs (Saddoud et al., 2007; Mota et al., 2013). Although these studies report excellent biological performance, unfortunately, they do not focus on the systematic differences

between the filtration performance and membrane fouling in single stage and two-stage AnMBRs. Our results suggest that, from a filtration point of view, it seems beneficial to acidify the wastewater in a pre-fermenter and then feed it to an AnMBR. This may limit the extensive growth of acidogens in the subsequent staged AnMBR and improve the sludge filterability. Moreover, complete separation of acidogens cultivated in the first stage by a membrane unit may be another option. It must be noted that reactor R-3, receiving acidified substrate without acidogens, offered the best sludge filterability. On the other hand, other parameters, such as F:M and SMP, besides the presence of acidogens were also found important parameters influencing sludge filterability. Moreover, it should also be kept in mind that the real fouling behavior in AnMBRs may be completely different due to membrane properties and operating conditions.

5.4 Conclusions

It can be concluded that substrate acidification degree indirectly affects sludge filterability. Rapidly fermentable substrate promoted proliferation of acidogens and this decreased median particle size and hydrophobicity of the sludge. Filterability of R-3, receiving acidified substrate, was superior compared to the others in terms of CST, SRF and supernatant filterability. High F:M ratios stimulated the release of SMP which led to a gradual decrease in filterability of sludge. Interestingly, we could not validate the adverse effect of acidogens on short term critical flux, but their effect on long term cake compaction and fouling development should be further investigated.

6. EFFECT OF NITROGEN DEFICIENCY ⁵

6.1 Introduction

Cheese whey, a by-product remaining after the precipitation and removal of milk casein and fats during the cheese making process, is either considered a resource of interest or a concentrated wastewater following the different points of view. Whey basically represents 85-95% of the milk volume and typically contains lactose (4.5-5% w/v), soluble proteins (0.6-0.8% w/v), lipids (0.4-0.5% w/v), lactic acid (0.05% w/v) and mineral salts (8–10% of dry matter) (Siso, 1996). Although it is possible to recover many alternative products from whey, such as i) condensed or powdered whey, ii) whey protein concentrate, iii) lactose and its derivatives, many small to medium scale industries do not have the technical know-how or economic power to apply these valorization technologies. Thus, this makes it necessary to consider an efficient way for the treatment of whey as a wastewater stream (Malaspina et al., 1996; Mockaitis et al., 2006).

When no valorization is possible, cheese whey is considered a very concentrated wastewater characterized by a high chemical and biological oxygen demand (COD and BOD), low pH and alkalinity. The whey wastewater is highly biodegradable (~99%) and the main portion of the COD can be attributed to the lactose content coming from the milk (Siso, 1996; Malaspina et al., 1996). Anaerobic digestion is considered the state of the art technology for the treatment of high strength industrial wastewaters such as whey. However, due to its high organic matter content cheese whey may impair biomass granulation in high rate anaerobic reactors and may cause process instabilities (Malaspina et al., 1996; Mockaitis et al., 2006). Methanogenesis seems to be the rate limiting step for the anaerobic treatment of cheese whey, since the whey tends to

⁵ This chapter was submitted to “Water Research” journal for peer review:

Dereli, R.K., Wang, X., van der Zee, F.P., van Lier, J.B. (2015) Biological Performance and Sludge Filterability of Anaerobic Membrane Bioreactors Under Nitrogen Limited Conditions.

rapidly acidify due to its very high biodegradability and low alkalinity (Ghaly, 1996; Mockaitis et al., 2006).

Anaerobic membrane bioreactors (AnMBRs) have become very popular in the last decade for the treatment of specific waste streams due to their many advantages over conventional high rate anaerobic reactors (Chapter 2). They provide complete sludge retention, very high COD removal efficiency and excellent effluent quality, free of suspended solids. Despite these advantages, fouling, the common problem that plagues membrane processes, is the most important drawback of AnMBRs which limit their wide-spread implementation. Membrane fouling can be defined as clogging of membrane pores and/or the formation of a dense cake layer on the membrane surface, due to the accumulation of organic and inorganic foulants, resulting in a subsequent flux decrease. Fouling is a very complex phenomenon, and its extent of manifestation depends on many parameters, such as substrate characteristics, mixed liquor properties, bioreactor design and operating conditions, membrane properties and operation (Meng et al., 2009). Most of the time, it is difficult to identify a single parameter that determines the degree of fouling, because all these parameters are interrelated to each other.

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

through ultrafiltration (Gillies, 1974). Thus, anaerobic treatment would logically require supplementation of nitrogen as a nutrient (Yilmazer and Yenigun, 1999). Although the biomass yield of anaerobic sludge is very low, it is reported that for carbohydrate type readily biodegradable substrates, i.e. whey; the yield can reach up to $0.2 \text{ g VSS} \cdot \text{g}^{-1} \text{ COD}_{\text{removed}}$ (Kalyuzhnyi and Davlyatshina, 1997; Fernandez et al. 2011). This is mainly due to rapid growth of acidogenic microorganisms that are characterized by a higher biomass yield compared to methanogens. Jeison and van Lier (2007a) reported that acidogenic biomass, abundantly growing as single cells, determines the sludge filterability and plays an important role in membrane fouling in AnMBRs.

Nutrient stress can be used to limit the growth of microorganisms and shift their metabolic pathways to produce desired end products. For instance, nitrogen starvation was proven as a useful strategy in enhanced biological phosphorus removal, bioplastic production, hydrogen production through dark fermentation and microalgae cultivation for biodiesel production processes (Rawat et al., 2013; Markou and Nerantzis, 2013; Argun et al., 2008). Nitrogen limitation stimulates polyhydroxyalkanoates (PHA) storage of bacteria and lipid accumulation of microalgae in these processes. Nitrogen deficit may induce metabolic changes on microorganisms and limit biomass synthesis. This may change sludge production and characteristics, product formation and species distribution which may have consequences on not only treatment efficiency and reactor stability but also on sludge filterability and fouling propensity in AnMBRs. Possibly, imposed nitrogen limitation may be used to suppress the abundant growth of (single cell) acidifying organisms, thus minimizing fouling and enhancing the membrane flux. However, too low N concentrations, may adversely affect the overall digestion process.

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

6.2 Materials and Methods

6.2.1 Reactor setup and operation

Two lab-scale cross flow AnMBRs with 10 L effective volume were operated under mesophilic conditions. Reactors were equipped with tubular ultrafiltration membranes (Pentair X-Flow) with a pore size of 0.03 μm . Membrane surface area was 0.014 m^2 . A cross-flow velocity of 0.5 $\text{m}\cdot\text{s}^{-1}$ was imposed with a peristaltic pump (Watson Marlow 530U) and the permeate suction and backwash was conducted with a small sized peristaltic pump (Watson Marlow, 120U). A detailed schematic presentation of the reactors can be found in Chapter 3. Daily biogas production, TMP and pH data were registered online. The pH of the reactors was controlled with a stand-alone controller (HACH LANGE SC-1000) and a dosing pump (KNF Stepdos O8 RC) for caustic addition. The reactors were named R-1 (N limited) and R-2 (N supplemented) related to the nitrogen addition to the substrate. R-1 and R-2 were operated for 155 and 170 days, respectively. Both reactors had more than sufficient phosphorus for biomass growth.

6.2.2 Experimental methods

6.2.2.1 Analytical methods

Chemical oxygen demand (COD) and total phosphorus (TP) concentrations were determined with Hach-Lange Kits. Total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total kjeldahl nitrogen (TKN) and ammonium nitrogen were measured according to Standard Methods (APHA, 1998). Soluble parameters were measured after centrifuging the sludge at 17500 g for 10 minutes and subsequently filtering the supernatant with 0.45 μm disposable filters. Volatile fatty acids (VFAs) were determined with a gas chromatograph with the specifications given in Chapter 3. Extracellular polymeric compounds (EPS) and soluble microbial products (SMP) were extracted and measured according to the methods described in Chapter 4. Each sample was measured in duplicates.

6.2.2.2 Substrate characteristics

Whey permeate obtained from a cheese production plant was used as substrate in the study. As a result of precipitation and removal of proteins during cheese production, the nitrogen content of whey was quite low. In order to test the effect of nitrogen on sludge filterability, nitrogen was supplemented with the addition of urea to the feed of R-2. The COD:TKN ratio of the substrates fed to R-1 and R-2 was 190 and 50, respectively. The detailed compositions of the substrates fed to each reactor are given in Table 6.1. The feed was almost a completely soluble and rapidly fermentable substrate. It had high polysaccharide content originating mainly from lactose present in the milk.

Table 6.1 : Feed characterization of R-1 and R-2 (mean±standard deviation).

Parameters	Unit	R-1	R-2
TCOD	g·L ⁻¹	26.1±1.3	29.2±3.3
SCOD	g·L ⁻¹	25.4±0.9	28.9±3.3
TKN	mg·L ⁻¹	136±14	600±135
NH ₄ ⁺ -N	mg·L ⁻¹	27±6	45±14
TSS	mg·L ⁻¹	730±315	460±400
VSS	mg·L ⁻¹	700±360	340±215
TP	mg·L ⁻¹	350±30	415±45
pH	-	5.1±0.2	5.3±0.5
Soluble protein	g·L ⁻¹	1.3±0.3	1.4±3.2
Soluble polysaccharide	g·L ⁻¹	11.2±2.2	14.1±0.6

6.2.2.3 Sludge filterability

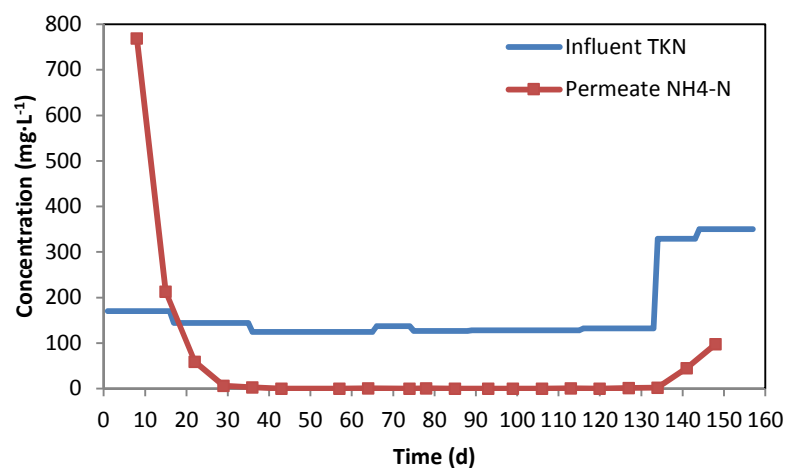
The critical flux was determined according to flux-step method with 15 minutes of step length and 2 L·m⁻²·h⁻¹ step height. A slope of $dP/dt \geq 1$ mbar·min⁻¹ was chosen according to weak definition of critical flux (Le-Clech et al., 2003) to confirm that the critical flux was reached. Capillary suction time (CST) and specific resistance to filtration (SRF) were measured following the procedures described in Chapter 4. Laser diffraction analysis (Beckman Coulter LS230) was used to determine particle size distribution of the sludge between 0.4 and 2000 µm.

6.3 Results and Discussion

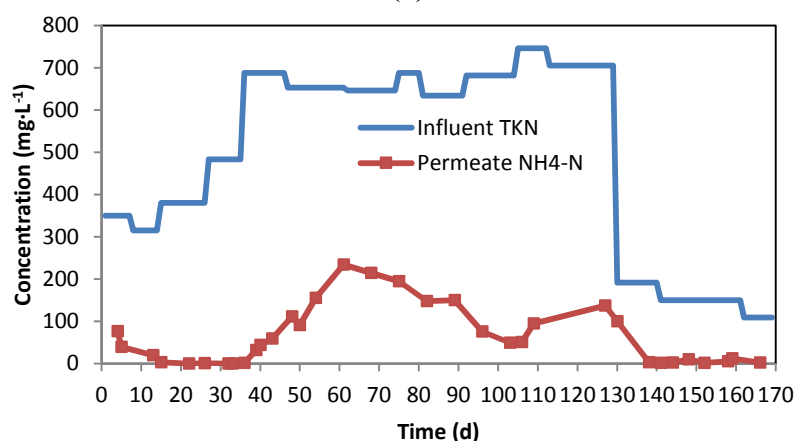
6.3.1 Biological performance

The influent and permeate nitrogen concentrations in both reactors are shown in Figure 6.1. The lack of nitrogen in R-1 permeate confirmed that that treatment of cheese whey with high COD:TKN ratio was indeed nitrogen limited. During operation, until 134th

day almost no ammonium nitrogen was detected in the permeate of R-1. Moreover, no nitrogen was detected in R-2 permeate until the feed TKN concentration was increased up to 700 mg·L⁻¹. Then, the permeate nitrogen concentration gradually increased up to 230 mg·L⁻¹ and leveled around 100 mg·L⁻¹ until 130th day when the feed TKN concentration was sharply reduced.



(a)



(b)

Figure 6.1 : Feed TKN and permeate ammonium nitrogen concentrations of R-1 (a) and R-2 (b).

An unstable performance was observed in R-1 fed with nitrogen limited substrate (Figure 6.2). Several attempts were done to increase the organic load and maintain it at around 5 kg COD·m⁻³·d⁻¹ but VFA accumulation restricted the performance. Speece (1996) reported a remarkable decrease in acetate utilization rate when the NH₄-N concentration was below 70 mg·L⁻¹. However, in our present case propionic acid was the most dominant VFA building up in R-1. Although the exact reason for that is not clear, the lack of nitrogen either hinders propionate oxidation or hydrogen consumption by the hydrogenotrophic methanogens. Efficient propionate conversion

requires strict collaboration of syntrophic bacteria in order to maintain sufficiently low hydrogen partial pressures for propionate oxidation to occur. Until 115th day, high COD concentrations up to 4000 mg·L⁻¹ were observed in the permeate. During this instable period 55±8% of permeate COD originated from accumulated VFA. At the last stage of operation, the OLR was fixed to 2 kg COD·m⁻³·d⁻¹ between 115th and 158th days. Moreover, TKN concentration of the feed was increased to 340 mg·L⁻¹ between 134 to 158th days (Figure 6.1). As a result of the decreased organic load, a stable performance was observed and permeate COD concentrations of 80±20 mg·L⁻¹ could be achieved without any VFA accumulation.

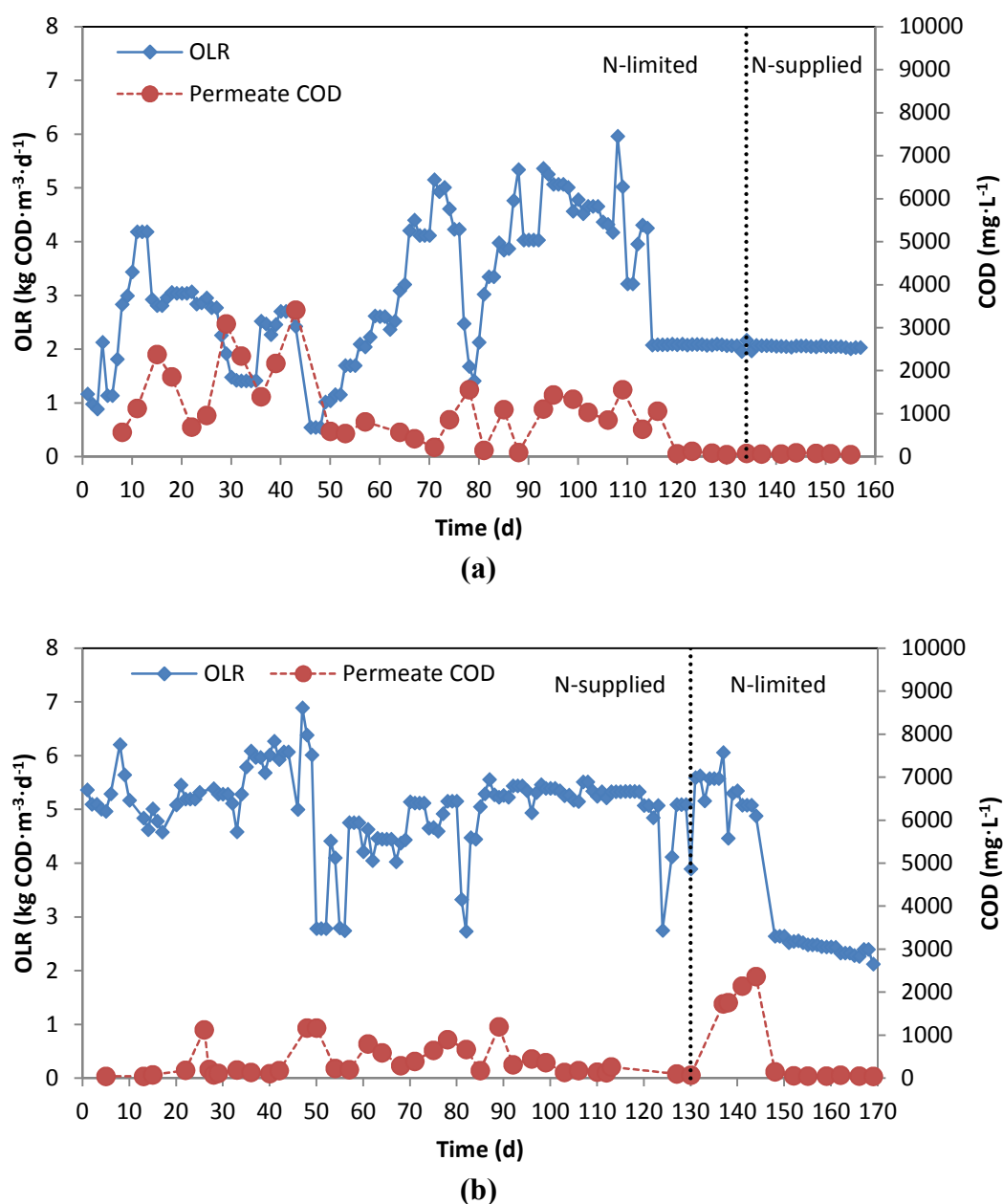


Figure 6.2 : Applied load and permeate COD concentrations in R-1 (a) and R-2 (2).

A more stable performance was observed in R-2 (Figure 6.2). Compared to R-1, a higher organic load could be applied with occasional VFA peaks. Nevertheless, the VFA concentrations were much lower than R-1. In the last period of the study, the TKN concentration of the R-2 feed was gradually decreased to $110 \text{ mg}\cdot\text{L}^{-1}$ between 130th to 169th days (Figure 6.1). Interestingly, VFA concentrations in the permeate gradually increased up to $2500 \text{ mg COD}\cdot\text{L}^{-1}$ within 10 days. Propionate was the only VFA accumulating in the reactor at this period. Consequently, the applied organic load had to be decreased to $2 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ and reactor regained its stability. This result clearly indicates that feed COD:TKN ratio has a strong effect on biological treatment performance and stability of anaerobic reactors. Interestingly, after 1 month of operation the sludge color in R-2 turned grayish, likely due to abundant bacterial growth, whereas the sludge in R-1 remained black.

The main operational problem in R-2 was severe sludge foaming which was never observed in R-1. Several studies mentioned about foaming problems during anaerobic treatment of carbohydrate rich wastewaters (Brooks et al., 2008; Suhartini et al., 2014). Kougias et al. (2014) reasoned that to the dominance of acidogenic bacteria such as *Lactobacillus*, *Bacillus*, *Thermotoga*, *Micrococcus* and *Pseudonocardia*. These species are known to produce foam promoting compounds such as proteins, lactic acid, extracellular water-soluble bio-surfactants and lipopeptides which may be extensively released to the bulk liquor under high shear conditions of an AnMBR. The supplementation of nitrogen together with a rapidly degradable disaccharide, i.e. lactose, would promote the growth of the mentioned acidogenic bacteria.

The operating conditions and treatment performance of R-1 and R-2 are summarized in Table 6.2. A high COD removal efficiency could be achieved in both reactors thanks to membrane filtration which removed all particulate matter from the effluent. Nitrogen supplementation significantly increased the biomass yield in R-2 compared to R-1. Due to high sludge yield observed in R-2, the SRT was decreased to 50 days in order to maintain the TSS concentration around $40 \text{ g}\cdot\text{L}^{-1}$. Moreover, due to increased biomass synthesis a lower methane yield per gram COD fed in to the reactor was observed in R-2.

Anaerobic treatment requires a balanced microbial consortium in terms of acidogens, acetogens and methanogens in order to achieve a stable reactor performance. Due to their rapid growth and high biomass yield, the acidogens would consume the major

part of the nitrogen fed to R-1 and as a consequence may limit the productivity of other slow growing microorganisms in the anaerobic food chain. This may be a reason for the poor performance of R-1.

Table 6.2 : Operating conditions and performance comparison of the reactors (mean±standard deviation).

Parameter	Unit	R-1	R-2
SRT	d	300	50
OLR	kg COD·m ⁻³ ·d ⁻¹	3.0±1.3	5.0±0.8
HRT	d	10.6±5.1	6.0±1.3
F/M ratio	kg COD·kg VSS ⁻¹ ·d ⁻¹	0.14±0.04	0.18±0.02
Permeate COD	mg·L ⁻¹	1260±990	380±360
Permeate VFA	g COD·L ⁻¹	600±590	320±390
COD removal efficiency based on permeate quality	%	96±3	98±2
Methane yield	Nm ³ CH ₄ ·kg ⁻¹ COD _{fed}	0.33±0.05	0.28±0.02
Sludge yield	g VSS·g ⁻¹ COD _{removed}	0.03±0.01	0.19±0.03

6.3.2 Sludge characteristics

6.3.2.1 Particle size distribution

The median particle size in R-1 and R-2 was 25 µm and 13 µm, respectively. Interestingly, R-1 sludge showed a unimodal PSD, whereas the PSD of R-2 sludge was bimodal (Figure 6.3). This may be due to the proliferation of acidogens which prefer dispersed growth at lower COD:TKN ratio. Jeison and van Lier (2007a) reported that acidogens grew to a large extent as individual cells. In Chapter 5, it was validated that the presence of acidogens shifted the overall PSD of the bulk sludge to more small sized particles.

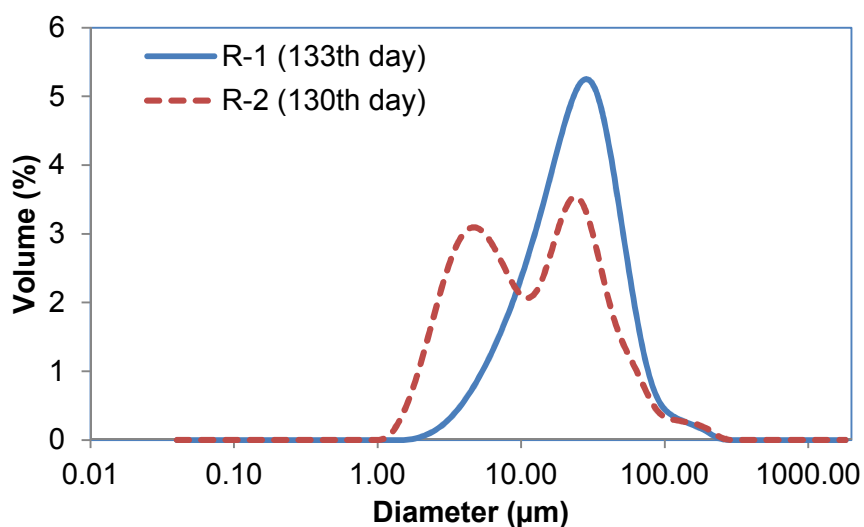


Figure 6.3 : Particle size distribution in different reactors.

6.3.2.2 Soluble microbial products

The SMP concentrations monitored in R-1 and R-2 throughout the study are shown in Figure 6.4. After two months of operation, the polysaccharide type SMP in the reactor R-1 decreased from 100 to around 35 mg·L⁻¹. This may be due to the adaptation of biomass to the prevailing reactor conditions and substrate. The decrease in OLR to 2 kg COD·m³·day on 115th day led to a slight decrease in polysaccharides. On the other hand, when the nitrogen concentration in the feed was increased after 134th day, the polysaccharides concentration tended to increase. The protein type SMP started to accumulate in the reactor R-1 after 30th day until 64th day. This may be due to undesired dosing of caustic which gradually increased the pH up to 8.3 between 15th and 28th days because of pH probe malfunctioning. The proteinaceous SMP started to decrease after day 65 and stabilized at very low concentrations till the end of study.

After an initial increase, the polysaccharide type SMP concentrations stabilized at 40 mg·L⁻¹ until 87th day in reactor R-2. Although the protein concentrations were relatively low compared to R-1, high protein concentrations were observed between 40th and 70th days in R-2. On 88th day, we observed a very serious bulking event which led to a rapid jump of both polysaccharide and protein SMP. After that, the proteinaceous SMP concentrations decreased and dropped below detection limit for the rest of the study. Switching from N-supplied to N-limited feed in reactor R-2 led to a gradual increase in polysaccharides between 130th and 140th days. However, an organic load of 5 kg COD·m⁻³·d⁻¹ could not be maintained due to VFA accumulation under N-limited conditions. Thus, the organic load had to be reduced which caused a decrease in polysaccharide concentrations.

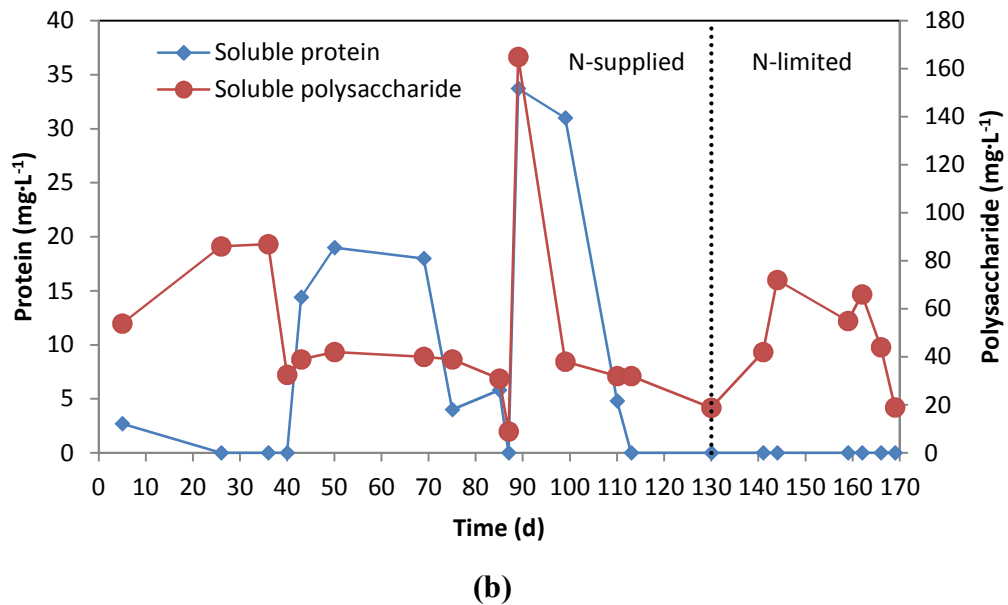
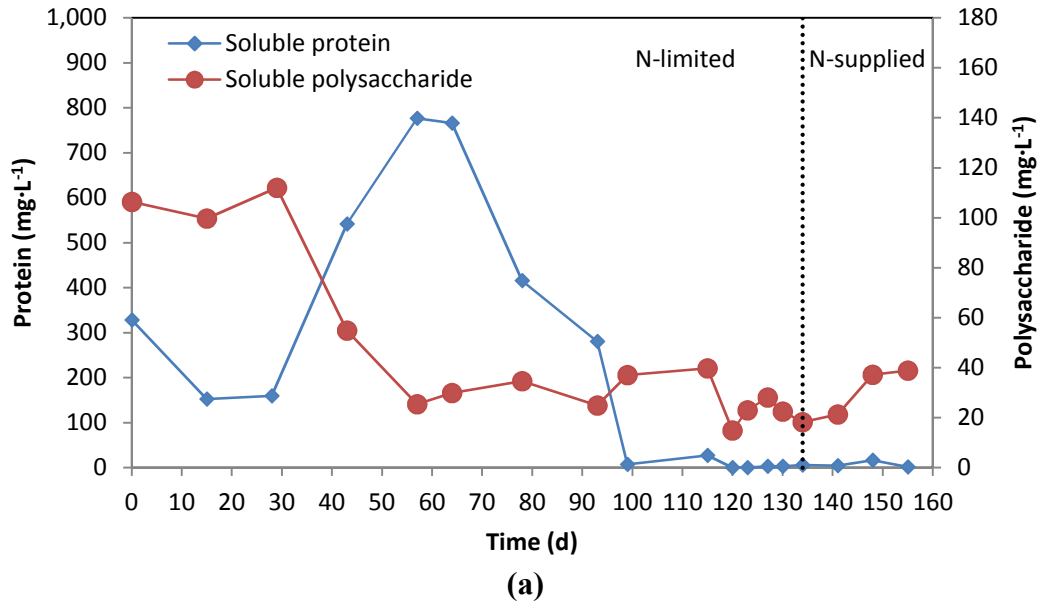
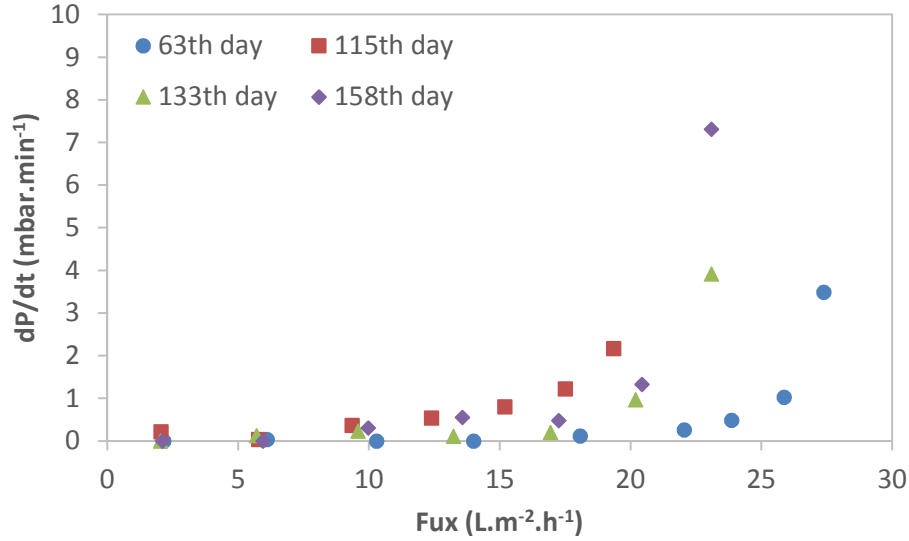


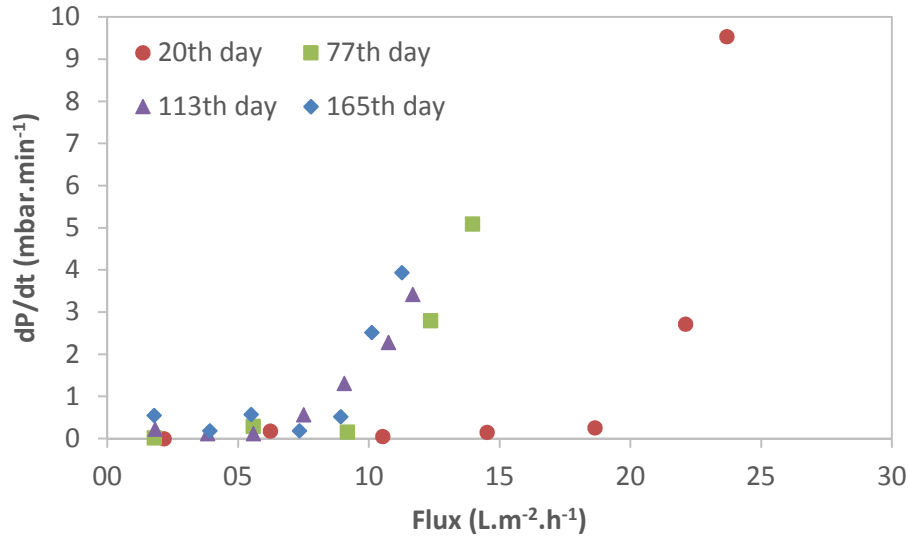
Figure 6.4 : Soluble microbial products measured in R-1 (a) and R-2 (b).

6.3.3 Sludge filterability

During operation, the critical flux of R-2 decreased from 20 to 9 L·m⁻²·h⁻¹ within 2.5 months and remained stable till the end of the experimental study (Figure 6.5). On the other hand, the steady state critical flux measured in R-1 was 20±2 L·m⁻²·h⁻¹ which was two times higher than that of R-2. Short term critical flux tests revealed that COD:TKN ratio has an important effect on sludge filterability.



(a)



(b)

Figure 6.5 : Fouling rate evolution, expressed as TMP increasing rate, in critical flux tests of R-1 (a) and R-2 (b).

In order to investigate the effect of COD:TKN ratio on sludge filtration characteristics, supplementary indicators such as CST and SRF were monitored throughout the study (Figure 6.6). These standard parameters allow an objective comparison of sludge filterability at different operating conditions (Chapter 4; Ersahin et al., 2014). CST and SRF parameters were clearly linked and showed a very high positive correlation in both reactors (Pearson correlation coefficient > 0.92 , $\alpha = 0.05$). Similarly, good correlation between these parameters was observed in Chapter 5. Since CST is a rapid and easy to measure parameter, it is suggested to use it as an indicator of sludge

filterability. This was also recommended by several authors (Pollice et al., 2008; Laera et al., 2009).

The increase in CST and SRF in R-1 between 15th and 30th days was attributed to failure of the pH meter. In general, the CST and SRF parameters decreased until 80th day and remained stable until 115th day. A decrease in the OLR after day 115, led to gradual decrease of sludge filterability indicated by increasing CST and SRF values in R-1. This may be due to sudden change of F:M ratio from 0.14 to 0.09 kg COD·kg VSS⁻¹·d⁻¹. F:M ratio was reported as an important parameter that affects sludge filterability and membrane fouling (Liu et al., 2012b). A lower F:M may stimulate substrate limitation and release of bacterial decay products. However, we did not detect an increase in proteinaceous and the polysaccharide type SMP. We observed a significant positive correlation between polysaccharide SMP and the sludge filterability indicators CST and SRF. On the other hand, the accumulation of proteins between 30th and 100th days did not seem to influence sludge filterability.

The sludge filterability in R-2 continuously deteriorated throughout operation. A pH shock of 8.6 at 55th day led to a rapid increase followed by a decrease in sludge filterability, meanwhile the system performance recovered within 10 days. The decrease in filterability coincided with the accumulation of proteinaceous SMP. Moreover, the decrease in the feed nitrogen concentration between 130th and 170th days seemed not to improve sludge filterability, although a further worsening was not observed. At the end of operation, the normalized CST and SRF parameters reached to 70 s·L·g⁻¹ TSS and 1700 E¹² m·kg⁻¹, respectively. These values were several times higher compared to what was measured in R-1.

Acidogenic bacteria are known to exert an adverse effect on sludge filterability. Jeison and van Lier (2007a) reported an increased rate of fouling and decreased critical flux in an AnMBR fed with partially acidified wastewater compared to the control reactor operated with VFA based wastewater. They attributed this to the single cell growth of acidogenic biomass, which results in a decrease in sludge overall particle size. In a further study, they validated that acidogens dominate the supernatant fraction of sludge which determines the overall filtration behavior and fouling propensity of bulk liquor (Jeison et al., 2009a). In Chapter 5 the effect of substrate acidification degree on sludge filterability was systematically evaluated and it was concluded that the acidogenic biomass also decreased sludge particle size and negatively affected filterability. We

observed a rapid deterioration of CST and SRF parameters, immediately following the startup of an anaerobic reactor treating non-acidified cheese whey. Nitrogen deprivation seemed to limit the overgrowth of acidogenic bacteria as indicated by low biomass yield in R-1. On the other hand, nitrogen supplementation induced high biomass growth yields, most plausibly due to the growth of acidogens on rapidly fermentable substrate. We observed an obvious impairment in all filterability parameters such as CST, SRF and critical flux in R-2 compared to R-1.

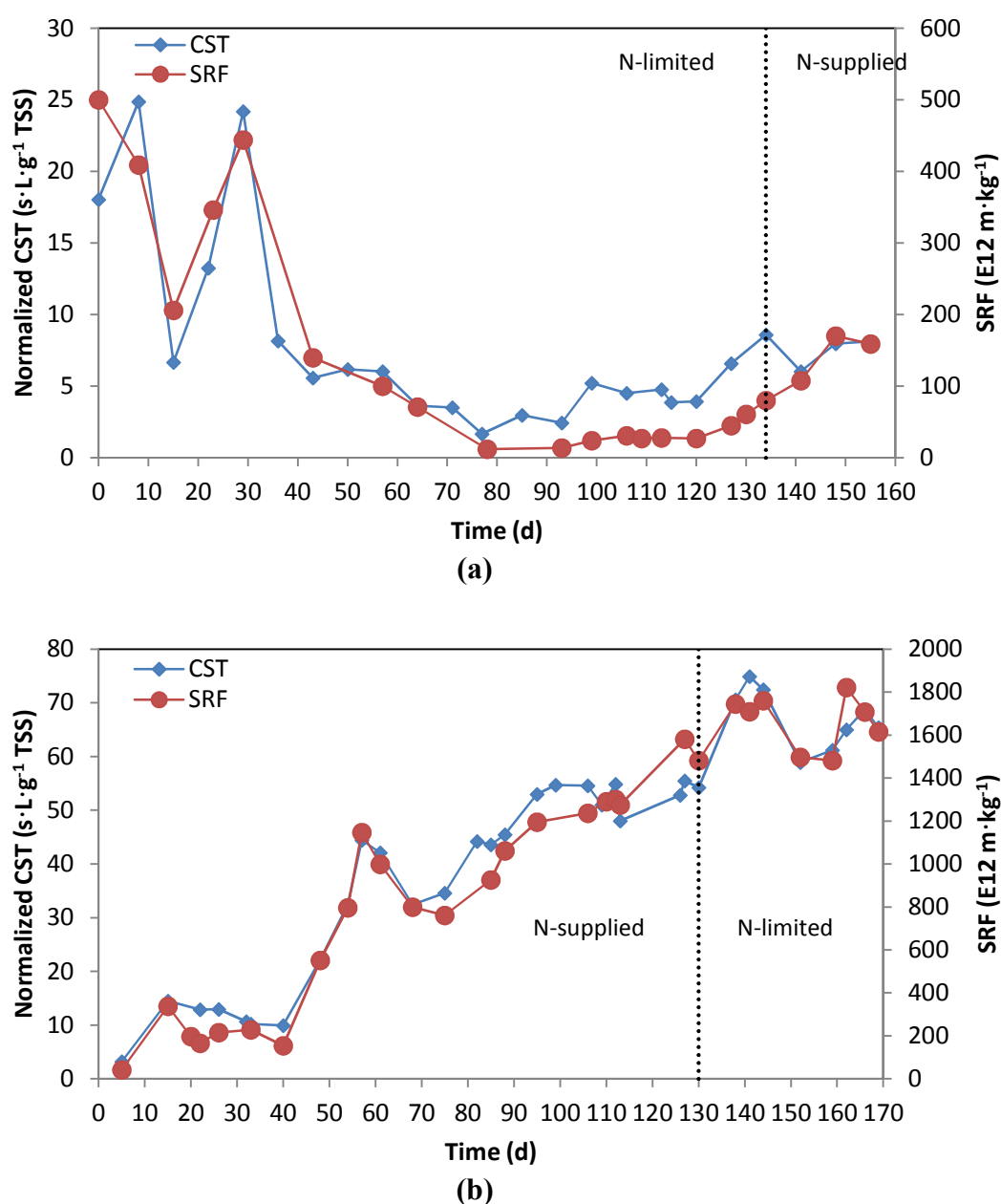


Figure 6.6 : Evolution of CST and SRF in R-1 (a) and R-2 (b).

The particle size analysis of R-2 sludge showed a bimodal distribution (Figure 6.3). Indeed, after centrifugation of bulk sludge at 17500g for 10 minutes, we observed two

distinct fractions in sludge pellet: a whitish milky top layer and a darker bottom layer. In fact, a similar observation was previously made by Gao et al. (2010b) who investigated the microbial species distribution of sludge fractions and cake layer of an AnMBR. In order to investigate the filterability of different sludge fractions, bulk sludge was separated into white and black solids. The SRF of bulk sludge, white solids and black solids were measured as 1150, 6000 and 770 $\text{E}^{12} \text{ m} \cdot \text{kg}^{-1}$, respectively. The cake resistances of white solids were much higher than the original sludge and black solids found at the bottom of pellet. The PSD analysis of white solids revealed that the median particle size (4 μm) was lower than the bulk sludge (Figure 6.7). The latter likely contributed significantly to the high cake resistance, since the SRF parameter is directly linked to the sludge particle size (Endo and Alanso, 2001). The SRF of black solids was significantly lower than the original sludge which means when the white solids are selectively removed an improvement in filterability may be achieved. Interestingly, the white solids were found rich with polysaccharide and poor with proteinaceous type EPS compared to bulk sludge and black solids (Table 6.3). On the contrary, Gao et al. (2010b) reported a higher proteinaceous EPS content compared to polysaccharide based EPS for light solids fraction in the sludge. This may be attributed to the differences in substrate composition used in both studies. They used a protein rich substrate which in our case it was mainly a disaccharide, lactose. The protein to polysaccharide (PN:PS) ratio was also low in the harvested white solids. Protein to polysaccharide ratio is known to affect sludge hydrophobicity and the membrane fouling propensity (Drews, 2010). Gao et al. (2010a) reported a reciprocal correlation between PN:PS ratio and membrane fouling rate.

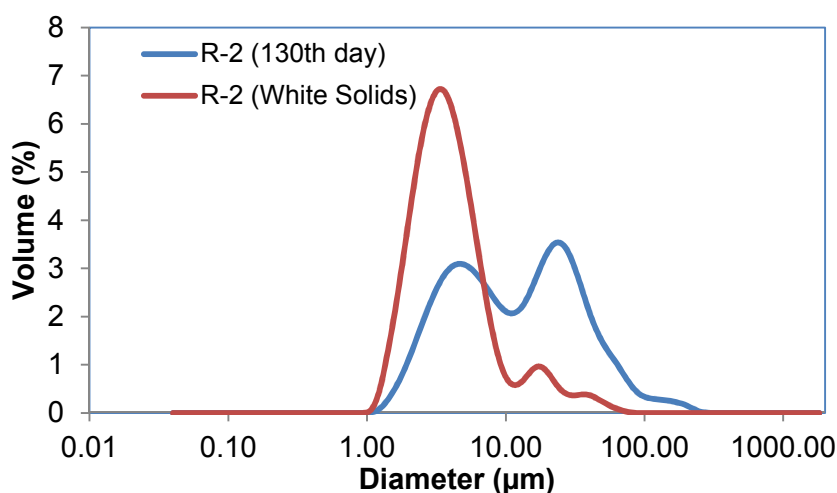


Figure 6.7 : Particle size distribution of bulk sludge and white solids in R-2.

Table 6.3 : EPS content of different sludge fractions.

Parameter	Unit	Bulk Sludge	White Solids	Black Solids
Protein	mg·g ⁻¹ VSS	37±2	30±1	37±3
Polysaccharide	mg·g ⁻¹ VSS	25±3	35±0.1	22±0.3
PN:PS	-	1.5	0.8	1.7

6.4 Conclusions

The results presented in this chapter clearly show that nitrogen deficiency limited the biological performance of R-1 indicated by accumulation of VFA. Particularly, propionic acid degradation seems to be influenced by the lack of nitrogen. Nitrogen limited sludge was characterized by a high filterability. On the other hand, supplementation of high nitrogen adversely affected sludge filterability indicated by CST, SRF and critical flux. Moreover, the sludge particle size decreased and two distinct fractions of sludge with different filterability characteristics became visually apparent: a whitish small sized fraction with a very low filterability and a dark bigger sized fraction characterized by a good filterability. Besides, nitrogen supplementation also promoted sludge foaming in R-2. The reason for poor sludge filterability was attributed to excessive growth of acidogenic biomass on readily available substrate with the surplus of nitrogen.

7. CONCLUSIONS

7.1 Introduction

This thesis focused mainly on the effects of substrate composition and solids retention time (SRT) on sludge filterability. Filterability at different operating conditions was assessed with parameters such as capillary suction time (CST), specific resistance to filtration (SRF), supernatant filterability and critical flux. The link between sludge characteristics such as soluble microbial products (SMP), particle size distribution, relative hydrophobicity and membrane fouling was evaluated. In this chapter, the main conclusions and outcomes of the thesis are summarized. Moreover, perspectives and recommendations for future research on anaerobic membrane bioreactors (AnMBRs) are given based on the experiences obtained in this research.

7.2 Conclusions from the Various Research Steps

7.2.1 Effect of SRT

Solids retention time is one of the state-of-the-art parameters for the operation of any biological process. In this thesis, a better substrate biodegradation efficiency was observed at increased SRTs, likely due to longer contact times of substrate with biomass (Chapter 3). On the other hand, longer residence time induced long chain fatty acid (LCFA) adsorption on microorganisms and consequent biomass activity loss was observed. Sludge activity deterioration was almost completely recoverable when the feeding was stopped and biomass was given sufficient time to degrade the accumulated LCFAs. From filtration point of view, the reactor operated with the shortest SRT exhibited the best sludge filterability (Chapter 4). This was attributed to the washout of fine particles such as colloids and SMP with the discarded sludge. The colloids tended to accumulate in the bulk liquor at higher residence times. Therefore, a compromise between substrate biodegradation efficiency and sludge filterability must be made in order to find the optimum SRT for AnMBRs.

7.2.2 Effect of substrate composition

Substrate characteristics have an indirect effect on the filtration process. The concentration and composition of SMP and EPS, and distribution of microbial species are strongly influenced by the composition of the substrate. In this thesis, two wastewater types with unique characteristics were used as substrate: corn-to-ethanol thin stillage and cheese whey. Thin stillage is a very strong wastewater with high COD, suspended solids and lipid concentrations. Cheese whey is an almost completely soluble and rapidly fermentable wastewater which contains mainly lactose.

7.2.2.1 Lipids

In Chapter 3 and 4, it was shown that high lipid containing wastewaters can be successfully treated by AnMBR process at moderate to high organic loading rates (OLRs) ($6\text{--}8 \text{ kg COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$) and with good membrane fluxes ($9\text{--}13 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$). Biomass retention problem often reported for granular sludge bed systems treating lipid rich wastewaters was certainly no issue for the AnMBRs. However, the biological process still suffered from LCFA accumulation and inhibition. Fortunately, the deterioration of activity due to LCFA accumulation on biomass was reversible. These results indicate that AnMBRs require an operation strategy to improve their potential for the treatment of lipid rich wastewaters. Intermittent feeding regime suggested by several authors can be adopted to AnMBRs to shorten the adaptation time (Cavaleiro et al., 2008). Moreover, bioaugmentation with LCFA degrading species or previously acclimatized sludge can also help to increase biodegradation efficiency and decrease LCFA accumulation. In this thesis, it was shown that LCFA precipitated with bivalent cations such as calcium and magnesium present in the feed. This reduced the potential adverse effects of LCFA inhibition. Therefore, dosing calcium and magnesium to AnMBRs can help to prevent the adverse effects of LCFA on biomass at the expense of reduced bio-methanation efficiency.

It was shown that LCFA accumulation can strongly change the surface characteristics of sludge and makes it more hydrophobic. In Chapter 4, it was shown that a lesser degree of irreversible organic fouling was present on the membrane after physical removal of the cake layer. Sludge flocs interactions with the membrane surface are important especially at the initial stages of filtration (Le-Clech et al., 2006). Van den Broeck (2011) stated that hydrophobic flocs interacted less with the hydrophilic

membrane surface and caused less fouling. Yang et al. (2012a) reported better membrane performance when treating oil and surfactant rich wastewater compared to the control reactor. In general, high lipid containing wastewaters are avoided in membrane bioreactors due to the tendency of lipids to accumulate and cause fouling on polymeric membranes. However, the results presented in this thesis suggest that the hydrophobic interaction of lipids with biomass and membrane is a complex phenomenon and do not necessarily negatively impact the membrane filtration process.

7.2.2.2 Degree of substrate acidification

The effect of substrate acidification degree on sludge filterability was systematically evaluated in Chapter 5. It was shown that non-acidified rapidly degradable substrate, cheese whey, promoted the rapid growth of acidogenic biomass. The sludge filterability of the reactor receiving non-acidified whey rapidly deteriorated soon after the start-up. However, the reactor fed with acidified substrate maintained a good sludge filterability for the long term experimental period. These results confirm the findings of Jeison et al. (2009a) who reported that the acidogens present in sludge supernatant determined the attainable flux and filtration behavior of the bulk liquor. As previously reported by Jeison and van Lier (2007a) the acidogens tend to grow as single cells instead of forming large flocs, thus the proliferation of acidogens decreased the particle size and increased the hydrophilicity of sludge, both negatively impacting sludge filterability.

In addition to the effect of acidogens, the food to mass ratio (F:M) was also found as an important parameter influencing sludge filterability. High F:M stimulated the release of soluble microbial products and adversely affected filterability. Contrary to Jeison et al. (2009a), the critical flux of acidogenic sludge was found as high as the methanogenic reactor sludge. This was most plausibly due to the sequencing batch regime applied in the acidogenic reactor which inevitably selects for large and settleable sludge flocs.

7.2.2.3 Nitrogen limitation

Nitrogen is one of the essential nutrients for cell synthesis. Nitrogen limitation is generally well dealt with in full scale anaerobic reactors by adding urea to the influent flow if the influent characteristics require this. However, many agro-industrial

wastewaters do not need N additions due to low cell yield of anaerobic biomass. However, carbohydrate type rapidly degradable substrates may promote extensive growth of acidogenic microorganisms, which require a substantial amount of nitrogen, owing to their high growth yield which is up to 5 times higher than that of the methanogens. In Chapter 6, the effect of nitrogen limitation on biological performance and sludge filterability of AnMBRs was investigated. The lack of nitrogen exerted an adverse effect on the biological treatment performance and reactor stability. VFA accumulation, especially propionate, was observed even at low OLRs such as $3 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. This was attributed to the changing microbial metabolism under nitrogen deficiency. It was reported that hydrogen yield and production rate increased under nitrogen limited conditions. This may adversely affect the syntrophic propionate oxidation conversion, which requires a low hydrogen partial pressure.

On the other hand, supplementation of nitrogen negatively affected sludge filterability. This was reasoned to the over-growth of acidogenic biomass which was reported to impair filterability due to their single cell growth (Jeison and van Lier, 2007a; Jeison et al., 2009a). The sludge fed with nitrogen deprived feed exhibited a superior filterability in terms of CST, SRF and critical flux compared to the one receiving nitrogen supplemented feed.

7.3 Perspectives and Recommendations for Future Research

Anaerobic granular sludge bed reactors are well established and proven treatment systems for mainly soluble and easily degradable wastewaters (van Lier, 2008). Therefore, it is expected that they will continue to dominate the market for these wastewater types due to their low costs to performance ratio. In fact, AnMBRs are not expected to compete with sludge bed reactors in this application field. However, various types of wastewaters have more extreme characteristics, such as high particulate matter, lipid content, salinity and refractory/toxic compounds, which are known to hamper granulation (Chapter 2). Following the trends of industrial water reuse and developments towards zero-discharge production facilities, it is expected that quantity of more extreme wastewaters will increase in the near future. Therefore, it is anticipated that AnMBR technology is a growing niche treatment technology for these types of wastewaters. Moreover, AnMBRs can be used to retain specific microorganisms that can degrade target pollutants in difficult to treat wastewaters,

such as chemical wastewaters. These microorganisms may washout in granular bed systems due to their inferior adherence capacities and/or inability to form granules. However, membrane assisted biomass retention provides an excellent tool to cultivate and enrich these microorganisms in AnMBRs.

Integration of AnMBRs with other industrial processes also offers several opportunities. In this context, AnMBRs operated at strictly controlled acidogenic conditions is a quite interesting subject. AnMBR permeate rich with organic acids and free of suspended solids can be used to produce industrial products such as ethanol, butanol and bioplastics. Such applications are particularly of interest when high substrate concentrations are treated, thus when low membrane fluxes only have a low impact on the economics of the process.

Another application of AnMBRs is the possible production of largely disinfected, nutrient rich permeates. AnMBR systems could be coupled to innovative nutrient removal technologies to close the water and nutrient cycles in a more efficient and feasible way.

Although there has been a significant scientific interest in the past decade, AnMBRs are considered a commercially new treatment technology leading to reluctance at industries for full scale applications. The laboratory studies are certainly useful for determination of treatment performance and identification of potential problems. However, the membranes used in laboratory set-ups cannot represent the real performance of full scale membranes due to scale differences in hydraulics, shear distribution, pressure gradients and geometry. Thus, it is very difficult to extrapolate membrane performance of a lab-scale reactor to full scale system. In this respect, it is crucial to conduct pilot studies to obtain more reliable information about filtration performance.

Standard tools such as CST and SRF were found quite useful for the comparison of sludge filterability under different operating conditions. It is difficult to directly compare the TMP profile and/or membrane permeability of AnMBRs from different studies. This is mainly due to different membrane properties, operation conditions, presence of inorganic foulants and sludge characteristics. However, the standard filterability parameters provide an objective comparison of filterability and they can be used to quantify the effects of operating conditions. Especially, CST is a rapid and

very easy parameter. In this thesis, it is shown that it correlates well with the SRF parameter. The correlation between membrane fouling and sludge filterability parameters were rather weak. This may be attributed to the complexity of the fouling process or to the different conditions between the actual membrane filtration process and filtration tests. The lack of observed correlation does not imply no correlation at all, but the relation may more complex and non-linear. It was reported that it is impossible to characterize filterability and fouling with a single parameter, thus a set of parameters should be taken into consideration and needs to be evaluated. Moreover, filterability tests that can better represent the conditions applied in real filtration processes should be developed. In this respect, the Delft Filtration Characterization method (DFCm), which was successfully used by several researchers to quantify sludge filterability in full scale aerobic MBRs, (Geilvoet, 2010; Lousada-Ferreira, 2011; Krzeminski, 2013, Lousada-Ferreira et al., 2014), or comparable characterization methods, should be adopted for anaerobic applications.

AnMBR technology will certainly benefit from mathematical modeling studies for predicting the performance of both the biological process and the membrane filtration step subjected to fouling. In this context, a simplified version of IWA Anaerobic Digestion Model No.1 (Batstone et al., 2002) with the inclusion of SMP production can be a starting point. Moreover, computational fluid dynamic (CFD) modeling studies can be helpful to understand the particle to particle interactions and the effect of shear rate for mitigating membrane fouling (Yang et al., 2012b).

The overall results of this thesis clearly indicate that substrate composition strongly effect the relative hydrophobicity of sludge. Results showed that carbohydrate type of substrates promoted the rapid growth of acidogenic bacteria which were hydrophilic (Chapter 5). Moreover, LCFA accumulation modified the surface characteristics of biomass and made sludge more hydrophobic (Chapter 2). Additionally, it was reported that LCFA degraders were more hydrophobic species (Daffonchio et al., 1995; Nielsen et al., 2002). Hydrophobic interaction is regarded as the first step in colonization of bacteria on different surfaces (van Loosdrecht et al., 1987). Recently, it was shown that the microbial species distribution of the bulk sludge and cake layer accumulating on the membrane differ (Calderon et al., 2011; Lin et al., 2011). This suggests that microorganisms have different tendencies to accumulate on the membrane. The microbial tools such as fluorescence in situ hybridization (FISH), real time polymerase

chain reaction (RT-qPCR) and pyrosequencing would help to identify these pioneering species responsible for membrane fouling.

REFERENCES

- Abdurrahman, N.H., Rosli, Y.M., Azhari, N.H.** (2011). Development of a membrane anaerobic system (MAS) for palm oil mill effluent (POME) treatment. *Desalination*, 266(1-3), 208-212.
- Ahn, J.-H., Do, T.H., Kim, S.D., Hwang, S.** (2006). The effect of calcium on the anaerobic digestion treating swine wastewater. *Biochemical Engineering Journal*, 30(1), 33-38.
- Akram, A., Stuckey, D.C.** (2008a). Biomass acclimatization and adaptation during start-up of a submerged anaerobic membrane bioreactor (SAMBR). *Environmental Technology*, 29(10), 1053-1065.
- Akram, A., Stuckey, D.C.** (2008b). Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Process Biochemistry*, 43(1), 93-102.
- Al-Halbouni, D., Dott, W., Hollender, J.** (2009). Occurrence and composition of extracellular lipids and polysaccharides in a full-scale membrane bioreactor. *Water Research*, 43(1), 97-106.
- Alves, M.M., Mota Vieira, J.A., Alvares Pereira, R.M., Pereira, M.A., Mota, M.** (2001a). Effect of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part I: biofilm growth and activity. *Water Research*, 35(1), 255-263.
- Alves, M.M., Mota Vieira, J.A., Álvares Pereira, R.M., Pereira, M.A., Mota, M.** (2001b). Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: Oleic acid toxicity and biodegradability. *Water Research*, 35(1), 264-270.
- Alves, M.M., Picavet, M.A., Pereira, M.A., Sousa, D.Z., Cavaleiro, A.J.** (2007). Novel anaerobic reactor for the removal of long chain fatty acids from fat containing wastewater. WO/2007/058557.
- Alves, M.M., Pereira, M.A., Sousa, D.Z., Cavaleiro, A.J., Picavet, M., Smidt, H., Stams, A.J.M.** (2009). Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA). *Microbial Biotechnology*, 2(5), 538-550.
- Amaral, A.L., Pereira, M.A., da Motta, M., Pons, M.N., Mota, M., Ferreira, E.C., Alves, M.M.** (2004). Development of image analysis techniques as a tool to detect and quantify morphological changes in anaerobic sludge: II. Application to a granule deterioration process triggered by contact with oleic acid. *Biotechnology and Bioengineering*, 87(2), 194-199.
- APHA** (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

- Arabi, S., Nakhla, G.** (2008). Impact of protein/carbohydrate ratio in the feed wastewater on the membrane fouling in membrane bioreactors. *Journal of Membrane Science*, 324(1-2), 142-150.
- Araya, P., Aroca, G., Chamy, R.** (1999). Anaerobic treatment of effluents from an industrial polymers synthesis plant. *Waste Management*, 19(2), 141-146.
- Argun, H., Kargi, F., Kapdan, I.K., Oztekin, R.** (2008). Biohydrogen production by dark fermentation of wheat powder solution: Effects of C/N and C/P ratio on hydrogen yield and formation rate. *International Journal of Hydrogen Energy*, 33(7), 1813-1819.
- Bailey, A.D., Hansford, G.S., Dold, P.L.** (1994). The enhancement of upflow anaerobic sludge bed reactor performance using crossflow microfiltration. *Water Research*, 28(2), 291-295.
- Batstone, D.J., Keller J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A.** (2002). *Anaerobic Digestion Model No. 1 (ADM1) Scientific and Technical Report No.13*. International Water Association (IWA) Publishing, London, UK.
- Bérubé, P.R., Hall, E.R., Sutton, P.M.** (2006). Parameters Governing Permeate Flux in an Anaerobic Membrane Bioreactor Treating Low-Strength Municipal Wastewaters: A Literature Review. *Water Environment Research*, 78(8), 887-896.
- Blaszczyk, R., Gardner, D., Kosaric, N.** (1994). Response and recovery of anaerobic granules from shock loading. *Water Research*, 28(3), 675-680.
- Bouhabila, E.H, Aim, R.B, Buisson, H.** (2001). Fouling characterization in membrane bioreactors. *Separation and Purification Technology*, 22-23, 123-32.
- Brockmann, M., Seyfried, C.F.** (1997). Sludge activity under the conditions of cross-flow microfiltration. *Water Science and Technology*, 35(10), 173-181.
- Brooks, L., Parravicini, V., Svardal, K., Krois, H., Prendi, L.** (2008). Biogas from sugar beet press pulp as substitute of fossil fuels in sugar beet factories. *Water Science and Technology*, 58(7), 1497-1504.
- Buntner, D., Spanjers, H., van Lier, J.B.** (2014). The influence of hydrolysis induced biopolymers from recycled aerobic sludge on specific methanogenic activity and sludge filterability in an anaerobic membrane bioreactor. *Water Research*, 51, 284-292.
- Cadi, Z., Huyard, H., Manem, J., Moletta, R.** (1994). Anaerobic digestion of a synthetic wastewater containing starch by a membrane reactor. *Environmental Technology*, 15(11), 1029-1039.
- Calderón, K., Rodelas, B., Cabirol, N., González-López, J., Noyola, A.** (2011). Analysis of microbial communities developed on the fouling layers of a membrane-coupled anaerobic bioreactor applied to wastewater treatment. *Bioresource Technology*, 102(7), 4618-4627.
- Cavaleiro, A.J., Alves, M.M., Mota, M.** (2001). Microbial and operational response of an anaerobic fixed bed digester to oleic acid overloads. *Process*

Biochemistry, 37(4), 387-394.

- Cavaleiro, A.J., Pereira, M.A., Alves, M.** (2008). Enhancement of methane production from long chain fatty acid based effluents. *Bioresource Technology* 99(10), 4086-4095.
- Chang, I.-S., Lee, C.-H.** (1998). Membrane filtration characteristics in membrane-coupled activated sludge system - The effect of physiological states of activated sludge on membrane fouling. *Desalination*, 120(3), 221-233.
- Chang, I.-S., Kim, S.-N.** (2005). Wastewater treatment using membrane filtration-effect of biosolids concentration on cake resistance. *Process Biochemistry*, 40(3-4), 1307-1314.
- Charfi, A., Ben Amar, N., Harmand, J.** (2012). Analysis of fouling mechanisms in anaerobic membrane bioreactors. *Water Research*, 46(8), 2637-2650.
- Chen, Y., Cheng, J.J., Creamer, K. S.** (2008). Inhibition of anaerobic digestion process: A review. *Bioresource Technology*, 99(10), 4044-4064.
- Chernicharo, C.A.D.** (2007). *Biological Wastewater Treatment: Volume 4 - Anaerobic Reactors*. IWA Publishing, UK.
- Choo, K.H., Lee, C.H.** (1998). Hydrodynamic behavior of anaerobic biosolids during cross-flow filtration in the membrane anaerobic bioreactor. *Water Research*, 32(11), 3387-3397.
- Choo, K.H., Kang, I.J., Yoon, S.H., Park, H., Kim, J.H., Adiya S., Lee C.H.** (2000). Approaches to membrane fouling control in anaerobic membrane bioreactors. *Water Science and Technology*, 41(10-11), 363-371.
- Christian, S., Grant, S., McCarthy, P., Wilson, D., Mills, D.** (2010). The first two years of full-scale anaerobic membrane bioreactor (AnMBR) operation treating high-strength industrial wastewater. In: *Proceedings of IWA 12th World Congress on Anaerobic Digestion*, Guadalajara, Mexico.
- Chua, H., Hu, W.F., Yu, P.H. F., Cheung, M.W.L.** (1997). Responses of anaerobic fixed-film reactor to hydraulic shock loadings. *Bioresource Technology*, 61(1), 79-83.
- Cirne, D.G., Paloumet, X., Björnsson, L., Alves, M.M., Mattiasson, B.** (2007). Anaerobic digestion of lipid-rich waste—Effects of lipid concentration. *Renewable Energy*, 32(6), 965-975.
- Daffonchio, D., Thaveesri, J., Verstraete, W.** (1995). Contact angle measurement and cell hydrophobicity of granular sludge from upflow anaerobic sludge bed reactors. *Applied and Environmental Microbiology*, 61(10), 3676-3680.
- Defrance, L., Jaffrin, M.Y., Gupta, B., Paullier, P., Geaugey, V.** (2000). Contribution of various constituents of activated sludge to membrane bioreactor fouling. *Bioresource Technology*, 73(2), 105-112.
- Delrue, F., Stricker, A.E., Mietton-Peuchot, M., Racault, Y.** (2011). Relationships between mixed liquor properties, operating conditions and fouling on two full-scale MBR plants. *Desalination*, 272(1-3), 9-19.

- Demirel B, Scherer P.** (2011). Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass Bioenergy*, 35(3), 992-998.
- Dereli, R.K., Ersahin, M.E., Ozgun, H., Ozturk, I., Jeison, D., van der Zee, F., van Lier, J.B.** (2012a). Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters. *Bioresource Technology*, 122, 160-170.
- Dereli, R.K., Urban, D.R., Heffernan, B., Jordan, J.A., Ewing, J., Rosenberger, G.T., Dunaev, T.I.** (2012b). Performance evaluation of a pilot scale anaerobic membrane bioreactor (AnMBR) treating ethanol thin stillage. *Environmental Technology*, 33(13-15), 1511-1516.
- Diez V., Ramos C., Cabezas J. L.** (2012). Treating wastewater with high oil and grease content using an Anaerobic Membrane Bioreactor (AnMBR). Filtration and cleaning assays. *Water Science and Technology*, 65(10), 1847-1853.
- dos Santos, A.B., Cervantes, F.J., van Lier, J.B.** (2007). Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic biotechnology. *Bioresource Technology*, 98(12), 2369-2385.
- Drews, A.** (2010). Membrane fouling in membrane bioreactors - Characterization, contradictions, cause and cures. *Journal of Membrane Science*, 363(1-2), 1-28.
- Dubois M., Grilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.** (1956). Colorimetric methods for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356.
- Endo Y., Alonso, M.** (2001). Physical meaning of specific cake resistance and effects of cake properties in compressible cake filtration. *Filtration and Separation*, 38(7), 42-46.
- Enright, A.M., McHugh, S., Collins, G., O'Flaherty, V.** (2005). Low-temperature anaerobic biological treatment of solvent containing pharmaceutical wastewater. *Water Research*, 39(19), 4587-4596.
- Ersahin, M.E., Ozgun, H., Tao, Y., van Lier, J.B.** (2014). Applicability of dynamic membrane technology in anaerobic membrane bioreactors. *Water Research*, 48, 420-429.
- Ewing J.D., Taylor V., Rich B.** (2008). Development, design and operations of full scale anaerobic membrane bioreactor technology leads to better than secondary effluent from high strength industrial waste. In: *Proceedings of WEFTEC.08 Conference*, Chicago, Illinois, USA.
- Fakhru'l-Razi, A., Noor, M.J.M.M.** (1999). Treatment of palm oil mill effluent (POME) with the membrane anaerobic system (MAS). *Water Science and Technology*, 39(10-11), 159-163.
- Fang, H.H.P., Chen, T., Li, Y.Y., Chui, H.K.** (1996). Degradation of phenol in wastewater in an upflow anaerobic sludge blanket reactor. *Water Research*, 30(6), 1353-1360.

- Fernández, F.J., Villaseñor, J., Infantes, D.** (2011). Kinetic and stoichiometric modelling of acidogenic fermentation of glucose and fructose. *Biomass and Bioenergy*, 35(9), 3877-3883.
- Fuchs, W., Binder, H., Mavrias, R., Broun, R.** (2003). Anaerobic treatment of wastewater with high organic content using a stirred tank reactor coupled with a membrane unit. *Water Research*, 37(4), 902-908.
- Gao, W.J.J., Lin, H.J., Leung, K.T., Liao, B.Q.** (2010a). Influence of elevated pH shocks on the performance of a submerged anaerobic membrane bioreactor. *Process Biochemistry*, 45(8), 1279-1287.
- Gao, D.-W., Zhang, T., Tang, C.-Y.Y., Wu, W.-M., Wong, C.-Y., Lee, Y.H., Yeh, D.H., Criddle, C.S.** (2010b). Membrane fouling in an anaerobic membrane bioreactor: Differences in relative abundance of bacterial species in the membrane foulant layer and in suspension. *Journal of Membrane Science*, 364(1-2), 331-338.
- Gao, W.J., Leung, K.T., Qin, W.S., Liao, B.Q.** (2011a). Effects of temperature and temperature shock on the performance and microbial community structure of a submerged anaerobic membrane bioreactor. *Bioresource Technology*, 102(19), 8733-8740.
- Gao, W.J., Lin, H.J., Leung, K.T., Schraft, H., Liao, B.Q.** (2011b). Structure of cake layer in a submerged anaerobic membrane bioreactor. *Journal of Membrane Science*, 374(1-2), 110-120.
- Gao, W.J., Han, M.N., Qu, X., Xu, C., Liao, B.Q.** (2013). Characteristics of wastewater and mixed liquor and their role in membrane fouling. *Bioresource Technology*, 128, 207-214.
- Geilvoet, S.** (2010). The Delft Filtration Characterization Method: Assessing Membrane Bioreactor Activated Sludge Filterability. *PhD Thesis*, Delft University of Technology, Delft, the Netherlands.
- Ghaly, A.E.** (1996). A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two-stage reactor. *Bioresource Technology*, 58(1), 61-72.
- Gillies M.T.** (1974). *Whey Processing and Utilization: Economic and Technical Aspect*. Noyes Data Corp. New Jersey.
- Gonçalves, M.R., Costa, J.C., Marques, I.P., Alves, M.M.** (2012). Strategies for lipids and phenolics degradation in the anaerobic treatment of olive mill wastewater. *Water Research*, 46(6), 1684-1692.
- Grant, S., Christian, S., Vite, E., Juarez, V.** (2010). Anaerobic membrane bioreactor (AnMBR) pilot-scale treatment of stillage from tequila production. In: *Proceedings of IWA 12th World Congress on Anaerobic Digestion*, Guadalajara, Mexico.
- Grelier, P., Rosenberger, S., Tazi-Pain, A.** (2006). Influence of sludge retention time on membrane bioreactor hydraulic performance. *Desalination*, 192(1-3), 10-17.
- Grelot, A., Tazi-Pain, A., Weinrich, L., Lesjean, B., Grasmick, A.** (2009). Evaluation of a novel flat sheet MBR filtration system. *Desalination*, 236(1-3), 111-119.

- Grethlein, H.E.** (1978). Anaerobic digestion and membrane separation of domestic wastewater. *Journal of the Water Pollution Control Federation*, 50(4), 754-763.
- Guglielmi, G., Chiarani, D., Saroj, D.P., Andreottola, G.** (2010). Sludge filterability and dewaterability in a membrane bioreactor for municipal wastewater treatment. *Desalination*, 250(2), 660-665.
- Hanaki, K., Matsuo, T., Nagase, M.** (1981). Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. *Biotechnology and Bioengineering*, 23(7), 1591-1610.
- Hatamoto, M., Imachi, H., Ohashi, A. and Harada, H.** (2007). Identification and cultivation of anaerobic, syntrophic long-chain fatty acid-degrading microbes from mesophilic and thermophilic methanogenic sludges. *Applied and Environmental Microbiology*, 73(4), 1332-1340.
- Hawkes F.R., Donnely T., Anderson G.K.** (1995). Comparative performance of anaerobic digesters operating on ice-cream wastewater. *Water Research*, 29(2), 525-533.
- He, Y., Xu, P., Li, C., Zhang, B.** (2005). High-concentration food waste treatment by an anaerobic membrane bioreactor. *Water Research*, 39(17), 4110-4118.
- Ho, J., Sung, S.** (2009). Effects of solid concentrations and cross-flow hydrodynamics on microfiltration of anaerobic sludge. *Journal of Membrane Science*, 345(1-2), 142-147.
- Hu, A.Y., Stuckey, D.** (2007). Activated carbon addition to a submerged anaerobic membrane bioreactor: Effect on performance, transmembrane pressure, and flux. *Journal of Environmental Engineering*, 133(1), 73-80.
- Hulshoff Pol, L.W., de Castro Lopes, S.I., Lettinga, G., Lens, P.N.L.** (2004). Anaerobic sludge granulation. *Water Research*, 38(6), 1376-1389.
- Hwu, C., Donlon, B., Lettinga, G.** (1996). Comparative toxicity of long-chain fatty acid to anaerobic sludges from various origins. *Water Science and Technology*, 34(5-6), 351-358.
- Hwu, C.S.** (1997). Enhancing Anaerobic Treatment of Wastewaters Containing Oleic Acid. *PhD Thesis*, Wageningen University, Wageningen, the Netherlands.
- Hwu, C., Lettinga G.** (1997). Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme and Microbial Technology*, 21(4), 297-301.
- Hwu, C., Tseng S., Yuan C., Kulik Z., Lettinga G.** (1998a). Biosorption of long-chain fatty acids in UASB treatment process. *Water Research*, 32(5), 1571-1579.
- Hwu, C., van Lier, J.B., Lettinga, G.** (1998b). Physicochemical and biological performance of expanded granular sludge bed reactors treating long-chain fatty acids. *Process Biochemistry*, 33(1), 75-81.
- Ince, O., Anderson, G.K., Kasapgil, B.** (1995). Effect of Changes in Composition of Methanogenic Species on Performance of a Membrane Anaerobic

- Reactor System Treating Brewery Wastewater. *Environmental Technology*, 16(10), 901-914.
- Irani, R.R., Callis, E.F.** (1960). Metal complexing by phosphorus compounds. II. solubilities of calcium soap of linear carboxylic acids. *The Journal of Physical Chemistry*, 64(11), 1741-1743.
- Ismail, S.B., de La Parra, C.J., Temmink, H., van Lier, J.B.** (2010). Extracellular polymeric substances (EPS) in upflow anaerobic sludge blanket (UASB) reactors operated under high salinity conditions. *Water Research*, 44(6), 1909-1917.
- Jeison, D., van Lier, J.B.** (2006a). On-line cake-layer management by trans-membrane pressure steady state assessment in anaerobic membrane bioreactors for wastewater treatment. *Biochemical Engineering Journal*, 29(3), 204-209.
- Jeison, D., van Lier, J.B.** (2006b). Cake layer formation in anaerobic submerged membrane bioreactors (AnSMBR) for wastewater treatment. *Journal of Membrane Science*, 284(1-2), 227-236.
- Jeison D.** (2007). Anaerobic Membrane Bioreactors for Wastewater Treatment: Feasibility and Potential Applications. *PhD Thesis*, Wageningen University, Wageningen, the Netherlands.
- Jeison, D., van Lier, J.B.** (2007a). Thermophilic treatment of acidified and partially acidified wastewater using an anaerobic submerged MBR: Factors governing long term operational flux. *Water Research*, 41(17), 3868-3879.
- Jeison, D., van Lier, J.B.** (2007b). Cake formation and consolidation: Main factors governing the applicable flux in anaerobic submerged membrane bioreactors (AnSMBR) treating acidified wastewaters. *Separation and Purification Technology*, 56(1), 71-78.
- Jeison, D., Plugge, C., Pereira, A., van Lier, J.B** (2009a). Effects of the acidogenic biomass on the performance of an anaerobic membrane bioreactor for wastewater treatment. *Bioresource Technology*, 100(6), 1951-1956.
- Jeison, D, Telkamp, P, van Lier, JB.** (2009b). Thermophilic sidestream anaerobic membrane bioreactors: the shear rate dilemma. *Water Environment Research*, 81(11), 2372-2380.
- Kalyuzhnyi, S.V., Davlyatshina, M.A.** (1997). Batch anaerobic digestion of glucose and its mathematical modeling. I. Kinetic Investigations. *Bioresource Technology*, 59(1), 73-80.
- Kalyuzhnyi, S., de los Santos L.E., Martinez J.R.** (1998). Anaerobic treatment of raw and preclarified potato-maize wastewaters in a UASB reactor. *Bioresource Technology*, 66(3), 195-199.
- Kanai, M., Ferre, V., Wakahara, S., Yamamoto, T., Moro, M.** (2010). A novel combination of methane fermentation and MBR-Kubota submerged anaerobic membrane bioreactor process. *Desalination*, 250(3), 960-967.

- Kang, I.-J., Yoon, S.-H., Lee, C.-H.** (2002). Comparison of the filtration characteristics of organic and inorganic membranes in a membrane-coupled anaerobic bioreactor. *Water Research*, 36(7), 1803-1813.
- Kataoka, N., Tokiwa, Y., Tanaka, Y., Fujiki, K., Taroda, H., Takeda, K.** (1992). Examination of Bacterial Characteristics of Anaerobic Membrane Bioreactors in Three Pilot-Scale Plants for Treating Low-Strength Wastewater by Application of the Colony-Forming-Curve Analysis Method. *Applied and Environmental Microbiology*, 58(9), 2751-2757.
- Khongnakorn, W., Wisniewski, C., Pottier, L., Vachoud, L.** (2007). Physical properties of activated sludge in a submerged membrane bioreactor and relation with membrane fouling. *Separation and Purification Technology*, 55(1), 125-131.
- Kim, M., Ahn, Y.H., Speece, R.E.** (2002). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water Research*, 36(17), 4369-4385.
- Kim, Y., Mosier, N.S., Hendrickson, R., Ezeji, T., Blaschek, H., Dien, B., Cotta, M., Dale, B., Ladisch, M.R.** (2008). Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. *Bioresource Technology*, 99(12), 5165-5176.
- Kim, J., Shin, S.G., Han, G., O'Flaherty, V., Lee, C., Hwang, S.** (2011). Common key acidogen populations in anaerobic reactors treating different wastewaters: Molecular identification and quantitative monitoring. *Water Research*, 45(8), 2539-2549.
- Kimura, S.** (1991). Japan's Aqua Renaissance '90 Project. *Water Science and Technology*, 23(7-9), 1573-1582.
- Kleyböcker, A., Liebrich, M., Verstraete, W., Kraume, M., Würdemann, H.** (2012). Early warning indicators for process failure due to organic overloading by rapeseed oil in one-stage continuously stirred tank reactor, sewage sludge and waste digesters. *Bioresource Technology* 123, 534-541.
- Kougiass, P.G., De Francisci, D., Treu, L., Campanaro, S., Angelidaki, I.** (2014). Microbial analysis in biogas reactors suffering by foaming incidents. *Bioresource Technology*, 167, 24-32.
- Krzeminski, P.** (2013). Activated Sludge Filterability and Full Scale Membrane Bioreactor Operation. *PhD Thesis*, Delft University of Technology, Delft, the Netherlands.
- Laera, G., Pollice, A., Saturno, D., Giordano, C., Sandulli, R.** (2009). Influence of sludge retention time on biomass characteristics and cleaning requirements in a membrane bioreactor for municipal wastewater treatment. *Desalination*, 236(1-3), 104-110.
- Lalman, J.A., Bagley, D.M.** (2000). Anaerobic degradation and inhibitory effects of linoleic acid. *Water Research*, 34(17), 4220-4228.
- Lalman, J.A., Bagley, D.M.** (2001). Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids. *Water Research*, 35(12), 2975-2983.

- Laspidou, C.S., Rittmann, B.E.** (2002). A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Research*, 36(11), 2711-2720.
- Le Clech, P., Jefferson, B., Chang, I.S., Judd, S.J.** (2003). Critical flux determination by the flux-step method in a submerged membrane bioreactor. *Journal of Membrane Science*, 227(1-2), 81-93.
- Le-Clech, P., Chen, V., Fane, T.A.G.** (2006). Fouling in membrane bioreactors used in wastewater treatment. *Journal of Membrane Science*, 284(1-2), 17-53.
- Lee, W., Kang, S., Shin, H.** (2003). Sludge characteristics and their contribution to microfiltration in submerged membrane bioreactors. *Journal of Membrane Science*, 216(1-2), 217-227.
- Lefebvre, O., Moletta, R.** (2006). Treatment of organic pollution in industrial saline wastewater: A literature review. *Water Research*, 40(20), 3671-3682.
- Lefebvre, O., Vasudevan, N., Torrijos, M., Thanasekaran, K., Moletta, R.** (2006). Anaerobic digestion of tannery soak liquor with an aerobic post-treatment. *Water Research*, 40(7), 1492-1500.
- Lettinga, G., van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W., Klapwijk A.** (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, 22(4), 699-734.
- Liang, S., Liu, C., Song, L.** (2007). Soluble microbial products in membrane bioreactor operation: Behaviors, characteristics, and fouling potential. *Water Research*, 41(1), 95-101.
- Liang, S., Qu, L., Meng, F., Han, X., Zhang, J.** (2013). Effect of sludge properties on the filtration characteristics of self-forming dynamic membranes (SFDMs) in aerobic bioreactors: formation time, filtration resistance, and fouling propensity. *Journal of Membrane Science*, 436, 186-194.
- Liao, B.Q., Allen, D.G., Droppo, I.G., Leppard, G.G., Liss, S.N.** (2001). Surface properties of sludge and their role in bioflocculation and settleability. *Water Research*, 35(2), 339-350.
- Liao, B.-Q., Kraemer, J.T., Bagley, D.M.** (2006). Anaerobic membrane bioreactors: Applications and research directions. *Critical Reviews in Environmental Science and Technology*, 36(6), 489-530.
- Lin, H.J., Xie, K., Mahendran, B., Bagley, D.M., Leung, K.T., Liss, S.N., Liao, B.Q.** (2009). Sludge properties and their effects on membrane fouling in submerged anaerobic membrane bioreactors (SAnMBRs). *Water Research*, 43(15), 3827-3837.
- Lin, H.J., Xie, K., Mahendran, B., Bagley, D.M., Leung, K.T., Liss, S.N., Liao, B.Q.** (2010). Factors affecting sludge cake formation in a submerged anaerobic membrane bioreactor. *Journal of Membrane Science*, 361(1-2), 126-134.

- Lin, H., Liao, B.-Q., Chen, J., Gao, W., Wang, L., Wang, F., Lu, X.** (2011). New insights into membrane fouling in a submerged anaerobic membrane bioreactor based on characterization of cake sludge and bulk sludge. *Bioresource Technology*, 102(3), 2373-2379.
- Liu, Y., Liu, Z., Zhang, A., Chen, Y., Wang, X.** (2012a). The role of EPS concentration on membrane fouling control: Comparison analysis of hybrid membrane bioreactor and conventional membrane bioreactor. *Desalination*, 305, 38-43.
- Liu, Y., Liu, H., Cui, L., Zhang, K.** (2012b). The ratio of food-to-microorganism (F/M) on membrane fouling of anaerobic membrane bioreactors treating low-strength wastewater. *Desalination*, 297, 97-103.
- Lousada-Ferreira, M.** (2011). Filterability and Sludge Concentration in Membrane Bioreactors. *PhD Thesis*, Delft University of Technology, Delft, the Netherlands.
- Lousada-Ferreira, M., Krzeminski, P., Geilvoet, S., Moreau, A., Gil, J.A., Evenblij, H., van Lier, J.B., van der Graaf, J.** (2014). The Delft Filtration Characterization method (DFCm) as a tool to assess membrane bioreactor activated sludge filterability- 10 years of experience. *Membranes*, 4, 227-242 (doi:10.3390/membranes4020227).
- Lousada-Ferreira, M., van Lier, J.B., van der Graaf, J.H.J.M.** (2015). Impact of suspended solids concentration on sludge filterability in full-scale membrane bioreactors. *Journal of Membrane Science*, 476, 68-75.
- Lyko, S., Wintgens, T., Alhalbouni, D., Baumgarten, S., Tacke, D., Drensla, K., Janot, A., Dott, W., Pinnekamp, J., Melin, T.** (2008). Long-term monitoring of a full-scale municipal membrane bioreactor - Characterisation of foulants and operational performance. *Journal of Membrane Science*, 317(1-2), 78-87.
- Malaspina, F., Cellamare, L., Stante, L., Tilche, A.** (1996). Anaerobic treatment of cheese whey with a downflow-upflow hybrid reactor. *Bioresource Technology* 55(2), 131-139.
- Markou, G., Nerantzis, E.** (2013). Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. *Biotechnology Advances* 31(8), 1532-1542.
- Martin, I., Pidou, M., Soares, S., Judd, S., Jefferson, B.** (2011). Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. *Environmental Technology*, 32(9), 921-932.
- Martínez, E.J., Fierro, J., Sánchez, M.E., Gómez, X.** (2012). Anaerobic co-digestion of FOG and sewage sludge: Study of the process by fourier transform infrared spectroscopy. *International Biodeterioration and Biodegradation*, 75, 1-6.
- Massé, A., Spérandio, M., Cabassud, C.** (2006). Comparison of sludge characteristics and performance of a submerged membrane bioreactor and an activated sludge process at high solids retention time. *Water Research*, 40(12), 2405-2415.

- McAdam, E., Judd, S., Cartmell, E., Jefferson, B.** (2007). Influence of substrate on fouling in anoxic immersed membrane bioreactors. *Water Research*, 41(17), 3859-3867.
- Meabe, E., Déléris, S., Soroa, S., Sancho, L.** (2013). Performance of anaerobic membrane bioreactor for sewage sludge treatment: Mesophilic and thermophilic processes. *Journal of Membrane Science*, 446, 26-33.
- Meng, F., Zhang, H., Yang, F., Zhang, S., Li, Y. and Zhang, X.** (2006). Identification of activated sludge properties affecting membrane fouling in submerged membrane bioreactors. *Separation and Purification Technology*, 51(1), 95-103.
- Meng, F., Chae, S.-R., Drews, A., Kraume, M., Shin, H.-S., Yang, F.** (2009). Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research*, 43(6), 1489-1512.
- Metzger, U., Le-Clech, P., Stuetz, R.M., Frimmel, F.H., Chen, V.** (2007). Characterization of polymeric fouling in membrane bioreactors and the effect of different filtration modes. *Journal of Membrane Science*, 301(1-2), 180-189.
- Mikkelsen, L.H., Keiding, K.** (2002). Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water Research*, 36(10), 2451-2462.
- Miron, Y., Zeeman, G., van Lier, J.B., Lettinga, G.** (2000). The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. *Water Research*, 34(5), 1705-1713.
- Mockaitis, G., Ratusznei, S.M., Rodrigues, J.A.D., Zaiat, M., Foresti, E.** (2006). Anaerobic whey treatment by a stirred sequencing batch reactor (ASBR): effects of organic loading and supplemented alkalinity. *Journal of Environmental Management*, 79(2), 198-206.
- Moreau, R.A., Liu, K., Winkler-Moser, J.K., Singh, V.** (2011). Changes in lipid composition during dry grind ethanol processing of corn. *Journal of the American Oil Chemists' Society*, 88(3), 435-442.
- Mota, V.T., Santos, F.S., Amaral, M.C.S.** (2013). Two-stage anaerobic membrane bioreactor for the treatment of sugarcane vinasse: Assessment on biological activity and filtration performance. *Bioresource Technology*, 146, 494-503.
- Nederlandse norm (NEN) 6671** (1994). *Afvalwater en slib. Gravimetrische bepaling van het gehalte aan petroleumether extraheerbare olien en vetten*. Normalisatie-instituut, Nederlands, UCD 626.31:543.383.056.
- Neves, L., Pereira, M.A., Mota, M., Alves, M.M.** (2009a). Detection and quantification of long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids. *Bioresource Technology*, 100(1), 91-96.
- Neves, L., Oliveira, R., Alves, M.M.** (2009b). Fate of LCFA in the co-digestion of cow manure, food waste and discontinuous addition of oil. *Water Research*, 43(20), 5142-5150.

- Nielsen, P.H., Roslev, P., Dueholm, T.E., Nielsen, J.L.** (2002). *Microthrix parvicella*, a specialized lipid consumer in anaerobic-aerobic activated sludge plants. *Water Science and Technology*, 46(1-2), 73-80.
- Noureddini, H., Bandlamudi, S.R.P., Guthrie, E.A.** (2009). A novel method for the production of biodiesel from the whole stillage-extracted corn oil. *Journal of the American Oil Chemists' Society*, 86(1), 83-91.
- Omil, F., Mendez, R., Lema, J.M.** (1995). Anaerobic treatment of saline wastewaters under high sulphide and ammonia content. *Bioresource Technology*, 54(3), 269-278.
- Ozgun, H., Dereli, R.K., Ersahin, M.E., Kinaci, C., Spanjers, H., van Lier, J.B.** (2013). A review of anaerobic membrane bioreactors for municipal wastewater treatment: Integration options, limitations and expectations. *Separation and Purification Technology*, 118, 89-104.
- Padmasiri, S.I., Zhang, J., Fitch, M., Norddahl, B., Morgenroth, E., Raskin, L.** (2007). Methanogenic population dynamics and performance of an anaerobic membrane bioreactor (AnMBR) treating swine manure under high shear conditions. *Water Research*, 41(1), 134-144.
- Palatsi, J., Affes, R., Fernandez, B., Pereira, M.A., Alves, M.M., Flotats, X.** (2012). Influence of adsorption and anaerobic granular sludge characteristics on long chain fatty acids inhibition process. *Water Research*, 46(16), 5268-5278.
- Pan, J.R., Su, Y.-C., Huang, C., Lee, H.-C.** (2010). Effect of sludge characteristics on membrane fouling in membrane bioreactors. *Journal of Membrane Science*, 349(1-2), 287-294.
- Park, H., Choo, K., Lee, C.** (1999). Flux enhancement with powdered activated carbon addition in the membrane anaerobic bioreactor. *Separation Science and Technology*, 34(14), 2781-2792.
- Pereira, M.A., Mota, M., Alves, M.M.** (2002). Operation of an anaerobic filter and EGSB reactor for the treatment of an oleic acid-based effluent: Influence of inoculum quality. *Process Biochemistry*, 37(9), 1025-1031.
- Pereira M.A., Sousa D.Z., Mota M., Alves M.M.** (2004). Mineralization of LCFA associated to anaerobic sludge: Kinetics, enhancement of methanogenic activity and effect of VFA. *Biotechnology and Bioengineering*, 88(4), 502-511.
- Pereira, M.A., Pires, O.C., Mota, M., Alves, M.M.** (2005). Anaerobic biodegradation of oleic and palmitic acids: evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. *Biotechnology and Bioengineering* 92(1), 15-23.
- Pollice, A., Laera, G., Saturno, D., Giordano, C.** (2008). Effects of sludge retention time on the performance of a membrane bioreactor treating municipal sewage. *Journal of Membrane Science*, 317, 65-70.

- Qin, L., Zhang, G., Meng, Q., Zhang, H., Xu, L., Lv, B.** (2012). Enhanced submerged membrane bioreactor combined with biosurfactant rhamnolipids: Performance for frying oil degradation and membrane fouling reduction. *Bioresource Technology*, 126, 314–320.
- Quarmby, J., Forster, C.F.** (1995). A comparative-study of the structure of thermophilic and mesophilic anaerobic granules. *Enzyme and Microbial Technology*, 17(6), 493-498.
- Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Lata, K. and Kishore, V.V.N.** (2000). State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renewable and Sustainable Energy Reviews*, 4(2), 135-156.
- Ramos, C., Zecchino, F., Ezquerro, D., Diez, V.** (2014). Chemical cleaning of membranes from an anaerobic membrane bioreactor treating food industry wastewater. *Journal of Membrane Science*, 458, 179-188.
- Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F.** (2013). Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, 103, 444-467.
- Rinzema, A., van Lier, J., Lettinga, G.** (1988). Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme and Microbial Technology*, 10(1), 24-32.
- Rinzema, A., Alphenaar, A., Lettinga, G.** (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting acetate. *Journal of Chemical Technology and Biotechnology*, 46(4), 257-266.
- Rinzema, A., Alphenaar, A., Lettinga, G.** (1993). Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. *Process Biochemistry*, 28(8), 527-537.
- Rinzema, A., Boone, M., van Knippenberg K., Lettinga, G.** (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environment Research*, 66(1), 40-49.
- Rosenberg, M., Gutnick D., Rosenberg, E.** (1980) Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters*, 9(1), 29-33.
- Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.-C.** (2006). Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. *Water Research*, 40(4), 710-720.
- Roy, F., Albagnac, G., Samain, E.** (1985). Influence of calcium addition on growth of highly purified syntrophic cultures degrading long-chain fatty acids. *Applied Environmental Microbiology*, 49(3), 702-705.
- Saddoud A., Hassairi, I., Sayadi, S.** (2007). Anaerobic membrane reactor with phase separation for the treatment of cheese whey. *Bioresource Technology*, 98(11), 2102-2108.

- Saddoud, A., Sayadi, S.** (2007). Application of acidogenic fixed-bed reactor prior to anaerobic membrane bioreactor for sustainable slaughterhouse wastewater treatment. *Journal of Hazardous Materials*, 149(3), 700-706.
- Sainbayar, A., Kim, J.S., Jung, W.J., Lee, Y.S., Lee, C.H.** (2001). Application of surface modified polypropylene membranes to an anaerobic membrane bioreactor. *Environmental Technology*, 22(9), 1035-1042.
- Sam-Soon, P., Loewenthal, R.E., Wentzel, M.C., Marais, G.V.R.** (1991). A long-chain fatty acid, oleate, as sole substrate in upflow anaerobic sludge bed (UASB) reactor systems. *Water SA*, 17(1), 31-36.
- Savant, D.V., Abdul-Rahman, R., Ranade, D.R.** (2006). Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. *Bioresource Technology*, 97(9), 1092-1104.
- Sawalha, O., Scholz, M.** (2010). Modeling the relationship between capillary suction time and specific resistance to filtration. *Journal of Environmental Engineering*, 136(9), 983-991.
- Sayed, S., van Campen, L., Lettinga, G.** (1987). Anaerobic treatment of slaughterhouse waste using a granular sludge UASB reactor. *Biological Wastes*, 2(1), 11-28.
- Shen, Y., Zhao, W., Xiao, K., Huang, X.** (2010). A systematic insight into fouling propensity of soluble microbial products in membrane bioreactors based on hydrophobic interaction and size exclusion. *Journal of Membrane Science*, 346(1), 187-193.
- Siegrist, H., Hunziker, W., Hofer, H.** (2005). Anaerobic digestion of slaughterhouse waste with UF-membrane separation and recycling of permeate after free ammonia stripping. *Water Science and Technology*, 52(1-2), 531-536.
- Silvestre, G., Rodríguez-Abalde, A., Fernández, B., Flotats, X., Bonmati, A.** (2011). Biomass adaptation over anaerobic co-digestion of sewage sludge and trapped grease waste. *Bioresource Technology*, 102(13), 6830-6836.
- Singh, K., Burke, D., Grant, S.** (2010). Anaerobic flat sheet membrane bioreactor treating food processing wastewater: Pilot-scale performance. In: *Proceedings of IWA 12th World Congress on Anaerobic Digestion*, Guadalajara, Mexico.
- Siso, M.I.G.** (1996). The biotechnological utilization of cheese whey: A review. *Bioresource Technology*, 57(1), 1-11.
- Soto, M., Mendez, R., Lema, J.M.** (1992). Characterization and comparison of biomass from mesophilic and thermophilic fixed bed anaerobic digesters. *Water Science and Technology*, 25(7), 203-212.
- Speece, R.E.** (1996). *Anaerobic Biotechnology for Industrial Wastewaters*. Archae Press.

- Stroot, P.G., McMahon, K.D., Mackie, R.I., Raskin, L.** (2001). Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions - I. Digester performance. *Water Research*, 35(7), 1804-1816.
- Stuckey, D.C.** (2010). Anaerobic membrane reactors (Chapter 7). In: Fang, H.H.P. (Ed.), *Environmental Anaerobic Technology: Applications and New Developments*. Imperial College Press, London, UK.
- Suhartini, S., Heaven, S., Banks, C.J.** (2014). Comparison of mesophilic and thermophilic anaerobic digestion of sugar beet pulp: Performance, dewaterability and foam control. *Bioresource Technology*, 152, 202-211.
- Tao, Y., Gao, D.-W., Fu, Y., Wu, W.-M., Ren, Q.-J.** (2012). Impact of reactor configuration on anammox process start-up: MBR versus SBR. *Bioresource Technology* 104, 73-80.
- Tay, J.H., He, Y.X., Yan, Y.G.** (2000). Anaerobic biogranulation using phenol as the sole carbon source. *Water Environment Research*, 72(2), 189-194.
- Thiele, J.H., Chartrain, M., Zeikus, J.G.** (1988). Control of interspecies electron flow during anaerobic digestion: Role of flock formation in syntrophic methanogenesis. *Applied and Environmental Microbiology*, 54(1), 10-19.
- Tian, Y., Chen, L., Zhang, S., Zhang, S.** (2011). A systematic study of soluble microbial products and their fouling impacts in membrane bioreactors. *Chemical Engineering Journal*, 168(3), 1093-1102.
- Torres, A., Hemmelmann, A., Vergara, C., Jeison, D.** (2011). Application of two-phase slug-flow regime to control flux reduction on anaerobic membrane bioreactors treating wastewaters with high suspended solids concentration. *Separation and Purification Technology*, 79(1), 20-25.
- Trzcinski, A.P., Stuckey, D.C.** (2009). Continuous treatment of the organic fraction of municipal solid waste in an anaerobic two-stage membrane process with liquid recycle. *Water Research*, 43(9), 2449-2462.
- Trzcinski, A.P., Stuckey, D.C.** (2010). Treatment of municipal solid waste leachate using a submerged anaerobic membrane bioreactor at mesophilic and psychrophilic temperatures: Analysis of recalcitrants in the permeate using GC-MS. *Water Research*, 44(3), 671-680.
- Van den Broeck, R., Krzeminski, P., Van Dierdonck, J., Gins, G., Lousada-Ferreira, M., Van Impe, J.F.M., van der Graaf, J.H.J.M., Smets, I.Y., van Lier, J.B.** (2011). Activated sludge characteristics affecting sludge filterability in municipal and industrial MBRs: Unraveling correlations using multi-component regression analysis. *Journal of Membrane Science*, 378(1-2), 330-338.
- van Langerak, E.P.A., Ramaekers, H., Wiechers, J., Veeken, A.H.M., Hamelers, H.V.M., Lettinga, G.** (2000). Impact of location of CaCO_3 precipitation on the development of intact anaerobic sludge. *Water Research*, 34(2), 437-446.

- van Lier, J.B., Grolle, K.C.F., Stams, A.J.M., de Macario, E.C., Lettinga, G.** (1992). Start-up of a thermophilic upflow anaerobic sludge bed (UASB) reactor with mesophilic granular sludge. *Applied Microbiology and Biotechnology*, 37(1), 130-135.
- van Lier J.B., Tilche, A., Ahring, B.K., Macarie, H., Moletta, R., Dohanyos, M., Pol, L.W., Lens, P., Verstraete, W.** (2001). New perspectives in anaerobic digestion. *Water Science and Technology*, 43(1), 1-18.
- van Lier J.B.** (2008). High-rate anaerobic wastewater treatment: diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. *Water Science and Technology*, 57(8), 1137-1148.
- van Lier, J.B., Mahmoud, N., Zeeman, G.** (2008). Anaerobic Wastewater Treatment (Chapter 16). in: M. Henze, M.C.M. van Loosdrecht, G.A. Ekama, D. Brdjanovic (ed.), *Biological Wastewater Treatment, Principles, Modelling and Design*, IWA Publishing, London, UK.
- Van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, G., Zehnder, A.J.B.** (1987). The Role of Bacterial Cell Wall Hydrophobicity in Adhesion. *Applied and Environmental Microbiology*, 53(8), 1893-1897.
- van Zyl, P.J., Wentzel, M.C., Ekama, G.A., Riedel, K.J.** (2008). Design and start-up of a high rate anaerobic membrane bioreactor for the treatment of a low pH, high strength, dissolved organic wastewater. *Water Science and Technology*, 57(2), 291-295.
- Vyrides, I., Stuckey, D.C.** (2009). Saline sewage treatment using a submerged anaerobic membrane reactor (SAMBR): Effects of activated carbon addition and biogas-sparging time. *Water Research*, 43(4), 933-942.
- Vyrides, I., Stuckey, D.C.** (2010). Fouling cake layer in a submerged anaerobic membrane bioreactor (SAMBR) treating saline wastewaters: curse or a blessing? In: *Proceedings of IWA 12th World Congress on Anaerobic Digestion*, Guadalajara, Mexico.
- Wang, Z., Wu, Z., Yu, G., Liu, J., Zhou, Z.** (2006). Relationship between sludge characteristics and membrane flux determination in submerged membrane bioreactors. *Journal of Membrane Science*, 284(1-2), 87-94.
- Wang, H., Wang, T., Johnson, L.A., Pometto III., A.L.** (2008). Effect of corn breaking method on oil distribution between stillage phases of dry-grind corn ethanol production. *Journal of Agricultural and Food Chemistry*, 56(21), 9975-9980.
- Wilen, B.-M., Jin, B., Lant, P.** (2003). The influence of key chemical constituents in activated sludge on surface and flocculating properties. *Water Research*, 37(9), 2127-2139.
- Wilkie, A.C., Riedesel, K.J., Owens, J.M.** (2000). Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass and Bioenergy*, 19(2), 63-102.
- Winkler-Moser, J.K., Breyer, L.** (2011). Composition and oxidative stability of crude oil extracts of corn germ and distillers grains. *Industrial Crops and Products*, 33(3), 572-578.

- Wisniewski, C., Grasmick, A.** (1998). Floc size distribution in a membrane bioreactor and consequences for membrane fouling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 138(2), 403-411.
- Wu, Z., Wang, Z., Zhou, Z., Yu, G., Gu, G.** (2007). Sludge rheological and physiological characteristics in a pilot-scale submerged membrane bioreactor. *Desalination*, 212(1-3), 152-164.
- Xiao, K., Wang, X., Huang, X., Waite, T.D., Wen, X.** (2011). Combined effect of membrane and foulant hydrophobicity and surface charge on adsorptive fouling during microfiltration. *Journal of Membrane Science*, 373, 140-151.
- Xie, K., Lin, H.J., Mahendran, B., Bagley, D.M., Leung, K.T., Liss, S.N., Liao, B.Q.** (2010). Performance and fouling characteristics of a submerged anaerobic membrane bioreactor for evaporator condensate treatment. *Environmental Technology*, 31(5), 511-521.
- Xu, H., He, P., Yu, G., Shao, L.** (2011). Effect of ultrasonic pretreatment on anaerobic digestion and its sludge dewaterability. *Journal of Environmental Science*, 23(9), 1472-1478.
- Yang, K., Yu, Y., Hwang, S.** (2003). Selective optimization in thermophilic acidogenesis of cheese-whey wastewater to acetic and butyric acids: partial acidification and methanation. *Water Research*, 37(10), 2467-2477.
- Yang, B., Chen, G., Chen, G.** (2012a). Submerged membrane bioreactor in treatment of simulated restaurant wastewater. *Separation and Purification Technology*, 88, 184-190.
- Yang, J., Vedantam, S., Spanjers, H., Nopens, I., van Lier, J.B.** (2012b). Analysis of mass transfer characteristics in a tubular membrane using CFD modeling. *Water Research*, 46(15), 4705-4712.
- Yigit, N.O., Civelekoglu, G., Harman, I., Koseoglu, H., Kitis, M.** (2009). Effects of various backwash scenarios on membrane fouling in a membrane bioreactor. *Desalination*, 237(1-3), 346-356.
- Yilmazer, G., Yenigun, O.** (1999). Two-phase anaerobic treatment of cheese whey. *Water Science and Technology*, 40(1), 289-295.
- Yu, J., Pinder, K.L.** (1993). Intrinsic fermentation kinetics of lactose in acidogenic biofilms. *Biotechnology and Bioengineering*, 41(4), 479-488.
- Zhang, J., Padmasiri, S.I., Fitch, M., Norddahl, B., Raskin, L., Morgenroth, E.** (2007). Influence of cleaning frequency and membrane history on fouling in an anaerobic membrane bioreactor. *Desalination*, 207(1-3), 153-166.
- Zhang, Y., Yan, L., Chi, L., Long, X., Mei, Z., Zhang, Z.** (2008). Startup and operation of anaerobic EGSB reactor treating palm oil mill effluent. *Journal of Environmental Sciences-China* 20(6), 658-663.

CURRICULUM VITAE

Name Surname: Recep Kaan DERELI
Place and Date of Birth: Istanbul, Turkey / 24.05.1980
E-Mail: derelir@itu.edu.tr; rkaandereli@yahoo.com

EDUCATION:

B.Sc.: 2003, Yildiz Technical University, Civil Engineering Faculty, Environmental Engineering Department
M.Sc.: 2006, Istanbul Technical University, Environmental Engineering Department, Environmental Sciences and Engineering Programme

PROFESSIONAL EXPERIENCE AND REWARDS:

- 2010 TINCEL FOUNDATION Scholarship
- 2012 Turkish Academy of Sciences (TUBA) Integrated Doctorate Programme Scholarship (6 months)

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

Journal papers

- **Dereli, R.K.**, Wang, X., van der Zee, F.P., van Lier, J.B. (2015) Biological Performance and Sludge Filterability of Anaerobic Membrane Bioreactors Under Nitrogen Limited Conditions (*Submitted to a journal*).
- **Dereli, R.K.**, Loverdou, L., van der Zee, F.P., van Lier, J.B. (2015) A systematic study on the effect of substrate acidification degree and acidogenic biomass on sludge filterability. *Water Research* 82, 94-103.
- **Dereli, R.K.**, Heffernan, B., Grelot, A., van der Zee, F.P., van Lier, J.B. (2015) Influence of high lipid containing wastewater on filtration performance and fouling in AnMBRs operated at different sludge retention times. *Separation and Purification Technology* 139, 43-52.
- **Dereli, R.K.**, Grelot, A., Heffernan, B., van der Zee, F.P., van Lier, J.B. (2014) Implications of changes in solids retention time on long term evolution of sludge filterability in anaerobic membrane bioreactors treating high strength industrial wastewater. *Water Research* 59, 11-22.
- **Dereli, R.K.**, van der Zee, F.P., Heffernan, B., Grelot, A., van Lier, J.B. (2014) Effect of sludge retention time on the biological performance of anaerobic

membrane bioreactors treating corn-to-ethanol thin stillage with high lipid content. *Water Research* 49, 453-464.

- **Dereli, R.K.**, Ersahin, M.E., Ozgun, H., Ozturk, I., Jeison, D., van der Zee, F., van Lier, J.B. (2012) Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters. *Bioresource Technology* 122, 160-170.

Conference papers

- Grélot, A., **Dereli, R.K.**, van der Zee, F.P., van der Lubbe, J.G.M., Heffernan, B., van Lier, J.B. (2012). Performances of anaerobic membrane bioreactors treating thin stillage from bioethanol plants at different sludge retention times. *EUROMEMBRANE Conference 2012*. 23-27 September, London, England. (poster)
- Heffernan, B., van der Lubbe, J., Grélot, A., **Dereli, R.K.**, van der Zee, F.P. (2012). Economic and carbon footprint evaluation of thin stillage treatment in an AnMBR. *4th International Symposium on Energy from Biomass and Waste*. 12-15 November, Venice, Italy.
- Grélot, A., **Dereli, R.K.**, Heffernan, B., van der Zee, F.P., Pereboom, J. (2012). Anaerobic membrane bioreactor for upgrading the energy balance of bioethanol plants. *4th International Symposium on Energy from Biomass and Waste*. 12-15 November, Venice, Italy.

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS

Journal papers

- Yetilmezsoy, K., Ozgun, H., **Dereli, R.K.**, Ersahin, M.E., Ozturk, I. (2014) Adaptive neuro-fuzzy inference-based modeling of a full-scale expanded granular sludge bed reactor treating corn processing wastewater. *Journal of Intelligent and Fuzzy Systems* 28(4), 1601-1616.
- Ozgun, H., **Dereli, R.K.**, Ersahin, M.E., Kinaci, C., Spanjers, H., van Lier, J.B. (2013) A review of anaerobic membrane bioreactors for municipal wastewater treatment: Integration options, limitations and expectations. *Separation and Purification Technology* 118, 89-104.
- Ersahin, M.E., **Dereli, R.K.**, Ozgun, H., Ozturk, I., Roest, K., van Lier, J.B. (2012) A review on dynamic membrane filtration: Materials, applications and future perspectives. *Bioresource Technology* 122, 196-206.
- **Dereli, R.K.**, Yangin Gomec, C., Ozabali, A., Ozturk, I. (2012) The Feasibility of a centralized biogas plant treating the manure produced by an organised animal farmers union in Turkey. *Water Science and Technology* 66(3), 556-563.
- **Dereli, R.K.**, Urban, D.R., Heffernan, B., Jordan, J.A., Ewing, J., Rosenberger G.T., Dunaev T.I. (2012) Performance evaluation of a pilot scale anaerobic membrane bioreactor (AnMBR) treating ethanol thin stillage. *Environmental Technology* 33(13), 1511-1516.
- Ozgun, H., Karagul, N., **Dereli, R.K.**, Ersahin, M.E., Coskuner, T., Altinbas, M., Ozturk, I. (2012) Confectionery industry: A case study on treatability based

effluent characterization and treatment system performance. *Water Science and Technology* 66(1), 15-20.

- Grelot, A., **Dereli, R.K.**, Van Der Zee, F.P., Van Der Lubbe, J.G.M., Heffernan, B. (2012) Performances of anaerobic membrane bioreactors treating thin stillage from bioethanol plants at different sludge retention times. *Procedia Engineering* 44, 776-779.
- Ersahin, M.E., **Dereli, R.K.**, Ozgun, H., Donmez, B.G., Koyuncu, I., Altinbas, M., Ozturk, I. (2011) Source based characterization and pollution profile of a Baker's Yeast Industry. *Clean-Soil Air Water* 39(6), 543-548.
- Ersahin, M.E., Yangin Gomec, C., **Dereli, R.K.**, Ozturk, I. (2011) Biomethane production as an alternative bioenergy source from codigesters treating municipal sludge and organic fraction of municipal solid wastes. *Journal of Biomedicine and Biotechnology*, Article ID 953065, doi:10.1155/2011/953065.
- **Dereli, R.K.**, Ersahin, M.E., Ozgun, H., Ozturk, I., Aydin, A.F. (2010) Applicability of Anaerobic Digestion Model No.1 (ADM1) for a specific industrial wastewater: Opium alkaloid effluents. *Chemical Engineering Journal* 165(1), 89-94.
- Aydin, A.F., Ersahin, M.E., **Dereli, R.K.**, Sarikaya, H.Z. ve Ozturk, I. (2010) Long-term anaerobic treatability studies on opium alkaloids industry effluents. *Journal of Environmental Science and Health - Part A* 45(2), 192-200.
- **Dereli, R.K.**, Ersahin, M.E., Gomec, C.Y., Ozdemir O., Ozturk, I. (2010) Co-digestion of the organic fraction of municipal solid waste and primary sludge at a municipal wastewater treatment plant in Turkey. *Waste Management Research* 28, 404-410.

Conference papers

- Ozgun, H., Karagul, N., **Dereli, R.K.**, Ersahin, M.E., Coskuner, T., Ciftci, D.I., Ozturk, I., Altinbas, M., (2011). Confectionery industry: A case study on treatability based effluent characterization and treatment system performance. *In Proceedings of 8th IWA International Symposium on Waste Management Problems in AGRO Industries*, AGRO 2011, 22-24 June, Izmir-Turkey. (published in journal)
- **Dereli, R.K.**, Yangin Gomec, C., Ozabali, A. and Ozturk, I., (2011). The feasibility of a centralized biogas plant treating the manure produced by an organized farmers union in Turkey. *In Proceedings of 8th IWA International Symposium on Waste Management Problems in AGRO Industries*, AGRO 2011, 22-24 June, Izmir-Turkey. (published in journal)
- **Dereli R.K.**, Ersahin M.E., Ozgun H., Koyuncu I., Ozturk I, Yildiz S., (2010). Performance evaluation of a full scale membrane bioreactor and nanofiltration system treating landfill leachate. *In Proceedings of IWA Regional Conference and Exhibition on Membrane Technology and Water Reuse, IWA MTWR 2010*, 18-22 October, Istanbul – Turkey. (poster)
- **Dereli, R.K.**, Urban, D.R., Heffernan, B., Jordan, J.A., Ewing, J., Rosenberger, G.T. and Dunaev, T.I., (2011). Performance Evaluation of a Pilot Scale Anaerobic Membrane Bioreactor (AnMBR) treating ethanol thin stillage. *In Proceedings of*

8th IWA International Symposium on Waste Management Problems in AGRO Industries, AGRO 2011, 22-24 June, Izmir-Turkey. (published in journal)