

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

**ANAEROBIC MEMBRANE BIOREACTORS
FOR COST-EFFECTIVE
MUNICIPAL WATER REUSE**

Ph.D. THESIS

Hale ÖZGÜN

Department of Environmental Engineering

Environmental Sciences and Engineering Programme

OCTOBER 2015

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

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ
DELFT TEKNOLOJİ ÜNİVERSİTESİ ★ İNŞAAT MÜHENDİSLİĞİ VE YER
BİLİMLERİ ENSTİTÜSÜ

**EVSEL ATIKSULARIN ANAEROBİK MEMBRAN BİYOREAKTÖRLER İLE
MALİYET ETKİN OLARAK YENİDEN KULLANIMI**

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To my father, Hamdi ÖZGÜN..

Canım Babama..

FOREWORD

This section is the most difficult part of this thesis for me to write since there are lots of things that I would like to express and unfortunately, I am not so good at expressing my deepest feelings by words. Apparently, for me writing this section will be more tough than writing discussion parts of the thesis. But anyway, I will try my best..

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ABBREVIATIONS

AD	: Anaerobic Digestion
AMPTSII	: Automated Methane Potential Test System
AnMBR	: Anaerobic Membrane Bioreactor
BOD	: Biochemical Oxygen Demand
COD	: Chemical Oxygen Demand
CSTR	: Completely Stirred Tank Reactor
DNA	: Deoxyribonucleic Acid
DOC	: Dissolved Organic Carbon
EGSB	: Expanded Granular Sludge Bed
EPS	: Extracellular Polymeric Substances
F/M	: Food To Microorganism Ratio
FB	: Fluidized Bed
FO	: Forward Osmosis
GAC	: Granular Activated Carbon
GLS	: Gas-Liquid-Solids
HRT	: Hydraulic Retention Time
MBR	: Membrane Bioreactor
MF	: Microfiltration
MLSS	: Mixed Liquor Suspended Solids
OLR	: Organic Loading Rate
OSN	: Observed Species Number
OTU	: Operational Taxonomic Unit
P/C	: Protein/Carbohydrate Ratio
PAC	: Powdered Activated Carbon
PBS	: Phosphate Buffered Saline
PEI	: Polyetherimide
PES	: Polyethersulfone
PSD	: Particle Size Distribution
PTFE	: Polytetrafluoroethylene
PVDF	: Polyvinylidene Fluoride
PVP	: Polyvinylpyrrolidone
QIIME	: Quantitative Insights Into Microbial Ecology
rDNA	: Recombinant Deoxyribonucleic Acid
RDP	: Ribosomal Database Project
RO	: Reverse Osmosis
SMA	: Specific Methanogenic Activity
SMP	: Soluble Microbial Products
SRT	: Solids Retention Time
SS	: Suspended Solids
SUR	: Specific Ultrafiltration Resistance
TMP	: Transmembrane Pressure
TN	: Total Nitrogen
TOC	: Total Organic Carbon

TP	: Total Phosphorus
TS	: Total Solids
TSS	: Total Suspended Solids
UASB	: Upflow Anaerobic Sludge Blanket
UF	: Ultrafiltration
VFA	: Volatile Fatty Acid
VS	: Volatile Solids
VSEP	: Vibratory Shear Enhanced Processing
VSS	: Volatile Suspended Solids

SYMBOLS

CO₂	: Carbon Dioxide
D50	: Median Particle Size
J	: Permeate Flux
NaCl	: Sodium Chloride
NaHCO₃	: Sodium Bicarbonate
NaOCl	: Sodium Hypochlorite
NH₄-N	: Ammonium Nitrogen
P	: Membrane Permeability
PO₄³⁻	: Phosphate
R_{intrinsic}	: Intrinsic Resistance
R_{irrecoverable}	: Irrecoverable Resistance
R_{irreversible}	: Irreversible Resistance
R_{removable}	: Removable Resistance
R_T	: Total Filtration Resistance
η	: Dynamic Viscosity Of Water

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ANAEROBIC MEMBRANE BIOREACTORS FOR COST-EFFECTIVE MUNICIPAL WATER REUSE

SUMMARY

In recent years, anaerobic membrane bioreactor (AnMBR) technology has been increasingly researched for municipal wastewater treatment as a means to produce nutrient-rich, solids free effluents with low levels of pathogens, while occupying a small footprint. An AnMBR can be used not only for on-site wastewater treatment, but also for the generation of nutrient-rich irrigation water leading to reuse and recycling possibility for agricultural applications as well. Furthermore, biogas produced in the anaerobic process could potentially be used for minimising the energy requirements of AnMBR operation. Despite the mentioned advantages, the current state-of-the-art AnMBR technology for potential full-scale application raises some concerns related to energy requirements and investment costs for membrane fouling control, as well as the impact of high shear stress on biomass activity. Municipal wastewater in many countries can be characterised as low strength and is generated at high flows rates, related to the population served. However, ongoing efforts in water conservation and the implementation of source separated sewer systems may possibly change municipal wastewater characteristics in the near future.

Several factors involved in design and operation of AnMBR systems may influence membrane fouling, such as treatment plant configuration and membrane characteristics, feeding and biomass properties and operational conditions. Among them, reactor design is proposed as an important factor that can change the AnMBR sludge fouling propensity. To date, especially in the last decade, many studies have been conducted in which various types of anaerobic reactors, including completely stirred tank reactor (CSTR), upflow anaerobic sludge blanket (UASB) reactor, expanded granular sludge bed reactor (EGSB), were used in combination with various types of membranes. Among them, a membrane coupled UASB reactor may be a promising approach to overcome problems related with fouling since the membrane is only subjected to supernatant filtration and not by bulk sludge.

The purpose of this thesis was to investigate the applicability of an innovative AnMBR configuration in order to produce a pathogen-free but nutrient-rich effluent for use in agricultural irrigation, concomitantly enabling energy recovery. With the realization of this study, flux enhancement by controlling the total solids (TS) load to the membrane was the major starting point, bringing AnMBR technology for full-scale sewage treatment one step closer to realisation.

The AnMBR-Digester system as developed in this study may offer a solution for the mentioned challenges. The system consisted of a membrane integrated UASB reactor coupled to a parallel operating digester that can be operated at any required process temperature between 0-40 °C. The external cross-flow tubular membrane module contained 28 membrane fibers with a very small internal tube diameter of 1.5 mm in order to increase the filtration area for accommodating large sewage flows. Blocking

of the membrane module lumens is minimised by the preceding UASB reactor, which scavenges the large part of suspended solids (SS), having only the supernatant subjected to membrane filtration. Besides that, the combination of a UASB reactor and a sludge digester, the so-called UASB-Digester system, has been shown to be successful for mutual sewage treatment at low temperature and sludge stabilization. Therefore, membrane integration to the UASB-Digester system can be attractive for producing high quality and nutrient-rich effluents for reuse purposes under moderate climate conditions.

Based on this general aim, the optimum upflow velocity that will result in an effluent with good filterability is determined in a laboratory-scale UASB reactor for the case of membrane coupled UASB reactor systems. Filterability tests were carried out in order to assess the effect of upflow velocity on subsequent membrane performance. Results indicated a significant impact of upflow velocity on both biological performance and physicochemical effluent characteristics. Operation at a higher upflow velocity caused the washout of colloidal particles. Effluent characterization results coincided with filterability tests. Results showed that filterability of the effluent during the operation at 0.6 m/h was better than that during the operation at 1.2 m/h. The observed differences in protein/carbohydrate ratio (P/C) and particle size distribution (PSD), which play important roles in membrane fouling, lead to the hypothesis that upflow velocity is a critical parameter for effluent filterability in membrane coupled UASB reactors.

In subsequent experiments, the membrane was integrated into the UASB reactor and the system was operated as an AnMBR system with an upflow velocity of 0.6 m/h in order to understand the impact of membrane addition on both the biological performance and sludge characteristics. Membrane incorporation induced an accumulation of fine particles and a decrease in extracellular polymeric substances (EPS), resulting in a decrease in PSD and thus, a drop in sludge settleability. Deterioration of sludge settleability led to an increase in sludge washout, with a resultant increase in chemical oxygen demand (COD) and total suspended solids (TSS) concentrations in the UASB effluent. However, SS-free permeate with an average COD of 42 mg/L was obtained and despite the sludge bed deterioration, the average transmembrane pressure (TMP) value was 85 mbar during reactor operation, indicating that no severe membrane fouling occurred in the AnMBR.

Following that investigation, the AnMBR was operated at 15 °C in order to investigate whether the membrane coupled UASB is a technically feasible alternative in the treatment of municipal wastewater at lower temperatures. The results showed that membrane fouling at 15 °C was more severe than at 25 °C. Increased COD and soluble microbial products (SMP) concentrations, reduced particle's diameter, and higher turbidity in the UASB reactor effluent at lower temperature aggravated membrane fouling. However, treatment performance was not considerably affected by temperature possibly due to the retention capacity of membrane. Cake resistance was found responsible for over 40% of the total fouling at both temperatures. However, an increase was observed in the contribution of irreversible fouling resistance to the total filtration resistance (R_T) at lower temperature, related to the larger amount of fine particles in the UASB reactor effluent.

During the operation at 15 °C, differences were observed in sludge characteristics at different heights along the UASB reactor. The best location in the sludge bed for conveying the sludge from the UASB reactor to the digester needed to be

determined. Analysis over the height of the reactor with time showed that TS, volatile solids (VS), TSS and volatile suspended solids (VSS) concentrations in the reactor decreased with height, and highest COD concentration of 46 g/L was observed at the bottom of the reactor. The active biomass remained near the inlet of the reactor; whereas, non-active biomass consisted of loose, suspended particles and flocculents moved towards the top. This was confirmed by the high specific COD consumption rate near the inlet and the poor specific COD biodegradation in the remaining portions of the bioreactor. Apparently, the assumption of a completely mixed sludge bed behavior for the UASB reactor, being part of an AnMBR system, does not hold for this type of reactor systems even at low temperatures, which makes the location in the sludge bed from where the sludge is to be conveyed to the digester of operational importance. Considering the observed sludge bed stratification with regard to sludge stability and solids concentration, the sludge to be recirculated from the UASB reactor to the digester is recommended to be extracted from the sampling point where the sludge has the lowest stability. A low value of stability coincides with a high amount of anaerobic biodegradable organic compounds present in the sludge.

Finally, the impact of digester coupling on removal efficiency and filtration performance of the AnMBR was investigated. Digester incorporation remarkably influenced the characteristics of the UASB reactor effluent, resulting in a decrease in turbidity, soluble and total COD and an increase in median particle size (D50), which led to a substantial decrease in the R_T . Improved stability and specific methanogenic activity (SMA) of the sludge were achieved in the UASB reactor with an increase in total biogas production of the AnMBR-Digester system. The sludge recirculation from the sampling point having the lowest stability in the AnMBR-Digester system improved both the solids physical removal and the conversion, which confirms the importance of sludge transfer point selection, especially for the filtration performance of AnMBR-Digester systems.

Overall, it can be concluded that the UASB reactor is a suitable alternative for coupling membranes in AnMBR systems at 25 °C. Despite the sludge bed deterioration, SS-free permeate with an average COD of 42 mg/L was obtained and the average TMP of 85 mbar was maintained during the operation period, indicating no severe membrane fouling. However, this configuration is not found technically feasible at 15 °C, considering the deterioration of the filtration performance, which would be a bottleneck to the practical engineering application of AnMBRs. Thus, the filtration performance of the investigated single stage AnMBR was limited by the low temperature. In that situation, the results revealed the high potential of the new AnMBR-Digester configuration for treating municipal wastewater at 15 °C, as it couples wastewater treatment and sludge stabilization. Under optimized sludge recirculation conditions, the integrated AnMBR-Digester system represents an efficient technology to mitigate rapid membrane fouling for the low temperature applications of membrane coupled UASB reactors.

EVSEL ATIKSULARIN ANAEROBİK MEMBRAN BİYOREAKTÖRLER İLE MALİYET ETKİN OLARAK YENİDEN KULLANIMI

ÖZET

Evsel atıksudan anaerobik membran biyoreaktör teknolojisi kullanılarak yüksek besli madde, düşük patojen içeriği olan ve katı madde içermeyen su eldesi günümüzde yoğun olarak araştırılmaktadır. Anaerobik membran biyoreaktörler diğer teknolojilere göre daha az yer kaplamakta olup, sadece atıksu arıtma amaçlı değil, aynı zamanda besli madde içeriği yüksek su eldesi amacıyla da kullanılmaktadır. Böylece evsel atıksuların tarımsal sulama amaçlı yeniden kullanımına olanak sağlanmaktadır. Ayrıca anaerobik prosesler ile elde edilen biyogaz anaerobik membran biyoreaktör işletiminde gerekli olan enerji ihtiyacını azaltmaktadır. Belirtilen avantajlarının dışında, anaerobik membran biyoreaktör teknolojisi tam ölçekli uygulamalarda membran tıkanma problemine yol açmaktadır. Tıkanmaya bağlı olarak oluşan yüksek enerji gereksinimi ve yatırım maliyeti bu teknolojinin dezavantajları arasında sıralanabilir. Ayrıca anaerobik membran biyoreaktörlerde biyokütle üzerinde yüksek kesme kuvveti söz konusu olmaktadır. Evsel atıksular birçok ülkede düşük kuvvetli ve hizmet edilen nüfusa bağlı olarak yüksek debide oluşmaktadır. Ancak su korunumu ve ayrık kanalizasyon sistemlerinin kurulması ile ilgili devam eden çalışmalar yakın gelecekte evsel atıksu karakterizasyonunda değişimlerin olabileceğini göstermektedir.

Arıtma tesisi konfigürasyonu, membran özellikleri, çamur ve giriş atıksu karakterizasyonu ve işletme koşulları gibi tasarım ve işletme ile ilgili birçok faktör anaerobik membran biyoreaktörlerde tıkanma potansiyeline etki etmektedir. Bunların arasında, reaktör dizaynı çamurun tıkanma potansiyelini etkileyen en önemli faktörlerden biridir. Özellikle son on yılda tam karışimli reaktör, yukarı akışlı havasız çamur yataklı reaktör ve genişlemiş granüler çamur yataklı reaktörler gibi farklı birçok anaerobik reaktör tipinin farklı membran türleri ile birlikte kullanıldığı pek çok çalışma yapılmıştır. Bu alternatifler arasında yukarı akışlı havasız çamur yataklı reaktörlerde çamur yerine reaktör üst suyu membrana ulaşmaktadır. Dolayısıyla, tıkanma ile ilgili problemleri azaltması açısından bu kombinasyon avantajlı görülmektedir.

Bu çalışmanın amacı evsel atıksudan tarımsal sulamada kullanıma amaçlı düşük patojen yüksek besli madde içeriği olan su eldesini ve enerji geri kazanımını sağlayacak yenilikçi bir anaerobik membran biyoreaktör konfigürasyonunun uygulanabilirliğinin araştırılmasıdır. Bu çalışmanın gerçekleştirilmesi ile membrana ulaşacak olan toplam katı madde yükü kontrol edilerek akı artışı sağlanacak ve anaerobik membran biyoreaktörlerin tam ölçekli evsel atıksu arıtımında kullanımına yönelik önemli bir adım atılmış olacaktır.

Anaerobik membran biyoreaktör ve çürütücü içeren sistem evsel atıksu arıtımında farklı sistemlerde karşılaşılabilecek birçok dezavantajı önleyebilecek potansiyelindedir. Önerilen sistemde; yan akım membran ünitesi, yukarı akışlı havasız çamur yataklı reaktör ve buna paralel olarak çalışacak çürütücü kullanılacaktır. Çürütücü 0 ile 40 °C arasında istenilen sıcaklıkta işletilebilir. Sistemde kullanılacak olan membran modülü tübüler olarak dizayn edilmiş olup, modül içerisinde 28 adet ince fiber membran bulunmaktadır. Membran fiberlerinin çapı yüksek debili evsel atıksular için filtrasyon alanını artırma amaçlı oldukça düşük olacak şekilde 1.5 mm seçilmiştir. Membran öncesinde yukarı akışlı havasız çamur yataklı reaktör kullanılması büyük katı maddelerin reaktörde tutularak sadece reaktör üst sıvısının membrana ulaşmasını sağlayacak; böylece modül girişinde tıkanma önlenmiş olacaktır. Bunun yanı sıra, yukarı akışlı havasız çamur yataklı reaktör ve çürütücü kombinasyonu düşük sıcaklıklarda evsel atıksu arıtımı ve çamur stabilizasyonu açısından ideal bir sistem olarak öne sürülmektedir. Bu kombinasyona membran ilavesinin yapılması ile özellikle ılıman iklimli bölgelerde evsel atıksuyun tarımsal sulama amaçlı yeniden kullanımı için yüksek kalitede besi madde içeriği yüksek su elde edilmesi hedeflenmektedir.

Bu genel amaç doğrultusunda tezin ilk aşamasında laboratuvar ölçekli yukarı akışlı havasız çamur yataklı reaktörde çıkış akımında en yüksek filtre edilebilirliği sağlayacak optimum yukarı akış hızının belirlenmesi hedeflenmiştir. Yukarı akışlı havasız çamur yataklı reaktör farklı yukarı akış hızlarında işletilmiş, bu hızların çıkış suyu üzerindeki etkileri araştırılmıştır. Yukarı akış hızının membran performansı üzerindeki etkisinin incelenebilmesi için filtre edilebilirlik testleri gerçekleştirilmiştir. Sonuçlar; yukarı akış hızının sistemin biyolojik performansı ve çıkış suyunun fizikokimyasal özellikleri üzerinde oldukça büyük etkisinin olduğunu göstermektedir. Yüksek yukarı akış hızı kolloidal maddelerin sistemden kaçışına neden olmaktadır. Yukarı akışlı havasız çamur yataklı reaktörde çıkış suyu karakteristiği filtre edilebilirlik testleri ile paralel sonuçlar vermiştir. Sonuçlar çıkış suyunun filtre edilebilirliğinin 0.6 m/sa yukarı akış hızında, 1.2 m/sa yukarı akış hızına göre daha yüksek olduğunu göstermektedir. Yukarı akışlı havasız çamur yataklı reaktör çıkış suyunda farklı yukarı akış hızlarında protein/karbondidrat oranında ve partikül boyut dağılımında gözlenen büyük değişimler yukarı akış hızının çıkış suyu filtre edilebilirliği açısından anaerobik membran biyoreaktörlerde oldukça kritik bir parametre olacağını göstermektedir.

Çalışmanın ikinci aşamasında; membran yukarı akışlı havasız çamur yataklı reaktöre entegre edilmiş ve sistem bir önceki çalışmada seçilen 0.6 m/sa'lik yukarı akış hızında anaerobik membran biyoreaktör olarak işletilmiştir. Bu çalışmadaki amaç, membran ilavesinin sistemin biyolojik performansı ve çamur karakterizasyonu üzerindeki etkisini incelemektir. Membran ilavesi ile küçük partiküllerin sistemde birikmesi ve hücre dışı polimerik maddelerin azalması sonucu çamurun partikül boyut dağılımında azalma gözlenmiştir. Çamurun partikül boyut dağılımında gözlenen azalma çamurun çökelme özelliğinin bozulmasına neden olmuştur. Çamurun çökelme özelliğinin bozulması ile üst suya biyokütle kaçışı gerçekleşmiş, buna bağlı olarak yukarı akışlı havasız çamur yataklı reaktör çıkış suyunda kimyasal oksijen ihtiyacı ve askıda katı madde konsantrasyonlarında artış gözlenmiştir. Çıkış suyunda gözlenen bu artışa rağmen membran süzüntü akımında kimyasal oksijen ihtiyacı konsantrasyonu 42 mg/L olup, süzüntü katı madde içermemektedir. Ayrıca çamurun çökelme özelliğindeki bozulmaya rağmen, membran ilavesi sonrası

anaerobik membran biyoreaktörde transmembran basınç değeri 85 mbar civarında olmuş, ciddi bir membran tıkanma problemi ile karşılaşılmamıştır.

Üçüncü aşamada anaerobik membran biyoreaktör sisteminin düşük sıcaklıkta evsel atıksu arıtımında uygulanabilirliği araştırılmıştır. Bu amaç doğrultusunda; sistem 15 °C’de işletilmeye başlanmıştır. Sonuçlar membran tıkanma potansiyelinde 15 °C’de, 25 °C’ye göre ciddi seviyede artış olduğunu göstermiştir. Düşük sıcaklıkta yukarı akışlı havasız çamur yataklı reaktör çıkış suyunda artan kimyasal oksijen ihtiyacı ve çözünmüş mikrobiyal ürün konsantrasyonu, düşük partikül boyutu ve yüksek bulanıklık değerleri membran tıkanmasında artışa neden olmuştur. Filtrasyon performansında gözlenen değişime rağmen, arıtma performansında düşük sıcaklığa bağlı herhangi bir değişim gözlenmemiştir. Bu durum membranın alıkoyma kapasitesi ile ilişkilendirilebilir. Her iki sıcaklıkta da keke bağlı membran direnci toplam direncin %40’ından fazlasını oluşturmuştur. Ancak düşük sıcaklıkta yukarı akışlı havasız çamur yataklı reaktör çıkış suyunda artan küçük partiküller gözenek tıkanmasına bağlı oluşan direncin toplam filtrasyon direncine oranında artışa neden olmuştur.

Anaerobik membran biyoreaktörün 15 °C’de işletilmesi sırasında yukarı akışlı havasız çamur yataklı reaktör boyunca çamur karakterizasyonunda değişim gözlenmiştir. Bu durum çürütücü ilave edildiğinde, reaktörler arası çamur transferinin gerçekleştirileceği noktanın belirlenmesi açısından önemlidir. Reaktör boyunca farklı yüksekliklerden alınan çamur numunelerinde gerçekleştirilen analizler toplam ve uçucu katı madde ve toplam ve uçucu askıda katı madde konsantrasyonlarının reaktör yüksekliğine bağlı azaldığını ve en yüksek kimyasal oksijen ihtiyacı konsantrasyonunun 46 g/L olacak şekilde reaktörün en alt seviyesinde ölçüldüğünü göstermektedir. Aktif biyokütle reaktör girişine yakın bölgelerde yer alırken, aktivitesi düşük gevşek askıda ve flok yapıda partiküller reaktörün üst kısmına doğru hareket etmiştir. Bu durum reaktör girişine yakın yerlerdeki yüksek spesifik kimyasal oksijen ihtiyacı tüketim hızı ve reaktörün geri kalan bölümlerindeki düşük kimyasal oksijen ihtiyacı parçalanma hızı ile desteklenmektedir. Sonuçlar, düşük sıcaklıkta dahi membran ilavesi sonrası gerçekleşen çamur çökme özelliğinde gözlenen bozulmanın, yukarı akışlı havasız çamur yataklı reaktörü tam karışimli reaktöre dönüştürmediğini göstermektedir. Düşük sıcaklık koşullarında reaktör boyunca farklı seviyeler arası değişim devam etmiştir. Dolayısıyla; çürütücüye transfer edilecek olan çamurun yukarı akışlı havasız çamur yataklı reaktörün hangi seviyesinden alınacağı işletim açısından kritik bir karar haline gelmiştir. Çamur yatağı boyunca çamur stabilizasyonu ve katı madde konsantrasyonuna bağlı analizler gerçekleştirilmiş ve yukarı akışlı havasız çamur yataklı reaktörden çürütücüye transfer edilecek olan çamurun stabilite değerinin en düşük olduğu noktadan transfer edilmesine karar verilmiştir. Düşük stabiliteye sahip çamur yüksek miktarda biyolojik olarak parçalanabilir organik madde içermektedir.

Çalışmanın son aşamasında, çürütücü ilavesinin sistemin arıtma ve filtrasyon performansı üzerindeki etkisi araştırılmıştır. Çürütücü ilavesi sonrası, yukarı akışlı havasız çamur yataklı reaktör çıkış suyunda çözünmüş ve toplam kimyasal oksijen ihtiyacı konsantrasyonlarında ve bulanıklık değerlerinde azalma, partikül boyut dağılım sonuçlarında ise artış gözlenmiştir. Bu değişimler toplam filtrasyon direncini önemli ölçüde azaltmıştır. Ayrıca çürütücü ilavesi sonrası yukarı akışlı havasız çamur yataklı reaktörde çamurun stabilite ve spesifik metanojenik aktivite değerlerinde artış gözlenmiştir. Sistemin toplam biyogaz miktarında artış gerçekleşmiştir. En düşük stabiliteye sahip noktadan çamur transferinin yapılması

sonucu sistemde katı madde giderim ve parçalanma potansiyelinde gözlenen olumlu yöndeki değişimler çamur transfer noktasının seçiminin özellikle filtrasyon performansı açısından oldukça kritik olduğunu göstermektedir.

Genel olarak sonuçlar değerlendirildiğinde; yukarı akışlı havasız çamur yataklı reaktörlerin membran ile entegrasyonunun 25 °C için oldukça uygun olduğu görülmüştür. Çamur çökeltme özelliğinde gözlenen bozulmaya rağmen, girişte 530 ± 30 mg/L olan ortalama kimyasal oksijen ihtiyacı, membran çıkışında 42 mg/L'ye düşmüş ve katı madde içermeyen bir süzüntü elde edilmiştir. Ayrıca sistemin ortalama transmembran basınç değeri 85 mbar olarak belirlenmiş, ciddi seviyelerde membran tıkanma problemi gözlenmemiştir. Ancak, sistem 15 °C'de işletildiğinde, arıtma performansı 25 °C'deki ile aynı olmakla birlikte, filtrasyon performansında ciddi seviyelerde bozulma gözlenmiştir. Bu durum sistemin mühendislik uygulamaları açısından kullanılmasını büyük ölçüde engellemektedir. Özetle, 15 °C'de sistemin filtrasyon performansında düşük sıcaklığa bağlı bozulmalar belirlenmiştir. Çürütücü ilavesi sonrası gerçekleşen olumlu değişimler anaerobik membran biyoreaktör ve çürütücü konfigürasyonunun atıksu arıtımı ve çamur stabilizasyonunu birlikte gerçekleştirerek, 15 °C'de dahi evsel atıksu arıtımı için yüksek bir potansiyeli olduğunu göstermektedir. Anaerobik membran biyoreaktör ve çürütücü konfigürasyonu optimum çamur transfer noktası seçilerek işletildiğinde, düşük sıcaklıklarda yukarı akışlı havasız çamur yataklı reaktör ve membran entegre sistemlerde gerçekleşen ani membran tıkanma problemi önlenmektedir.

1. INTRODUCTION

1.1 Background

Demographic growth, urbanization, higher living standards and technological advance have led to an unprecedented increase in water demand, not only for municipal use but also for agricultural and industrial use. In many places fresh water is not sufficient to meet the water demand. Therefore, reclamation and use of treated wastewater for various purposes such as urban, irrigational and industrial purposes are increasingly recognized as essential strategies, especially for those areas suffering from deficiencies in the water balance, such as various European and Mediterranean regions. Reuse projects also exist in Japan, USA, Israel, and Australia (Zanetti et al., 2010; Agraftioti and Diamadopoulos, 2012; Bunani et al., 2013; Tang et al., 2013; Xiao et al., 2013).

Wastewater treatment and use of effluents have two major advantages including the reduction of the environment contamination, and hence the health risks, and conserving the limited freshwater reserves. Taking into account that high water consumption increases the volume of wastewater generated, municipal wastewater is one of the main alternative sources for water reclamation and reuse. However, the presence of chemicals and microbial contaminants in the municipal wastewater threatens ecosystem safety and human health (Saddoud et al., 2006). In consequence, municipal wastewater should be subjected to appropriate treatment to reduce the health and ecological risks of reclaimed water (Ma et al., 2013).

Globally a major demand exists for more efficient municipal wastewater treatment with a high effluent quality and reduced energy consumption, which will lead to cost effective treatment systems with effluent reuse for irrigation. Throughout different alternatives, anaerobic treatment systems have major potentials to meet the market demand with almost no energy requirement meanwhile biogas is produced for potential use. Anaerobic treatment is an energy generating process, in contrast to aerobic systems that generally demand a high energy input for aeration purposes. In

addition, the lower anaerobic waste biosolids production compared with aerobic treatment, reduces the costs and difficulties associated with biosolids management. However, anaerobic sewage treatment systems only partly remove the organic pollutants, with COD removal efficiencies reaching 70-80% in full-scale reactors (Chernicharo et al., 2015; van Lier et al., 2010). In addition, they have a limited feasibility for the treatment of sewage at low temperatures ($<15\text{ }^{\circ}\text{C}$), while nutrient removal needs to be addressed in subsequent systems.

Among anaerobic treatment technologies, the anaerobic membrane bioreactor (AnMBR) is a promising technology as a means to retain all the biomass in the reactor more effectively and achieve highly efficient solids-liquid separation, producing superior effluent qualities. In addition, AnMBRs appear suitable for the treatment of wastewaters with high organic suspended solids (SS) content, because particles are confined inside the reactor, allowing their degradation (Fuchs et al., 2003; Hu and Stuckey, 2007; Lin et al., 2010; Lin et al., 2011). The AnMBR has gained popularity in recent years, and more attention has been focused on the development of this technology for the treatment of high-concentration wastewater, mainly food and beverage industry wastewaters, or for the treatment of swine manure (Fakhru'l-Razi and Noor, 1999; Fuchs et al., 2003; He et al., 2005; Zhang et al., 2007; Meabe et al., 2013). With respect to domestic sewage treatment, the production of solids-free, nutrient-rich effluents with a low content of pathogenic organisms is mentioned as a feature of major interest applying AnMBRs (Liao et al., 2006; Kocadagistan and Topcu, 2007; Ho and Sung, 2010; Gao et al., 2010; Liu et al., 2012).

1.2 Problem Statement, Objectives and Structure of the Thesis

1.2.1 Problem statement and research approach

Despite continuous developments in the field of AnMBR technology, the state-of-the-art of this technology is not yet appropriate for municipal wastewater treatment to reach re-use quality effluents, due to high energy and investment costs and high shear stress on the biomass. It has been mainly applied for industrial or high strength wastewater treatment.

A radical different AnMBR design is required to facilitate further development and optimization of AnMBR technology for municipal wastewater treatment. Thus, an innovative configuration of AnMBR will be investigated in order to solve the bottlenecks of AnMBRs and enable energy recovery and effluent use for irrigation (Figure 1.1). The system consists of an upflow anaerobic sludge blanket (UASB) reactor coupled with a parallel-operated digester, which can be operated at any required process temperature. Membrane integration with the UASB-Digester system can be attractive for producing high quality and nutrient-rich effluents for reuse purposes under moderate climate conditions.

The UASB within this concept improves primary sedimentation, sludge thickening and methanogenesis. A UASB reactor coupled to a membrane is also useful for decreasing the SS concentration of the effluent being sent to the membrane because the sludge bed would entrap 70-80% of the particulate matter by adsorption and biodegradation (Kataoka et al., 1992; An et al., 2009a; Wu et al., 2009; Zhang et al., 2011). Most of the sludge is kept in the reactor instead of circulated across the membrane. Thus, the membrane flux may become less dependent on the reactor mixed liquor suspended solids (MLSS) concentration, possibly leading to increased membrane fluxes. Using a UASB instead of a completely stirred tank reactor (CSTR) reduces both the shear on the biomass and the required membrane surface and thus the costs. Most of the sludge will be retained in the sludge bed of the UASB reactor, so there is no shear impact on the sludge mass due to membrane filtration. It is anticipated to feed the membranes with a maximum of 1 g/L total solids (TS).

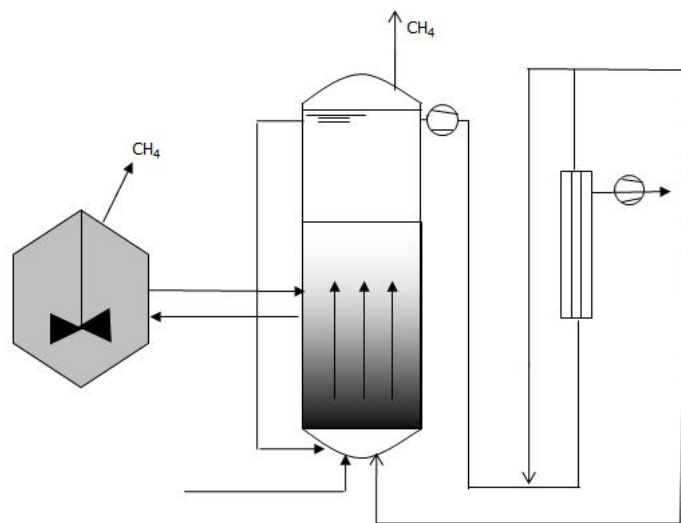


Figure 1.1 : The schematic representation of the AnMBR-Digester system.

Municipal wastewater in many countries is generated at high per capita flow rates. For treating these high flows large membrane surface areas are required, increasing the capital costs of the membrane system. External cross-flow tubular membrane modules that contain membrane fibers with a very small internal tube diameter of only 1.5 mm are one hand very compact and on the other hand characterised by a large membrane surface area and thus in principle very suitable to treat the high sewage flows. Moreover, if the filtration step is preceded by a UASB reactor, then TS level in the membrane unit is drastically reduced, which will reduce the clogging and fouling potential of the membrane feed. In fact, the reduced clogging potential enables the use of smaller membrane diameters and a simpler membrane process.

Since municipal wastewaters are often characterised by low temperature, membrane integrated UASB systems must be adapted to overcome these temperature limitations. The performance of UASB reactors at low temperature climates (5-20 °C) is highly limited by the hydrolysis of entrapped solids that accumulate in the sludge bed, reducing the overall solids retention time (SRT). A low SRT will result in a low sludge stability and thus, a low specific methanogenic activity (SMA), resulting in a poor soluble chemical oxygen demand (COD) removal. In the proposed system, a digester will be coupled to the membrane integrated UASB system. The solids that are entrapped in the sludge bed of the UASB reactor, are conveyed to the digester, which is operated at optimal temperature conditions, i.e. 35 °C. The solids are then further degraded, and the methanogens-enriched digested sludge is re-circulated to the UASB reactor, thereby providing additional methanogenic capacity to improve soluble COD removal at low temperatures (Mahmoud et al., 2004; Mahmoud, 2008; Zhang et al., 2013). Thus, the digester within this concept is expected to improve sludge stabilization by methanation, as well as the sludge dewatering characteristics, resulting in energy production and a continuous inoculation of the UASB reactor with methanogens-enriched biomass. The characteristics of the sludge that needs to be recirculated are crucial to the viability of the AnMBR-Digester system. Optimization of the sludge recirculation needs to be done carefully in AnMBR-Digester systems in order to maintain complete conversion of biodegradable dissolved COD (Mahmoud, 2002; Zhang et al., 2012).

Overall, the investigated system will be a cost-effective technology with reduced investment and operational costs and energy consumption. No energy will be

required for aerating the bioreactor and this will be a major step towards a positive net energy balance. Compared to conventional anaerobic treatment, the treated water is free of SS, has a log 4-5 lower levels of pathogenic organisms and has low concentrations of soluble COD. With ultrafiltration (UF), the effluent will be virtually free of pathogens, so it can be reused for unrestricted irrigation purposes. The envisaged technology is further characterised by extremely low energy consumption rates and biogas production (Liao et al., 2006; Kocadagistan and Topcu, 2007; Ho and Sung, 2010). The envisaged system could be further optimised towards net energy producing wastewater treatment plant.

1.2.2 Research objectives

In this study, the applicability of an innovative configuration of AnMBR will be investigated in order to produce pathogen-free and nutrient-rich effluents for use in irrigation, meanwhile energy is recovered. With the realization of this study, flux enhancement in AnMBR technology by controlling the total suspended solids (TSS) load to the membrane unit will be the major output of the study, bringing AnMBR technology for full-scale sewage treatment one step closer to realisation. Based on this general aim, specific aims were identified as follows:

- ✓ Identify the optimum upflow velocity that will result in an effluent with good filterability for the case of membrane coupled UASB systems
- ✓ Understand the impact of membrane addition on both the biological performance and sludge characteristics of a UASB reactor in an AnMBR system treating municipal wastewater
- ✓ Investigate the relationship between the characteristics of the UASB effluent and the filtration performance of the AnMBR at different operational temperatures
- ✓ Identify the best location in the sludge bed for conveying the sludge from a low temperature UASB reactor to the digester
- ✓ Investigate the impact of the digester coupling on the removal efficiency and filtration performance of the AnMBR system

1.2.3 Thesis outline

The objectives mentioned above have been addressed in eight chapters and the chapters are structured as follows (Figure 1.2):

Chapter 2 critically evaluates the potential of AnMBR technology for municipal wastewater treatment with a focus on different types of anaerobic reactors that are coupled with membranes. Various types of membrane coupled anaerobic sludge bed reactors, such as UASB and expanded granular sludge bed (EGSB), are discussed as an alternative approach to the more conventional AnMBRs consisting of CSTR type bioreactors with either internal or external membrane units. Current anaerobic bioreactor membrane process integration alternatives reported in the literature are evaluated with proposals for future applications. In addition, the chapter discusses the impact of various factors on both biological and filtration performances of AnMBRs including strengths and limitations. Finally, possible uses of treated municipal wastewater are discussed.

Chapter 3 deals with the effect of upflow velocity on biological removal efficiency and effluent filterability in a laboratory-scale UASB reactor. Upflow velocities of 1.2, 0.6 and 1.2 m/h are applied in three successive stages over a total operation period of 116 days. The effect of upflow velocity on the effluent quality is assessed not only on biological based parameters such as organic matter removal but also on physical characteristics. Filterability tests are carried out during each stage in order to assess the effect of upflow velocity on subsequent membrane performance. From the obtained results, an optimum upflow velocity for the UASB unit was identified that will result in an effluent with good filterability for subsequent membrane filtration. The information obtained from this study is valuable for optimizing the hydraulic conditions in membrane coupled UASB systems in order to achieve stable effluent quality and to minimize membrane fouling potential.

Chapter 4 presents the impact of membrane incorporation for effluent extraction on the performance of a UASB reactor in terms of system performance and sludge characteristics. A laboratory-scale system was operated in two different operational stages: (i) before membrane incorporation as a sole UASB reactor and (ii) after membrane incorporation as an AnMBR system. The impact is evaluated by monitoring and comparing several indicators including COD removal efficiency,

TSS and soluble microbial products (SMP) concentrations in the UASB effluent and sludge particle size distribution (PSD), sludge SMA and microbial community analysis before and after membrane addition. This study is supposed to provide a fundamental understanding of how a UASB system is affected by the elimination of selective sludge washout, as well as to give a clue on the operation of a UASB reactor as part of an AnMBR system.

Chapter 5 focuses on the relationship between the effluent characteristics and filtration performance of an AnMBR. For this purpose, UF membrane coupled to a UASB reactor is operated continuously for nearly 126 days at two operational temperatures (25 °C and 15 °C). The effect of temperature on the overall performance of the AnMBR system is determined in order to investigate whether the membrane coupled UASB is a technically feasible alternative in the treatment of municipal wastewater at 15 °C. Pyrosequencing is used to compare the microbial community structure including both archaeal and bacterial communities and the relative abundance of microbial species at different temperatures. The results provide valuable new insights into the fouling mechanisms and would consequently further advance knowledge on the behavior of membrane coupled UASB system at low temperatures.

Chapter 6 provides a comparison of the sludge characteristics along the height of a UASB reactor in terms of sludge morphology, activity and stability. The main aim of this study is to identify the best location (i.e. where sludge is of lowest stability and/or highest concentration) in the sludge bed for conveying the sludge from the low temperature UASB reactor to the digester. Within this concept, the sludge profile was investigated by collecting sludge samples along the different heights of the UASB-AnMBR treating municipal wastewater. The sludge characteristics were comparatively evaluated in terms of solids content, PSD, sludge morphology, SMA and stability. Besides, pyrosequencing was employed to analyze samples from each location in order to compare the microbial community composition including both archaeal and bacterial communities and the relative abundance of microbial species. The microbial community compositions elucidated in this study provide new insights into understanding anaerobic wastewater treatment and shed some light on the application potentials of UASB reactors in AnMBR concepts. The overall information obtained from this study is valuable for the selection of the location

through which sludge will be transferred between UASB and digester in membrane coupled UASB systems.

Chapter 7 evaluates the applicability of the proposed configuration. In the study, the anaerobic sewage treatment using an AnMBR-Digester system was investigated at a sewage temperature of 15 °C. It provides the impact of digester incorporation on treatment and filterability performance of the AnMBR. The UASB reactor was firstly operated without incorporating the digester aiming at monitoring of the overall AnMBR performance, i.e. (i) treatment performance, (ii) filtration performance and (iii) sludge quality. This is of particular importance to have base line records to be used as reference values to assess the achievements obtained from incorporating a digester. Afterwards, the single-stage UASB reactor was modified to a UASB-Digester system by incorporating a digester operated at 35 °C in order to assess the performance of the AnMBR-Digester system. The characteristics of the sludge that is recirculated are crucial to the viability of the AnMBR-Digester system. Therefore, the optimum sampling location for sludge transfer is selected. The overall information obtained from this study is valuable for the operation and improvement of the AnMBR-Digester system treating municipal wastewater at low temperature.

Chapter 8 concludes the thesis with a general discussion and summarizes the conclusions, along with directions for future research.

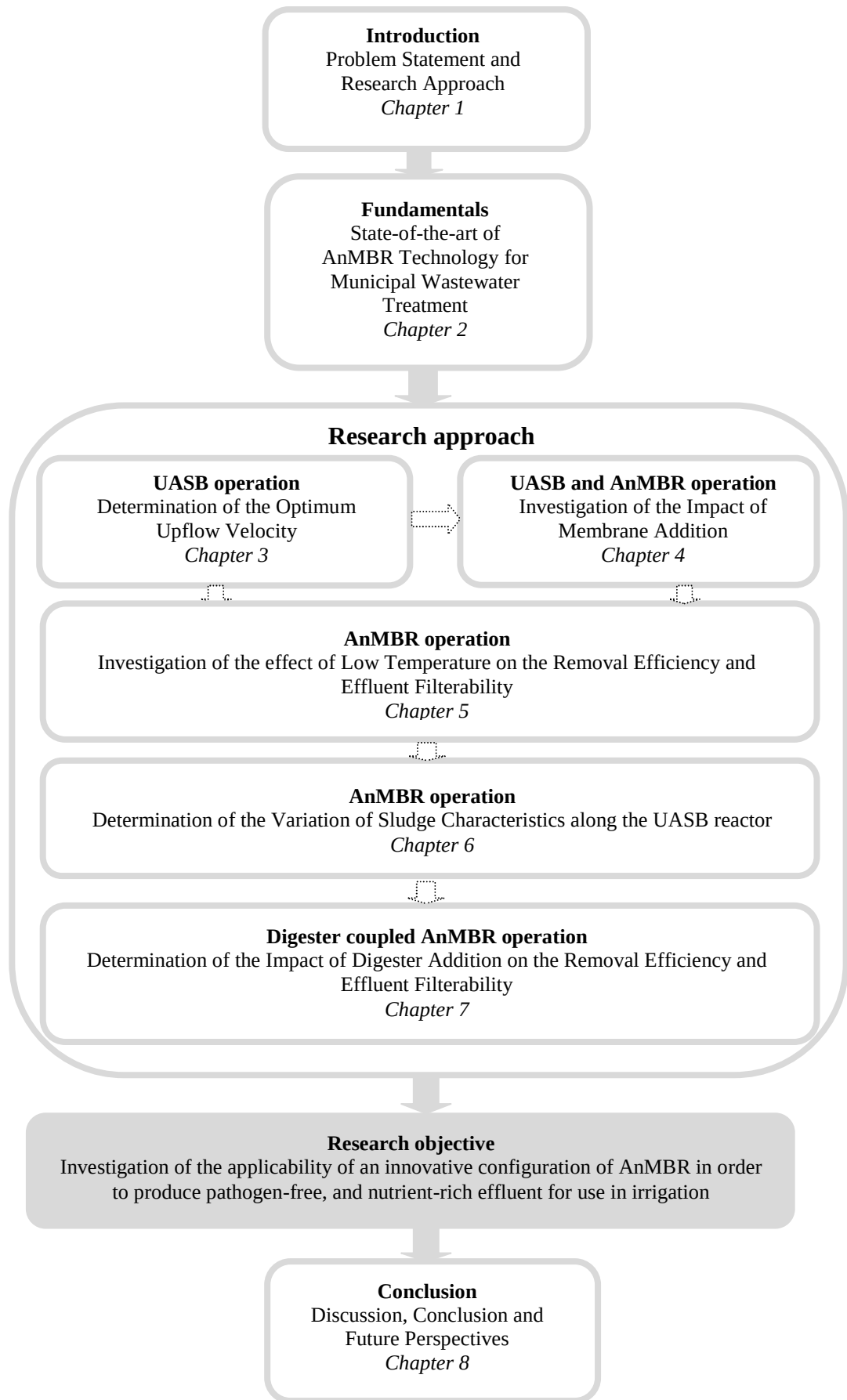


Figure 1.2 : Schematic representation of the research approach and thesis outline.

2. ANAEROBIC MEMBRANE BIOREACTORS FOR MUNICIPAL WASTEWATER TREATMENT: INTEGRATION OPTIONS, LIMITATIONS AND EXPECTATIONS⁽¹⁾

2.1 Introduction

Municipal wastewater is the most abundant type of wastewater that falls into the category of low-strength waste streams, characterized by low organic strength and high particulate organic matter content (van Lier, 2008). If recoverable, municipal wastewater treatment plants have the potential to become net producers of renewable energy, converting the chemically bound energy content in the organic pollutants of raw municipal wastewater to useful energy carrier (Shizas and Bagley, 2004). Therefore, selection of an appropriate energy recovery technology that can convert the inherent energy in wastewater into a renewable energy source has become more important.

Anaerobic technology has drawn considerable attention for municipal wastewater treatment. In addition to the energy that can be recovered from methane-rich biogas, the application of anaerobic processes distinctly reduces the overall energy demand for municipal wastewater treatment because no aeration energy is required for mineralizing the organics. Moreover, anaerobic processes produce mineralized nutrients in the form of ammonia and orthophosphate enabling direct agricultural use of the effluent for ferti-irrigation. The most important concern for further improving the energy sustainability and resource preservation is the identification of an appropriate anaerobic technology and methods for full recovery of the solubilized methane from treated effluents (Shizas and Bagley, 2004; Liao et al., 2006).

⁽¹⁾ This chapter is based on:

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.

The retention of slow growing anaerobic biomass was the most important challenge in the earlier development of an appropriate reactor technology for the anaerobic treatment of municipal wastewater. Treatment of municipal wastewater by different types of anaerobic processes has drawn considerable attention of various researchers (Singh and Viraraghavan, 1998; Lettinga et al., 2001; Chan et al., 2009). The invention of the UASB reactor in early 1970s by Lettinga and his colleagues was a milestone in anaerobic wastewater treatment (Lettinga et al., 1980). The major success of the UASB reactor lies in its ability to retain a high concentration of biomass due to formation of a thick dense sludge bed, which, in dependence to wastewater characteristics, may consist of well settleable methanogenic sludge granules. Formation of well settleable sludge allows the decoupling of hydraulic retention time (HRT) and SRT so that efficient treatment can be carried out at high organic loading rates (OLRs) with a significant decrease in reactor size (Singh and Viraraghavan, 1998). Owing to the high influent flows and low organic matter content, municipal wastewater is treated at ambient temperatures in both aerobic and anaerobic applications worldwide (van Lier et al., 2010), especially in developing countries with (semi) tropical climates such as Brazil, India and Colombia. However, municipal wastewater treatment by anaerobic systems in more temperate climates is still considered a challenge since municipal wastewater belongs to the complex wastewater category due to the high fraction of particulate organic material, moderate biodegradability and its low strength (Lettinga et al., 2001). Under low temperature (<20 °C) conditions, hydrolysis of particulate matter into dissolved molecules becomes the rate-limiting step, which results in the accumulation of SS in the reactor and a decrease in organic matter conversion efficiency together with a decrease in methanogenic activity (van Lier et al., 1997; Kalogo and Verstraete, 1999; Lettinga et al., 2001; Martinez-Sosa et al., 2011a). Moreover, due to low substrate affinity of anaerobic biomass compared to aerobic bacteria it is practically very hard to achieve low effluent COD concentrations and to fulfill the environmental regulations for wastewater reclamation and reuse (Salazar-Pelaez et al., 2011a).

With ever growing application experiences from aerobic membrane bioreactors (MBRs) (Santos et al., 2011), AnMBR technology has also started to be researched for municipal wastewater treatment as a possible alternative to the conventional

anaerobic treatment processes (Martin et al., 2011). In AnMBRs, biomass can be effectively retained inside the reactor providing optimal conditions for organic matter degradation without any carry-over of SS. By incorporating membranes to anaerobic municipal wastewater treatment, superior effluent quality in terms of COD, SS and pathogen counts can be achieved in comparison with conventional anaerobic processes, and a stable treatment performance can be obtained to meet stringent discharge standards (Liao et al., 2006; Kocadagistan and Topcu, 2007; An et al., 2009a; Ho and Sung, 2010). It was reported that AnMBRs provide a possibility for the agricultural use of the treated effluent for non-potable purposes in many regions suffering from water shortage (Martinez-Sosa et al., 2011b). Agricultural use of treated effluents generally demands extensive pathogen removal along with the availability of macronutrients. Since macronutrients such as ammonium and orthophosphates are not removed by anaerobic bioprocesses and pathogens can be retained by the membrane unit (Saddoud et al., 2006; Ellouze et al., 2009), permeates of AnMBRs are certainly of interest for agricultural use. Information about a selected number of guidelines on irrigation water quality can be found in the study of Norton-Brandao et al. (2013). In addition to achieving high effluent qualities, a shorter start-up period is required for AnMBRs in comparison to UASB systems, which is one of the major advantages in the treatment of especially low-strength wastewaters. Start-up periods of 6 and 12 days have been reported in the study of Hu and Stuckey (2006) and Lin et al. (2011), respectively, whereas this period was in the range of one to several months for UASB systems (Álvarez et al., 2006a).

Despite the mentioned advantages, there are still critical obstacles such as low flux, membrane fouling, high capital and operational costs that limit the extensive use of AnMBRs (Kocadagistan and Topcu, 2007; Hu and Stuckey, 2007; Zhang et al., 2010). AnMBRs are generally operated at higher biomass concentrations compared to aerobic MBRs, impacting rheology and thus, reactor hydraulics and pumping. High MLSS concentrations also result in a more rapid and dense cake layer build up in comparison to aerobic MBRs, requiring frequent physical cleaning, interval operation, and likely sub-critical flux operation, in order to sustain the flux (Chang et al., 2002; Liao et al., 2006; Jeison and van Lier, 2006a; Visvanathan and Abeynayaka, 2012). Operational costs related to energy requirements for gas/liquid recirculation for membrane fouling control and chemical costs required for

membrane cleaning are still heavy burdens on the economic feasibility of AnMBRs. However, membrane acquisition and/or replacement costs have decreased significantly due to a decline in membrane module costs (Judd, 2008; Santos and Judd, 2010; Santos et al., 2011).

Despite above constraints, AnMBR is identified as a potential anaerobic process of interest for treating municipal wastewater, whereby, various studies indicate that treatment performances are seemingly dependent on the chosen process configuration (Liao et al., 2006). Different types of anaerobic bioreactors, including CSTR, UASB, EGSB, etc., have been investigated in combination with various types of membranes. However, the most optimal process configuration, i.e. anaerobic bioreactor type and the coupling of the bioreactor with the membrane module, yet needs to be determined. Recently, several more general review papers on AnMBR systems have been published, discussing the AnMBR feasibility (Smith et al., 2012; Stuckey, 2012; Skouteris et al., 2012; Lin et al., 2013). This chapter focuses on the perspectives of the various types of membrane coupled anaerobic sludge bed reactors, such as UASB and EGSB, as an alternative approach to the more conventional AnMBRs consisting of CSTR type bioreactors with either internal or external membrane separation devices. The review chapter evaluates the current anaerobic bioreactor-membrane process integration alternatives reported in the literature, with proposals for future applications. Besides, many aspects of current AnMBR applications for the treatment of municipal wastewater are discussed in detail including treatability and filterability. Moreover, limitations and difficulties encountered in anaerobic treatment and the possible use of the treated municipal wastewater are discussed together with reasonable solution perspectives.

2.2 Integration Possibilities of Membranes with Different Types of Anaerobic Reactors for Municipal Wastewater Treatment

Membranes can be coupled to various anaerobic reactor types such as CSTRs, UASB and EGSB reactors, in different configurations for the treatment of municipal wastewater. Table 2.1 and Table 2.2 present the performance of different AnMBR applications for the treatment of municipal wastewater with regard to both biological performance and membrane aspects, respectively. However, information regarding the membrane performance is quite limited in most of the studies. Section 2.2

discusses both the advantages and disadvantages of different integration possibilities of membranes with several types of anaerobic reactors.

2.2.1 Completely stirred tank reactor

By far, CSTR is the most common anaerobic process researched in AnMBR systems (Kocadagistan and Topcu, 2007; Hu and Stuckey, 2007; Grundestam and Hellstrom, 2007; Ho et al., 2007; Vyrides and Stuckey, 2009; Ho and Sung, 2010; Martinez-Sosa et al., 2011a; Gimenez et al., 2011) in analogy to the type of bioreactors used in aerobic MBRs. In general, CSTRs are operated at equal HRT and SRT without any internal biomass retention device. In-reactor biomass concentration can be increased by applying a secondary clarifier with return flow, resulting in an anaerobic contact process, or a membrane separation device. Simple flow-through CSTRs, without sludge separation, are characterized by very low loading rates, resulting in long retention times and, consequently, large reactor volumes. However, in membrane coupled CSTRs, the complete retention of solids in the reactor decouples SRT from HRT, which leads to an increase in biomass concentration and thus increase in conversion rates alleviating the rate limiting step, e.g. hydrolysis/solubilization of solids and/or methanogenesis. Often CSTRs are coupled to external cross-flow membranes, resulting in high bioreactor liquid turnover rates, leading to a well-mixed flow regime. In addition, as a result of the prevailing high shear stress and intense mixing, an AnMBR setup might even increase the biological methane potential of the substrate (Liao et al., 2006).

Notwithstanding its advantages, CSTRs directly expose the membrane to bulk sludge, which results in heavy membrane fouling and low fluxes since effluent solids concentration of CSTRs is equal to the bulk solids concentration (Liao et al., 2006). The high solids concentration subjected to membrane filtration exacerbates cake deposition in CSTR configuration, either with pressure-driven or vacuum-driven membranes. Moreover, sludge recirculation through the membrane feed pump, especially for external cross-flow membranes, results in a substantial decrease in the mean particle size (Choo and Lee, 1998). On one hand, disruption of particles may positively impact hydrolysis, but on the other hand it may negatively impact the juxtapositioning of acetogens and methanogens, limiting the required interspecies hydrogen transfer for attaining a high SMA (Brockmann and Seyfried, 1997).

Table 2.1 : Treatment performance of membrane coupled conventional anaerobic treatment processes for municipal wastewater treatment.

Reactor Type/ Module Configuration	Volume	Temperature	Wastewater Source	Influent COD	HRT	OLR	SRT	COD Removal (based on membrane effluent)	MLSS	Biogas Production	Reference
-	(L)	(°C)	-	(mg/L)	(h)	(kg COD/m ³ .day)	(day)	(%)	(g/L)	(L CH ₄ /g COD _{removed})	-
CSTR/Side-stream	8850	25	Raw	200 ^a	89-120	1.4-2 ^b	144	84.5 ^c	-	-	Kiriyama et al. (1992)
CSTR/Side stream	850	22	Raw	637	14.4	0.94	∞ ^d	92 ^e	-	0.12	Grundestam and Hellstrom (2007)
CSTR/Side-stream	-	25	Synthetic	500- 1000	-	-	∞ ^d	97	9.6-16	-	Ho et al. (2007)
CSTR/Submerged	3	35	Synthetic	460	3, 6, 12, 24	-	100	90-95	4.3-4.8	-	Hu and Stuckey (2007)
CSTR/Submerged	3	35	Synthetic	465	8-20	-	250	99 ^f	2-3 ^g	-	Vyrides and Stuckey (2009)
CSTR/Side-stream	4	15-25	Synthetic	500	12	1	-	>85	-	-	Ho and Sung (2010)
CSTR/Submerged	6	-	Synthetic	425	12	-	∞ ^d	83	4.6	-	Achilli et al. (2011)
CSTR/Submerged	900	-	Raw	445	6-21	-	70	87	6-22	0.069	Gimenez et al. (2011)
CSTR/Submerged	60	30	Raw	322	10	1	∞ ^d	88	6.4-9.3	0.24	Lin et al. (2011)
CSTR/Submerged	350	35	Municipal+ Glucose	630	0.8	0.6-1.1	680	90	15	0.27	Martinez-Sosa et al. (2011a)
CSTR/Submerged	350	20	Municipal+ Glucose	630	0.8	0.6-1.1	680	82-90	19	0.23	Martinez-Sosa et al. (2011a)
CSTR/ Submerged	1300	33	Pre-treated	410	6-21	0.71	76	-	-	-	Gimenez et al. (2012)
CSTR/ Submerged	1300	21	Pre-treated	720	6-21	0.64	74	-	-	-	Gimenez et al. (2012)
Digester/Submerged	12.9	15-20	Raw	259.5	2.6	2.36	-	52-87	-	-	An et al. (2009b)
UASB/Side-stream	5400	Ambient	Pre-hydrolyzed	490	-	2.8 ^b	-	83	-	-	Kataoka et al. (1992)
UASB/ Side-stream	34	Ambient	Raw	185.6	5.5-10	0.3-0.9	∞ ^d	77-81	12-32	0.062-0.121	An et al. (2009a)
UASB/ Side-stream	15.1	35	Synthetic municipal	150 ^h	6	0.3 ⁱ	-	-	-	-	Wu et al. (2009)

Table 2.1 (continued) : Treatment performance of membrane coupled conventional anaerobic treatment processes for municipal wastewater treatment.

Reactor Type/ Module Configuration	Volume	Temperature	Wastewater Source	Influent COD	HRT	OLR	SRT	COD Removal (based on membrane effluent)	MLSS	Biogas Production	Reference
-	(L)	(°C)	-	(mg/L)	(h)	(kg COD/m ³ .day)	(day)	(%)	(g/L)	(L CH ₄ /g COD _{removed})	-
UASB/Submerged	45	10-15	Raw	302.1	8	-	-	56.6-57.7	5.9-19.8 ^g	-	Zhang et al. (2010)
UASB/Side-stream	10	30	Synthetic	500	24	5	50	96	-	-	Gao et al. (2010)
UASB/ Submerged	45	Ambient	Raw	298.4	8	-	-	63.4	-	-	Zhang et al. (2011)
Upflow anaerobic reactor/Side-stream	180	25	Pre-settled	540	12, 6, 4.5	1.08, 2.16, 4.32	∞ ^d	88	14-80	-	Lew et al. (2009)
EGSB/Submerged	4.7	11, 15, 20, 25	Synthetic	383-849	3.5, 4.6, 5.7	1.6-4.5	145	76-96	13-23	-	Chu et al. (2005)
Fluidized bed (FB) reactor/-	660	-	Pre-hydrolyzed solids from raw sewage	353	-	1.1 ^b	-	90	-	-	Kataoka et al. (1992)
Hydrolyzation reactor /Side-stream	500	30	Concentrated solids from raw sewage	353	5	-	-	-	-	-	Kataoka et al. (1992)
Hydrolyzation reactor-membrane-FB bioreactor/ Side-stream	1000	26	Raw	2187	-	-	-	98	-	-	Kimura (1991)
Hydrolyzation reactor UASB/Side-stream	5400	26	Raw	1144	-	-	-	94	-	-	Kimura (1991)
Jet flow anaerobic bioreactor/ Side-stream	50	37	Raw	685	15-60	0.23-2	140	88	0.5-10 ^g	-	Saddoud et al. (2007)
Hybrid upflow anaerobic bioreactor/Submerged	17.7	Ambient	Raw	97.5-2600	4-6	0.5-12.5	150	97	16-22.5	0.13-0.42 ^j	Wen et al. (1999)

^a mg biochemical oxygen demand (BOD)/L; ^b kg BOD/m³.day; ^c Volatile suspended solids (VSS) removal; ^d No discharge except sampling; ^e Total organic carbon (TOC) removal; ^f Dissolved organic carbon (DOC) removal; ^g g VSS/L; ^h mg TOC/L; ⁱ g TOC/L.day; ^j m³/m³.day.

Table 2.2 : Membrane performance of membrane coupled conventional anaerobic treatment processes for municipal wastewater treatment.

Reactor Type/Module Configuration/Membrane Configuration	Membrane Type	Material	Pore Size (μm)	Filtration Area (m^2)	Flux ($\text{L}/\text{m}^2\cdot\text{h}$)	Transmembrane pressure (TMP) (kPa)	Cross-flow velocity (m/s)	Gas Sparging (L/min)	Reference
-	-	-	(μm)	(m^2)	($\text{L}/\text{m}^2\cdot\text{h}$)	(kPa)	(m/s)	(L/min)	-
CSTR/Side-stream/Capillary organic membrane	-	-	-	7.6	-	-	-	-	Kiriyama et al. (1992)
CSTR/Side-stream/Vibratory shear enhanced processing (VSEP)	-	Polytetrafluoroethylene (PTFE) Teflon	0.45	1.59	-	-	-	-	Grundestam and Hellstrom (2007)
CSTR/Side-stream/Tubular	-	PTFE laminated non-woven filter	10	0.015	5-12	3.45-20.7	0.1-0.2	-	Ho et al. (2007)
CSTR/Submerged/-	Microfiltration (MF)	-	0.4	-	10-20	up to 30	-	-	Hu and Stuckey (2007)
CSTR/Submerged/Flat sheet	-	Polyethylene	0.4	0.1	5-10	30-45	-	5	Vyrides and Stuckey (2009)
CSTR/Side-Stream/Tubular	MF	PTFE	1	0.09	5	6.9-55.2	-	-	Ho and Sung (2010)
CSTR/Submerged/Flat sheet	MF	Polyolefine	0.4	0.1	10.5	-	-	8	Achilli et al. (2011)
CSTR/Submerged/Hollow fiber	-	-	0.05	30	10	8	-	0.23 ^a	Gimenez et al. (2011)
CSTR/Submerged/Flat sheet	-	Polyvinylidene fluoride (PVDF)	140 ^b	0.6	12	-	-	5	Lin et al. (2011)
CSTR/Submerged/Flat sheet	UF	Polyethersulfone (PES)	0.038	3.5	7	17.7	0.026	62 ^a	Martinez-Sosa et al. (2011a)
CSTR/Submerged/Hollow fiber	UF	-	0.05	30	-	-	-	-	Gimenez et al. (2012)
Digester/Side-stream/Tubular	-	Non-woven fabric (Polyethylene terephthalate)	0.64	0.98	5	up to 30	-	-	An et al. (2009b)
UASB/External/Capillary	UF	Polyvinylalcohol	15 ^b	100	-	-	-	-	Kataoka et al. (1992)
UASB/Side-stream/Tubular	-	Polyacrylonitrile	-	0.2	10.5	<50	0.4-1.45	-	An et al. (2009a)
UASB/Side-stream/Flat sheet	MF	PVDF	0.22	0.05	25	30	-	-	Wu et al. (2009)
UASB/Submerged/Flat type	Dynamic	Dacron mesh	61	-	65	-	-	-	Zhang et al. (2010)
UASB/Side-stream/Flat sheet	UF	PVDF coated with a Polyether block amide	100 ^b	0.052	8-12	-	-	-	Gao et al. (2010)
UASB/Submerged/Flat sheet	-	Dacron mesh (Dynamic membrane)	61	-	65	25	-	-	Zhang et al. (2011)

Table 2.2 (continued) : Membrane performance of membrane coupled conventional anaerobic treatment processes for municipal wastewater treatment.

Reactor Type/Module Configuration/Membrane Configuration	Membrane Type	Material	Pore Size	Filtration Area	Flux	Transmembrane pressure (TMP)	Cross-flow velocity	Gas Sparging	Reference
-	-	-	(μm)	(m^2)	($\text{L}/\text{m}^2\cdot\text{h}$)	(kPa)	(m/s)	(L/min)	-
Upflow anaerobic reactor/Side-stream/Hollow fibre	MF	-	0.2	4	3.75, 7.50, 11.25	<19.6 ^c	-	-	Lew et al. (2009)
EGSB/Submerged/ Hollow fibre	-	Polyethylene	0.1	0.1	-	-	-	-	Chu et al. (2005)
FB reactor/External-pressure/Hollow fiber	MF	Polyethylene	0.1	54	-	-	-	-	Kataoka et al. (1992)
Hydrolyzation reactor/Internal-pressure/Tubular	UF	Polyacrylonitrile	13 ^b	0.94	-	-	-	-	Kataoka et al. (1992)
Hydrolyzation reactor-membrane-FB bioreactor/Side-stream/Hollow fiber	MF	Polyethylene	0.1	54	24	108	1	-	Kimura (1991)
Hydrolyzation reactor UASB/Side-Stream/Capillary	-	Polyvinylalcohol-Polysulfone	-	100	16	147	0.7	-	Kimura (1991)
Jet flow anaerobic bioreactor/Side-stream/-	UF	-	100 ^b	1	3.5-13	100-200	3	-	Saddoud et al. (2007)
Hybrid upflow anaerobic bioreactor /Submerged/Hollow fiber	-	Polyethylene	0.03	0.3	5-10	<70	-	-	Wen et al. (1999)

^a $\text{m}^3/\text{m}^2\cdot\text{h}$; ^b kDa, molecular weight cut off; ^c Driven by water level difference.

Martinez-Sosa et al. (2011a) studied an AnMBR system consisting of an external UF membrane coupled to a CSTR for the treatment of municipal wastewater and obtained an effluent that can be used for agricultural irrigation. An innovative process, i.e. vibrating membranes coupled to a CSTR, was proposed by Grundestam and Hellstrom (2007) for the treatment of municipal wastewater. They achieved high TOC removal efficiency of 92%. Authors subsequently applied reverse osmosis (RO) as post treatment in order to concentrate the nutrients for further use on agricultural lands. Ho and Sung (2010) reported high COD removal efficiencies for membranes coupled CSTRs treating synthetic municipal wastewater. Gimenez et al. (2011) tested hollow fiber membranes in an AnMBR system consisting of a CSTR and submerged membranes immersed in an external chamber and achieved a COD removal efficiency of 90% with a flux of 10 L/m².h.

2.2.2 High-Rate anaerobic reactors

In high-rate anaerobic reactors such as sludge bed systems and anaerobic filters, biomass is retained either by the formation of granular and/or thick flocculent sludge or by attachment to a support material. Effluent SS concentration is significantly lower than the biomass concentration in the reactor, which makes them feasible for high hydraulic loadings. For instance, sludge bed systems are characterized by TSS concentrations ranging between 20-40 kg/m³ reactor volume (van Lier et al., 2008). As in these reactors biomass is not directly subjected to membrane filtration, dense cake layer formation and consolidation will be less apparent in comparison with CSTRs when combining these reactors with a membrane module. Therefore, high rate anaerobic reactors may offer a good opportunity for being combined with membranes, especially if very low SS concentration is required in the effluent and retention of biomass is required under presence of toxicity related to discharges of industrial wastewater in the municipal sewer systems, or hydraulic overload events (Liao et al., 2006). However, the impact of membrane application on sludge immobilization and granule stability is not yet well understood.

2.2.2.1 Upflow anaerobic sludge blanket reactor

UASB reactors coupled to membrane separation can be a reasonable option to decrease the SS concentration being sent to the membrane since the sludge bed would entrap most of the particulate matter by adsorption and biodegradation

(Kataoka et al., 1992; An et al., 2009a; Wu et al., 2009; Zhang et al., 2011). All biological processes take place inside the dense sludge bed in the bottom of the UASB reactor (van Lier et al., 2008). The physical removal of particulate organics by settling, adsorption and entrapment in the sludge bed of the UASB makes it appropriate for the first treatment step of municipal wastewater, which is generally characterized by a high particulate COD/soluble COD ratio. In fact, UASB reactors can be upfront used as biofilters before membrane treatment, which prevents the membrane from excessive exposure to high SS concentrations. For instance, while a UASB reactor had a biomass concentration of 20-30 g/L, SS concentration in the effluent was below 1 g/L (Kleerebezem and Macarie, 2003). An et al. (2009a) reported TSS concentrations in the range of 11-32 g/L in a UASB reactor; whereas, TS concentration in the effluent was less than 50 mg/L. Obviously, the efficiency of solids entrapment determines the amount and properties of solids leaving the UASB with the effluent. In this context, HRT and upflow velocity in membrane coupled UASB reactors seem to be the critical parameters determining the efficiency and effluent fouling propensity. High SRTs and OLRs can be maintained by applying UASB reactors, without increasing the effluent solids concentration that will be subjected to membrane filtration. Thus, membrane flux may become less dependent on the reactor MLSS concentration, possibly leading to high membrane fluxes. There are many studies that focus on the fouling potential in AnMBR systems including UASB reactors coupled to membranes. Liao et al. (2006) suggested that membrane coupled UASB systems may decrease the capital cost of UASB reactors by eliminating the need for a gas-liquid-solids (GLS) separator in a UASB. Moreover, organic fouling may be reduced by increasing the SRT which leads to high biomass concentrations in the reactor and, through increased entrapment in the bed, lower COD concentrations in the effluent of the UASB reactor (Liao et al., 2006). Obviously, with increased biogas production the absence of a GLS separator will likely lead to high sludge carry over to the effluent.

Despite the expected more efficient membrane filtration step, for the treatment of municipal wastewater under sub-mesophilic conditions, hydrolysis of the retained particulates likely becomes the rate-limiting step and particulate matter accumulation in the sludge bed will occur, subsequently resulting in activity loss (Lettinga et al., 2001). Furthermore, a thinner and less porous cake layer on the membrane surface

can be expected, possibly leading to more serious pore clogging problems in UASB coupled AnMBRs, since the membrane would be exposed directly to only fine particles instead of a range of particle sizes. According to the previous results particularly the small (submicron) particles determined the operational flux (Jeison et al., 2009a; Jeison et al., 2009b). Therefore, the degree of small particle retention by the sludge bed will be of prime importance for the feasibility of coupled membrane filtration.

In some studies, researchers used membrane filtration as a polishing step after UASB systems without returning the concentrate stream to the bioreactor as it is applied in AnMBRs (Herrera-Robledo et al., 2010; Salazar-Pelaez et al., 2011a; Herrera-Robledo et al., 2011; Salazar-Pelaez et al., 2011b) (Figure 2.1). These configurations can be regarded as tertiary filtration. This approach has the advantage of easier control of hydraulics in the UASB reactor and the preservation of the dilution rate as bacterial selection criterion. However, it may have the disadvantage of high SS concentrations exposure to the membrane because SS would be concentrated in the concentrate collection tank.

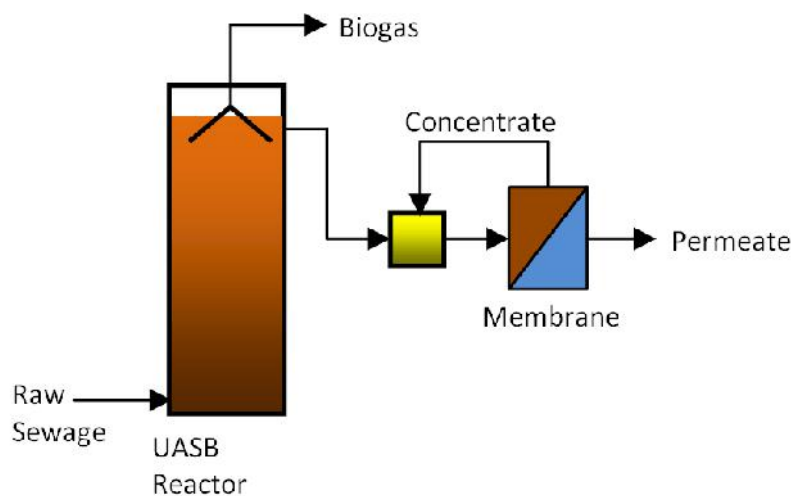


Figure 2.1 : Membrane filtration used as a post-treatment step after UASB reactors.

2.2.2.2 Expanded granular sludge bed reactor

UASB reactors treating sewage at low and moderate temperatures are sometimes characterized by a poor mixing regime, which causes a decrease in soluble COD treatment efficiency. To solve this problem, tall reactors with a small footprint and external or internal effluent recirculation are being applied, i.e. the so-called EGSB reactors (van der Last and Lettinga, 1992). Bench-scale and (semi-)pilot studies have

shown that an EGSB reactor is an attractive alternative, especially for the treatment of low strength wastewaters at ambient temperatures, due to the efficient biomass-substrate contact induced by the applied high upflow velocity (de Man et al., 1988; Kato et al., 1997; Lettinga et al., 1999). Besides, higher OLRs can be applied in EGSB reactors compared to UASB reactors. However, SS are limitedly removed from the wastewater and sludge washout would occur in the system due to high upflow velocity. Therefore, anaerobic treatment applying EGSB systems is restricted to pre-settled sewage only. As an alternative to pre-settling, Chu et al. (2005), as the sole example in literature, proposed a membrane unit that is capable of retaining the SS inside the EGSB reactor. In that study, the membrane module is submerged in the upper part of the EGSB reactor and the applied high upflow velocity was beneficial in the reduction of membrane fouling due to the increased hydraulic shear stress on the membrane surface (Chu et al., 2005). Nonetheless, cake resistance governed the achievable flux over the hollow fiber membranes, whereas pore blocking only was of minor importance. Based on the high removal efficiencies even at low temperatures, the use of EGSB reactors in AnMBRs was suggested as a potential technology for treating municipal wastewater at ambient temperature (Chu et al., 2005). Following their reasoning, submerged membrane configurations are found more appropriate than external configurations for the use of EGSB reactors in AnMBRs. However, membrane integration eliminates the hydraulic selection pressure required for granulation, by avoiding the washout of flocculent sludge with poor immobilization characteristics. Therefore, no granulation is expected in EGSB reactors coupled to membrane filtration, which would decrease the settleability of the biomass on the long-term operation.

2.2.2.3 Other reactor types

In addition to the most popular high rate reactors, i.e. UASB and EGSB reactors, other reactor types such as hybrid upflow anaerobic systems (Wen et al., 1999) and jet flow anaerobic reactors (Saddoud et al., 2006) were also used for the treatment of municipal wastewater at low temperatures.

Wen et al. (1999) operated a hybrid upflow anaerobic system coupled to a membrane unit for 200 days. The hybrid system consisted of an anaerobic bioreactor column in which the bottom part was used as a sludge bed zone and the upper filter part was

packed with fine fibres to prevent sludge washout. They obtained a high COD removal efficiency (>97%) with an effluent COD concentration up to 20 mg/L. It was also reported that high OLRs (0.5-12.5 kg COD/m³.d) could be applied successfully.

A jet flow anaerobic reactor (Saddoud et al., 2006) was coupled to membrane filtration for the treatment of municipal wastewater. Circulation of the liquid inside this type of reactor by using an inner tube and a nozzle system provides a good homogenization inside the reactor. By using an UF membrane coupled to the jet flow anaerobic reactor, total removal of pathogens and high quality effluent were obtained of interest for agricultural usage (Saddoud et al., 2006).

2.3 Alternative Integration Possibilities of AnMBRs in Municipal Wastewater Treatment Flow Schemes

In view of cost-effective municipal wastewater treatment, which also aims at recovery of resources (nutrients), production of energy, and the use of the treated effluent, integrated system approach deserves more attention than solely the coupling of a specific anaerobic reactor type to membrane units. By that approach, the optimum way of closing nutrient and water cycles should be determined. Some alternative integration options for AnMBRs in conventional wastewater treatment schemes can be found in the literature. However, innovative possibilities should be continuously developed for energy and nutrient recovery.

For municipal wastewater treatment at low (~15 °C) and/or fluctuating temperatures (15-25 °C), a two-step system including a UASB reactor combined with an additional sludge digester was shown advantageous to prevent activity loss in the sludge bed due to slowly degradable particulate matter accumulation (Mahmoud et al., 2004). In this system, the non-degraded particulate COD entrapped in the sludge bed of the UASB reactor is further stabilized in a separate CSTR type digester operated under optimal mesophilic conditions (30-35 °C). The UASB-Digester setup is proposed to overcome the hydrolysis limitation induced by low temperatures and to reduce non-stabilized particulate matter accumulation in the sludge bed. By recirculating the digested solids to the UASB reactor, the sludge bed SMA is increased resulting in enhanced removal of soluble organics (Mahmoud et al., 2004). Membrane integration to the UASB-Digester system can be attractive for producing

high quality and nutrient-rich effluents for reuse purposes under moderate climate conditions (Figure 2.2(a)).

Since anaerobic processes are particularly of interest for concentrated waste stream, upconcentration of sewage using forward osmosis (FO) technology is currently receiving increasing interest (Zhao et al., 2012). FO uses an osmotic pressure gradient to drive water across a semi-permeable membrane from the low osmotic pressure side (feed solution) to the high osmotic pressure side (draw solution). Owing to the absence of pressure driven water transport, FO membranes are not susceptible for the traditional sewage fouling propensities (Achilli et al., 2009; Zhao et al., 2012). FO processes may be used as a pretreatment to concentrate municipal wastewater in coastal areas where the sea water can be used as a draw solution (Achilli et al., 2009; Cath et al., 2010; Zhao et al., 2012). Alternatively, closed loop draw solutions can be used, however, at the expense of energy consumption for draw solution regeneration. For the optimization of energy and other operational costs, appropriate draw solutes and concomitant regeneration methods are being researched (McGinnis and Elimelech, 2008; Elimelech and Phillip, 2011). A decrease in wastewater flow is regarded as the benefit of the FO based concept resulting in small treatment systems with anaerobic digestion (AD) as the main bio-stabilization process (Holloway et al., 2007; Zhao et al., 2012). A possible integration of the FO unit in an AnMBR treatment system is given in Figure 2.2(b). Interestingly, a current Dutch project researches the possibility to extract fresh water from sewage using FO, whereas the AD-recovered energy from the concentrated sewage is used for re-concentrating the draw solution. The energy needed for the desalination part of the total process could be obtained by digestion of the concentrated sewage. However, further research should be done in order to clarify the sufficiency of the produced energy to drive the whole integrated concept (Roest et al., 2010).

Due to the low COD concentrations and concomitant high influent flow rates, high membrane surface areas and/or high fluxes are required for municipal wastewater treatment, using membrane technology. Therefore, a pretreatment step such as (advanced) solid/liquid separation in municipal wastewater is of interest when AnMBR systems are considered for municipal wastewater treatment. Consequently, AnMBRs may take the role of sludge digesters in municipal wastewater treatment systems as presented in Figure 2.2(c). A similar flow scheme was proposed by Sutton

et al. (2011). The latter authors carried out a model based feasibility study with mass balance calculations for a conceptual flow diagram of a municipal wastewater treatment plant consisting of aerobic and anaerobic MBRs and innovative nutrient recovery processes such as ammonia removal by using zeolite, and orthophosphate removal by a reactive filtration system. They proposed to shuttle the largest fraction of organic carbon in the wastewater into solids slurry and to further digest the organic solids in an AnMBR. Modeling and simulation results were used to optimize the design of the system with respect to energy usage and solids production and to obtain cost savings. Minimization of residual solids production and maximization of the energy produced resulted in cost reduction. However, results are theoretical and emphasis should be given to membrane performance, validating the concept with pilot and full-scale studies.

Another alternative for AnMBR integration as a sludge digester is depicted in Figure 2.2(d). The main difference between Figure 2.2(c) and 2.2(d) is the presence of a membrane coupled hydrolysis reactor in Figure 2.2(d). The hydrolysis reactor can be used for solubilization of particulate organic matter which may accumulate in the UASB reactor. The generated volatile fatty acids (VFAs) can be further converted to methane in a subsequent high rate anaerobic system such as membrane coupled UASB reactor or can be used for other purposes. This approach was investigated at pilot-scale by Kiriya et al. (1992) and Kiriya et al. (1994) who concentrated raw sewage by a filter unit and fed the thickened sludge to a hydrolysis reactor equipped with external cross-flow membranes. VSS reduction rate increased from 45% to over 80% by the addition of a membrane module to the hydrolysis reactor. The pre-acidified wastewater was further treated in a UASB reactor. However, no information about the membrane performance was given in the study. Kataoka et al. (1992) operated a two stage system consisting of a hydrolysis reactor and a FB reactor for the treatment of municipal wastewater. Both reactors were equipped with external membrane units. The membrane coupled FB reactor was operated at an OLR of $1.1 \text{ kg BOD/m}^3 \cdot \text{d}$ with 91% BOD removal.

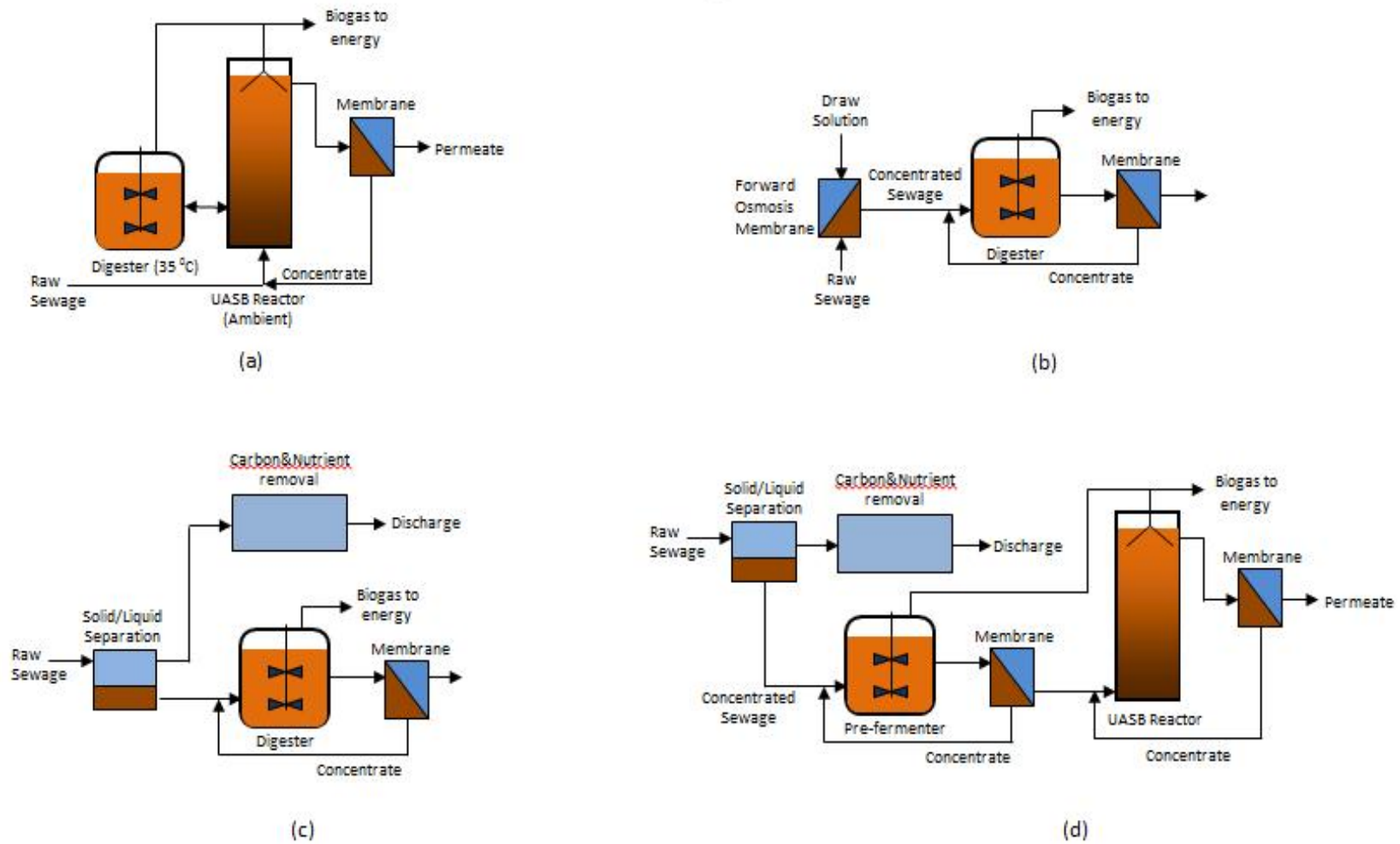


Figure 2.2 : Alternative integrated flow schemes of AnMBRs.

In case nutrient removal is required instead of recovery for reuse, all proposed flow schemes can be modified by adding innovative nutrient removal systems such as Sharon/Anammox (van Dongen et al., 2001; van Kempen et al., 2001) and struvite recovery (Marti et al., 2008; Pastor et al., 2010). Grundestam and Hellstrom (2007) proposed to treat AnMBR effluents by RO membranes in order to achieve high nutrient recovery and water reuse. The system was operated at 22 °C without any heating and a high permeate quality was obtained. Removal efficiencies obtained by the proposed system were 91%, 99% and >99% for Kjeldahl nitrogen, phosphorus and TOC, respectively. Total energy consumption of the system was reported as 3-6 kWh/m³.

2.4 Factors Affecting the Treatment Performance of AnMBRs for Municipal Wastewater Treatment

Optimization of AnMBR processes generally focus on either the improvement of biological efficiencies (Visvanathan et al., 2000) or on enhancing the membrane based separation process (Huang et al., 2008; Dereli et al., 2012). Few authors research the interaction between both processes (Chu et al., 2005; Vyrides and Stuckey, 2009). In subsequent paragraphs the impact of the various factors, including the key operational parameters such as temperature, HRT, upflow velocity, OLR as well as sludge characteristics, and addition of adsorbents, on both biological and physical aspects are further discussed.

2.4.1 Operational conditions

2.4.1.1 Temperature

The temperature dependence of biological reaction rates carries vital importance for the overall efficiency of a biological treatment process. Generally, the activity of microorganisms in a biological process decreases when the temperature decreases, which consequently results in a decrease in COD removal efficiencies. Besides its effect on the metabolic activities of the microbial population, temperature has a profound effect on factors such as biogas solubility, solubility of (in)organic compounds, and the settling characteristics of the biological solids due to water viscosity changes (Tchobanoglous and Burton, 2003).

Chu et al. (2005) investigated the effect of temperature on the performance of membrane-coupled EGSB reactor at moderate and low temperatures. Temperature strongly affected the rate of the anaerobic conversion process and a decrease in both COD removal efficiency and activity was observed with the decrease in temperature from 25 °C to 11 °C. However, the contribution of the membrane to the COD removal efficiency increased from 8.8% to 14.2% with the decrease in temperature, which demonstrated the advantage and robustness of membrane-coupled reactors in maintaining high treatment efficiency even at low temperatures due to the complete retention of particulate COD and biomass. This is in agreement with findings of Ho and Sung (2010) who also investigated the effect of temperature in the treatment of municipal wastewater by AnMBRs. Two identical AnMBRs were operated at 25 °C and 15 °C in parallel for 112 days. They reported that the physical removal capacity by the membrane compensated for the decreased SMA and biological removal rate at 15 °C by rejecting soluble organics. Wen et al. (1999) also observed that AnMBRs treating municipal wastewater had a strong tolerance to temperature fluctuations between 12 °C and 26 °C. They obtained 88% COD removal even at 12 °C. Martinez-Sosa et al. (2011a) reported that bioreactor temperature impacted methane recovery in an anaerobic externally submerged MBR treating municipal wastewater. COD removal efficiencies close to 90% were achieved at both 35 °C and 20 °C. A stable cake layer formation on the membrane surface may compensate differences in biological organic matter degradation rate at different temperatures. Although methane recovery at 20 °C is lower owing to the higher solubility of methane at lower temperatures, interestingly, the methane content of the biogas was higher at low temperatures due to the gas solubility difference of carbon dioxide (CO₂) and methane at 20 °C compared to 35 °C. Lower solubility of methane in comparison to CO₂ at 20 °C resulted in a major proportion of CO₂ in the liquid phase leaving the reactor and an increase in the proportion of methane from 80% to 88% in the gas phase. Gimenez et al. (2012) also investigated the effect of temperature on methane recovery efficiency in an AnMBR system treating municipal wastewater. They obtained slightly lower values at 20 °C in comparison to 33 °C due to a reduction in the treatment efficiency and an increase in the gas solubility.

2.4.1.2 Organic loading rate

AnMBR processes have the advantage of tolerating changes in organic loading similar to its tolerance to the fluctuations in temperature. OLRs ranging from 0.3 to 12.5 kg COD/m³.day have been applied in AnMBRs treating municipal wastewater (Table 2.1). Wen et al. (1999) confirmed that an excellent effluent quality could be obtained by the combination of an anaerobic bioreactor with a membrane unit in spite of high organic loading fluctuations between 0.5 and 12.5 kg COD/m³.day, which generally would cause perturbations in conventional anaerobic reactors. Accordingly, Lin et al. (2011) observed relative stability in the permeate COD regardless of the fluctuations in the influent COD. The quality of the effluent was also stable in the study of An et al. (2009a) in spite of the fluctuations in OLR. They stated that AnMBR could achieve more stable effluent quality in comparison to a conventional anaerobic process. Moreover, it was shown that the biogas yield increased linearly with an increase in the organic loading.

2.4.1.3 Hydraulic retention time

HRT is an important parameter from an economic perspective as it has a strong influence on capital costs, considering that shorter HRTs allow smaller reactors (Salazar-Pelaez et al., 2011a). Therefore, the influence of HRT on the biological removal efficiency of AnMBRs treating municipal wastewater has been investigated by various researchers. Among them, Hu and Stuckey (2007) studied an AnMBR operated at 35 °C and concluded that COD concentrations both in the reactor and permeate increased slightly with a decrease in HRT due to an increased organic load. Chu et al. (2005) also studied the effect of HRT at various temperatures on the treatment performance of a membrane-coupled EGSB reactor. They found that COD removal efficiency was independent of HRT at temperatures higher than 15 °C. However, an increase in COD removal efficiency was observed with an increase in HRT at 11 °C, indicating the significance of HRT at low temperatures. HRT was controlled as an independent parameter from upflow velocity in the study of Chu et al. (2005) by introducing effluent recirculation to the reactor. However, for upflow reactors without recirculation, effects of HRT and upflow velocity can be evaluated dependently as they are inversely proportional to each other as in the study of An et al. (2009a). They reported an increase in the TOC removal efficiency when the HRT

of a membrane coupled UASB reactor was decreased from 10 h to 5.5 h. They explained this as a result of improved wastewater distribution in the sludge bed and better contact between biomass and substrate at higher upflow velocity. Based on the results, it can be hypothesized that there is an optimum HRT for each case, which is determined by many factors such as system hydraulics, wastewater characteristics and sludge properties, to provide both efficient biological removal and filtration performance.

2.4.1.4 Upflow velocity

Upflow velocity is an important parameter having two opposing effects on the biological removal efficiency in upflow reactors. An increase in upflow velocity may enhance mixing providing better substrate-biomass contact. On the other hand, increasing upflow velocity may deteriorate the removal efficiency by exceeding the settling velocity of particles, resulting in the detachment of the captured solids due to high hydraulic shear force (Mahmoud et al., 2003). Different upflow velocities were applied in Chu et al. (2005)'s study by using effluent recirculation in a membrane-coupled EGSB reactor and a better COD removal performance was achieved at higher upflow velocities which supported the former hypothesis of Mahmoud et al. (2003). Besides, a significant increase in COD removal efficiency was observed with an increase in upflow velocity at 11 °C in comparison with a slight increase at 25 °C, which demonstrated the importance of adequate hydraulic mixing at lower temperatures.

2.4.2 Sludge characteristics

Biomass characteristics, bacterial flora, e.g. the fraction of slow-growing bacteria, and nutritional requirements, are mainly dependent on the type and operational conditions of bioreactors (Kataoka et al, 1992). There are several reports asserting an activity loss of biomass, especially of propionate degraders, in AnMBRs (Brockmann and Seyfried, 1997; Padmasiri et al., 2007). This may be due to lysis of the cells under high shear or disruption of juxta-positioning of the hydrogen producing bacteria and hydrogenotrophic methanogens, enlarging the interspecies hydrogen transfer distance. On the other hand, Jeison et al. (2009b) preserved both methanogenic and acetogenic activities in a cross-flow AnMBR system applying liquid superficial velocities of 1-1.5 m/s and gas slug upflow velocities of 0.1 m/s.

SMA of the cross-flow AnMBR sludge using propionate as the substrate were even higher than those of a parallel operating UASB system. Other studies focus on differences in microbial species composition or activity profiles between the fouling layer and biomass suspension of AnMBRs treating domestic sewage (Ho and Sung, 2010; Gao et al., 2010). Ho and Sung (2010) found that SMA of the biomass attached to the membrane surface was lower compared to the bulk sludge in an AnMBR treating municipal wastewater. Thus, the attached sludge did not play a significant role as a biofilm for biological removal compared to suspended sludge. Zhang et al. (2011) also investigated the characteristics of the fouling layer and the bulk sludge in a dynamic AnMBR system in which separation is achieved by a dynamic cake layer that is formed by bulk sludge. Microorganism communities were found to be different from the bulk sludge, and activity of microorganisms in the dynamic layer was found lower than bulk sludge due to the compactness of cake layer which resulted in suppressed mass transfer.

Lin et al. (2011) investigated the effect of sludge concentration on biological treatment of AnMBRs and observed a relative stability in the permeate COD regardless of the fluctuations in sludge concentrations between 6.4 and 9.3 g MLSS/L.

2.4.3 Addition of adsorbents

Powdered activated carbon (PAC) and zeolites can be added into AnMBRs in order to adsorb soluble organic compounds. By this way, these materials reduce organic fouling, enhance membrane flux and also affect biological treatment performance (Akram and Stuckey, 2008). Hu and Stuckey (2007) investigated the effect of both PAC and granular activated carbon (GAC) addition on the performance of AnMBRs. The addition of PAC resulted in a significant increase in COD removal efficiency, whereas only limited effect was observed after GAC addition. This was attributed to the larger surface of PAC per unit mass compared to GAC, thus more colloids and macromolecules could attach to PAC from the bulk solution. VFAs were only limitedly absorbed to the activated carbon. Interestingly, the SMA of the sludge growing in the AnMBR without activated carbon addition was lower compared to that from the AnMBR with activated carbon addition. This finding was explained by the support surface provided by activated carbon in order to protect the biomass from

high shear conditions. Besides, Vyrides and Stuckey (2009) observed an increase in DOC removal after the addition of PAC due to the adsorption of high molecular weight compounds.

2.5 Factors Affecting the Membrane Performance of AnMBRs for Municipal Wastewater Treatment

Flux decline, especially in long-term operation, seems to be the most critical constraint for the applicability and feasibility of AnMBRs for municipal wastewater, since it plays a major role in the determination of the required membrane area. A number of factors are associated with flux decline. Therefore, the research of membrane fouling behavior and mechanisms requires the understanding of several factors such as membrane characteristics, operational conditions and sludge properties. So far, membrane fouling in AnMBRs has not been fully understood due to the complex nature of membrane foulants and diversity of operational conditions, membrane materials, configurations and wastewaters in different studies. Multiple and complex interactions occur between physical and biological factors not only in real plants but also in controlled lab-systems. Therefore, fouling phenomena should be investigated under well-defined combinations of parameters and conditions which individually might even present opposing effects (Drews, 2010). Having outlined the key parameters affecting biological treatment performance, major factors that affect filterability in AnMBRs for municipal wastewater treatment will now be discussed.

2.5.1 Membrane characteristics

2.5.1.1 Material

Membrane material characteristics may affect the degree of fouling in AnMBRs; e.g. organic and inorganic membranes may show different fouling behaviors. Kang et al. (2002) reported that cake layer formation was the main mechanism for fouling of organic membranes, whereas inorganic precipitation, mainly struvite, played the key role in the fouling of inorganic membranes.

Gao et al. (2010) observed differences in fouling rates and foulant layer composition even for two different organic membrane materials. They found that fouling of uncoated polyetherimide (PEI) UF membranes occurred faster than PVDF UF

membranes coated with polyether block amide, reaffirming the significance of membrane material on fouling. Furthermore, they determined significant differences in bacterial composition in the fouling layers of different membrane materials. Although *Bacteroidetes* were absent in the fouling layer of PVDF membrane, they were present on PEI membrane. Observations of Gao et al. (2010) showed that membrane material may affect the interactions between different microbial species and membrane surface, and thus the fouling phenomena, and support the value of integrating membrane material science with deep understanding of the adhesion and biofilm-formation potential of different microbial species.

2.5.1.2 Module type and configuration

Various membrane configurations such as flat sheet, hollow fiber, and tubular membranes are applied in AnMBRs, using different types of module configurations such as submerged/immersed and external cross-flow systems (Liao et al., 2006). Submerged AnMBRs can also be used in different configurations including directly immersed into the bioreactor or immersed in a separate membrane tank. Generally, for submerged AnMBRs flat sheet and hollow fiber membranes are preferred, whereas external cross-flow AnMBRs can be configured with tubular membranes as well (Liao et al., 2006). Different hydrodynamic conditions in submerged and external cross-flow AnMBRs have different effects on the bulk sludge properties, attainable flux and membrane fouling due to the extent of applicable shear rate (Berube et al., 2006). AnMBRs configured with external cross-flow membranes may provide a higher flux (van Lier and Jeison, 2010) and need a lower membrane area in comparison to their submerged counterparts. However, energy required for the cross-flow pumps would be high due to the high flow to be pumped for providing enough hydraulic shear force. Martin-Garcia et al. (2011) compared specific energy demands for different configurations of AnMBR treating municipal wastewater and found that 0.3 kWh/m³ permeate and 3.7 kWh/m³ permeate were needed for submerged and external cross-flow configurations, respectively. The amount of pumping energy may be decreased by using small diameter tubular membranes since the packing area of the membrane module can be increased. On the other hand, high hydraulic shear force may also disrupt anaerobic biomass and generate small particles, which result in significant membrane fouling. Besides, biological activity of anaerobic biomass may decrease due to the high hydraulic shear force (Lin et al., 2010).

An et al. (2009a) investigated the effect of different tubular membrane diameters, i.e. 3.0, 1.9, 1.2 mm, on the filtration performance of an external cross-flow AnMBR treating municipal wastewater. The results showed that the variations of TMP were reciprocally correlated with the variations in tube diameter. This phenomenon was related to the particle accumulation that occurred in the lumen. A smaller diameter tube was simply blocked by large particles, which caused serious membrane clogging in comparison to the tubes with larger diameter. The blockage of small diameter tubes resulted in an uneven flux distribution along the membrane module and an increase in the local flux, which finally caused more serious membrane clogging in comparison to the tube with larger diameter.

2.5.2 Operational conditions

2.5.2.1 Shear rate

Removing the deposited cake layer from the membrane surface is very important to achieve a steady operation in AnMBRs. Cross-flow and biogas sparging may be regarded as principle mechanisms to provide shear over the membrane surface in order to limit the deposition of particles and restrict their interaction with the membrane (Liao et al., 2006). 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 shear rate (Lin et al., 2010). Although increasing shear rate can help to reduce fouling and cake resistance, and improve flux, there appears to be a certain limit for both cross-flow velocity and gas sparging rate beyond which it would practically have little or no added benefit. Choo et al. (2000) indicated that cake layer resistance can be decreased by increasing cross-flow velocity. However, at a Reynolds number of 2000, a plateau was reached and no further reduction in the resistance could be obtained. The same is true for the gas sparging rate. Xie et al. (2010) reported that the critical flux of a submerged AnMBR increased and the fouling rate decreased when the biogas sparging rate was increased from 10 to 25 L/m².min. However, they indicated that there was a practical limit above which further increasing the biogas sparging rate provided little added benefit. Similar results, also for submerged AnMBRs, were presented by Jeison and van Lier (2006a).

Moreover, high shear rates may also stimulate the break-down 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. This results in the formation of a dense consolidated cake layer that is very hard to remove. This phenomenon has been called as the shear rate dilemma (Jeison et al., 2009a). An et al. (2009a) investigated the effect of cross-flow velocity on fouling in a UASB reactor equipped with tubular membranes. They concluded that a low TMP can be maintained for a long time at high cross-flow velocities. However, this operation condition led to a high irreversible fouling due to the deposition of small sized particles on the membrane surface and inside the pores during long-term operation.

Duration and frequency of applied shear rate may also have an effect on filterability. Vyrides and Stuckey (2009) reported that switching from continuous biogas sparging to intermittent mode (10 min ON/5 min OFF) resulted in a small increase in TMP. However, it improved the DOC removal of submerged AnMBR due to the formation of a thicker cake layer on the membrane surface. Under these conditions a higher rate of biodegradation was observed because the substrate was in contact with a thicker biofilm and more of the higher molecular weight solutes were retained until they could be degraded.

2.5.2.2 Flux

Choice of the operational flux is very important in terms of fouling management. Operation below the critical flux, which depends on membrane characteristics, operating conditions and sludge characteristics, is an effective approach to avoid severe fouling within a filtration system (Jeison and van Lier, 2006b; Meng et al., 2009). Martinez-Sosa et al. (2011b) reported that an AnMBR treating municipal wastewater could be operated stable for a long time at a critical flux of 7 L/m².h with a biomass concentration of 14.8 g TSS/L and a gas sparging velocity of 62 m/h. Increasing flux to 10 L/m².h and 12 L/m².h resulted in an unstable operation due to the high fouling rate that could not be controlled even at higher gas sparging velocities.

2.5.2.3 Operation mode

The operation mode of membrane systems is another important factor influencing fouling in AnMBRs. Generally, backwashing and/or relaxation are used as strategies

to decrease fouling. Moreover, optimization of backwash and relaxation duration and/or frequency plays an important role in flux enhancement. Lew et al. (2009) emphasized the necessity of backwash frequency optimization for fouling reduction. They obtained similar fouling rates for 15 and 30 minutes backwash frequencies; however, higher fouling rate was observed at a higher backwash interval of 60 min. Chu et al. (2005) also dealt with finding optimal operating modes and observed an increase in permeability and enhancement in the permeate flux (J) recovery with increasing relaxation time. During the relaxation period, efficient cake layer removal from the membrane surface was achieved. Gimenez et al. (2011) operated a pilot-scale AnMBR equipped with hollow fiber membranes for the treatment of municipal wastewater and obtained a flux of 10 L/m².h at an MLSS concentration of 22 g/L. They prevented fouling with the help of a rather complex membrane operation mode including backwash, relaxation and degasification cycles. An et al. (2009a) compared backwash, relaxation and continuous filtration modes and maintained low TMP for a longer time in backwash mode in comparison to relaxation or continuous filtration modes.

2.5.2.4 Temperature

Temperature affects not only the rate of the biodegradation process but also the viscosity of the filtered liquid and the solubility of various compounds and gases. Since under practical conditions sewage temperatures cannot be altered, the operational temperature is of significant importance for municipal wastewater treatment using AnMBRs. Martinez-Sosa et al. (2011a) stated that the operational temperature of an AnMBR is related to the observed membrane fouling. Although they operated the system at a flux of 7 L/m².h at 35 °C, they observed an increase in the fouling rate at 20 °C, possibly due to TSS and soluble COD accumulation and a higher viscosity in the bioreactor. Decrease in temperature and increase in solids content were proposed as possible explanations for viscosity increase. The fouling rate was reported as 0.14 mbar/d at 35 °C, whereas it was 2.61 mbar/d at 20 °C.

2.5.2.5 Upflow velocity

The upflow velocity in (submerged) AnMBRs generally has a positive impact on filterability mainly due to increased shear stress. Chu et al. (2005) operated an EGSB reactor equipped with a submerged membrane module and observed an increase in

permeability with the increase in upflow velocity likely due to the shear effect of higher upflow velocities. However, on the long-term, a flux decline was observed indicating a decreased contribution of upflow velocity to fouling control, suggesting the inadequacy of shear force induced by high upflow velocity to combat cake layer formation. Possibly, the observed phenomenon can be attributed to strong adhesion of the foulants to the membrane surface and an increased thickness of the cake layer.

2.5.2.6 Solids retention time

SRT has been identified as the main parameter influencing flux with values typically dropping at higher SRTs. Operation of anaerobic systems at ambient temperatures becomes feasible only if SRT is approximately twice as high as that under mesophilic conditions (Kashyap et al., 2003). As a rule of thumb, SRT should be at least three times the doubling of the slowest growing organism responsible for bioconversion (van Lier et al., 2008). High capability of solids retention in membrane systems makes membranes ideally suited for anaerobic treatment of municipal wastewaters especially at low temperatures when the degradation rate of SS and colloidal materials is the rate limiting step (Zhang et al., 2010). Since the particulate organics would also be retained in the reactor, they can eventually be further hydrolyzed and degraded (Zhang et al., 2010). However, although membrane processes result in solids retention independently of temperature (Liao et al., 2006), the activity limitation of anaerobic microorganisms at low temperature might yield high colloidal and soluble solids in anaerobic effluents, resulting in an increase in membrane fouling propensity (Herrera-Robledo et al., 2011). SRT of more than 140 days was reported as a possible cause of severe membrane fouling and flux decline (Saddoud et al., 2007). Huang et al. (2008) reported that longer SRTs (from 30 to infinite days) produced higher protein/carbohydrate ratio (P/C) in extracellular polymeric substances (EPS) and lower P/C in SMP, which resulted in serious fouling. However, Herrera-Robledo et al. (2010) investigated the effect of SRT for both short and long-term operations for the system that used UF membrane as a polishing step after a UASB reactor. It was found that fouling rate and effluent quality were not dependent on SRT. Their observations were based on trials for SRT of 60 and 100 days in short-term operation. Sudden changes in TMP and flux were observed for both SRTs. During long-term operation (500 h), they observed that sudden increases in TMP and decreases in flux occurred in shorter filtration period

(e.g. 140 hours) with SRT of 60 days than with SRT of 100 days (e.g. 175 hours). This leads to the assumption that a more rigid and strong fouling layer structure was developed in the system having long SRTs.

2.5.2.7 Hydraulic retention time

Variations in HRT may change MBR fouling propensity. A few researchers have reported on the effect of HRT in membrane coupled sludge bed reactor types treating municipal wastewaters. Among them, An et al. (2009a) reported that a decrease in HRT from 10 h to 5.5 h resulted in a decrease in solids removal efficiency of the bioreactor. However, owing to the membrane separation, the reactor performance was quite stable. Besides, Lew et al. (2009) observed that membrane fouling was positively correlated to the particulate matter concentration reaching the membrane.

2.5.3 Sludge characteristics

Currently EPS, both bound and soluble, is often mentioned as the sludge factor of prime importance in relation to membrane fouling. Soluble EPS is often also referred to as SMP (Meng et al., 2009). Membrane inner pore accumulation and sorption of EPS and SMP favor biomass attachment and cake layer formation, possibly leading to severe fouling (Zhang et al., 2010; Herrera-Robledo et al., 2011). Operational parameters such as SRT, OLR, temperature, pH and shear rate are the most important factors influencing both the concentration and composition of SMP and EPS.

EPS excreted from the microbial cells was considered to have a significant effect on the fouling due to both an increase in viscosity of the mixed liquor and an increase in the filtration resistance (Nagaoka et al., 1996). Chu et al. (2005) measured the amount of EPS from both granules in an AnMBR and sludge on the membrane surface and claimed that EPS affected the cake resistance by filling the void spaces between the particles in the cake layer resulting in a drastic reduction in the flux. Viscosity of the liquor in the anaerobic reactor, which was equal to the water viscosity, did not change with operating time. Apparently, the impact of EPS is only on filtration resistance and not on liquid viscosity. An et al. (2009b) observed that EPS extracted from the cake layer consisted of mainly protein-like and humic acid-like substances. Moreover, Gao et al. (2010) found that EPS mainly consisted of proteins and was the main reason of fouling in AnMBRs treating municipal wastewater. An et al. (2009b) reported that the support layer (nonwoven fabric)

surface was covered with a rough and dense layer consisting of mainly protein, clay materials and inorganic elements such as magnesium, aluminium, calcium, silisium, and iron.

SMP was grouped into two predominant fractions including high and low molecular weight SMP in Herrera-Robledo et al. (2011)'s study. High molecular weight SMP was associated with long SRT, incomplete organic matter hydrolysis and rate limitations of anaerobic microorganisms at low temperatures, e.g. 20 °C. Low molecular weight SMP have the propensity to pass freely through membrane pores. However, it can also adsorb onto the membrane surface leading to an intermediate blocking or deposit inside the pores resulting in standard blocking.

Besides, microbial community composition is effective on fouling. Gao et al. (2010) found differences in community composition between the cake layer and biomass suspension for a membrane coupled UASB reactor. They detected a bacterium within phylogenetic division OP11 at higher abundance in the cake layer compared to the suspension. Moreover, some bacteria such as *Bacteroidetes* were not found in the fouling layer although they existed in the suspension. These results may indicate that some species play a direct role in fouling, e.g. by attaching to the membrane surface, while others, including some that likely play a major role in the metabolism of influent organics, play a less important or indirect role. When this hypothesis is true, it means that cells are selectively incorporated within the fouling layer. Then, isolation and characterization of representatives of these species may provide useful information for biofouling control.

2.5.4 Addition of flux enhancers

The interest in flux enhancers that can act through a number of different phenomena such as adsorption of SMP, coagulation, crosslinking between flocs and SMP has greatly increased (Drews, 2010). Various additives such as activated carbon, polyelectrolytes, coagulants and flocculants can be used to improve the flux and to reduce fouling in MBRs (Hu and Stuckey, 2007; Wu et al., 2009).

Wu et al. (2009) investigated poly-aluminum chloride as a flux enhancer and found that addition of 10 mg/L poly-aluminum chloride decreased fouling significantly in comparison to other tested adsorbents/coagulants including PAC, zeolite, and polyamide. In the study of Hu and Stuckey (2007), the effects of PAC and GAC

addition on TMP and flux were investigated in an AnMBR. In terms of both TMP values and variations, better performances were achieved with activated carbon that constantly scoured deposited particles from the membrane surface. Besides, a shift to a relatively higher range in PSD was observed in an AnMBR with activated carbon addition. On the other hand, PSD shifted to a lower size range in the AnMBR without activated carbon addition, which probably led to pore clogging. Maintaining stable TMP and reducing fouling are more pronounced with PAC than with GAC due to the higher surface area of PAC. Moreover, rigid PAC particles may also make the cake layer more porous since they are larger than the bioflocs. Besides, Vyrides and Stuckey (2009) observed a reduction in biofilm (gel layer) resistance and thus, TMP reduction after the addition of PAC. However, there appears to be an optimum dose for PAC addition. Extensive addition can decrease the flux due to increased viscosity of the sludge (Akram and Stuckey, 2008). Akram and Stuckey (2008) performed flux experiments at a constant TMP and observed a significant flux improvement from 2 L/m².h to 9 L/m².h with PAC addition of 1.67 g/L due to the adsorption of fine colloids and dissolved organics, and the formation of a thin cake layer on the membrane surface. However, flux decreased with the addition of 3.4 g/L PAC as a result of the viscosity increase. Due to the significant effects of additives on mass transfer properties and biochemical environment and concerns related to cost effectiveness, the optimum dosage of additives should be determined above which no further or even adverse impacts can occur (Drews, 2010). Most of the studies about the effect of flux enhancers were done in short-term experiments so far. Therefore, the feasibility of continuous addition of these chemicals during long-term operation of AnMBRs treating municipal wastewater still needs to be assessed.

2.6 Cleaning Methods

The level and degree of membrane fouling depend on many factors such as membrane operation, reactor type, membrane configuration, substrate type and sludge characteristics. The cures and cleaning methods can be various depending on the nature of fouling. Membrane cleaning can either be done physically or chemically.

Physical cleaning is closely related to membrane operation such as regular backwash, relaxation or short-term increase in shear rate to remove the cake layer accumulated

on the membrane surface. In addition, membranes can also be physically removed from the membrane tank to be cleaned ex-situ, e.g. by applying water jets. Cake layer formation on the membrane surface was found to play the major role in the increase of membrane resistance and decrease of flux in AnMBRs (Jeison and van Lier, 2006a). As a matter of fact, Martinez-Sosa et al. (2011b) reported that the efficiency of physical cleaning in an AnMBR was close to 100% which indicated that irreversible fouling was not notable or even did not exist.

In most literature, cleaning is generally understood as ‘cleaning in place’, a chemical cleaning procedure done with alkali, oxidants or acid cleaning agents. The cleaning in place efficiency depends on many factors, such as foulant type, substrate type, membrane material, cleaning procedure, i.e. type of chemical, chemical concentration, soaking/flushing duration, temperature, and frequency.

Generally, an alkali cleaning solution such as sodium hydroxide and oxidants such as hydrogen peroxide and sodium hypochlorite (NaOCl) are effective in removing organic foulants such as organic matter with carboxylic and phenolic groups, proteins and polysaccharides, whereas acid solutions (hydrochloric acid, sulfuric acid, citric acid) have been extensively used to remove inorganic foulants such as metal hydroxides and divalent cations (Liao et al., 2006; Porcelli and Judd, 2010). Investigating municipal wastewater, Chu et al. (2005) applied chemical cleaning for permeability recovery and compared two cleaning agents including only NaOCl and the combination of NaOCl and sulfuric acid in terms of permeability recovery. No significant difference was observed between two washing methods. Chemical cleaning with NaOCl and backwashing methods were insufficient to remove membrane biofouling in a membrane coupled UASB reactor (Calderon et al., 2011). Especially methanogenic Archaea remained undisturbed by the chemical cleaning. Instead of these conventional cleaning methods, some advanced methods including nitric oxide cleaning, enzymatic disruption of exopolymers, bacteriophages, etc. were proposed as effective cleaning techniques (Gao et al., 2010; Calderon et al., 2011). Moreover, membrane material characteristics play a critical role on the application of different chemicals for cleaning, i.e. PP membranes are not chlorine tolerant or PVDF membranes cannot resist high pHs over 11 (Porcelli and Judd, 2010).

The latest experience gained from aerobic MBRs treating municipal wastewater suggests that frequent maintenance cleaning (weekly or monthly) at mild chemical

concentrations can prevent permeability loss during long-term operation. That is, long-term operation without any chemical cleaning results in residual membrane fouling that is difficult to remove with chemicals (Zhang et al., 2007; Ayala et al., 2011). Moreover, frequent cleaning with dilute solutions consumes ~30% less reagents than infrequent cleaning that is applied i.e. quarterly or biennially using high-strength reagents (Ayala et al., 2011).

One of the greatest concerns about using chemicals for membrane cleaning is the effect of cleaning agents on membrane lifetime and integrity. Ayala et al. (2011) suggested that a membrane lifetime of 6-7 years can be easily sustained in aerobic MBRs without a significant loss of permeability and integrity if regular chemical cleaning is practiced. Inorganic fouling that causes irreversible fouling during long-term operation can decrease the membrane lifetime in AnMBR applications. Kang et al. (2002) identified struvite as the most important inorganic foulant especially for inorganic membranes. This would imply that inorganic fouling should not be underestimated in AnMBRs. However, despite the fact that there are many investigations aimed at understanding the fouling mechanisms in membrane systems, the research on chemical cleaning is very limited to either qualitative measurements, such as characterization of membrane foulants and their cleaning removal efficiency, or permeability recovery observed after cleaning (Porcelli and Judd, 2010). Thus far, chemical cleaning is neither fully understood nor given the required importance, which is critical for the development of better cleaning agents and protocols, i.e. cleaning conditions, sequence and method (Porcelli and Judd, 2010). More research should be conducted on membrane cleaning in order to fully understand the interaction between chemicals, foulants and membranes. This would definitely help to develop better cleaning practices and procedures for AnMBRs.

2.7 Economic Feasibility of AnMBRs for Municipal Wastewater Treatment

Economic efficiency is a key decisive criterion for the selection of a process among competing technologies. High membrane surface areas are required for the treatment of municipal wastewater since influent flow rates are generally high. Therefore, AnMBRs are particularly of interest if the (agricultural) use of the treated wastewater is considered. Within this concept, a feasibility study should be performed based on the energy consumption of pumps and extra costs of additional membrane system

devices and cleaning agents (Kocadagistan and Topcu, 2007). However, limited information is available in the literature about the economic efficiency of AnMBRs for municipal wastewater treatment.

The reduced flux is known as the main factor determining the economic feasibility of membrane processes (Lee et al., 2001). This unavoidable disadvantage of AnMBR technology is associated with an increase in operational costs, which arises from the requirement of higher suction pressure, more intensive biogas recycling, larger membrane surface area and more frequent membrane cleaning and replacement (Meng et al., 2009; Herrera-Robledo et al., 2010).

Lin et al. (2011) assessed the economic feasibility of a submerged AnMBR system for municipal wastewater treatment based on overall costs. Overall costs are represented by the sum of capital costs including the costs of membranes, tanks and plant equipment and operational costs which mainly comprise the costs of power, sludge disposal and chemicals. It was reported that membrane costs account for the largest fraction (72%) followed by the costs of tank construction and screens among capital costs. Since membrane costs are linearly correlated to the applicable flux, the observed low fluxes remain the obstacle for the potential application of submerged AnMBRs in municipal wastewater treatment. For instance, similar to aerobic MBRs, coarse screening followed by a fine screen should be considered in full-scale systems in order to protect the membranes from damage and maximize membrane life (Lin et al., 2011). Gas scouring energy represents the most significant operational cost. Operational costs for aerobic MBRs were found three times higher than those for AnMBRs due to the increased blower energy and sludge disposal costs. Moreover, the operational costs for the AnMBRs can be partly offset by the use of produced methane as an energy source (Liao et al., 2006). Martin et al. (2011) also reported that energy requirement associated with fouling control in AnMBRs was 2-3 times lower in comparison to the energy demands of aerobic MBRs. However, due to the lower fluxes reported in literature for AnMBRs, the capital costs associated with membranes might be higher compared to aerobic MBRs. They reported a wide variation in energy demand (0.03 to 3.57 kWh/m³) in submerged AnMBRs. Achilli et al. (2011) compared the operational costs of an aerobic MBR and AnMBR treating municipal wastewater and found that the operational costs of the AnMBR were lower than that of the aerobic MBR due to excess sludge management in the aerobic MBR.

However, the AnMBR system required a longer acclimation time for stable operation than the aerobic MBR. Lin et al. (2011) also performed a sensitivity analysis including the effect of variations in several aspects such as HRT, flux, membrane price, flow, membrane lifetime, interest and specific gas demand per unit of membrane, and reported that influent flow exerted the largest impact on the total life cycle costs since it determined the capacity and footprint of the system. Besides, costs are very sensitive to changes in the applicable flux, membrane price and membrane lifetime followed by the moderate effect of interest. In contrast, HRT and specific gas demand per unit of membrane were found to influence the costs to a lesser extent. With the rapid development and implementation of AnMBR technology especially in full-scale systems, more research on economic analysis is required.

2.8 Problems Encountered and Future Perspectives

Upgrading of existing anaerobic treatment processes for municipal wastewater with membranes can be of vital importance, especially when high effluent quality and/or use of treated effluents are considered. AnMBR effluents are pathogen-free, which makes them suitable for agricultural use (Saddoud et al., 2006; Ellouze et al., 2009). However, the wide application of the AnMBRs in domestic utilities, especially in full-scale systems, has not yet become a reality, which is partly because of system novelty and reluctance owing to membrane fouling problems (Chu et al., 2005). Therefore, AnMBR systems will likely benefit from the development of efficient technologies to prevent fouling.

Inorganic fouling by the precipitation of struvite, potassium ammonium phosphate and/or calcium carbonate may be one of the major concerns in fouling due to the release of ammonia and phosphate (PO_4^{3-}) from organic nitrogen and phosphorus during AD and pH increases as a result of changes in CO_2 partial pressure and alkalinity generation in AnMBRs (Liao et al., 2006). Salazar-Pelaez et al. (2011b) estimated that struvite would be undersaturated in municipal wastewaters with lower concentrations of ammonium nitrogen ($\text{NH}_4\text{-N}$), PO_4^{3-} and magnesium compared to industrial wastewaters and struvite precipitation would unlikely occur. However, not only the concentration of these ions but also the membrane properties can play a significant role in struvite precipitation (Kang et al., 2002). Therefore, this

phenomenon which may cause irreversible fouling should be further investigated with long-term studies in AnMBRs treating municipal wastewater.

Other major concerns about using treated municipal wastewater in agriculture are related with the removal of toxicity and endocrine disrupting chemicals. Industrial discharges to municipal sewer systems can cause serious problems such as toxicity and organic shock loads in the end-of-pipe wastewater treatment plants. Saddoud et al. (2006) using an AnMBR equipped with a cross-flow UF module investigated the treatability of municipal wastewater comprising industrial discharges containing toxic substances. The AnMBR process was found to be inefficient, in terms of unstable biogas production rate and composition, due to the high fluctuations in the industrial toxicants. Besides, Ellouze et al. (2009) observed a residual toxicity in wastewaters treated with AnMBRs due to the toxic soluble compounds from industrial discharges into the sewer, which passed through the membrane. However, the permeate obtained from the AnMBR was found to be significantly less toxic than the effluent of conventional treatment processes, such as aerated lagoon and activated sludge, based on micro-toxicity and phyto-toxicity analysis. Various studies address the removal of endocrine disrupting chemicals in municipal wastewater treatment by MBR systems (Cases et al., 2011; Tadkaew et al., 2011; Boonyaroj et al., 2012). In aerobic MBRs, phenolic compounds, phthalates and estrogens can be more effectively removed in comparison to conventional activated sludge systems through biodegradation, adsorption and membrane rejection mechanisms (Cases et al., 2011). However, there exists very little information about their fate and biodegradation in AnMBRs (Ho et al., 2007). Obviously, more research should be conducted on the fate of these priority pollutants in AnMBR systems.

Salinity can be another problem for the treatment of municipal wastewater, especially for coastal residential areas with improper infrastructure which allows the infiltration of sea water into sewer systems. Vyrides and Stuckey (2009) examined the performance of a submerged AnMBR treating saline municipal wastewater with high fluctuations in salinity up to 35 g sodium chloride (NaCl)/L. They reported 99% removal of DOC at 35 g NaCl/L, however the removal efficiency inside the reactor was very low (40-60% DOC). Their results indicated that the retention of SMPs and colloidal COD in the reactor was due to membrane rejection. But in this situation

more attention should be given to fouling control in order to have a feasible treatment technology.

When agricultural use of the treated effluent is not considered, a nutrient removal step is generally required to comply with discharge standards. Many studies report the effluent nutrient concentration in AnMBRs treating municipal wastewater. Lin et al. (2011) did not observe any removal of nitrogen or total phosphorus (TP) in an AnMBR treating municipal wastewater as expected. Herrera-Robledo et al. (2011) also did not observe any decrease in ammonia concentration due to filtration. Interestingly, Kocadagistan and Topcu (2007) observed phosphorus removal up to 81% in an AnMBR treating municipal wastewater by membrane retention. Similar results with regard to phosphorus removal together with calcium, magnesium and iron were reported by Herrera-Robledo et al. (2011). Moreover, Saddoud et al. (2007) reported high nitrogen and phosphorus removal, such as 60% and 30% respectively, in an AnMBR which may not be explained solely by the uptake of these macronutrients by biomass growth. Removal of these elements with low molecular sizes in comparison to the membrane nominal cut-off may be attributed to their sorption by the biofouling layer or even chemical precipitation. These contradictory results are mainly due to the differences in membrane rejection capabilities of the systems in different studies.

More research needs to be directed towards the applicability of AnMBR to low-temperature municipal wastewaters. A significant reduction in the hydrolysis rate of solids is typical at ambient temperatures due to the low activity of anaerobic microorganisms (Lettinga et al, 2001; Zhang et al., 2010). Methane loss is another important issue of concern especially at low temperature processes since methane solubility in the liquid phase increases with decreasing temperature. Gimenez et al. (2012) proposed biogas-assisted mixing as a limitation for super-saturation and a guarantee for a minimum concentration of dissolved methane in the effluent of AnMBR systems.

Alternative membrane materials, reactor types, and membrane integration possibilities and configurations for AnMBR technology in municipal wastewater treatment schemes are of interest to further develop. Dynamic membrane technology that focuses on the use of meshes or fabrics as a support material instead of real membranes is gaining interest in the AnMBR applications for municipal wastewater

treatment. Application of dynamic membrane systems will significantly reduce capital costs associated with the purchase and renewal of membranes (Zhang et al., 2010; Ersahin et al., 2012). So far, there have been several attempts to utilize dynamic membrane filtration in AnMBR systems and the results in terms of removal efficiency were very promising and comparable to conventional membranes (Ho et al., 2007; An et al, 2009b; Zhang et al, 2010; Zhang et al., 2011). However, more research should be conducted to understand the formation mechanisms of dynamic membrane (cake) layer and to effectively control it for beneficial use of filtration.

2.9 Conclusion

AnMBR permeate may be used in agriculture or the nutrients in the permeate may be recovered in order to get full benefit from the advantages offered by this technology. Combination of membranes with different types of anaerobic high-rate reactor configurations such as UASB and EGSB reactors, which have already been defined as appropriate technologies for the treatment of dilute wastewaters, should be further investigated. So far, integration with UASB reactors seems to be promising since UASB reactors provide a pre-elimination of SS by entrapment and biodegradation in the sludge bed. This may reduce the SS load to the membrane and limit the membrane fouling due to cake layer formation. On the other hand, SMP and colloids can be more important in fouling of the membranes in this type of configuration. As a possible alternative, applying solid-liquid separation as a pretreatment and digesting the concentrated slurry in an AnMBR can significantly reduce the initial investment cost for membranes and bioreactor. For especially full-scale applications, long-term reliability and operability of AnMBRs in municipal wastewater treatment need to be further investigated.

3. EFFECT OF UPFLOW VELOCITY ON THE EFFLUENT MEMBRANE FOULING POTENTIAL IN MEMBRANE COUPLED UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS⁽²⁾

3.1 Introduction

The AnMBR is a potential process for treatment of municipal wastewater (Hu and Stuckey, 2007; Vyrides and Stuckey, 2009; Martin et al., 2011). Research results indicate the technology is feasible, particularly in moderate climate regions when high effluent quality and/or agricultural use of treated effluents is required. The performance of AnMBRs is affected by many factors including operational parameters, sludge characteristics, membrane location and bioreactor type/configuration (Liao et al., 2006). Possible configurations of AnMBRs include CSTR, UASB and EGSB reactors, etc., coupled with a membrane module (Liao et al., 2006; Ozgun et al., 2013a).

Research on AnMBRs, including membrane coupled UASB reactors, has received considerable interest recently, leading to several studies investigating their potential for the treatment of municipal wastewaters (Gao et al., 2010; Liu et al., 2012). Extensive research to investigate the microbial communities in the UASB reactors (Qui et al., 2013) and the biofouling layer on membranes (Calderon et al., 2011) has also been conducted and identification of the species was proposed as an important factor for biofouling control in membrane coupled UASB systems. Calderon et al. (2011) analyzed the structure and diversity of the prokaryotic communities in the biofouling layer of a membrane coupled UASB reactor and pointed out the importance of particular microbial groups such as *Sphingomonadaceae* bacteria and

⁽²⁾ This chapter is based on:

Ozgun, H., Ersahin, M.E., Tao, Y., Spanjers, H., van Lier, J.B. (2013) Effect of upflow velocity on the effluent membrane fouling potential in membrane coupled upflow anaerobic sludge blanket reactors. *Bioresource Technology* 147, 285-292.

methanogenic Archaea in the occurrence of biofouling. Due to the resistance of these microbial groups to standard chemical cleaning, use of alternative antifouling strategies was suggested as a promising approach for improving biofouling control and prevention.

In membrane coupled UASB systems, the sludge bed at the bottom of the UASB reactor acts as a biofilter prior to the membrane unit by entrapping most of the particulate matter via adsorption and biodegradation (Seghezzo et al., 1998; Ozgun et al., 2013a). Higher fluxes between 10.5-65 L/m².h could be achieved with UASB reactor coupled systems in comparison to CSTR coupled membrane systems (Wu et al., 2009; An et al., 2009a). The membrane unit can be located either just above the sludge bed in a UASB reactor or in a subsequent stage. In a membrane coupled UASB system, the membrane is only exposed to the supernatant of the sludge bed. Therefore, the fouling propensity of the effluent plays a major role in the stable operation of such reactor systems (Martin-Garcia et al., 2011).

One of the important indicators of the effluent quality for membrane filtration processes is filterability, which determines the loss of filtration performance over time due to membrane fouling (Janssen, 2011). Good filterability, a requirement for good performance and a trouble-free operation of AnMBRs, will lead to only a limited increase in membrane resistance, and thus, a long-term operation at low operational costs. Studies on AnMBRs show high variability in filterability (Spagni et al., 2010) because it is influenced by many factors including the properties of the feed water (Kang et al., 2003), membrane material/cake layer (Yun et al., 2006), sludge characteristics (Zhang et al., 2010) and operational conditions such as shear rate, SRT, temperature, HRT, operation mode and upflow velocity (Drews et al., 2008).

For membrane coupled UASB systems, the efficiency of solids entrapment determines the amount and properties of the solids leaving the UASB reactor with the effluent. In this context, upflow velocity in membrane coupled UASB reactors would seem to be the critical variable determining the reactor efficiency and effluent fouling propensity (Mahmoud et al., 2003; Ozgun et al., 2013a). The applied upflow velocity can have two opposing effects on the biological removal efficiency in upflow reactors. An increase in upflow velocity may result in an increase in the biological removal efficiency due to the increased interaction between the substrate

and the sludge. Conversely, high hydraulic shear force applied at high upflow velocity may also deteriorate the removal efficiency due to detachment of the captured solids (Mahmoud et al., 2003). Gonçalves et al. (1994) reported that SS removal efficiency decreased from 70% to 51% when upflow velocity increased from 0.9 m/h to 3.4 m/h in an upflow anaerobic reactor treating sewage. Moreover, TSS and VSS removal efficiencies were found inversely proportional to the upflow velocity in a UASB reactor treating settled sewage at moderate temperatures (van Haandel and Lettinga, 1994; Seghezzo et al., 2002; Souza et al., 2006). Since upflow velocity has a significant impact on the stable operation of UASB reactors, an optimum upflow velocity should be applied in UASB reactors enabling the proper distribution of gas pockets gathered in the sludge bed and providing a good contact between substrate and biomass.

Until now, a large number of related scientific studies have focused on the effect of upflow velocity on solely biological performance of the upflow reactors. However, for the cases of membrane coupled UASB systems, there is no information in the literature about its effect on filterability, a parameter that is closely related to membrane fouling potential of the effluent. This study investigated this influence of upflow velocity applied to a UASB reactor used for the treatment of municipal wastewater. In addition to this general objective, the specific aim is to identify the optimum upflow velocity that will result in an effluent with good filterability values for the case of membrane coupled UASB systems. The effect of upflow velocity on the effluent quality was assessed not only on biological based parameters such as organic matter removal but also on physical characteristics. Possible relationships between physical characteristics and filterability of the effluent were determined. The information obtained from this study is valuable for optimizing the hydraulic conditions in membrane coupled UASB systems in order to achieve a stable effluent quality and to minimize membrane fouling potential.

3.2 Materials and Methods

3.2.1 Wastewater source

Synthetic municipal wastewater was used as feed. Macronutrient and micronutrient solution compositions were slightly modified from the recipes in the studies of Aiyuk and Verstraete (2004) and Martin et al. (2010), respectively. The feed solution was

prepared in concentrated form and stored at 4 °C. It was always thoroughly mixed and diluted with tap water before being fed into the reactor. The composition of the concentrated substrate solution and the characterization of the synthetic municipal wastewater at the UASB inlet are presented in Table 3.1 and Table 3.2, respectively.

Table 3.1 : Composition of the concentrated synthetic municipal wastewater.

Macronutrient Solution			Micronutrient Solution		
Compound	Unit	Value	Compound	Unit	Value
Urea	mg/L	1200	FeCl ₃ .6H ₂ O	mg/L	1000
NH ₄ Cl	mg/L	2000	CoCl ₂ .6H ₂ O	mg/L	1000
CH ₃ COONa.3H ₂ O	mg/L	7400	MnCl ₂ .4H ₂ O	mg/L	250
Ovalbumin	mg/L	450	CuCl ₂ .2H ₂ O	mg/L	15
MgSO ₄ .7H ₂ O	mg/L	180	ZnCl ₂	mg/L	25
KH ₂ PO ₄ .3H ₂ O	mg/L	1400	H ₃ BO ₃	mg/L	25
CaCl ₂	mg/L	264.9	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	mg/L	45
Starch	mg/L	6400	Na ₂ SeO ₃ .H ₂ O	mg/L	50
Milk Powder	mg/L	1500	NiCl ₂ .6H ₂ O	mg/L	25
Yeast Extract	mg/L	600	EDTA	mg/L	500
Sunflower Oil	mg/L	1000	HCl 36%	mL/L	0.5
Micronutrients	mL/L	26.6	Resazurin Sodium Salt	mg/L	250
			Yeast Extract	mg/L	1000

Table 3.2 : Characterization of the synthetic municipal wastewater at the UASB inlet.

Parameter	Unit	Concentration (Average±Range)
COD	mg/L	530±30
Soluble COD	mg/L	159±25
Total nitrogen (TN)	mg/L	54±5.2
NH ₄ -N	mg/L	36±5.5
TP	mg/L	12±0.8
Alkalinity	mg CaCO ₃ /L	300±40
TSS	mg/L	230±25
Turbidity	NTU	75±15
pH	-	7.4±0.2

3.2.2 Seed sludge

The reactor was seeded with flocculent anaerobic sludge obtained from a pilot-scale UASB reactor treating black water (Sneek, the Netherlands). The characteristics of the seed sludge are presented in Table 3.3.

Table 3.3 : Characteristics of the seed sludge.

Parameter	Unit	Value
TS	mg/L	22000±300
Volatile Solids (VS)	mg/L	16900±235
TSS	mg/L	20200±75
VSS	mg/L	16900±225
COD	mg/L	27100±330
pH	-	7.9
Capillary Suction Time	s	285
SMA	g CH ₄ -COD/g VS.d	0.3

3.2.3 Experimental setup

The experiments were performed using a laboratory-scale UASB reactor (Figure 3.1) with an effective volume of 7 L. The system was equipped with feed, recycle and effluent pumps (Watson Marlow 120U/DV), pH and temperature sensors (Elscolab, M300 ISM), and gas meter (Ritter, Milligas Counter MGC-1 PMMA). A three-phase separator (Figure 3.2) was installed at the top part of the reactor to separate the biogas from the mixed liquor as well as to retain suspended particles in the reactor. The reactor was equipped with sampling ports at different heights for sample withdrawal. During the experiments, pH was in the range of 6.8 to 7.1. The temperature of the jacketed reactor was controlled at 25±2 °C by a thermostatic water bath (Tamson Instruments, the Netherlands). The experimental system was connected to a computer running LabView software in order to control all pumps and collect pH, temperature and biogas flow data on-line.

3.2.4 Experimental procedure

Before operation, the seed sludge was acclimated in the UASB reactor at 0.6 m/h for 40 days to obtain steady biogas production and stable sludge bed height. During the study, the UASB reactor was continuously fed for a total of 116 days at different upflow velocity conditions. Upflow velocities of 1.2 m/h, 0.6 m/h, and 1.2 m/h were applied successively for periods of 33, 52 and 31 days, respectively. The third experimental period, where an upflow velocity of 1.2 m/h was reestablished after the period with 0.6 m/h, was included to verify whether the effect of upflow velocity on effluent quality in the laboratory-scale UASB reactor was reproducible. During entire experiment, HRT was fixed at 6 h, and the corresponding OLR was 2 kg

COD/m³.day. Total COD, soluble COD, TSS, turbidity, TN, NH₄⁺-N and TP analyses were performed on the influent and the effluent samples. TSS analysis was also performed on samples taken from the middle of the upper phase of the UASB reactor over the sludge bed, which is referred to as supernatant sample. SMP, PSD and filterability analyses were performed on the effluent samples.

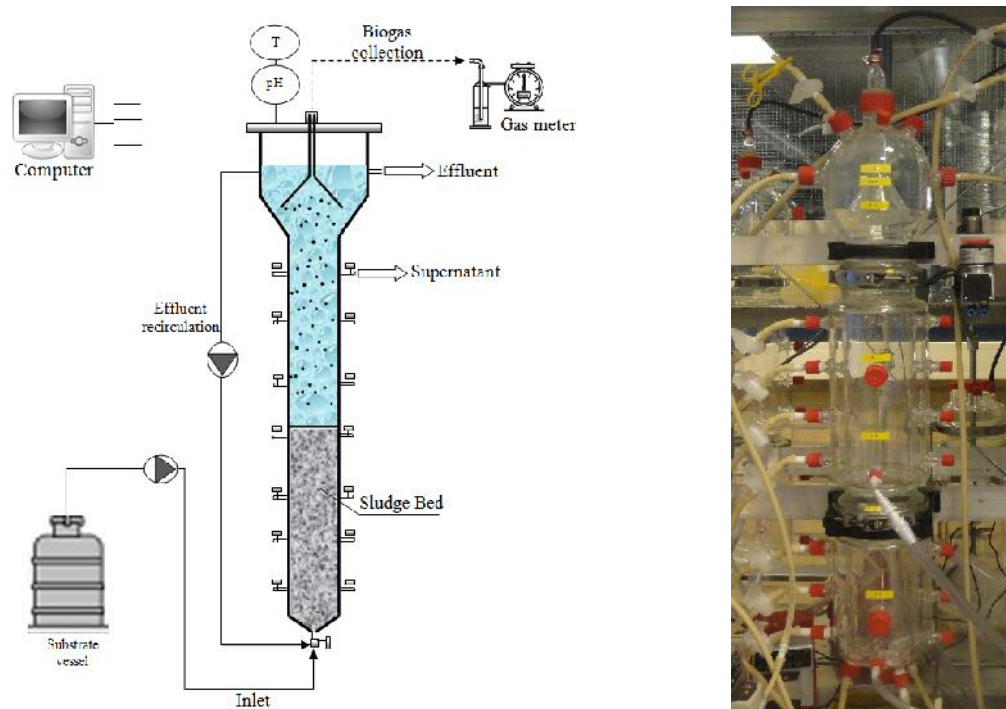


Figure 3.1 : Schematic diagram of the UASB reactor.



Figure 3.2 : A three-phase separator.

3.2.5 Analytical methods

COD, NH₄⁺-N, TN, TP, TSS, VSS, TS, VS and alkalinity were measured according to Standard Methods (APHA, 2005). Each analysis was performed in triplicate. The samples for soluble COD measurement were filtered through a 0.45 µm filter. The turbidity and pH were measured by HACH 2100N Turbidimeter and by a WTW

multi720 pH meter, respectively. The PSD of the effluent was assessed by a Mastersizer 2000 (Malvern Instruments, Hydro 2000 MU) which has a detection range of 0.02-2000 μm . Laser diffraction technique was used in order to measure the size of the particles. A volume of 5 ml effluent was freshly sampled, filtered through a 0.45 μm filter and diluted 4 times with phosphate buffered saline (PBS) (pH=7.2) for SMP analyses. The phenol-sulfuric acid method was used to quantify polysaccharides (Dubois et al., 1956). The concentration of protein was measured using the Bradford method (Bradford, 1976). The methane content in the biogas was measured using a Varian 3800 gas chromatograph equipped with a flame ionization detector (Varian, Palo Alto, CA). Statistical calculations were processed by using Minitab 16 software (Minitab Inc., USA).

3.2.6 Filterability and reversibility test

The Specific Ultrafiltration Resistance (SUR) test, developed at Delft University of Technology (Roorda, 2004), was used to test the filterability of the UASB effluent. SUR is defined as the filtration resistance per unit of filtered feed water per m^2 membrane area and it is calculated from the slope of a filtration curve, i.e. ratio of filtration time and filtered volume vs. total volume of the filtered water, that is measured within 30 min of filtration over an UF membrane at a constant temperature and at a constant TMP (Janssen et al., 2008). Low SUR values indicate relatively high filterability, whereas high SUR values indicate the poor filterability.

Reversibility is described as the extent to which the filtration resistance is returned back to the original value after applying a hydraulic cleaning. The reversibility of the fouling layer is calculated from the ratio between the change in resistance and time caused by the cleaning of the membrane ($\Delta R/\Delta t$).

The equipment (Figure 3.3) for the filterability and reversibility tests consisted of three pressurized buffer tanks (A, B and C) with a volume of 10 L. Pressurized buffer tank A was filled with demineralized water and pressurized buffer tank B was filled with UASB reactor effluent. These pressurized buffer tanks were connected to the membrane module by means of a three-way valve in between. The membrane module contained three capillary UF membranes (Norit X-Flow) prepared from PES and polyvinylpyrrolidone (PVP) with internal diameters of 0.8 mm and MWCOs of 150–200 kDa. The permeate is collected in the pressurized buffer tank C. To

maintain a TMP of 0.5 bar through the membrane module, two pressure control devices were installed to keep the pressures of buffer tank A and B under 1.5 bar and the pressure of buffer tank C under 1.0 bar. A digital pressure difference measuring device was placed to check and log the TMP over the membrane module. The permeate flow was continuously measured by a mass flowmeter. A data acquisition program was used to store and display the TMP, flux, temperature and resistance.

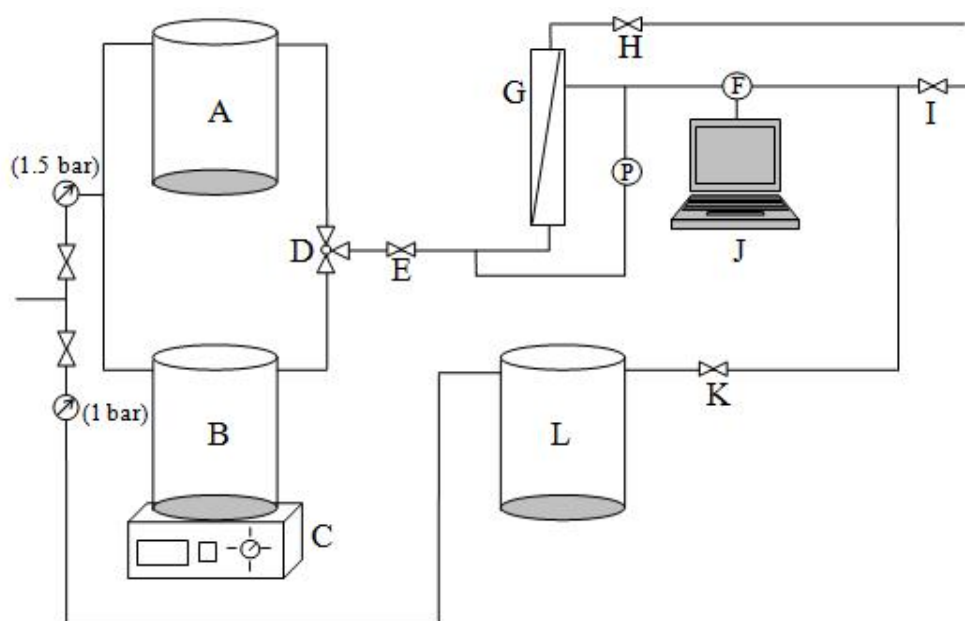


Figure 3.3 : Schematic diagram of the SUR measurement experimental setup (A: demineralized water vessel; B: wastewater vessel; C: magnetic stirrer; D: three-way valve; E, H, I, K: valves; F: flow meter; G: membrane module; J: computer with measuring program; L: permeate vessel; P: pressure difference sensor).

3.3 Results and Discussion

3.3.1 Effect of upflow velocity on system performance

Total and soluble COD concentrations in the effluent at different upflow velocities are shown in Figure 3.4. After the 40 day acclimation period at an operating upflow velocity of 0.6 m/h, the measuring period started at Day 0 with an increased upflow velocity of 1.2 m/h. Average total and soluble COD concentrations during this first period were 215 ± 10 mg/L and 63 ± 9 mg/L, respectively. After the decrease in upflow velocity from 1.2 m/h to 0.6 m/h in the second period, a gradual decrease was observed in total COD concentrations to the range of 130-160 mg/L. Soluble COD concentrations were found to be similar at both upflow velocities. Average total COD removal efficiencies of 60% and 72% were obtained at upflow velocities of 1.2 m/h and 0.6 m/h, respectively.

Upflow velocity was set to 1.2 m/h again at the start period on Day 88, in order to determine if the effect of upflow velocity on total COD concentration is reproducible. During this period, an increase in total COD concentration was observed again, similar to the first period of 1.2 m/h. The observed increase in total COD concentrations at an upflow velocity of 1.2 m/h may be either attributed to the release of colloidal and suspended particles from the sludge bed or the decrease of hydrolysis rate.

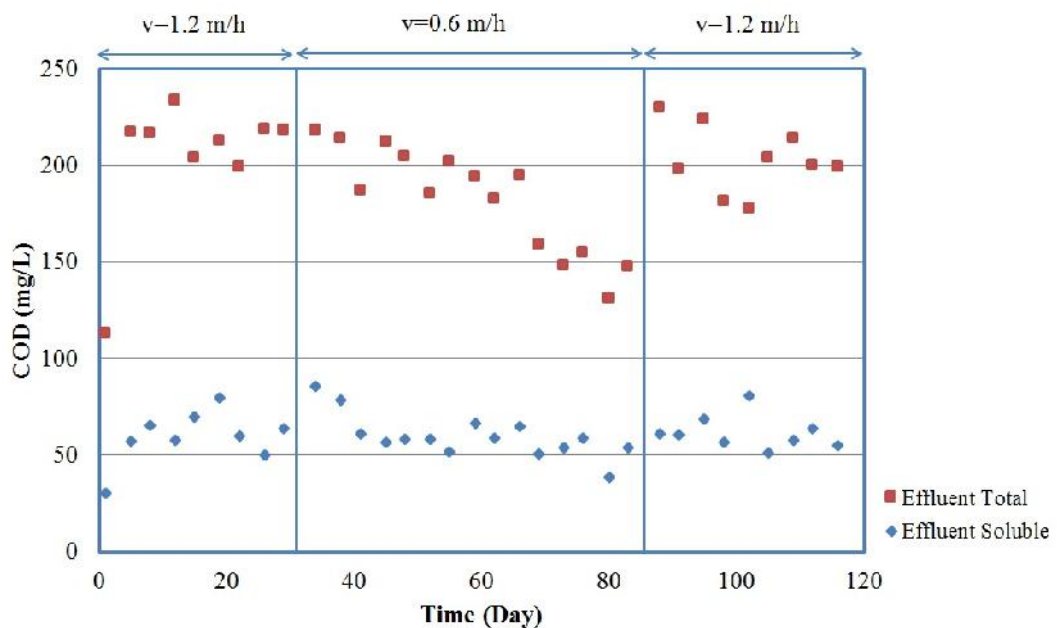


Figure 3.4 : Total and soluble COD concentrations in the effluent.

The difference in biogas production (Figure 3.5) observed during each period with different upflow velocities was found to be insignificant ($p>0.05$). Average methane content of the biogas was $61\pm5.7\%$ throughout the study, and methane yields of 0.13 ± 0.02 L $\text{CH}_4/\text{g COD}_{\text{removed}}$ and 0.11 ± 0.01 L $\text{CH}_4/\text{g COD}_{\text{removed}}$ were achieved at upflow velocities of 1.2 m/h and 0.6 m/h, respectively. The almost stable biogas production throughout the whole study in spite of the larger variations in total COD removal efficiency indicated that upflow velocity influences the release of colloidal and suspended particles from the sludge bed rather than the biodegradation of COD in the reactor.

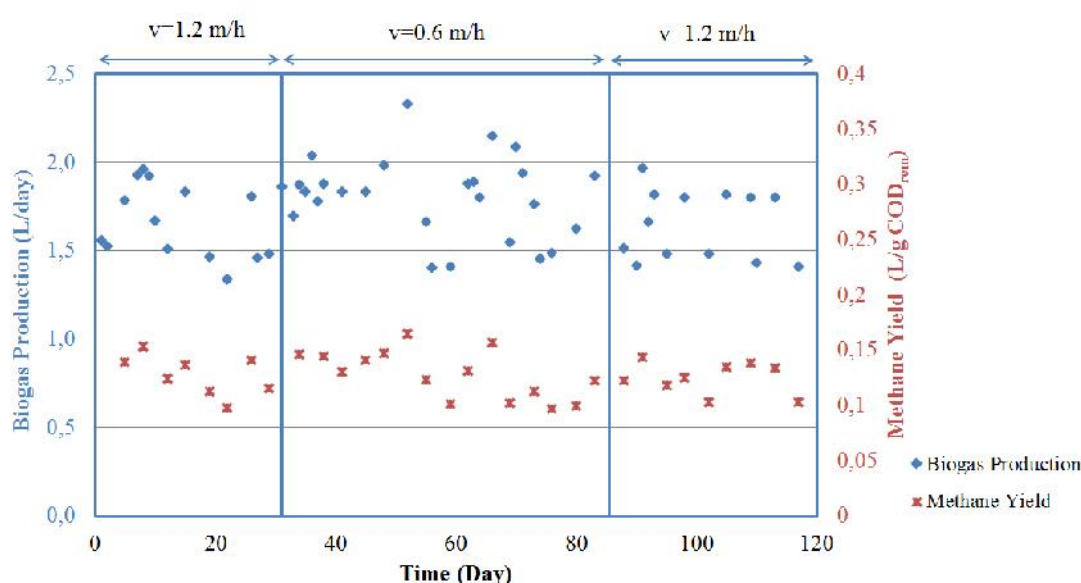


Figure 3.5 : Biogas production at different upflow velocities.

Operation at a higher upflow velocity may promote the release and washout of colloidal or particulate organic matter that would reside in the sludge bed at lower upflow velocity. In order to confirm this assumption, TSS and turbidity were measured both in the supernatant and effluent of the UASB reactor. A considerable decrease from the range of 230-270 mg/L to 90-110 mg/L was observed in the TSS concentrations of the supernatant when upflow velocity was decreased from 1.2 m/h to 0.6 m/h. However, there was no significant change ($p>0.05$) in TSS concentrations in the effluent as a result of the upflow velocity change: Average TSS concentrations in the effluent were 91 ± 29 mg/L and 81 ± 13 mg/L at 1.2 and 0.6 m/h upflow velocities, respectively (Figure 3.6). This finding may be attributed to the effective separation capability of the GLS separator at the top of the UASB reactor. With the increase in upflow velocity, particles present in the supernatant will rise to the top

but are prevented from rinsing with the aid of GLS separator. The average turbidity in the effluent was found to be 55 ± 5 NTU at the upflow velocity of 1.2 m/h, whereas it decreased to 32 ± 4 NTU at the upflow velocity of 0.6 m/h (Figure 3.7). Variations observed in effluent turbidity ($p < 0.05$) at different upflow velocities seem contradictory to TSS results which do not show significant differences between each upflow velocity. Observed results are attributed to the washout of colloidal particles from the system instead of suspended particles as turbidity is an indicator of both suspended and/or colloidal particles.

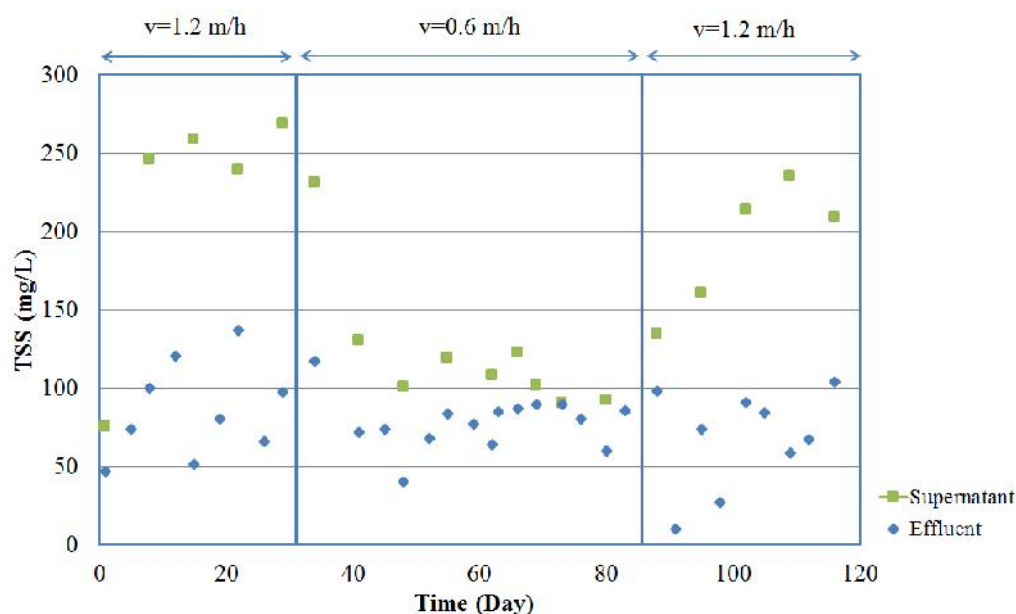


Figure 3.6 : TSS concentration in the supernatant and effluent.

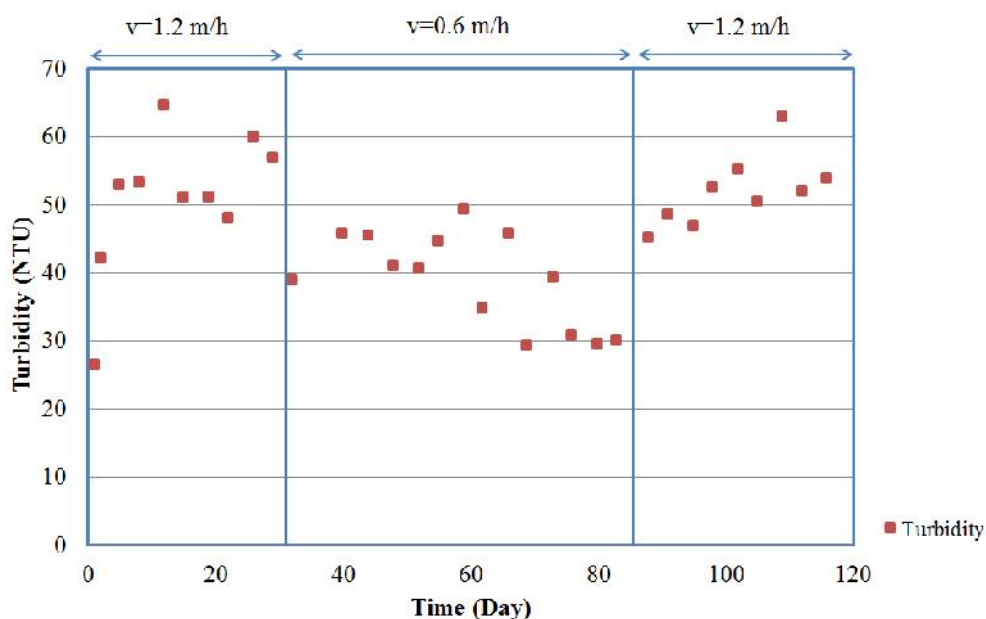


Figure 3.7 : Turbidity of the effluent.

$\text{NH}_4^+\text{-N}$, TN (Figure 3.8) and TP (Figure 3.9) concentrations were also measured in the UASB reactor effluent at different upflow velocities. TN mainly i.e. >75% consisted of ammonium. The ratio of $\text{NH}_4^+\text{-N}/\text{TN}$ in the effluent was higher at the upflow velocity of 0.6 m/h than the one obtained at 1.2 m/h, indicating again the washout of colloidal protein-like materials at higher upflow velocity. TP concentration was stable between 10.9-12.9 mg/L throughout the whole study period.

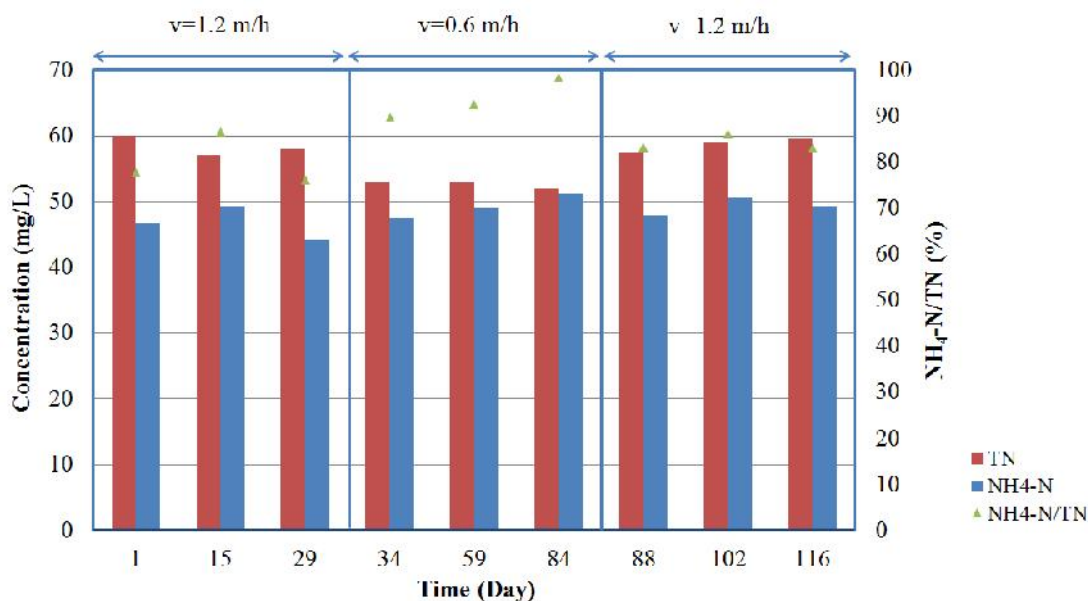


Figure 3.8 : TN and $\text{NH}_4\text{-N}$ concentrations in the effluent.

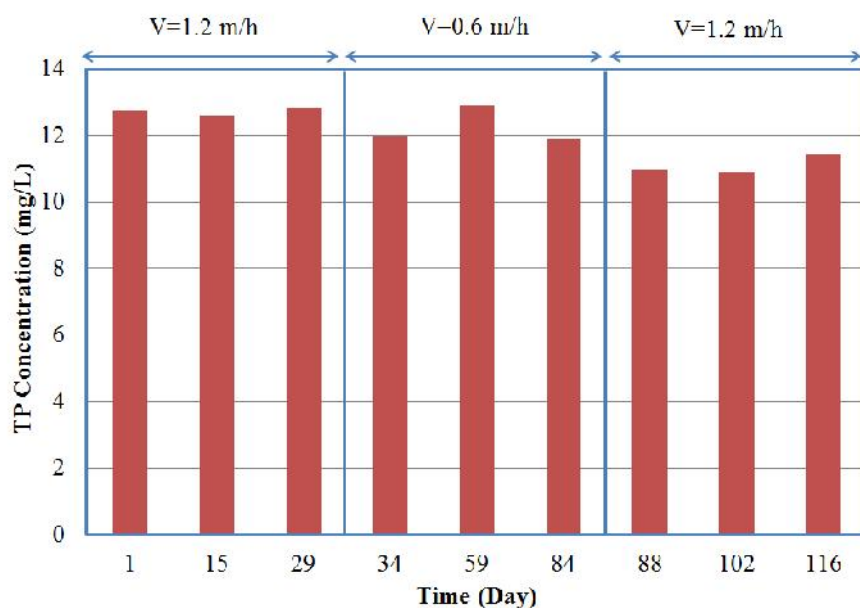


Figure 3.9 : TP concentrations in the effluent.

The presence of SMPs is considered an important factor which can play a role in membrane fouling by causing cake or gel layer compacting or even pore clogging (Jarusutthirak and Amy, 2006; Meng et al., 2009; Huang et al., 2011; Zhang et al., 2011). Aquino et al. (2006) found that SMP was the main contributor of pore clogging in AnMBRs, hence, SMP is likely have a negative impact on the filterability of the UASB effluent. Fouling due to SMP and colloids may be relatively more important in membrane coupled UASB systems than in membrane coupled CSTR systems because of selective accumulation of SMPs on the membrane in the former system (Ozgun et al., 2013a). In order to investigate the impact of SMP carry over at different upflow velocity, the concentrations of protein and polysaccharide were measured in the effluent (Figure 3.10). The protein concentration significantly decreased at the upflow velocity of 0.6 m/h and was under the detection limit (2.5 mg/L) in the samples obtained from Day 54 to Day 85, indicating that at low upflow velocity protein may be better metabolized or retained inside the reactor than at higher upflow velocity. This was supported by the observation that protein concentration again increased after upflow velocity was raised back to 1.2 m/h. Unlike the effluent protein concentration, effluent polysaccharide concentration was almost unaffected by the upflow velocity.

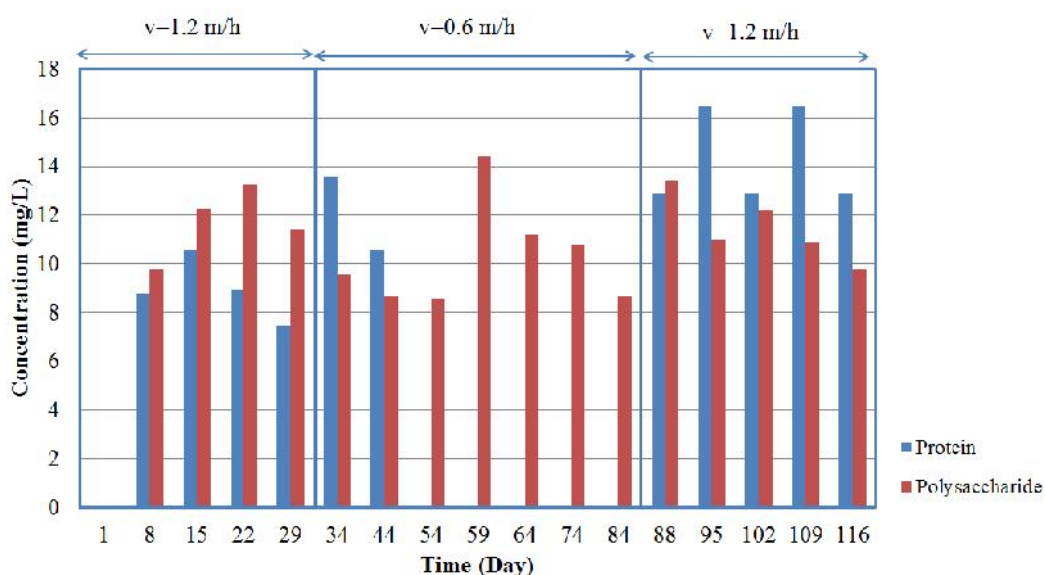


Figure 3.10 : SMP concentrations in the effluent.

Meng et al. (2006) indicated that P/C is an important indicator of the fouling potential of an effluent fed to a membrane. They showed that proteins adhere more easily to a membrane surface and are more likely to induce membrane fouling as

they are more hydrophobic than carbohydrates. Therefore, a high P/C would indicate the probability of more severe membrane fouling. Huang et al. (2011) also found a direct relationship between P/C and fouling. Due to the decrease in protein concentration at lower upflow velocity shown in this study, the P/C of the effluent decreased and thus, less fouling may be expected in a membrane coupled UASB reactor by applying at low upflow velocity in the sludge bed.

Figure 3.11 presents PSD curves in the effluent at different upflow velocities. The effluent at the upflow velocity of 0.6 m/h was more homogenous in terms of PSD showing more unevenly distributed peaks (Figure 3.11-b), whereas the PSD at an upflow velocity of 1.2 m/h was more heterogeneous showing more and lower peaks.

At an upflow velocity of 1.2 m/h, there was a shift to the left part of the distribution pattern and an apparent decrease in the median particle size (D50) was observed, whereas at an upflow velocity of 0.6 m/h peaks shifted to the right part of the PSD curve and an increase in D50 was observed. From these results, it was concluded that at the higher upflow velocity, small sized particles were washed out more vigorously, resulting in an effluent having a D50 of 4.9 μm , whereas a D50 of 108 μm was measured after the operation period of 0.6 m/h.

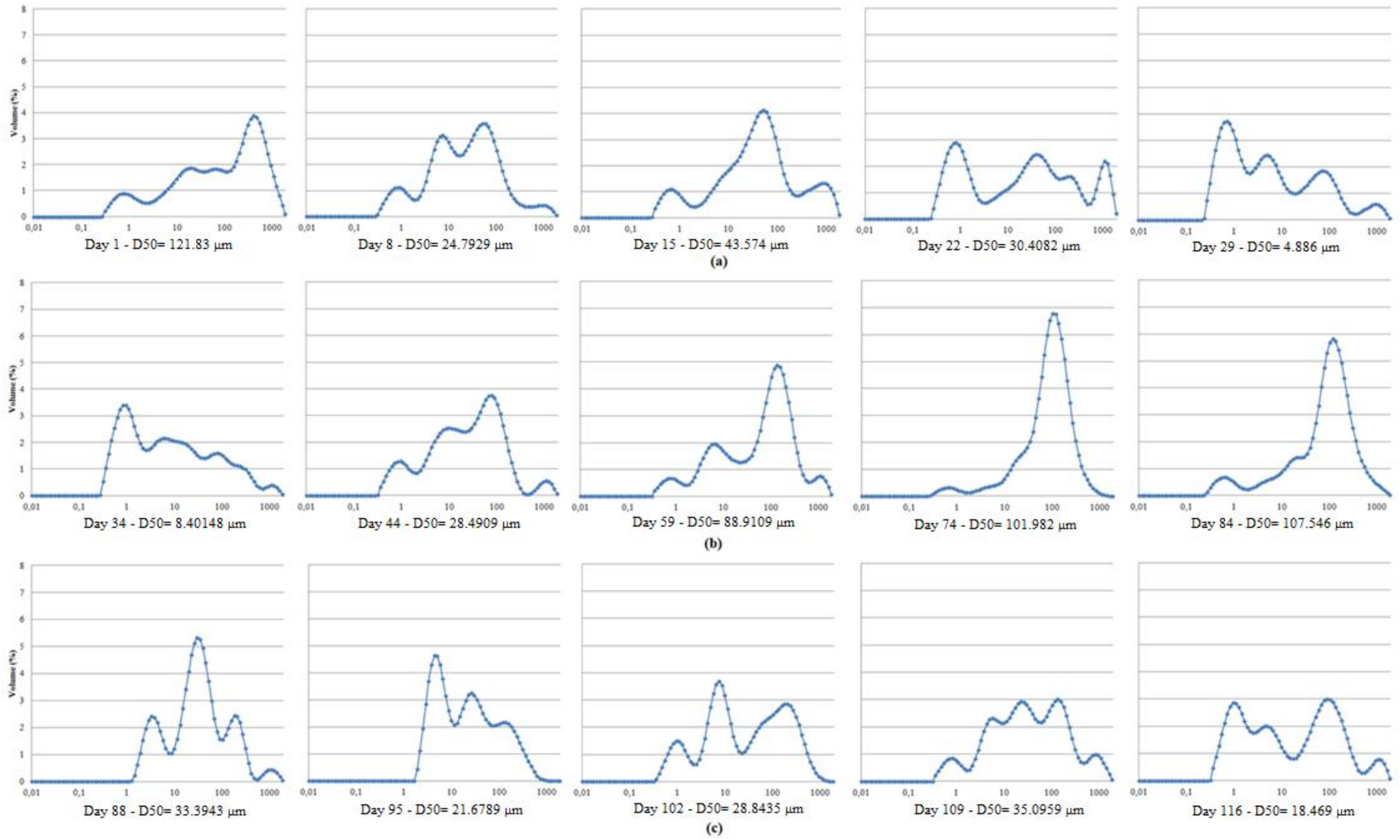


Figure 3.11 : PSD at different upflow velocities (a) $v=1.2$ m/h, (b) $v=0.6$ m/h, (c) $v=1.2$ m/h.

3.3.2 Filterability and reversibility at different upflow velocities

In order to investigate the relationship between filterability and effluent characteristics at different upflow velocities, the SUR of samples obtained at different upflow velocities was measured (Figure 3.12). Figure 3.12 shows that the SUR values varied between $50.10^{12} \text{ m}^{-2}$ and $300.10^{12} \text{ m}^{-2}$ over the whole experimental period. Increased SUR values with an increasing trend were obtained during the operation at 1.2 m/h, whereas a decreasing trend was observed during the operation at 0.6 m/h, which was an indicator of better filterability at lower upflow velocity. Filterability deterioration at high upflow velocity was related to the existence of colloidal material and a heterogeneous PSD during the operation at 1.2 m/h. This means that at the upflow velocity of 0.6 m/h, the average filterability of the UASB effluent was better and more appropriate for membrane filtration in comparison to the effluent obtained at 1.2 m/h.

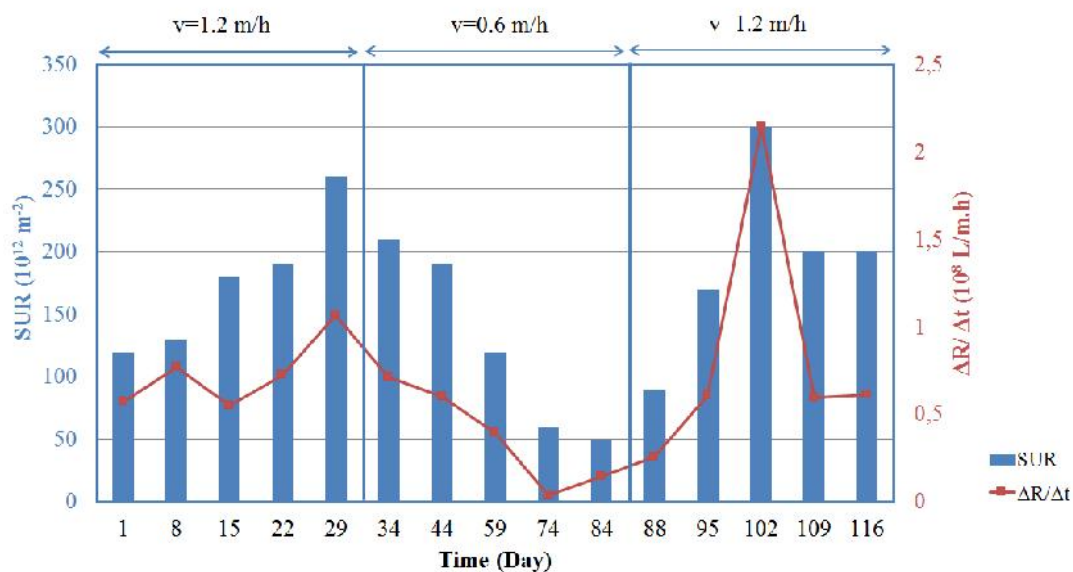


Figure 3.12 : SUR and $\Delta R/\Delta t$ values at different upflow velocities.

Reversibility of the UASB effluent was also measured at different upflow velocities in order to determine the extent to which filtration resistance is returned back to the original value after applying a hydraulic cleaning (Figure 3.12). A high value for $\Delta R/\Delta t$ indicates a low reversibility, whereas a high reversibility is indicated by a low value for $\Delta R/\Delta t$. The reversibility and SUR values of the UASB effluent were reciprocally correlated. This relation is also endorsed by Roorda (2004). The increase in upflow velocity from 0.6 m/h to 1.2 m/h resulted in a decreased reversibility,

which can be an indicator of unsteady process performance of the membrane unit after prolonged periods of operation.

3.4 Conclusion

Upflow velocity is an important variable which affects the effluent characteristics of a UASB, especially the physical characteristics and PSD. Results suggest that when applying a high sludge bed upflow velocity in a UASB coupled membrane system, there will be an increased load of small particles to the membrane. Effluent characterization results coincide with the filterability and reversibility tests. The increased effluent particle size seen at the lower upflow velocity was associated with a better filterability. Overall the results of this study clearly indicate that selection of an appropriate upflow velocity plays a major role in controlling the fouling potential, which is one of the most important challenges for stable operational process performance of membrane coupled UASB reactors.

4. IMPACT OF MEMBRANE ADDITION FOR EFFLUENT EXTRACTION ON THE PERFORMANCE AND SLUDGE CHARACTERISTICS OF UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS⁽³⁾

4.1 Introduction

Recently, there has been a growing interest in increasing cost-efficiency and overall sustainability of municipal wastewater treatment systems in addition to their goal to protect receiving surface water and human health. As a result, municipal wastewater is more and more considered as a source of energy, nutrients and water (Mo and Zhang, 2012). When water reclamation for agricultural reuse is targeted, conventional aerobic and anaerobic treatment schemes are being upgraded to mainstream processes that are preferably capable of producing nutrient-rich and solids-free effluents with a high retention of pathogens (Saddoud et al., 2006).

With growing concerns over climate change associated with fossil-fuel utilization, the anaerobic treatment of municipal wastewater is receiving increased attention. Compared with the widely used aerobic biological process for municipal wastewater treatment, the anaerobic biological process has significant advantages including no energy input required for aeration and energy recovery through methane production, lower sludge production and most nutrients (nitrogen and phosphorus) remaining in the effluent suitable for agricultural and landscaping irrigation reuse. Despite these advantages, the perceived notion is that an anaerobic biological process is inappropriate for municipal wastewater treatment with relatively low concentration of organics and a significant particulate fraction, which make it technically

⁽³⁾ This chapter is based on:

Ozgun, H., Gimenez, J.B., Ersahin, M.E., Tao, Y., Spanjers, H., van Lier, J.B. (2015) Impact of membrane addition for effluent extraction on the performance and sludge characteristics of upflow anaerobic sludge blanket reactors treating municipal wastewater. *Journal of Membrane Science* 479, 95-104.

challenging to achieve a high OLR with good effluent quality (Wei et al., 2014). However, within the alternatives to consider, the combination of anaerobic biological treatment with membranes stands out as a promising alternative that may overcome the drawbacks of the conventional anaerobic processes for municipal wastewater through the excellent sludge-water separation by a membrane, which results in high biomass and high OLR and organics removal and high quality effluent with lower amount of particles and pathogens. Municipal wastewater is found to be a potential source of energy (McCarty et al., 2011). AD of wastewater can produce methane gas, which can be used by methane-driven engine to generate electricity. Anaerobic processes not only produce methane as a renewable source of bioenergy but also consume less energy for operation than aerobic systems. Complete anaerobic treatment of municipal wastewater has the potential to achieve net energy production while meeting stringent effluent standards (Dutta et al., 2014). In addition, the lower anaerobic waste biosolids production compared with aerobic treatment reduces the costs and difficulties associated with biosolids management (Yoo et al., 2012). In anaerobic sewage treatment systems, the plant macronutrients ammonium and orthophosphates are solubilized from organic matter, reaching effluent concentrations even higher than influent values. In addition, a superior effluent quality, in terms of COD, SS and pathogen counts, can be achieved by incorporating membranes to the anaerobic sewage treatment systems (Brindle and Stephenson, 1996; Liao et al., 2006). The main disadvantage of anaerobic biological treatment with membranes is high energy usage to reduce the fouling problem. In order to reduce energy costs for membrane fouling control, UASB reactor has been proposed. Coupling of UASB with membrane reactor was found to potentially reduce the membrane energy cost. To date, the UASB reactor is the most commonly applied technology for anaerobically treating municipal sewage (Chernicharo, 2007; Noyola et al., 2012). UASB effluent extraction via membrane skids offers interesting potentials, since membrane separation is then challenged by solely sludge supernatant, due to the entrapment of most of the particulate matter by adsorption and biodegradation in the sludge bed. As a consequence, membrane fluxes may become less dependent on the reactor MLSS concentration, possibly leading to high membrane fluxes compared to AnMBR consisting of CSTRs (Ozgun et al., 2013a). Besides that, Chu et al. (2005), as the sole example in literature, proposed a membrane unit that is capable of retaining the SS inside the EGSB reactor. In that

study, the membrane module is submerged in the upper part of the EGSB reactor and the applied high upflow velocity was beneficial in the reduction of membrane fouling due to the increased hydraulic shear stress on the membrane surface (Chu et al., 2005). Nonetheless, cake layer resistance governed the achievable flux over the hollow fiber membranes, whereas pore blocking only was of minor importance. Based on the high removal efficiencies even at low temperatures, the use of EGSB reactors in AnMBRs was also suggested as a potential technology for treating municipal wastewater at ambient temperature (Chu et al., 2005). However, membrane integration eliminates the hydraulic selection pressure required for granulation, by avoiding the washout of flocculent sludge with poor immobilization characteristics. Therefore, no granulation is expected in EGSB reactors coupled to membrane filtration, which would decrease the settleability of the biomass on the long-term operation.

A number of studies have been published assessing the performance of membrane coupled UASB reactors for the treatment of municipal wastewater (Gao et al., 2010; Herrera-Robledo et al., 2010; Salazar-Pelaez et al., 2011a; Salazar-Pelaez et al., 2011b; Liu et al., 2012). In these studies, membranes have been coupled with UASB reactors in two different configurations: one employs membranes as a part of an AnMBR system, and the other uses the membrane unit as a polishing step after the UASB reactor. In most of the studies (Herrera-Robledo et al., 2010; Herrera-Robledo et al., 2011; Salazar-Pelaez et al., 2011a; Salazar-Pelaez et al., 2011b), membranes have been applied as a polishing step after UASB reactors. In such setup, the concentrate stream does not return to the bioreactor, preserving the liquid dilution rate as a bacterial selection pressure factor. In such setup, the hydraulics in the UASB reactor are not altered. However, the down-side of the approach is SS accumulation in the membrane tank located after the UASB reactor, leading to a progressive increase in SS loading on the membrane unit. Only few studies (Gao et al., 2010; Liu et al., 2012) have employed membranes as a part of an AnMBR system with concentrate flow recycle to the UASB reactor. In this case, concentrate flow recycle leads to nearly absolute biomass retention and allows for operation at high SRTs. Jeison et al. (2008) demonstrated the feasibility of AnMBRs including CSTRs for enhanced treatment of wastewaters containing particulate organic matter because they offer the possibility of TS retention. However, for UASB-based AnMBR

systems, the membrane barrier eliminates the hydraulic selection pressure required for a sludge bed with good settling properties in the UASB reactor. Additionally, the usually high concentration of partly degradable particulate matter in municipal wastewater, which can be entrapped in the sludge bed, may further hamper the applicability of UASB reactors in an AnMBR system configuration.

Membranes in UASB-based AnMBR systems are either located as side-stream (Gao et al., 2010) in which the concentrate is recycled back to the bioreactor or submerged (Liu et al., 2012). Gao et al. (2010) operated an AnMBR system consisting of side stream membranes coupled with a UASB reactor and focused on the relative abundance comparison of microbial species on the cake layer and biomass suspension. On the other hand, Liu et al. (2012) carried out a study in which the impact of food to microorganism ratio (F/M) on system performance was evaluated by using two AnMBR systems with membrane units placed at the top of the UASB reactor. However, neither of these studies investigated the impact of membrane addition on the performance of UASB reactor by comparing the system performance before and after membrane addition.

The goal of this study is to investigate the impact of membrane addition on both the system performance and sludge characteristics of a UASB reactor in an AnMBR system treating municipal wastewater. This impact is evaluated by monitoring and comparing several indicators including COD removal efficiency, TSS and SMP concentrations in UASB effluent and sludge PSD, sludge activity and microbial community analysis before and after membrane addition. This study is supposed to provide a fundamental understanding of how a UASB system is affected by the elimination of selective sludge washout, as well as to give a clue on the operation of a UASB reactor as part of an AnMBR system.

4.2 Materials and Methods

4.2.1 Wastewater source

In the present study, synthetic municipal wastewater was used as feed. Wastewater is the same as the one described in Chapter 3 for synthetic municipal wastewater. The composition of the concentrated substrate solution and the characterization of the

synthetic municipal wastewater at the UASB inlet were already presented in Chapter 3 in Table 3.1 and Table 3.2, respectively.

4.2.2 Seed sludge

The reactor was seeded with flocculent anaerobic sludge obtained from a pilot-scale UASB reactor treating black water (Sneek, the Netherlands). The characteristics of the seed sludge were presented in Table 3.3 in Chapter 3.

4.2.3 Experimental setup

The experimental setup consisted of a laboratory-scale UASB reactor with an effective volume of 7 L coupled with an external membrane module. The experimental period was divided into two stages: (i) before membrane incorporation, when the UASB reactor was operated as the sole process and (ii) after membrane incorporation, when the system was operated as an AnMBR. Before membrane incorporation (Figure 4.1(a)), the system was equipped with feed, recycle pump and effluent pump (Watson Marlow 120U/DV). After membrane incorporation, the AnMBR stage was initiated by directing the UASB effluent through an external cross-flow tubular module containing 28 UF (0.03 μm pore size) membrane fibers (Pentair X-Flow) (Figure 4.1(b)), and returning the concentrate to the UASB reactor. Each membrane fiber had an internal diameter of 1.5 mm and a length of 80 cm, with an effective filtration area of 0.0038 m^2 . PES UF membranes with a pore size of 30 nm were used in the AnMBR. Peristaltic pumps (Watson Marlow 120U/DV) were used for circulating the flow between the UASB reactor and the membrane module, and obtaining permeate through the membrane. Furthermore, recirculation over the membrane surface was applied by a peristaltic pump (Watson Marlow 620UN/R) in order to maintain a cross-flow velocity of 1 m/s across the membrane surface independently of the circulation flow between the UASB reactor and the membrane module. This concentrate circulation flow rate was adjusted enabling a stable upflow velocity in the UASB reactor. Pressure sensors (AE Sensors, ATM -800/+600 mbar, the Netherlands) were installed in the membrane feed, concentrate and permeate lines in order to measure the TMP. The biogas was collected by means of a three-phase separator installed at the top of the UASB reactor. Biogas flow rate was measured with a gas meter (Ritter, Milligas Counter MGC-1 PMMA). Temperature was controlled by means of a water bath (Tamson Instruments, the Netherlands).

Temperature and pH inside the bioreactor were monitored on-line with a probe combined with a transmitter (Elscolab, M300 ISM). A computer accessible via LabView software (LabVIEW 10.0.1, National Instruments) was used to control the pumps and collect the data.

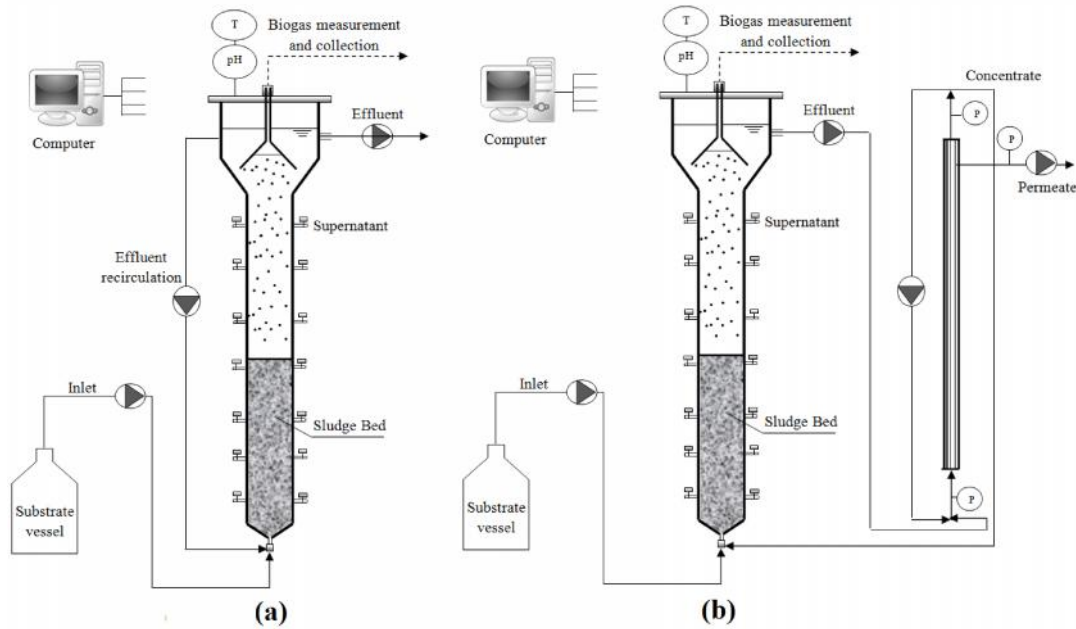


Figure 4.1 : The layouts of the experimental setups (a) UASB reactor and (b) AnMBR system.

4.2.4 Experimental procedure

The laboratory-scale experimental study was conducted during 126 days: the UASB operation stage before membrane incorporation lasted 63 days, and was followed by the AnMBR operation stage after the addition of membrane, which also lasted 63 days. The system was operated at an average HRT of about 6 h and an average OLR of 2 kg COD/m³.day at each stage. The upflow velocity of the UASB reactor was chosen as 0.6 m/h based on the results of Chapter 3 (Ozgun et al., 2013b) and kept constant during the whole experimental period. The average pH and temperature of the system remained stable around 6.9 and 25 °C, respectively. Membrane operation consisted of alternating between 3 minutes filtration and 20 seconds backwash, at a membrane flux of 12.3 L/m².h during the AnMBR operation stage. The cross-flow velocity of the membrane was 1 m/s. Backwash was applied by reversing the flow direction, keeping the flux at the same value as during filtration. Membrane cleaning including physical, base and acid cleaning steps was also carried out to recover the permeability. During membrane cleaning, the membranes were first physically

cleaned by flushing with tap water to remove cake layer. Following that, the membranes were chemically cleaned by soaking sequentially in 200 ppm NaClO-0.4% Divos solution for 30 minutes and 1% (w/v) citric acid solution for 1 hour.

4.2.5 Analytical methods

Frequent analyses were performed to assess the characteristics of the wastewater, effluent, permeate and sludge.

4.2.5.1 Experimental methods applied to effluent and permeate samples

COD and TSS concentrations were determined according to Standard Methods (APHA, 2005). Each analysis was performed twice a week in triplicate. The samples for soluble COD measurement were filtered through a 0.45 μm filter before analysis. VFA concentrations were measured weekly using a Focus GC (Thermo electron corporation) connected with flame ionization detector. A 30 m-long column (Hewlett Packard HP INNOWAX) with an internal diameter of 0.25 mm and a film thickness of 0.25 μm were used to separate VFAs. For SMP analysis, a volume of 5 ml effluent was freshly sampled weekly, filtered through a 0.45 μm filter and diluted 4 times with PBS (pH=7.2). The phenol-sulfuric acid method was used to quantify polysaccharides (Dubois et al., 1956). The concentration of protein was measured using the Bradford method (Bradford, 1976).

4.2.5.2 Experimental methods applied to sludge samples

PSD of the sludge was determined monthly by a Mastersizer 2000 (Malvern Instruments, Hydro 2000 MU), which has a detection range of 0.02-2000 μm . Laser diffraction technique was used to measure the size of the particles. For assessing the SMP content of sludge samples, a volume of 5 ml sludge was washed by PBS (pH=7.2) and centrifuged at 7000g for 7 minutes at 4 °C. The supernatant was filtered using a 0.45 μm filter and the filtrate was collected for SMP analysis. The pellet was re-suspended and vigorously washed with 10 ml PBS and then ultrasonicated at 40 kHz (Cole-Parmer Ultrasonic, the Netherlands) for 3 minutes. A high speed centrifuge (17000g) was applied for 20 minutes under 4 °C and the supernatant obtained was filtered using a 0.45 μm filter for EPS assessment. The phenol-sulfuric acid method was used to quantify polysaccharides (Dubois et al.,

1956). The concentration of protein was determined using the Bradford method (Bradford, 1976).

SMA of the sludge was determined monthly by using an Automated Methane Potential Test System (AMPTSII, Bioprocess Control, Sweden) (Li et al., 2011a). The Automated Methane Potential Test is characterized by a high sensitivity allowing on-line measurement of very low biomethane flows during the SMA test. This test system works with the same principles as conventional methane potential tests. The advantage of Automated Methane Potential Test over the conventional tests is that the gas volume measurements and data logging are fully automatic during the methane potential test (Figure 4.2).



Figure 4.2 : AMPTSII (1- Data storing, 2- Glass reactors with mixing and water bath, 3- CO₂ fixation unit, 4- Flow cell with methane production).

SMA tests were performed at 35 °C. Both samples and blanks were analyzed in triplicate. SMA tests were carried out in 500 mL serum bottles (with a working volume of 400 mL), which were filled with sludge and growth medium. The bottles were sparged with nitrogen gas to remove oxygen from the headspace. The growth medium for the blanks consisted of a mixture of macronutrients (6 mL of the stock solution, per liter of growth medium), trace elements (0.6 mL of the stock solution, per liter of growth medium) and phosphate buffer solution (10 mM). The growth medium for the samples additionally included 0.5 g COD/L as sodium acetate.

The nutrient stock solution consisted of (g/L): NH₄Cl (170), CaCl₂·2H₂O (8), MgSO₄·7H₂O (9) and the trace element stock solution contained (g/L): FeCl₃·4H₂O (2), CoCl₂·6H₂O (2), MnCl₂·4H₂O (0.5), CuCl₂·2H₂O (30), ZnCl₂ (50), H₃BO₃ (50), (NH₄)₆Mo₇O₂₄·4H₂O (90), Na₂SeO₃·5H₂O (100), NiCl₂·6H₂O (50), EDTA (1), HCl

36% (1 mL/L), Resazurine (0.5). The pH buffer stock solution was composed of $K_2HPO_4 \cdot 3H_2O$ (45.65 g/L) and $NaH_2PO_4 \cdot 2H_2O$ (31.20 g/L). The sludge and growth medium amounts were determined by setting a sludge VS amount to substrate COD amount ratio of 2:1.

Stability of sludge samples was tested monthly by obtaining the specific ultimate methane production. Stability tests were carried out in serum bottles with a volume of 120 mL. The anaerobic medium was prepared by dissolving 3.5 g/L sodium bicarbonate ($NaHCO_3$) in tap water without the addition of extra nutrients. The bottles were sealed with butyl rubber stoppers and aluminum caps. The head space was flushed with $N_2:CO_2$ (70:30%) mixture. The sludge was kept in the bottle at 35 °C without substrate addition in order to degrade the accumulated particulate matter in the sludge. During the test, the anaerobically degradable compounds were converted to biogas. Biogas production was monitored in terms of pressure increase with a pressure transducer (Centre Point Electronics PSI-30). Experiments lasted till the cumulative biogas production reached to a plateau. Methane content in the biogas was measured. The stability of the sludge was calculated as the ultimate methane COD produced divided by the sludge COD. Therefore, a high value indicates that a sludge is poorly stabilized and a high amount of anaerobic biodegradable organic compounds is still present and vice versa.

Microbial community structure was investigated using 454-pyrosequencing. UASB sludge samples were taken before and after membrane addition. All samples (seed sludge and UASB sludge) were washed twice with PBS and then centrifuged at 10000g for 3 minutes. The supernatant was removed in order to reduce biomass decay during sludge storage. All samples were stored at -25 °C until deoxyribonucleic acid (DNA) extraction.

16S recombinant deoxyribonucleic acid (rDNA) of sludge samples were extracted using a MoBio UltraClean Microbial DNA isolation kit (MoBIO Laboratories, Inc., CA, USA) following the protocol suggested by the manufacturer. In order to increase the efficiency in DNA isolation process, a combination of heat, detergent and mechanical force was applied. A minor modification including twice bead-beating (5 minutes) and heating (65 °C, 5 minutes) was applied to the protocol in sequence in order to enhance the lysis efficiency of microbial cells. DNA isolation was confirmed

by agarose gel electrophoresis and the concentration of DNA was measured using Nanodrop 1000 equipment (Thermo Scientific, Waltham, MA, USA).

Pyrosequencing of the 16S rDNA gene was performed at Research and Testing Laboratory (Lubbock, TX, USA) using a Roche454 GS-FLX system (454 Life Science, Branford, CT, USA) with titanium chemistry. Universal primers U515F (GTG CCA GCM GCC GCG GTA A) and U1071R (GAR CTG RCG RCR RCC ATG CA) (Wang and Qian, 2009) were used. By testing on Ribosomal Database Project (RDP) (Maidak et al., 1997), both forward and reverse primers target over 90% bacterial and archaeal 16S rDNA.

Post-processing and analysis of pyrosequencing results, including chimera removal, taxonomic classification and microbial diversity calculation (Chao1, Shannon index and observed species), were performed by combining different programs from the Quantitative insights into microbial ecology (QIIME) pipeline, version 1.6.0 (Caporaso et al., 2010).

4.3 Results and Discussion

4.3.1 Impact of membrane incorporation on UASB effluent

Figure 4.3 shows the total and soluble COD concentrations in the effluent of the UASB reactor before and after membrane incorporation. The average total COD concentration in the effluent before membrane incorporation was 149 ± 5.9 mg/L. After the membrane was coupled with the UASB reactor, total COD concentration in the effluent increased dramatically, reaching concentrations up to 2420 mg/L at the initial AnMBR operation stage. Afterwards, the effluent total COD concentration decreased and remained stable within the range of 750-1000 mg/L, indicating that a steady state condition was achieved. The increase in total COD concentration after membrane incorporation can be either attributed to the observed increased solids washout, indicating that significant quantities of sludge could not be retained in the sludge bed, or to the accumulation of intermediate products of the anaerobic degradation process. The latter might result from a biological process imbalance, allowing a part of influent COD to leave the UASB reactor before being completely degraded to the end products.

An increase in soluble COD concentration from 49 ± 6.8 mg/L to 122 ± 9.8 mg/L in the UASB effluent was also observed after membrane incorporation (Figure 4.3). The soluble fraction of the effluent might contain residual degradable and non- or slowly biodegradable influent substrate, SMP and intermediate products such as VFA. Therefore, this increase could be either related to soluble products of microbial origin or residual substrate and substrate degradation intermediates.

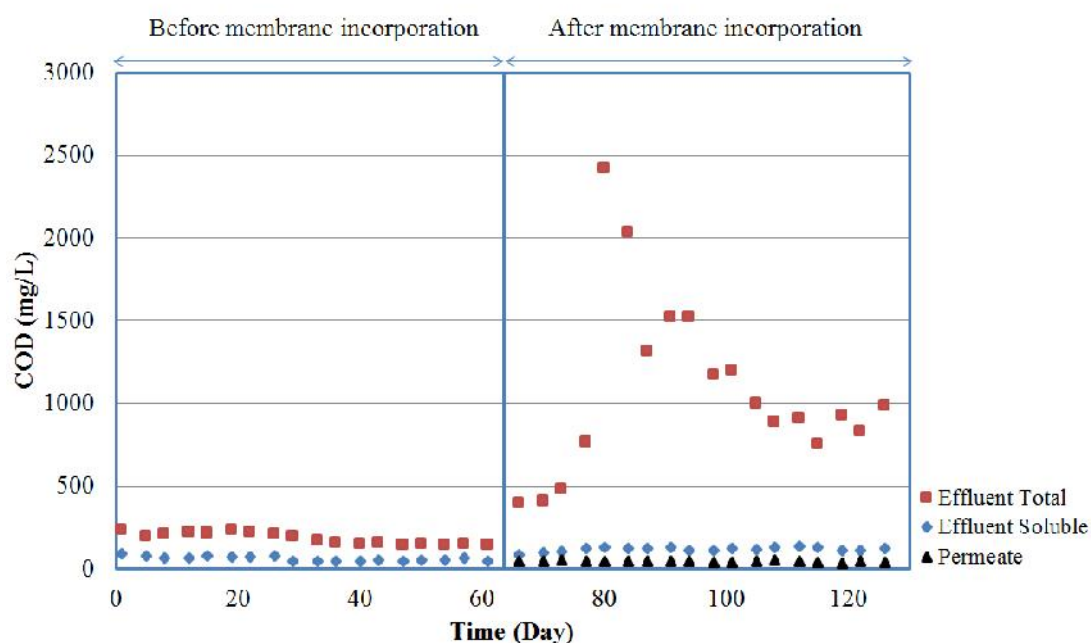


Figure 4.3 : Total and soluble COD concentrations in the UASB effluent and final permeate.

TSS was also measured both in the supernatant and effluent of the UASB reactor (Figure 4.4). Samples taken from the middle of the upper section above the sludge bed of the UASB reactor are referred as supernatant (Figure 4.1). As a result of membrane incorporation, average TSS concentrations in the effluent increased from 74 ± 10 mg/L to 387 ± 48 mg/L, which is in agreement with the total COD increase observed after membrane addition (Figure 4.3). Similar trends observed in total COD and TSS concentration profiles on the basis of a fixed COD/TSS ratio confirmed the buildup of sludge and/or suspended particles inside the system due to a complete retention of small and colloidal flocs that otherwise would have been evacuated from a conventional UASB reactor. Accumulation of fine particles in the system resulted from both the elimination of selective sludge washout in the system due to the complete retention by the membrane leading to recirculation of the membrane rejects into the system and abrasive effect of the recirculation pump used for maintaining the

cross-flow velocity over the membrane leading to particle breakage and fragmentation. After steady state conditions were achieved in the AnMBR, the difference between effluent and supernatant TSS concentrations decreased from 38% to 6%, which is also an indicator of GLS separator shortcoming for sludge washout prevention. However, despite the deterioration of the sludge bed, UASB reactor still acts like a biofilter before membrane treatment, which prevents the membrane from excessive exposure to high TSS concentrations. An average TSS concentration below 0.5 g/L was achieved in the UASB effluent after membrane incorporation, which is quite low in comparison to the ones in AnMBRs consisting of CSTRs.

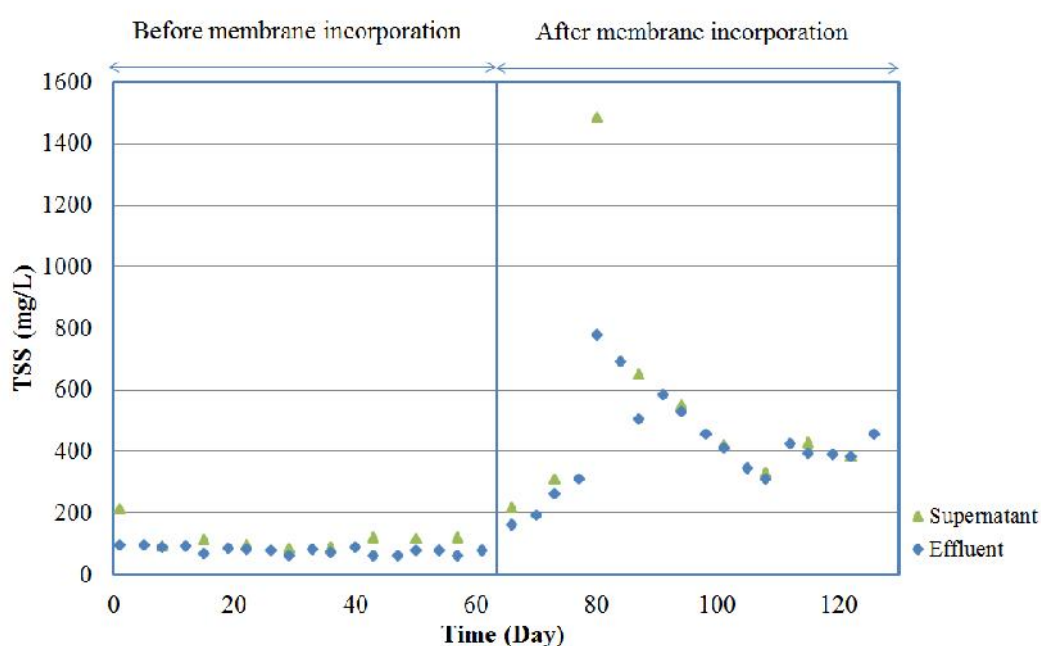


Figure 4.4 : TSS concentrations in the supernatant and effluent.

In order to measure the soluble compounds that resulted in this increase, SMP (Figure 4.5) and VFA (Table 4.1) concentrations were measured both in the UASB effluent and permeate. SMP consists of polysaccharide and protein. Protein concentration was under the detection limit (2.5 mg/L) in the UASB effluent obtained from Day 46 to Day 62 before membrane incorporation. Polysaccharide concentration was almost stable, with an average value of 15.3 ± 4.1 mg/L. As shown in Figure 4.5, membrane incorporation initially led to an increase in SMP production. Protein and polysaccharide concentrations in the effluent rapidly increased to 80 mg/L and 40 mg/L, respectively. Stuckey (2012) reported that under any type of stress SMP production can increase dramatically in an anaerobic culture. Therefore,

the rapid increase in SMP concentrations could be correlated with incomplete hydrolysis of solids in the UASB reactor that caused stress in microorganisms, given the limitations of anaerobic metabolism for particulate matter accumulation. Besides, this dramatic increase in protein and polysaccharide concentrations may be related with biomass associated products which are produced during cell lysis resultant from sludge recirculation. The recirculation pump used for maintaining the cross-flow velocity over the membrane may have had an abrasive effect that caused cell lysis and release of organic material from cell lysis may result in an increase in protein and polysaccharide concentrations in the UASB effluent. Following this initial increase, a decrease in protein and polysaccharide concentrations was observed, which was in agreement with the trends for COD and TSS concentrations, and is likely due to the adaptation to the new conditions or emergence of new microbial populations. However, protein and polysaccharide concentrations were still higher than those observed before membrane incorporation, with average values of 64 ± 11.1 mg/L and 26 ± 11.2 mg/L, respectively. The operational change in the system induced by the membrane incorporation apparently affected the microbes, inducing a higher SMP secretion. A decrease was observed in polysaccharide to protein in ratio after membrane incorporation in the UASB effluent.

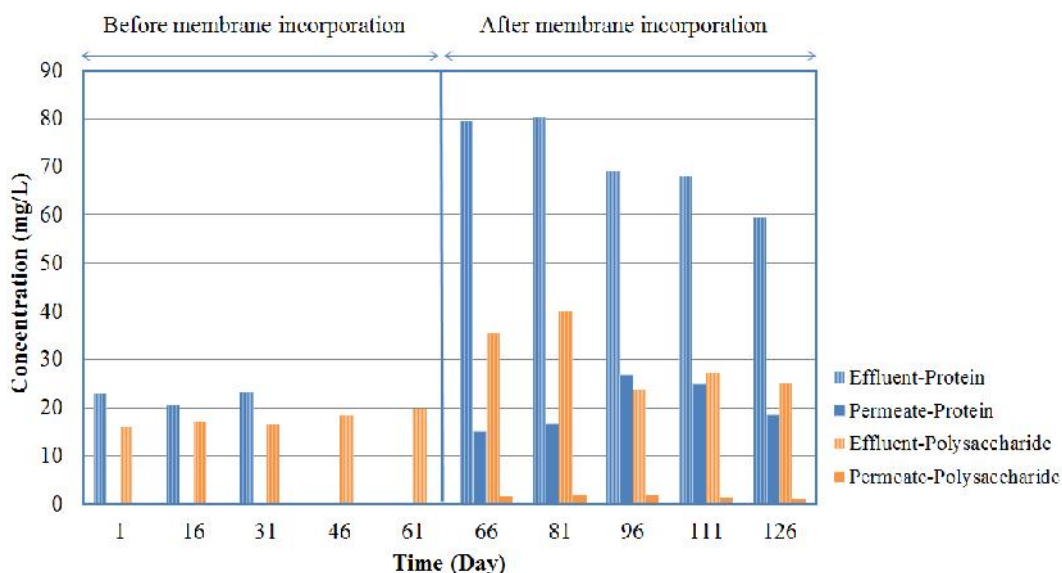


Figure 4.5 : SMP concentrations in the UASB effluent and final permeate.

In contrast to the significant increases observed in SMP concentrations after membrane incorporation, only a slight increase was observed in VFA concentrations from an average value of 2 mg/L before membrane incorporation to 13.5 mg/L after

membrane incorporation, which can be an indicator of soluble fraction increase coming mostly from microbial origin rather than residual substrate or substrate intermediate. Acetic acid represented the largest proportion of the VFA in the effluent and permeate.

Table 4.1 : VFA concentrations in the effluent and permeate before and after membrane incorporation.

Time	Stage	VFA (mg/L)															
		Effluent								Permeate							
		C2	C3	i- C4	C4	i- C5	C5	C6	Total	C2	C3	i- C4	C4	i- C5	C5	C6	Total
Day-1	BM ^a	3	0	0	0	0	0	0	3								
Day-16	BM	2	0	0	0	0	0	0	2								
Day-31	BM	2	0	0	0	0	0	0	2								
Day-46	BM	2	0	0	0	0	0	0	2								
Day-61	BM	2	0	0	0	0	0	0	2								
Day-66	AM ^b	14	0	0	0	0	0	0	14	12	0	0	0	0	0	0	12
Day-81	AM	16	1	1	1	1	9	0	29	13	1	1	1	1	9	0	26
Day-96	AM	9	2	1	1	1	14	0	28	8	2	1	1	1	14	0	27
Day-111	AM	13	0	1	1	1	1	0	17	12	0	0	1	1	1	0	15
Day-126	AM	8	1	0	0	0	1	0	10	7	1	0	0	0	1	0	9

^a Before membrane incorporation.

^b After membrane incorporation.

4.3.2 Impact of membrane incorporation on the overall system performance

Soluble COD concentration in the UASB effluent (122 ± 9.8 mg/L) was found to be higher than the permeate COD concentration value (42 ± 4.4 mg/L). The pore size of the filter used for obtaining the sample for soluble COD determination ($0.45 \mu\text{m}$) was approximately 10 times larger than the average pore size of the membrane ($0.03 \mu\text{m}$). This indicated that an important fraction of soluble COD (i.e. $<0.45 \mu\text{m}$) was retained by the UF membrane. VFA concentrations in the permeate were almost equal to those of the effluent because of their very low molecular weight. As expected, the membrane had no considerable effect on VFA removal. In contrast to VFA, the membrane was able to retain SMP. The average protein and polysaccharide concentrations in the permeate were 20 mg/L and less than 2 mg/L, respectively. The retention of protein by the membrane (66%) turned out to be lower than the retention of polysaccharide (95%). These results indicate that more soluble protein was discharged from the system when it was operated as an AnMBR, whereas more polysaccharide was observed in the effluent when the system was operated as a sole UASB.

As a general comparative evaluation of system performance before and after membrane incorporation, regardless of the increases in COD and TSS concentrations in the UASB effluent, SS-free (non-detected) AnMBR permeate was obtained after membrane incorporation with an average COD of 42 mg/L, standing for an overall COD removal efficiency of 92% in the AnMBR system (Figure 4.3), whereas total COD removal efficiency was approximately 72% before membrane incorporation. The OLR was kept constant at 2 kg COD/m³.d during the whole period. However, the COD in the effluent of the system, i.e., UASB effluent before the membrane addition and permeate after membrane addition, decreased from 149±5.9 mg/L to 42±4.4 mg/L, resembling an increase in the COD removal efficiency of about 20%, from 10.1±0.2 g COD/d to 12.9±0.1 g COD/d. The higher COD removal efficiency achieved after membrane addition, compared with the COD removal efficiency before membrane addition, can be attributed to the complete retention of all particulate and colloidal matter and biomass inside the reactor by the membrane. Average TN, NH₄-N and TP concentrations in the permeate were 57 mg/L, 54 mg/L and 11.8 mg/L. The results clearly demonstrate that AnMBRs are not sufficient for removing nutrient from wastewater. Therefore, a nutrient removal, when necessary, can be achieved in a separate post-treatment step. Average pHs of the overflow from UASB before and after membrane addition were 7.22±0.27 and 7.08±0.11, respectively. The rise in VFAs in the UASB effluent was accompanied by a drop in pH to 7.08±0.11 after membrane addition. Permeate pH was 7.10±0.26 after membrane addition.

The variations of TMP with operation time are shown in Figure 4.6. Membrane cleaning was carried out both at the middle (Day 94) and end (Day 126) of the AnMBR operation stage to recover the membrane permeability (P). The membrane was cleaned only once after its incorporation as the reactor was operated for 63 days. A slow increase was observed in TMP with operation time as membrane flux was kept at about 12.3 L/m²h.

The sudden TMP rise observed at the initial stage (Day 79) might be related to the sudden and sharp increase observed in both effluent COD and TSS concentrations (Figure 4.3 and Figure 4.4) just after operation, which resulted in the accumulation of particles in the lumen. The small diameter membrane tubes that were selected in order to increase the filtration area were simply blocked by particles, especially by

the bigger ones and this would result in an uneven flux distribution across the bundle of tubes and then an increase in TMP at Day 79, which eventually caused more serious membrane fouling. After steady state conditions were achieved, low average TMP values were maintained, which is in agreement with the results of stable UASB effluent characteristics having relatively low average TSS concentrations below 0.5 g/L. TMP remained quite stable with an average value of 87 mbar during AnMBR operation. Thus, membrane flux may become less dependent on the reactor MLSS concentration, possibly leading to low TMPs.

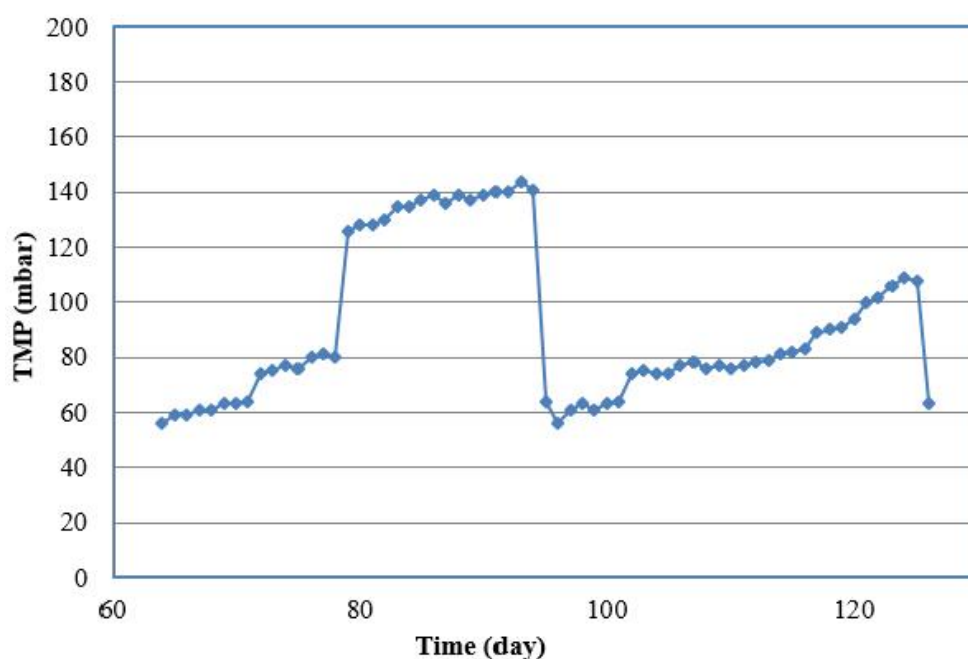


Figure 4.6 : Filtration performance of the AnMBR system.

4.3.3 Impact of membrane incorporation on sludge properties

In order to investigate the mechanisms behind the enhancement in overall system performance, sludge characteristics were also monitored during each operational stage. Considerable changes in sludge physical properties were observed during the experimental period, mainly characterized by a decrease in PSD, coinciding with the period after membrane incorporation. The success of UASB technology lies in its capability to establish a dense sludge bed (Seghezzo et al., 1998) and the settling ability of the sludge bed is related to the selective washout of the dispersed sludge, which results in an increased growth of retained sludge agglomerates. When membranes are coupled with the UASB process, this selection pressure is eliminated. Under conditions of high selection pressure, light and dispersed sludge will be

washed out while heavier sludge particles can be retained in the UASB reactor; whereas under low selection pressure conditions as in this case, growth will take place mainly as dispersed sludge, which leads to the formation of a bulking type of sludge (Hulshoff Pol et al., 2004).

Figure 4.7 presents PSD curves of the seed sludge, and UASB sludge before and after membrane incorporation. From Figure 4.7, it can be observed that the seed sludge had a broader PSD profile with D50 of 141 μm compared to sludge taken from the UASB under operation. Already after 1 day of operation, a notable washout of small particles was experienced. An increase in D50 value to 465 μm was observed before membrane incorporation, indicating that even small sized particles were washed out (Figure 4.7(a)). To the contrary, PSD curves after membrane addition shifted to the left and a decrease in the D50 was observed (Figure 4.7(b)). During the operation of the system as an AnMBR, fine sludge particles such as non-settling particles, small particles including colloidal substances and macromolecules of SMP could not be washed out and were returned to the UASB reactor with the concentrate flow, thereby decreasing the D50 of the sludge in the UASB reactor to 138 μm . Besides retention of non-settling particles and small particles, the recirculation pump used for maintaining the cross-flow velocity over the membrane may have had an abrasive effect that caused particle breakage and fragmentation. The decrease in particle size resulted in a reduction of the settling capacity of the sludge, supporting the assumption that the settling capacity depends on the PSD of the sludge, i.e. larger particles yield better settling properties. The deterioration of the settling capacity of the sludge promoted the sludge washout from the UASB reactor (Figure 4.8), which increased COD and TSS concentrations in the UASB effluent.

SMP and EPS were also analyzed in the sludge bed (Table 4.2). For the SMP of the sludge, the protein amount decreased from an average of 11.4 mg/g VSS to 5.1 mg/g VSS after membrane addition, whereas the polysaccharide concentration increased from an average of 1.3 mg/g VSS to 2.0 mg/g VSS. For the EPS, both protein and polysaccharide amounts decreased by 81% and 97%, respectively. EPS are crucial for integrating cells into flocs and granules. Therefore, the decrease in EPS amount might indicate a higher possibility of looser structures of flocs and granules, contributing to the decrease in sludge particle size and also to the increase in SMP in the effluent. For SMP, the increase in polysaccharide amount in the sludge might be

related with the increase in polysaccharide concentration in the effluent (Figure 4.5) after membrane incorporation. However, it is intriguing to find the simultaneous decrease of protein in sludge along with the increase of protein in the effluent (Figure 4.5). Results indicate that a large amount of bound protein from the sludge leaked into the effluent and rose to the top of the UASB reactor and even the decrease in protein concentration of SMP led to an increase in protein concentration in the effluent. All these results show that the membrane addition had obviously affected the EPS and SMP production in sludge, as well as the floc and granule structures

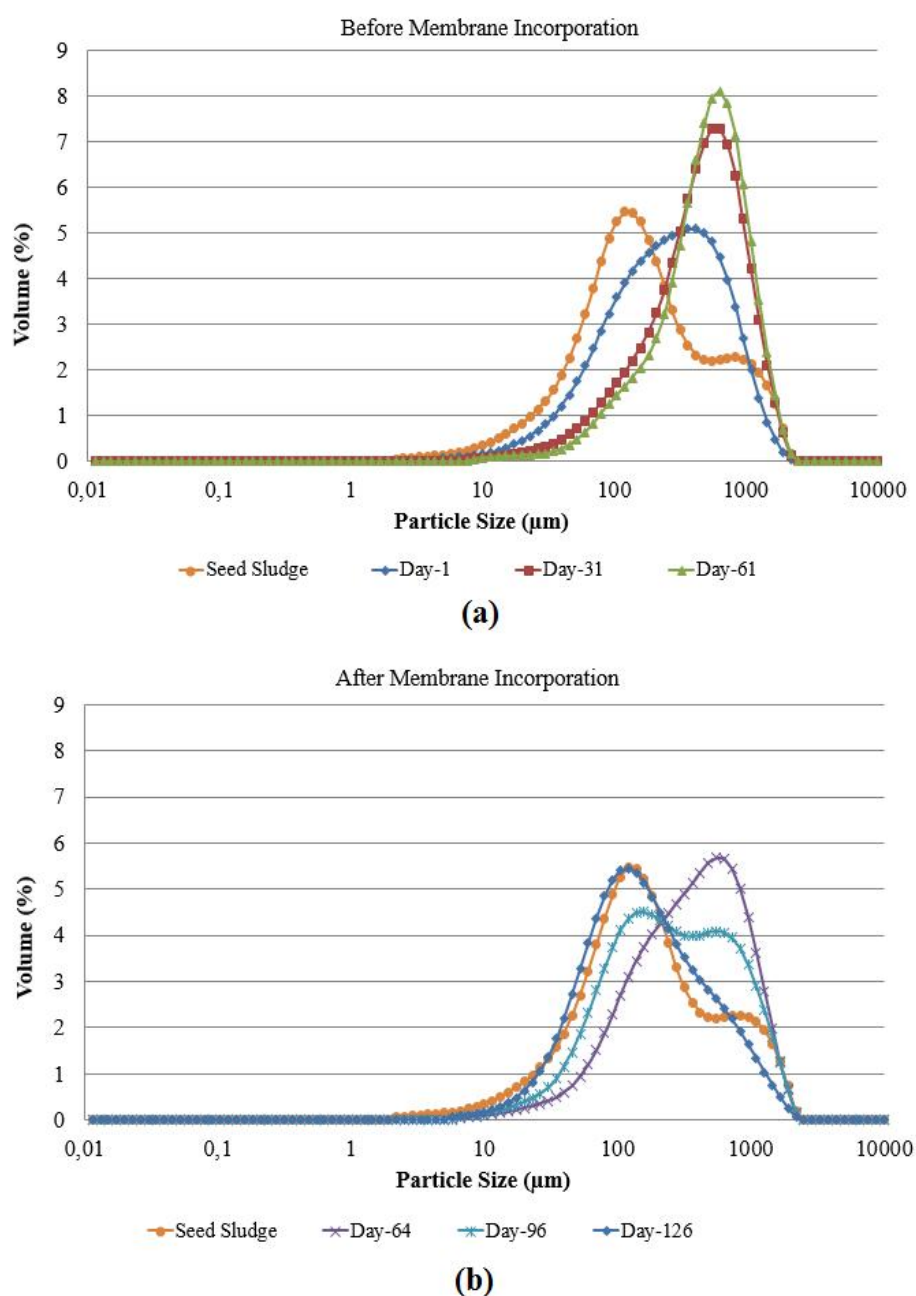


Figure 4.7 : PSD of the seed and UASB reactor sludge (a) before and (b) after membrane incorporation.

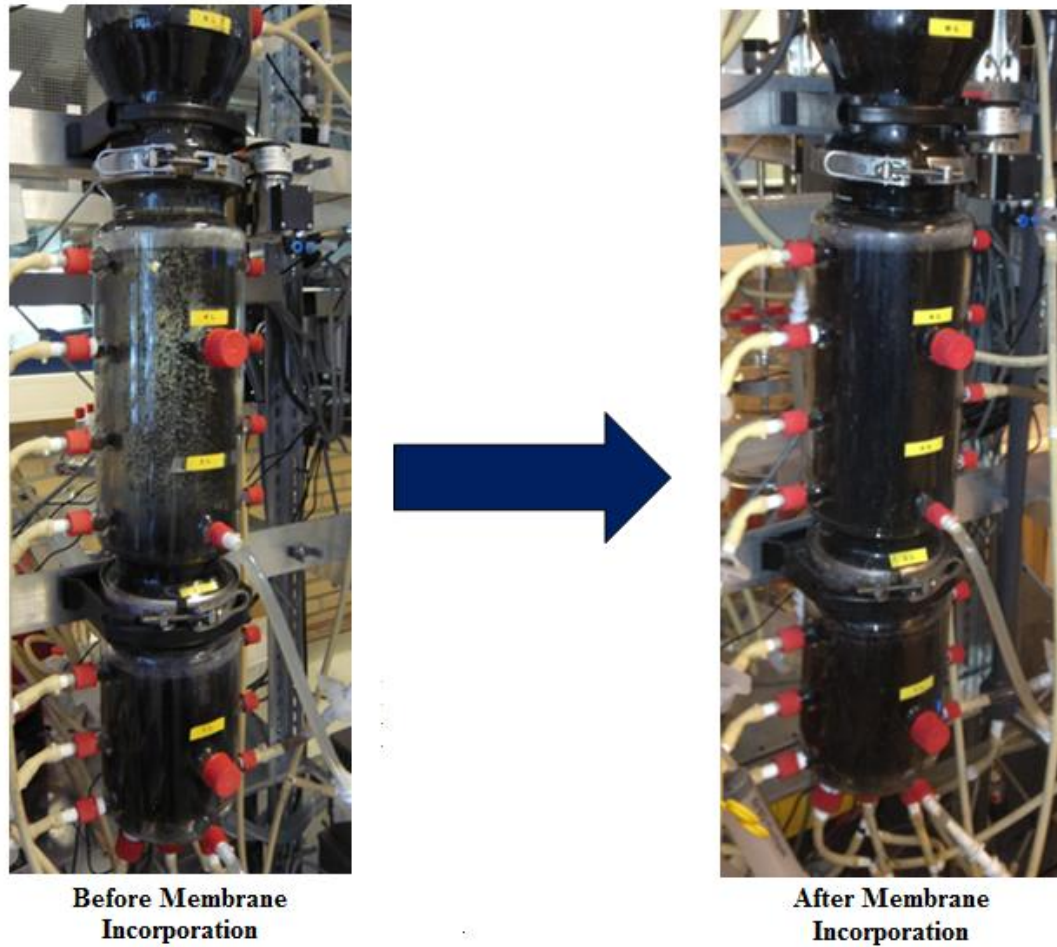


Figure 4.8 : Sludge settleability before and after membrane incorporation.

Table 4.2 : SMP and EPS amounts of the sludge in the UASB reactor.

Time	SMP		EPS	
	Protein (mg/g VSS)	Polysaccharide (mg/g VSS)	Protein (mg/g VSS)	Polysaccharide (mg/g VSS)
Day-1	11.5	1.2	21.4	5.1
Day-31	11.2	1.3	17.6	4.5
Day-61	11.4	1.4	17.4	4.3
Day-81	4.8	1.3	3.7	0.3
Day-96	6.1	2.1	3.7	0.2
Day-126	4.6	2.5	3.4	1.0

The impact of membrane incorporation on the SMA and stability of the UASB sludge is shown in Table 4.3. After membrane addition, the SMA of the sludge decreased by 24%, from 0.70 ± 0.06 to 0.53 ± 0.02 g CH_4 -COD/g VS.day. However, activity decrease was not expected due to the increases observed in both COD removal efficiency and methane production. In the bio-assays, the substrate COD to inoculum VS ratio was maintained at 2:1. The drop in the SMA can likely be

attributed to influent particulate matter accumulation within the system, due to the presence of a physical barrier, i.e. membrane, retaining all non- or slowly degradable polymers. Therefore, the same amount of VS used as inoculum would contain a lower amount of organisms, which would show a lower overall activity regardless of a decrease in the activity. In order to test this assumption, stability tests were performed. The sludge stability decreased from 0.35 to 0.48 g CH₄-COD/g COD after membrane incorporation. Apparently, after membrane incorporation, more biodegradable solids accumulated in the sludge bed.

Table 4.3 : SMA and stability of sludge in the UASB reactor before and after membrane incorporation.

Time	SMA (g CH ₄ -COD/g VS.day)	Stability (g CH ₄ -COD/g COD)
Day-1	0.76±0.02	0.32±0.02
Day-31	0.64±0.05	0.38±0.01
Day-61	0.70±0.05	0.36±0.01
Day-81	0.52±0.03	0.45±0.003
Day-96	0.55±0.06	0.49±0.01
Day-126	0.53±0.02	0.49±0.01

Collected samples were used to pyrosequence the former region of 16S rDNA gene using the 454 GS-FLX sequencer before and after membrane addition. A total of 11167 reads were obtained. The overall estimations of microbial diversity based on phylogenetic diversity index, Shannon-Wiener index, Chao1 and Observed species number (OSN) (Table 4.4) exhibited a great increase after membrane incorporation, demonstrating that both richness and evenness of sludge bed microbial community increased after membrane incorporation in UASB. From a microbial ecological perspective, adding a membrane implies, directly or indirectly, a wider niche for UASB microorganisms. Firstly, the membrane module functions as a direct retention of potentially-easily-washed-out microorganisms. After addition of membrane, the recycled concentrate brings the poorly settled microbial groups to the bottom section of the UASB and mixes with the surrounding sludge. Thus, all species are kept inside, regardless of their abundance. However, the membrane has also an indirect effect to microbial community as it retains otherwise-washed-out particulates that are substrates to some specific bacteria.

Table 4.4 : Microbial diversity index.

Biodiversity Index	Inoculum	UASB Sludge Before Membrane	UASB Sludge After Membrane
Phylogenetic Diversity Index	21.3	19.2	26.2
Shannon-Wiener Index	6.2	6.0	6.7
Chao1 Richness	422.3	540.3	803.1
OSN	326	312	396

Pyrosequencing analysis showed a drastic change in the bacterial community after membrane incorporation (Figure 4.9). In the initial UASB seed sludge, *Bacteroidetes* was the most abundant bacterial phylum (755 sequences, 36% of total bacteria), followed by *Firmicutes* (695, 33%), *Proteobacteria* (240, 11.5%), *Chloroflexi* (136, 6.5%), *Acidobacteria* (107, 5.2%) and other phyla with percentages lower than 2% (Table A.1, Appendix A). This means that the bacterial community at the starting point was very diverse and sequences belonged to more than ten phyla of bacteria. However, the sequences belonging to *Firmicutes*, *Acidobacteria*, *Synergistetes*, *Planctomycetes*, *Fusobacteria*, *Deferribacteres* and *Thermotogae* decreased dramatically after start-up period. This is reasonably to expect due to both substrate composition changes and washout conditions. The inoculated sludge was previously fed with black water in a pilot UASB reactor, which contained a wider variety of organic compounds compared to the laboratory-scale UASB, serving a more diverse microbial community. After membrane incorporation, an increase was again observed in some phyla such as *Firmicutes*, *Planctomycetes* and *Fusobacteria*.

The genus *Cytophaga* was further enriched and occupied the predominant position, followed by the genus *Flavobacterium* after membrane integration (Figure 4.9). The change in *Cytophaga* and *Flavobacterium* might be related with the change in the feed from black water to synthetic wastewater since similar increasing trends were sustained after membrane addition. *Cytophaga* genus predominance could be associated with the increase in small (organic) particles after membrane incorporation, as evidenced by PSD results (Figure 4.7). *Cytophaga* has been reported to grow anaerobically utilizing simple carbon sources such as glucose and starch, with final products such as acetic, propionic and succinic acids, in approximately equimolar quantities, plus a small amount of lactic acid (Bachmann, 1955). On the other hand, the slight increase in VFA (mainly acetic acid) observed after membrane addition (Table 4.1) could also be related with the large dominance

of genus *Cytophaga* (Oh et al., 2004; Chen and Dong, 2005). Another common bacterial genus that are closely involved in hydrolysis and acidogenesis (Rademacher et al., 2012; Zheng et al., 2013) -*Clostridium*- became the third largest population after membrane addition, with a 141% increase in abundance (Figure 4.9). However, *Clostridium* had a decreasing trend before membrane addition which was related with the change in the feed. It has been reported that some members of the genus *Clostridium* are also involved in acetogenesis and syntrophic acetate oxidation (Westerholm et al., 2012). Some bacterial genus, which were dominant during the first part of the experiment, such as *Desulfomicrobium*, *Bellilinea*, *Aeromonas* and *Dechloromonas* lost their dominance after membrane addition (Figure 4.9, Table A.1, Appendix A). As to *Bacteroides*, a commonly reported protein/carbohydrates-dependent genus (Yu et al., 2012; Li et al., 2013), its further decrease in abundance after membrane addition could be either linked to the change in the feed or to the observation that the EPS amount in the sludge bed decreased by 81% (protein) and 97% (polysaccharide) after membrane addition (Table 4.2).

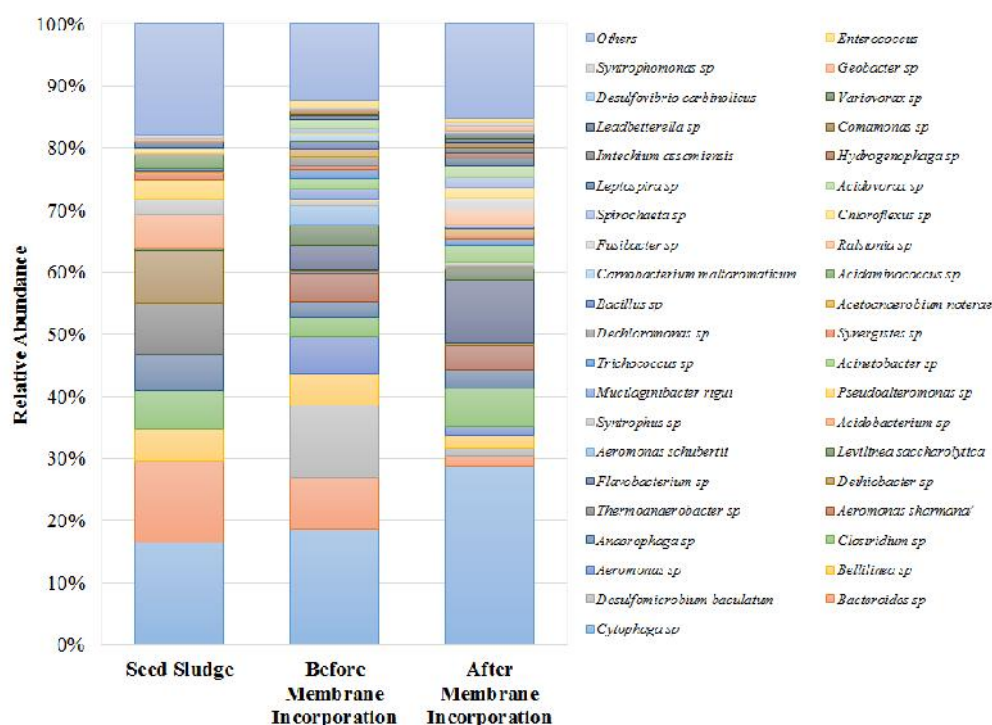


Figure 4.9 : Relative abundance of major bacterial genera.

The archaeal community structure was also significantly affected by the membrane addition. Eight different species were identified in the seed sludge. Members of the genus *Methanobrevibacter* comprised 43.8% of the total genera, followed by

Methanobacterium, *Methanolinea* and *Methanosaeta*, which accounted for 14.6% (Table A.2, Appendix A). However, after inoculating the laboratory-scale UASB reactor, a clear shift in the archaeal community appeared. The number of species decreased from eight to six, indicating that the archaeal community had a lower richness during the UASB operation stage compared to the seed sludge.

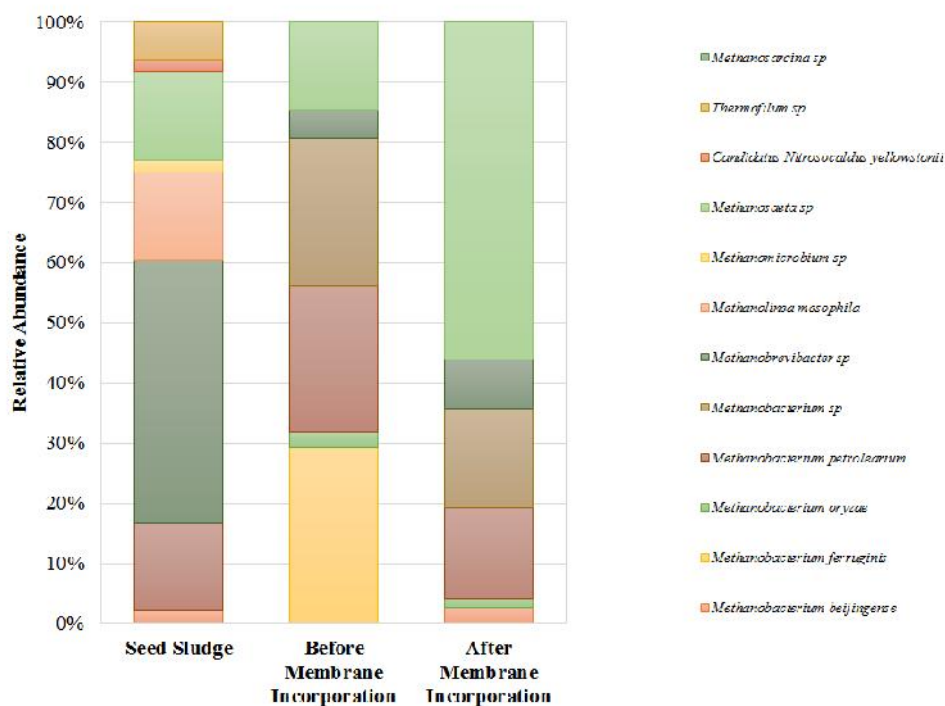


Figure 4.10 : Relative abundance of all archaeal species.

The relative abundances of *Methanobrevibacter* and *Methanolinea*-like methanogens diminished and the relative abundance of *Methanobacterium*-like methanogens increased in the sludge. After membrane incorporation, the species belonging to the genus *Methanosaeta* (acetoclastic methanogens) (Sousa et al., 2013) became dominant (56.2% in relative abundance) while before it only accounted for less than 15% (Figure 4.10, Table A.2, Appendix A). The previously dominant *Methanobacterium* species (hydrogenotrophs) decreased by 45%. It is well known that acetate is an important substrate for methanogenesis in anaerobic reactors, and the archaeal genus *Methanosaeta* is representative of acetoclastic methanogens. In addition, it was reported that *Methanosaeta* sp. contributed to the stable performance of the anaerobic bioreactors (Talbot et al., 2008) and they are believed to be competitive in established methanogenic communities (Bandara et al., 2012). Therefore, the predominance of *Methanosaeta* can be explained by means of the VFA accumulation (mainly acetic acid) observed during the AnMBR operational

stage. Similar results have also been reported in other studies (Siggins et al., 2011; Ziganshin et al., 2013). Additionally, the increase in methane production after membrane incorporation could be attributed to the further degradation of retained particulate organic matter.

4.4 Conclusion

Coupling a membrane to a UASB reactor resulted in significant changes in both physical and biological aspects, mainly due to the elimination of hydraulic dilution as bacterial selection pressure factor. After membrane addition, the sludge PSD decreased, coinciding with the complete retention of colloidal and fine particles by the membrane. In addition, the EPS content of the sludge decreased and the abrasive effect of the membrane recirculation pump caused particle fragmentation. Decrease in the sludge particle size deteriorated the sludge bed settleability, which led to a higher sludge washout, resulting in an increase in COD, TSS and SMP concentrations of the UASB effluent. However, enhancement in overall system performance was observed in terms of higher COD removal efficiency and methane production after membrane incorporation due to the complete retention of all particulate and colloidal matter and biomass inside the reactor by the membrane. Besides, TMP values remained quite stable during AnMBR operation indicating no severe membrane fouling. Pyrosequencing analysis showed that membrane incorporation induced population dynamics in both bacterial and archaeal species in the sludge. A decrease was observed in the sludge activity after membrane incorporation, in spite of the increases observed in both COD removal efficiency and methane production. Stability tests confirmed the observation that the decrease in SMA was related to the particulate matter accumulation. Overall, it is impossible to get an effluent that fulfills the regulations for water reuse by using solely a UASB reactor for treatment. However, a pathogen-free but nutrient-rich permeate was achieved during the AnMBR stage with quite stable TMP values. Therefore, the results obtained in this study clearly supported the applicability of a membrane-coupled UASB reactor for municipal wastewater treatment, in spite of the sludge bed deterioration.

5. IMPACT OF TEMPERATURE ON FEED-FLOW CHARACTERISTICS AND FILTRATION PERFORMANCE OF AN UPFLOW ANAEROBIC SLUDGE BLANKET COUPLED ULTRAFILTRATION MEMBRANE⁽⁴⁾

5.1 Introduction

With the growing interest to produce nutrient-rich and hygienically safe effluents from municipal wastewater, there is an increasing interest to assess the feasibility of AnMBRs as treatment alternative. Municipal wastewater is characterized by a low organic strength, implying low biomass growth rate and a significant amount of particulate organics that have to be hydrolyzed. These characteristics constrain the effective hold-up of biomass in the bioreactor, especially in reactors in which flocculent biomass can be easily washed out such as UASB and EGSB reactors (Martinez-Sosa et al., 2011a). The combination of a membrane separation unit and an anaerobic bioreactor allows efficient retention of biomass (Gao et al., 2009; Tao et al., 2012), eventually resulting in efficient hydrolysis of particulate organics and advanced sludge stabilization at long SRTs (An et al, 2009b; Zhang et al., 2011). Therefore, AnMBR is proposed as a promising technology for the treatment of municipal wastewaters, with the added benefits of low sludge production, high quality effluents with a very low content of pathogenic organisms but a high fertilizing value, absence of additional costs for aeration, generally associated with the conventional activated sludge process (Lin et al, 2011). However, thus far, the development of AnMBR systems for municipal sewage treatment is challenged by

⁽⁴⁾ This chapter is based on:

Ozgun, H., Tao, Y., Ersahin, M.E., Zhou, Z., Gimenez, J.B., Spanjers, H., van Lier, J.B. (2015) Impact of temperature on feed-flow characteristics and filtration performance of an upflow anaerobic sludge blanket coupled ultrafiltration membrane treating municipal wastewater. *Water Research* 83, 71-83.

membrane fouling that increases the energy costs, reduces the membrane lifetime, and makes the system less efficient (Nguyen et al., 2013).

Many attempts have been made to prevent or reduce membrane fouling (Martin-Garcia et al., 2011; Salazar-Pelaez et al., 2011b). Briefly, several factors involved in design and operation may influence fouling such as setup configuration and membrane characteristics, feeding and biomass properties and operational conditions (Gao et al., 2010; Salazar-Pelaez et al., 2011b). Among them, reactor design is proposed as an important factor that can change the AnMBR fouling propensity (Ozgun et al., 2013b). Most of AnMBRs studied employ CSTR because of the ease of construction and use (Liao et al., 2006). While CSTR configurations feature the general AnMBR advantages, they are characterized by exposing the membrane to the bulk MLSS, which easily leads to fouling (Liao et al., 2006). The MLSS of a CSTR AnMBR reactor is commonly >10000 mg/L, while the effluent from a UASB AnMBR is 300-550 mg TSS/L (Cho and Fane, 2002; Liao et al., 2006). Exposure to higher solids concentrations in CSTR designs usually increases the rate of cake formation in comparison to reactor designs that retain biomass. Rapid cake formation requires more maintenance and leads to longer down times (i.e. periods of non-filtration) and thus lower overall fluxes. Besides, more intimate exposure of the biomass to the membranes may induce EPS production, enhancing membrane fouling (Liao et al., 2006). Given these considerations, UASB reactors have recently attracted more interest to become the anaerobic bioreactor as a part of AnMBRs (Gao et al., 2010; Liu et al., 2012; Ozgun et al., 2015a). Membrane coupled UASB reactors may be a promising approach to overcome problems related with excessive fouling. High fluxes are expected in membrane coupled UASB configurations since the membrane is only challenged by the supernatant and not by the bulk sludge. Lower reversible fouling has been reported for a membrane coupled UASB reactor system in comparison to membrane coupled CSTR system for the treatment of black water (van Voorthuizen et al., 2008).

It is well known that anaerobic processes strongly depend on operational temperature that has a substantial effect on the conversion rate of organic matter and consequently on the quality of the effluent. Temperature can also impact the solubility of various compounds and gases and viscosity of sludge that has a great influence on the membrane filtration performance. In fact, several studies (Wen et

al., 1999; Ho and Sung, 2010; Martinez-Sosa et al., 2011a; Robles et al., 2013) have focused on the effect of operational temperature on the performance of AnMBRs treating municipal wastewater. Ho and Sung (2010) reported that AnMBRs operated at 25 °C and 15 °C, treating synthetic municipal wastewater achieved more than 85% COD removal due to the physical retention of pollutants by the membrane, although the methanogenic activity was suppressed at lower temperature. Martinez-Sosa et al. (2011a) also observed that a pilot-scale AnMBR treating municipal wastewater achieved 90% COD removal during operation for 100 days under both mesophilic and psychrophilic conditions showing a strong tolerance to temperature changes. Particularly, under sub-mesophilic conditions, hydrolysis of the retained particulates likely becomes rate-limiting and in UASB reactors particulate matter accumulation in the sludge bed results in a sludge activity loss (Lettinga et al., 2001). Under these conditions, the degree of small particle retention by the sludge bed will be of prime importance for the feasibility of UASB coupled membrane filtration systems (Ozgun et al., 2013a). However, regarding UASB-membrane configurations, research on particle retention in the sludge bed at lower temperatures and the effect of temperature on filtration performance are difficult to find in literature.

Therefore, in the present research, a UASB coupled membrane system treating municipal wastewater was operated for nearly 126 days at 25 °C and 15 °C in order to investigate the relationship between the characteristics of the UASB effluent and the filtration performance of the AnMBR at different operational temperatures. The effect of temperature on the overall performance of the AnMBR system was determined in order to investigate whether the membrane coupled UASB is a technically feasible alternative in the treatment of municipal wastewater at 15 °C. In addition, the effect of temperature on the microbial communities was also determined. The results reported here provide valuable new insights into the fouling mechanisms and will consequently further advance knowledge on the feasibility of membrane coupled UASB systems at low temperatures.

5.2 Materials and Methods

5.2.1 Wastewater source

In the present study, synthetic municipal wastewater was used as feed, similar to that described in Chapter 3. The composition of the concentrated substrate solution and

the characterization of the synthetic municipal wastewater at the UASB inlet were already presented in Chapter 3 in Table 3.1 and Table 3.2, respectively.

5.2.2 Seed sludge

The reactor was seeded with flocculent anaerobic sludge obtained from a pilot-scale UASB reactor treating black water (Sneek, the Netherlands). The characteristics of the seed sludge were presented in Table 3.3 in Chapter 3.

5.2.3 Experimental setup

The experimental setup consisted of a laboratory-scale UASB reactor with an effective volume of 7 L coupled to an external membrane module. A schematic diagram of the experimental setup is depicted in Figure 5.1. Peristaltic pumps (Watson Marlow 120U/DV) were used for circulating the flow between the membrane module and UASB reactor and obtaining permeate through the membrane. Furthermore, recirculation over the membrane surface was applied by a peristaltic pump (Watson Marlow 620UN/R) in order to maintain a cross-flow velocity of 1 m/s across the membrane surface independently from the upflow velocity in the UASB reactor. Concentrate recycle flow-rate was adjusted to keep the upflow velocity stable in the UASB reactor. The biogas was collected by means of a three-phase separator installed at the top of the reactor. Biogas flow-rate was measured by a gas meter (Ritter, Milligas Counter MGC-1 PMMA). The operational temperature was controlled by using water baths. High temperature (TC16, Tamson Instruments, the Netherlands) and low temperature (TLC 15, Tamson Instruments, the Netherlands) water baths were used in order to maintain operational temperatures of 25 °C and 15 °C, respectively. Temperature and pH inside the bioreactor were monitored on-line by a probe combined with a transmitter (Elscolab, M300 ISM). TMP was monitored using high accuracy pressure sensors (AE Sensors, ATM - 800/+600 mbar, the Netherlands) installed in the membrane feed, concentrate and permeate lines. A computer accessible via LabView software was used to control the pumps and collect the data.

The external cross-flow tubular membrane module contained 28 membrane fibers with an internal diameter of 1.5 mm and a length of 80 cm. Cross-sectional and permeate outlet images of the membrane module are presented in Figure 5.2(a) and Figure 5.2(b), respectively. Pentair X-Flow (Enschede, the Netherlands) PES UF

membranes with a pore size of 30 nm were used in the AnMBR. The detailed characteristics of the membrane are listed in Table 5.1.

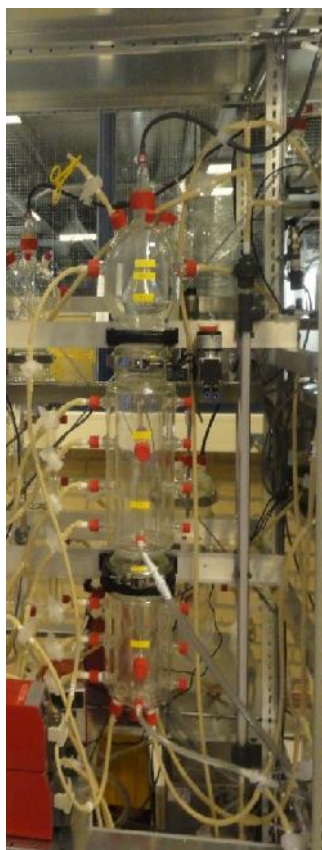
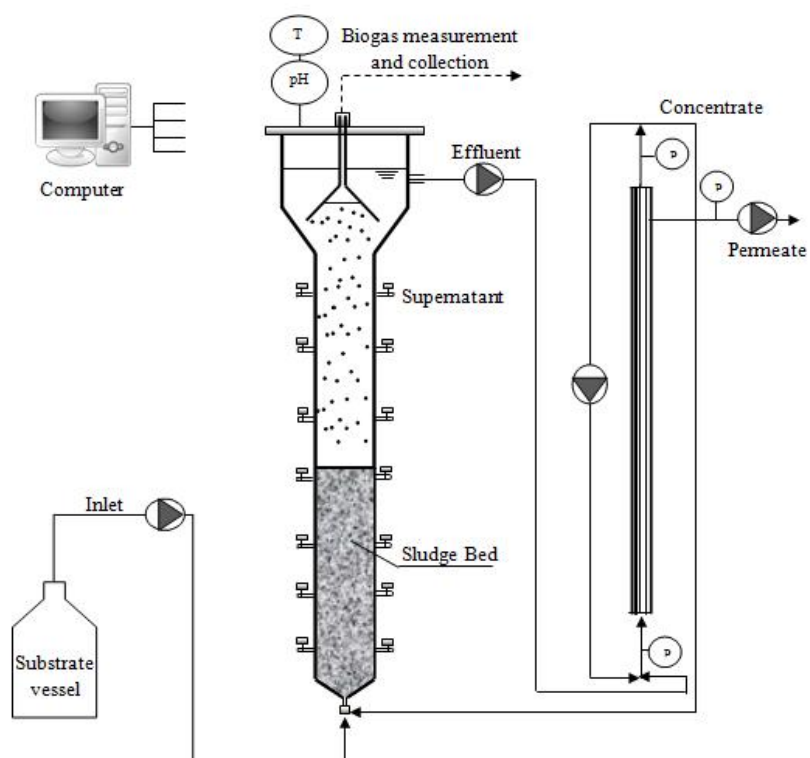


Figure 5.1 : Layout of the AnMBR, consisting of a UASB coupled UF unit.

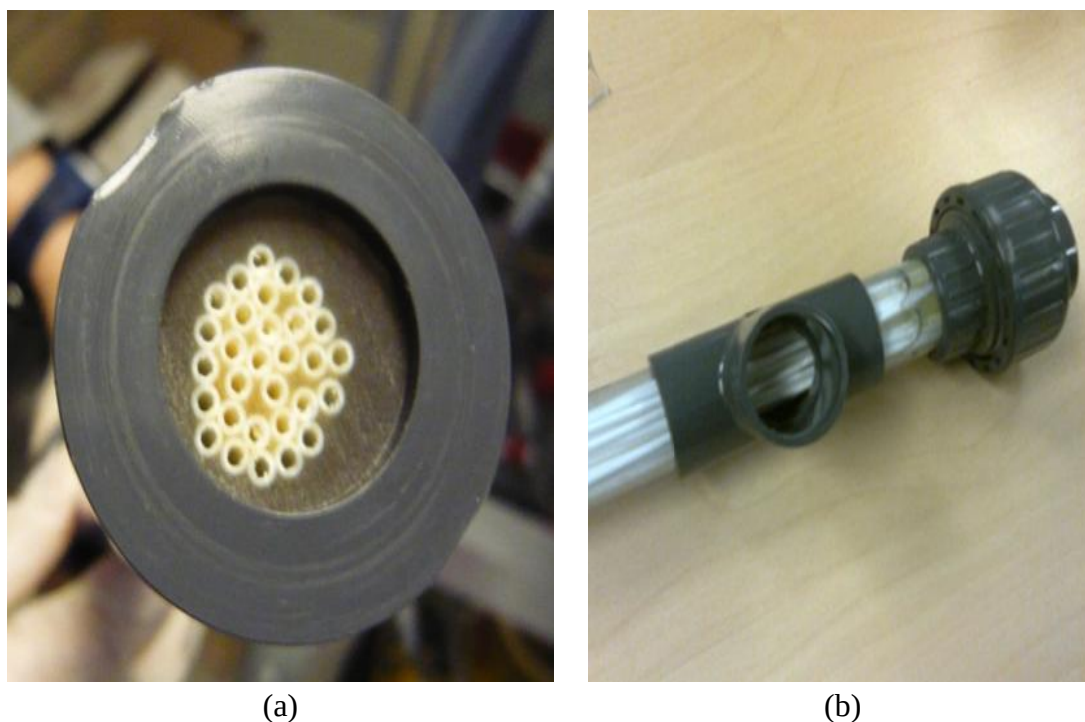


Figure 5.2 : Images of the UF membrane (a) Cross-sectional (b) Permeate outlet.

Table 5.1 : Specifications of the membrane.

Specifications	UFC M5 LE
Material	PES
Pore Size	30 nm
Structure	asymmetric/microporous
Membrane area	0.11 m ²
pH range	2-12
Chlorine exposure	250000 ppm·h
Temperature	1 – 80 °C
TMP	-300....+300 kPa

5.2.4 Experimental procedure

The experimental work was carried out over two successive periods. The reactor was operated in a continuous mode at 25 °C and 15 °C for 126 days. To isolate the impact of the temperature transients on the system performance, HRT (6 h), OLR (2 kg/m³.day) and upflow velocity (0.6 m/h) were maintained constant.

The AnMBR was operated in cycles consisting of 180 seconds of filtration, followed by 20 seconds of backwash. Backwash was applied by reversing the flow direction, keeping the flux at the same value as during filtration. The membrane flux was maintained constant at 12.3 L/m².h with a cross-flow velocity of 1 m/s.

5.2.5 Experimental methods

5.2.5.1 Analysis

COD and TSS were determined following Standard Methods (APHA, 2005). Each analysis was performed in triplicate. The samples for soluble COD measurement were filtered through a 0.45 μm filter before analysis. The turbidity was measured by HACH 2100N Turbidimeter. The PSD of the effluent was assessed by a Mastersizer 2000 (Malvern Instruments, Hydro 2000 MU) that has a detection range of 0.02-2000 μm . Laser diffraction technique was used to measure the size of the particles. For SMP analysis, a volume of 5 ml effluent was freshly sampled, filtered through a 0.45 μm filter and diluted 4 times with PBS (pH=7.2). The phenol-sulfuric acid method was used to quantify polysaccharides (Dubois et al., 1956). The concentration of protein was measured using the Bradford method (Bradford, 1976).

5.2.5.2 Critical flux determination

The critical flux was determined by applying a flux step method (Le Clech et al., 2003). The flux below which there is no flux decline and no fouling observed over time is defined as critical flux (Field et al., 1995; Ersahin et al., 2013). Critical flux determination consisted of successive filtration steps of 15 min, with flux increments of 5 $\text{L}/\text{m}^2\cdot\text{h}$. One minute of backwash was included between each cycle. In order to have similar membrane initial conditions, a cleaning protocol including physical, base and acid cleaning steps was followed every time the critical flux was determined or when new flux conditions were imposed. During each step, TMP was recorded with 30 seconds intervals. The critical flux was assumed to be exceeded when a clear discontinuity occurs in the slope of the TMP against time (Montgomery et al., 2001).

5.2.5.3 Filtration resistance

After long-term reactor operation, the fouled membranes were cleaned with a sequential cleaning procedure to calculate different fouling resistances and evaluate the characteristics of membrane fouling. Total filtration resistance (R_T) was assessed as a function of the TMP that was measured by pressure sensors located on the membrane feed, concentrate and permeate lines. R_T is calculated as the slope of the

measured TMP at each step versus the applied J, divided by the dynamic viscosity of water (η) by the equation (5.1):

$$R_T = \frac{\Delta P_T}{J} = R_{intrinsic} + R_{removable} + R_{irreversible} + R_{irrecoverable} \quad (5.1)$$

Then, the measured total membrane resistance represents the sum of various contributions including: the intrinsic resistance ($R_{intrinsic}$) of the membrane itself, the resistance due to cake layer formation that can be physically removed by flushing with water: Removable resistance ($R_{removable}$), the resistance due to pore-clogging that can be removed by chemical cleaning: Irreversible resistance ($R_{irreversible}$), and the one that cannot be easily reverted by neither physical nor chemical cleaning: Irrecoverable Resistance ($R_{irrecoverable}$).

$R_{intrinsic}$ test of the membrane was carried out by filtration of deionized water through the virgin membrane at the beginning of the experimental study. R_T determination was performed by measuring the J of deionized water using the fouled membrane. The membranes were first physically cleaned by flushing with tap water to remove the cake layer. The observed resistance, when filtering deionized water using the physically cleaned membrane, was taken as the sum of $R_{intrinsic}$ of the membrane, $R_{irreversible}$ and $R_{irrecoverable}$; by subtracting the obtained value from R_T , the $R_{removable}$ was obtained. Following that, the membranes were chemically cleaned by soaking sequentially in 200 ppm NaClO-0.4% Divos solution for 30 minutes and 1% (w/v) citric acid solution for one hour in order to calculate $R_{irrecoverable}$. $R_{irreversible}$ was obtained by subtracting the sum of $R_{intrinsic}$ of the membrane, $R_{removable}$, $R_{irrecoverable}$ from the R_T . After each step, the clean water permeability of the membranes was measured to determine the permeability recovery.

P ($L.h^{-1}.m^{-2}.bar^{-1}$) was calculated by the equation (5.2):

$$P = \frac{J[L/m^2.h]}{TMP[bar]} \quad (5.2)$$

5.2.5.4 Microbial community analysis

The microbial community structure was investigated using 454-pyrosequencing. UASB sludge samples were taken at different operational temperatures. Details

about sample preparation, DNA extraction, pyrosequencing and post analysis of pyrosequencing data can be found in Chapter 4.

5.3 Results and Discussion

5.3.1 Effluent characteristics

In order to clarify the relationship between the UASB effluent and the filtration performance of the AnMBR, the changes in the UASB effluent and AnMBR permeate characteristics, such as COD, SMP, TSS, turbidity and PSD, were evaluated at 25 and 15 °C.

Total and soluble COD concentrations in the effluent and permeate of the system during two runs are shown in Figure 5.3. A steady operational period was considered to be achieved once stable effluent COD concentrations and biogas production were obtained. Under steady operational conditions, the average COD concentration in the UASB effluent was 898 mg/L at 25 °C. The high COD concentrations in the UASB effluent can be ascribed to the membrane coupling, re-directing the reject water back to the UASB reactor, leading to a higher degree of mixing inside the UASB bulk. Upon the temperature decrease from 25 °C to 15 °C, the average effluent COD concentration increased to 1759 mg/L, possibly due to a less complete hydrolysis and a higher amount of accumulating SS. An increase in average soluble COD concentrations from 122 mg/L to 153 mg/L was also observed with the decrease in temperature. Generally, biological reactions proceed much slower at low temperatures (Lettinga et al., 2001). Furthermore, microbial communities performing the various steps of the anaerobic degradation pathway exhibit different responses to temperature changes, which can result in the accumulation of intermediary products of the anaerobic degradation as a result of unbalanced growth.

However, total COD removal efficiency, based on influent and permeate COD, was only slightly affected by the temperature change, with total COD removal efficiency higher than 89% even at 15 °C. Average total COD removal efficiency at 25 °C was 92% with an average COD concentration of 42 mg/L in the permeate. At the low temperature (15 °C), the permeate quality was slightly less (52 mg/L), with an average total COD removal efficiency of 90%. The exceptionally high COD removal efficiencies can likely be ascribed to the retention of biomass by the membrane unit.

Our results highlight the possible role of membranes in performance stability under temperature fluctuations, overall contributing to an improved permeate quality. A slight difference in the overall treatment performance of an AnMBR at lower temperatures has also been observed by Ho and Sung (2010) and it was attributed to the physical retention capacity of the membrane. Higher rejection of small particles or macromolecules by the membrane surface at 15 °C might result in the formation of cake/gel layer by internal and external membrane adhesion and interception.

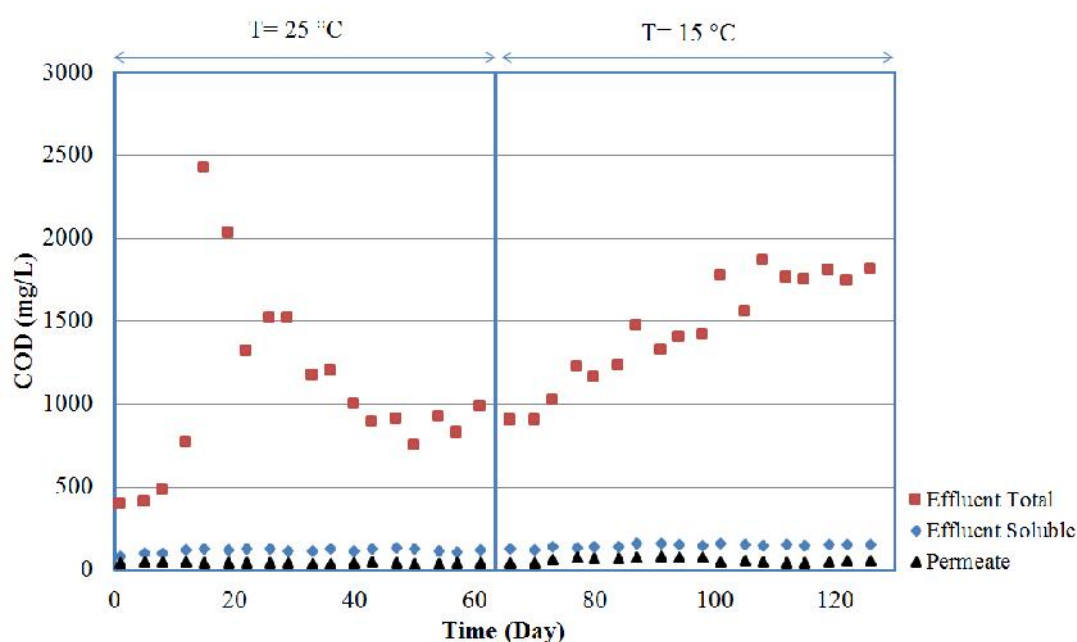


Figure 5.3 : Total and soluble COD concentrations in the UASB effluent and permeate.

Average soluble COD removal efficiencies, based on the soluble COD concentrations in the UASB effluent and permeate, were 65% and 67% at 25 °C and 15 °C, respectively. Since soluble COD entails all compounds below 0.45 μm , the observed soluble COD removal can be attributed to the nominal pore size of 0.03 μm , a further decreased porosity owing to cake layer formation, but also to possible internal and external adhesion, as well as possible degradation of organic compounds by the cake layer on the membrane surface.

In order to determine the effect of temperature on the release and washout of colloidal and suspended particles, TSS concentration and turbidity were measured in the effluent of the UASB reactor. A slight increase in UASB effluent TSS concentrations was observed from the range of 312-459 mg/L to 440-552 mg/L when

temperature was decreased from 25 °C to 15 °C. However, there was a considerable increase in UASB effluent turbidity, as a result of the temperature decrease: average turbidity in the effluent were 233 ± 27 NTU and 732 ± 46 NTU at 25 °C and 15 °C, respectively (Figure 5.4). Higher variations observed in effluent turbidity, which is contradictory to the lower variations in TSS concentration, might be attributed to the washout of colloidal particles from the UASB reactor instead of suspended particles at low temperature, as turbidity is an indicator of both suspended and/or colloidal particles.

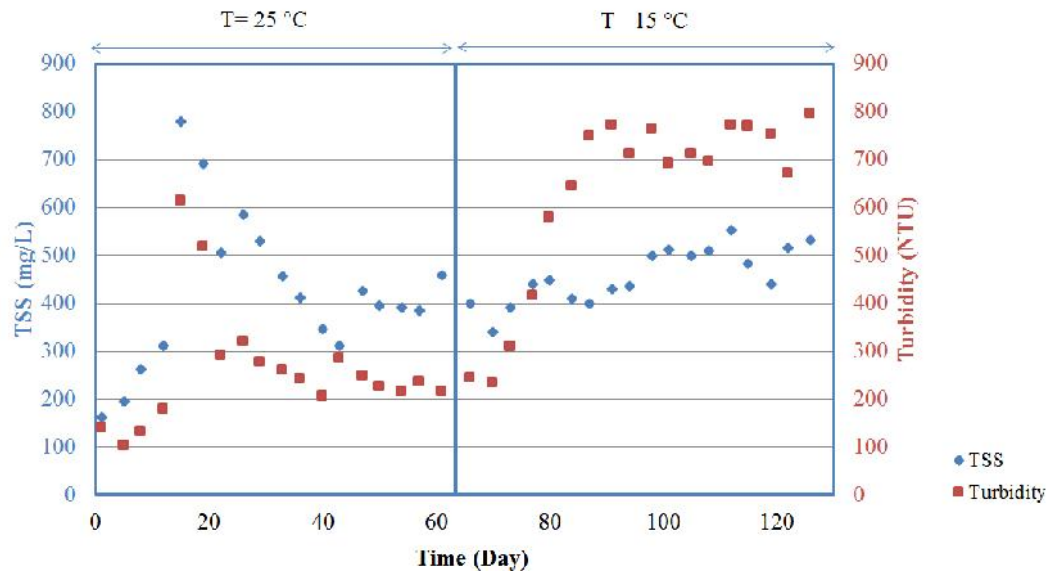


Figure 5.4 : TSS concentration and turbidity in the effluent.

In addition to the solids content, also the PSD of the UASB effluent has been measured during each operational period (Figure 5.5). Particle size is considered a crucial factor affecting particle deposition, since it can strongly determine the magnitude of back-transport mechanisms (Altmann and Ripperger, 1997; Jeison and van Lier, 2007). The range of D50 that was measured in the UASB effluent at 25 °C, was between 80 μm and 137 μm , whereas at 15 °C an apparent decrease in D50 was observed from 88 μm on Day 66 to 25 μm on Day 126. In addition, peaks shifted to the left part, i.e. smaller sizes, of the PSD curve when temperature was decreased to 15 °C. The distribution of volume percentage exhibited one-peak curves at 25 °C, which indicated that most of the particles in the UASB effluent had unimodal distribution. However, percent volume distribution at 15 °C showed two-peak curves with the emerge of a second peak curve at the left part of the distribution pattern, confirming the presence of smaller sized particles in the UASB effluent. Small

particles show a high tendency to deposit on the membrane surface induced by their low back-transport. Mahmoud et al. (2003) reported the substantial effect of temperature on the conversion of organic matter and the characteristics of the sludge bed. According to Lawler et al. (1986), large particles are broken and small particles are accumulated in the system, which results in a larger specific surface area when digestion does not work well. On the contrary, when digestion works well, particles of all sizes are reduced with a preferential removal of small particles (conversion into gaseous form) and the overall specific surface area will be reduced. Therefore, the observed change in the particle size at 15 °C might be related to the unbalanced disintegration rate of large composite influent particles into fractions of carbohydrates, proteins and lipids and the slow hydrolysis and solubilization rates of these products at low temperature. We therefore postulate that colloidal carbohydrates, proteins and lipids accumulated within the system, which contributed to the increase in carbohydrates and proteins in the effluent. In fact, the mentioned compounds are colloidal particles that reduced the D50, and increased the turbidity and suspended COD.

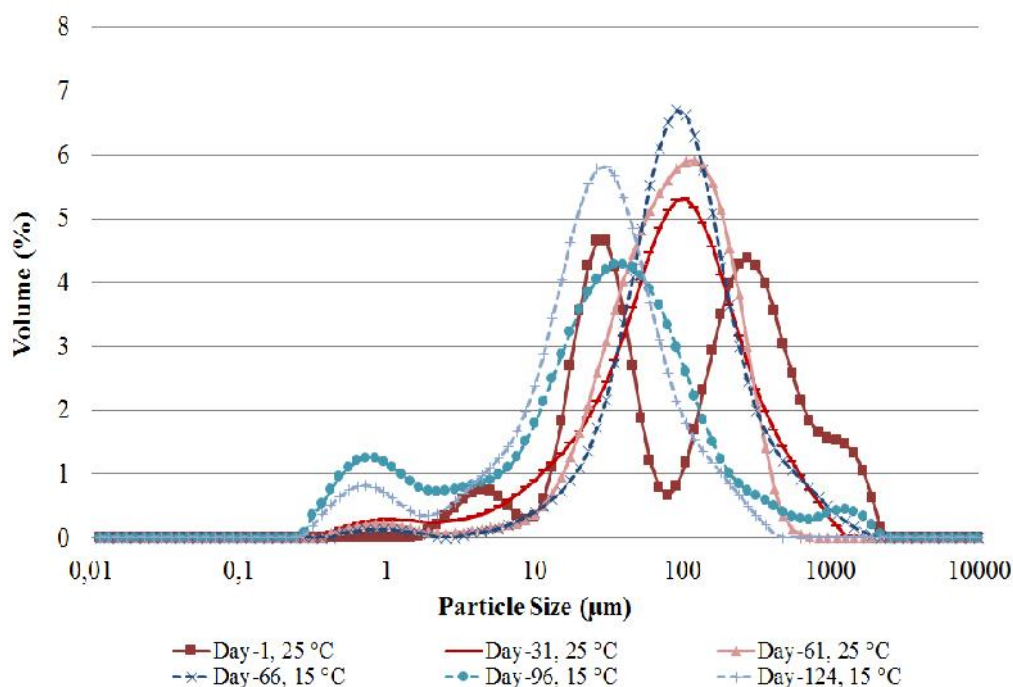


Figure 5.5 : PSD of the UASB effluent at different sampling days.

Figure 5.6 presents polysaccharide and protein concentrations of SMP in the UASB effluent and final permeate of the AnMBR system. Temperature decrease resulted in an increase in SMP concentration in the effluent of the UASB reactor, which might

be related to the decrease in SMP degradation rate due to kinetic considerations. Hence, the lower the temperature, the lower the removal rate of COD in the UASB reactor, and the higher the concentration of SMP. Furthermore, when the temperature decreased from 25 °C to 15 °C, although the release of SMP decreased due to the decrease in the rate of cell lysis and endogenous decay, the degradation rate of SMP also decreased with the temperature drop and resulted in an overall increase in SMP concentration.

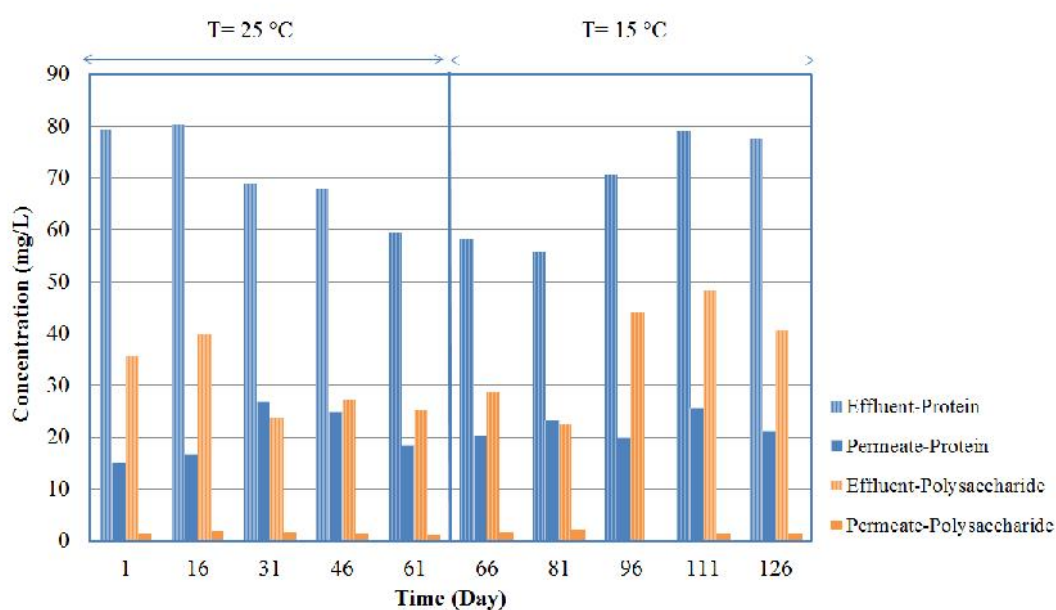


Figure 5.6 : SMP concentrations in the effluent and permeate.

Concentrations of both polysaccharide and protein were always higher in the UASB effluent than in the permeate; thus the membrane acted as a barrier for these compounds, part of which could be deposited on the membrane surface, contributing to its fouling. From Figure 5.6, it becomes clear that the membrane is more effective in retaining the polysaccharide-based SMP than the protein-based SMP. This result is consistent with the findings of Drews et al. (2006) and Salazar-Pelaez et al. (2011a).

Removal efficiency of SMP components were found to be slightly influenced by temperature. Rejection of SMP components increased from 66% to 70% for proteins and from 95% to 97% for polysaccharides with the decrease in temperature. The observed higher mass flux of SMP through the membrane at higher temperatures may be related with the increased diffusivity of the molecules at elevated temperatures enhanced by solubility increase and decrease in adhesive forces.

5.3.2 Microbial community

454-Pyrosequencing analysis revealed a slight effect of temperature on both the bacterial and archaeal communities. The communities' biodiversities were assessed based on α -diversity (Figure 5.7), in which the richness was indicated by Phylogenetic Diversity Index, Chao1 richness and OSN, while the evenness was shown by Shannon-Wiener Index. OSN and phylogenetic diversity slightly decreased when the temperature decreased from 25 °C to 15 °C, indicating that a decreased temperature may result in a decreased overall microbial diversity of an AD community (Figure 5.7). This is consistent with previous studies of Gao et al. (2011) and Lu et al. (2012).

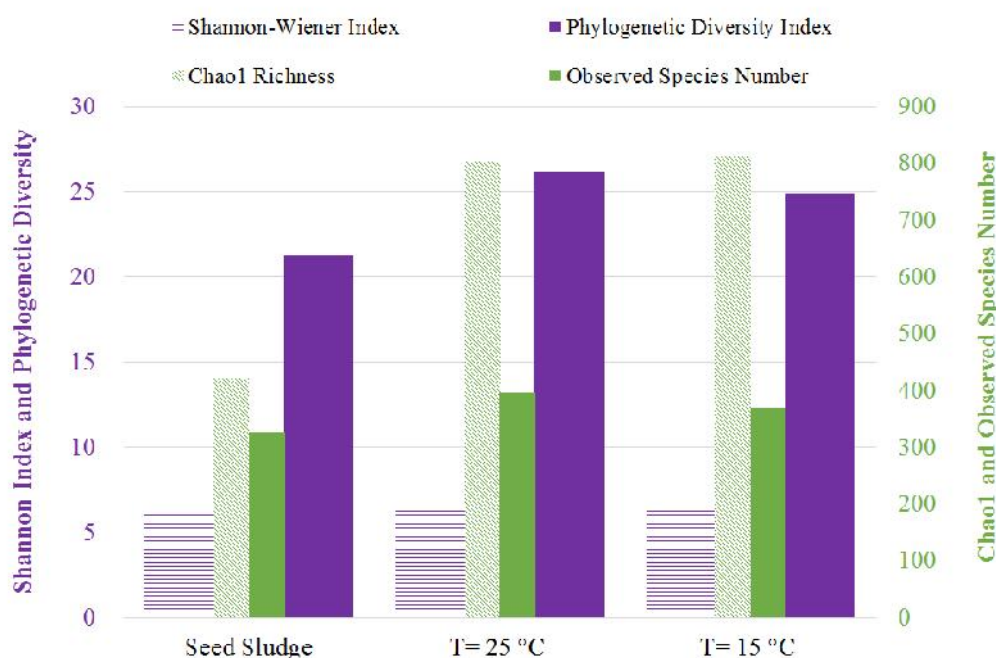


Figure 5.7 : Microbial diversity index.

The dominant bacterial phyla (Figure 5.8) and species (Figure 5.9) were apparently altered due to temperature decrease. From the results presented in Figure 5.8, it can be deduced that the phylum of *Bacteroidetes* was significantly more abundant at 25 °C than at 15 °C, showing relative abundances of 47% to 21%, respectively. In contrast, the abundance of phyla *Proteobacteria* and *Firmicutes* was about 50% and 100% higher, respectively, after the temperature drop (Figure 5.8). Although most of the microorganisms were retained in the sludge bed of the UASB and they were not directly in contact with membranes, it can be expected that bacterial spores and dispersed cells were lifted from the sludge bed and subsequently deposited on the

surface of the membrane. The pyrosequencing results showed that the *Firmicutes* strains, of which most are capable of yielding spores under stress conditions (Wunderlin et al., 2014), accounted for 50.1% in relative abundance in the top part of sludge bed at 15 °C, while such value at the same position at 25 °C was 18.2%. This suggests an upward movement of endospore-forming species to the top part of the sludge bed following the decrease in temperature, which possibly led to a higher biomass fraction in the UASB effluent. Those sub-micrometer-scale endospores are prone to washout, possibly contributing to membrane fouling.

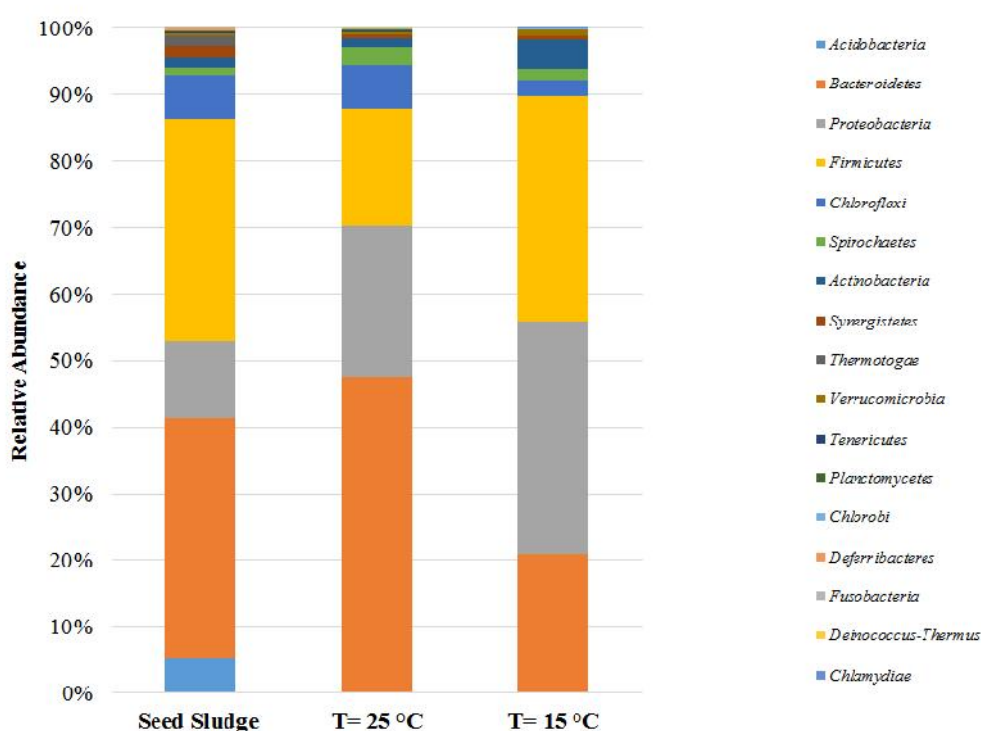


Figure 5.8 : Relative abundance of bacterial phyla.

Some mesophilic bacteria lost their pre-dominant position. For instance, the species belonging to genera *Cytophaga* decreased from 31% to 6% in abundance (Figure 5.9, Table B.1, Appendix B). These species are usually found in mesophilic (Li et al., 2011b; Kampmann et al., 2012) or thermophilic anaerobic reactors (Rademacher et al., 2012). Another commonly reported genera *Flavobacterium* decreased by more than half (from 8% to 3%) under low temperature (Figure 5.9, Table B.1, Appendix B). These genera are normally found to be dominant in mesophilic reactors (Cardinali-Rezende et al., 2009; Shin et al., 2010). Since genera *Cytophaga* were largely pre-dominant at 25 °C, the loss of dominance of this genus in the UASB reactor might promote the relative abundance of other bacteria at low temperature.

For example, the mesophilic lactic acid bacterium, *Lactococcus raffinolactis*, became dominant with abundance increased from 0.1% to 12.8%. *Lactococcus raffinolactis* is able to ferment galactosides (Boucher et al., 2003), which were available in the used synthetic wastewater made from milk powder. Compared to other lactic acid bacteria, *Lactococcus raffinolactis* is reported to grow faster when temperature ranges between 4 and 10 °C in natural environments (Hagi et al., 2004). To date, the only evidence of *Lactococcus raffinolactis* in a reactor treating wastewater was reported by (Lu et al., 2012) in a single-chamber microbial electrolysis cell for glucose fermentation at 4 °C, where 7/96 clones of *Lactococcus raffinolactis* were confirmed. Another two species, *Acinetobacter* sp. and *Dechloromonas* sp., were also found to increase in relative abundance by 4~20 fold at low temperature.

Like the bacterial community, also the archaeal community distinctly changed from the seed sludge conditions to the 25 °C and subsequent 15 °C conditions (Figure 5.10). The hydrogenotrophic methanogens, (that produce methane from formate, H₂ and CO₂) *Methanobrevibacter* sp, distinctly dropped from the seed sludge to the 25 °C reactor and almost vanished from the community when the reactor was operated at 15 °C (Figure 5.10, Table B.2, Appendix B). Apparently the prevailing conditions in the 15 °C reactor hamper the proliferation of this mesophilic archaea. Another hydrogenotrophic methanogen, *Methanobacterium petrolearium*, also lost its prevalence from 15% at 25 °C to about 3% at 15 °C (Figure 5.10, Table B.2, Appendix B). Previous studies have shown that the optimum growth temperature of *Methanobacterium petrolearium* is at 35 °C (Mori and Harayama, 2011). The observed decrease in the abundances of hydrogenotrophic methanogens at low temperature might be related to an increased competition with acetogenic hydrogen consumers at low temperatures (Nozhevnikova et al., 2001; Kotsyurbenko et al., 2001), suggesting a drop in relative importance of hydrogenotrophic methanogens with decreasing temperature. The latter contrasts to the hypothesis of Smith et al. (2012), that suggests that at low temperatures hydrogenotrophic methanogenesis may become more significant, because hydrogenotrophic methanogenesis is more exergonic at low temperature due to the increased solubility of hydrogen (Lettinga et al., 2001). Most of the hydrogen is generated during fermentation, which is limited by reduced hydrolysis and solubilization at low temperatures, leading to low hydrogen concentrations in the bulk liquid. Therefore, despite the enhanced

hydrogen solubility at low temperatures, the lower production of hydrogen might result in a substrate-limited hydrogenotrophic methanogenic population. In addition, owing to the higher hydrogen solubility, homo-acetogenesis may become more important under psychrophilic conditions, increasing the amount of COD that is channeled via acetate as precursor for methane (Nozhevnikova et al., 2001; Kotsyurbenko et al., 2001). Therefore, it is intriguing to find that *Methanosaeta sp* became even more dominant after the temperature decrease (from previously 56% to 84%). *Methanosaeta* is a typical genus of acetate-utilizing methanogens that is predominant in anaerobic reactors for methane production (Veiga et al., 1997). The role of these acetate-utilizing archaea, which utilize only acetate as the substrate for methanogenesis, has been proven to be able to out-compete other methanogens under low temperatures (Dhaked et al., 2010). For example, *Methanosaeta sp.* were found to be most numerous at low operating temperatures (5 °C-18 °C) in a fixed-bed reactor revealed by both qPCR and clone library (Zhang et al., 2012). In another study, it was found that *Methanosaeta sp.* showed an abundance of over 60% under psychrophilic temperature (15 °C) in a bench-scale AnMBR (Smith et al., 2013).

Figure 5.9 : Relative abundance of major bacterial species (bacterial species with abundance over 1%).

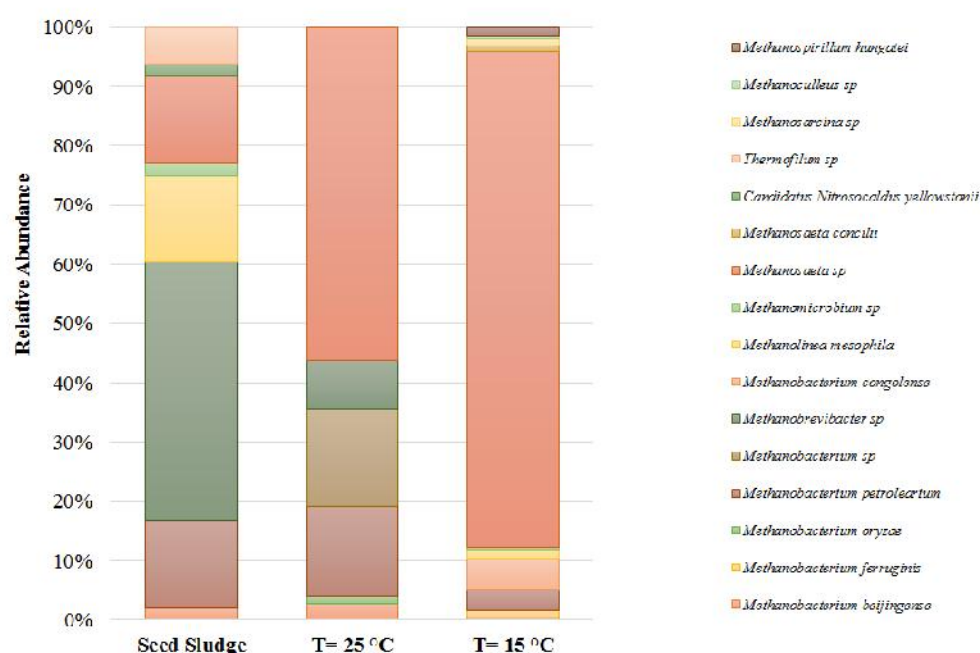


Figure 5.10 : Relative abundance of all archaeal species.

5.3.3 Critical flux tests

Flux and TMP trends obtained from the critical flux tests during the experimental study are given in Figure 5.11. For each test, a clear critical flux could be distinguished based on the sudden TMP increase at a certain flux. Following the flux-step method, the critical fluxes during operation at 25 °C were found to be 69.5, 41.3 and 53.8 L/m².h at Day 1, Day 31 and Day 61, whereas the critical fluxes decreased to 41, 38 and 34.3 L/m².h at Day 81, Day 96 and Day 126, respectively, during operation at 15 °C. The critical fluxes are much higher than most of those reported in the literature under anaerobic conditions using different reactor and membrane configurations (Gimenez et al., 2011; Wei et al., 2011; Martinez-Sosa et al., 2012; Fox and Stuckey, 2015), which likely can be attributed to the applied reactor configuration, preventing exposure of the bulk liquid to the membranes. The highest critical flux was obtained at 25 °C. The lower critical fluxes obtained at 15 °C in comparison to 25 °C, agreed with a concomitant higher turbidity, COD and SMP concentrations in the UASB effluent at 15 °C, confirming the negative impact of a low operational temperature on membrane fouling and thus reactor performance.

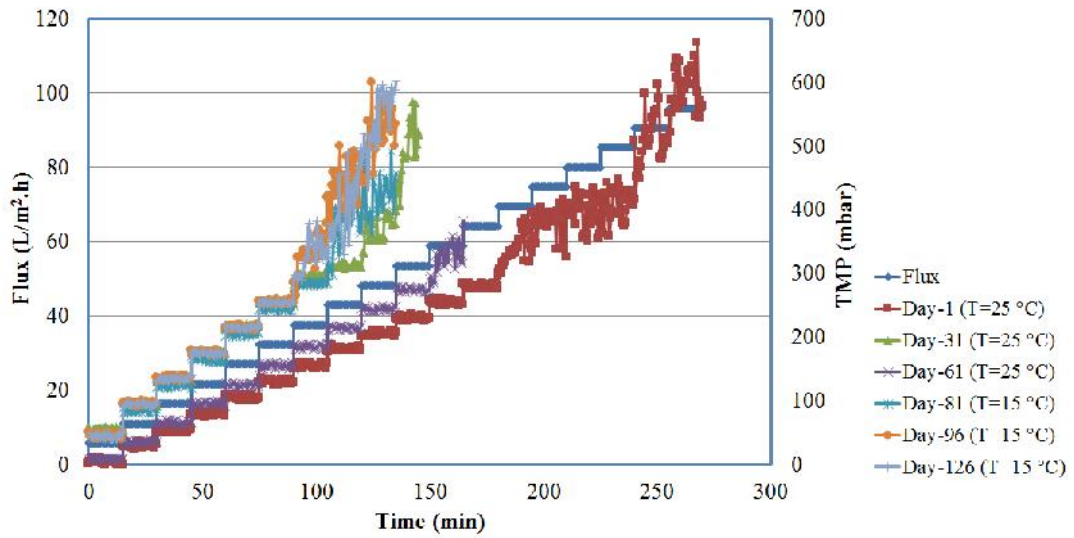


Figure 5.11 : Critical fluxes at different operating temperatures.

5.3.4 Filtration performance

The variations in TMP with operation time are shown in Figure 5.12. The results correlate the UASB effluent characteristics with the filtration performance of the entire AnMBR system. It can be observed that the TMP increased slowly with operation time, while keeping the membrane flux at about $12.3 \text{ L/m}^2\cdot\text{h}$ during the first 16 days. During each experimental period, membrane cleaning was carried out nearly every 30 days to recover the P, followed by another operation period. The sudden TMP rise observed on Day 16 resulted in a higher TMP increasing rate at the initial stage (Day 1-Day 31) in comparison to the second stage (Day 31-Day 63) of operation at 25°C . The sudden TMP increase coincides with the observed sudden and sharp increase in both UASB effluent COD and TSS concentrations. In addition, during the initial stage the UASB effluent was characterized by a wider PSD. The observed non-stable effluent characteristics during the initial stage resulted in the accumulation of particles in the lumen of the membrane unit. The small diameter membrane tubes that were selected in order to increase the filtration area were simply blocked by particles, especially by the bigger ones. In addition, particles with a lower diameter could easily accumulate in the lumen due to the reduction in nominal tube diameter. Such partial blockage would result in an uneven flux distribution across the bundle of membrane tubes. Moreover, the sudden increase in local flux at Day 16, could eventually cause more serious membrane fouling, in agreement with the observations made by Cho and Fane (2002), who also related the sudden rises in

TMP to the local flux increases. However, after stable operational conditions were achieved at 25 °C, it can be observed that the TMP increased only slowly with operation time and low TMP values were maintained. This stable filtration period is in agreement with the stable UASB effluent characteristics and selected flux that is below the critical flux at the same operation period. However, a gradual increase in the TMP was observed over time under subcritical flux conditions of 12.3 L/m².h during operation at 15 °C. Although the system was operated at fluxes below the critical flux for new or chemically-cleaned membranes, a rapid membrane fouling was observed. The relatively high critical flux values presented in Figure 5.11 can be attributed to short-term test operation. During the critical flux test, cross-flow conditions were applied for only a couple of hours. Apparently, for long-term operation periods, the predictive value of the assessed critical flux becomes insignificant, meaning that only much lower fluxes can be admitted for attaining stable membrane filtration. Nonetheless, the distinct difference between 25 °C and 15 °C suggests a decreased filtration performance at lower temperatures. Le Clech et al. (2003) concluded that the critical flux resulting from the flux-step method cannot be used to estimate the critical flux of systems operated continuously because this method is based on a short-term run. The findings of our study at 15 °C also confirmed this observation. Since TSS concentrations were very similar at both temperatures, the higher fouling rate at lower temperature was probably not associated with the TSS concentration. Particularly small particles, which were abundantly measured at the low temperature, have a high fouling propensity, likely related to the low back-transport (Martinez-Sosa et al., 2012). Presence of more SMP at low temperature could also have increased the possibility of external and internal pore clogging and biofilm growth on the membrane surface.

Our filtration performance results were obtained under laboratory-scale conditions. Although the laboratory-scale studies provide valuable information about the treatability and flux levels, the hydraulic conditions and pressure drops in a laboratory-scale membrane module can be significantly different from pilot- and full-scale membrane modules. Moreover, the applied shear rate and its effect on anaerobic biomass can significantly differ in laboratory and full-scale reactors due to the use of different pumps and valves. Therefore, laboratory-scale results should be validated with pilot- and full-scale studies since the latter ones can provide more

representative data in terms of attainable flux, TMP and fouling. To date, AnMBR application for domestic utilities in pilot- and full-scale systems has not yet become a reality, which is partly because of system novelty and reluctance owing to membrane fouling problems (Ozgun et al., 2013a). With the rapid development and implementation of AnMBR technology especially in full-scale systems, long-term reliability and operability of AnMBRs in municipal wastewater treatment need to be further investigated.

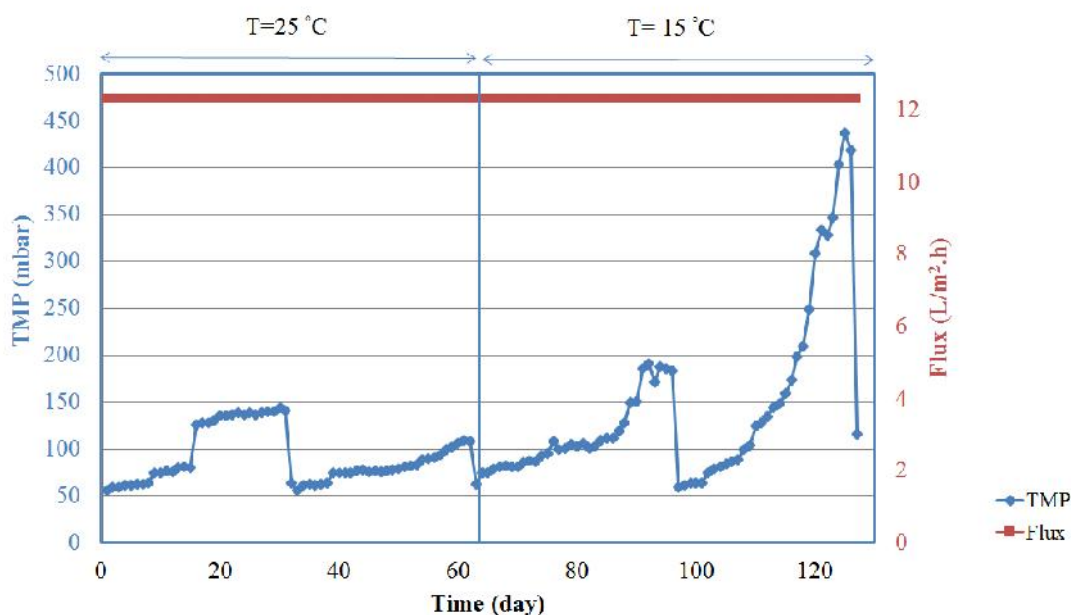


Figure 5.12 : Filtration performance of the AnMBR system.

5.3.5 Cleaning tests

In order to further control membrane fouling, applying an effective strategy to extend the stable filtration period, membrane filtration resistances were measured and summarized in Table 5.2. The results clearly showed that the filtration resistances at 25 °C were significantly lower than the ones at 15 °C when operated under similar hydrodynamic conditions. Indeed, R_T after the cleaning procedure was similar to the resistance of the virgin membrane at 25 °C. Higher contents of the foulants in UASB effluent and more fine particles tended to form a more dense and non-porous cake layer, giving rise to increased filtration resistance at 15 °C. Jeison and van Lier (2006a) and Massé et al. (2006) also reported that a reduction in the particle size resulted in a more compact cake layer structure and hence, a higher cake resistance. The latter results confirm our observations: the cake layer resulting from the material, which contains only small particles, showed a compact structure right from

the beginning of the filtration experiment. In contrast, the cake built up with bigger particles was more porous and therefore allowed a higher permeate flow. The TMP increase with time might be explained by cake consolidation, penetration of smaller particles into the porous cake layer.

For both experimental periods, $R_{\text{intrinsic}}$ of the membrane and the resistance caused by pore-clogging were found smaller compared to the cake layer resistance. Cake layer resistance accounted for most of the R_T , representing 51% and 43% of the R_T at 25 °C and 15 °C, respectively, which is consistent with the results obtained by Chu et al. (2005) (Figure 5.13). By far most of the sludge was retained in the sludge bed of the UASB module. However, some dispersed solids in the reactor apparently reached the membrane module and adhered to the membrane surface. It was clear that a foulant layer had built up on the surface, while the fouling of the membrane pores was insignificant. Irreversible fouling was less significant since it only represented around 20% and 35% of the total resistance at 25 °C and 15 °C, indicating the feasibility and importance of the fouling control by minimizing the cake resistance in AnMBRs. However, the importance of $R_{\text{irreversible}}$ increased at low temperature. As shown in Figure 5.13, an increase was observed in the contribution of the irreversible fouling resistance to the R_T of the system. The increase in irreversible fouling indicated more pore blocking, which is a drawback of extending the membrane operation period. Possibly, an increase in SMP resulted in the blocking of the membrane pores.

Table 5.2 : Total filtration resistances.

Procedure	Parameter	Unit	T=25 °C		T=15 °C	
			Day-31	Day-61	Day-96	Day-126
Virgin membrane	Permeability	L/(m ² .h.bar)	723	751	779	679
	Resistance	10 ¹² m ⁻¹	0.55	0.53	0.48	0.56
Before cleaning	Permeability	L/(m ² .h.bar)	187	214	119	100
	Resistance	10 ¹² m ⁻¹	2.02	1.80	3.16	3.69
After physical cleaning	Permeability	L/(m ² .h.bar)	385	434	194	184
	Resistance	10 ¹² m ⁻¹	0.98	0.89	1.9	2
After NaOCl-Divos solution cleaning	Permeability	L/(m ² .h.bar)	546	550	339	264
	Resistance	10 ¹² m ⁻¹	0.69	0.7	1.11	1.4
After citric acid cleaning	Permeability	L/(m ² .h.bar)	659	721	640	395
	Resistance	10 ¹² m ⁻¹	0.57	0.54	0.59	0.93

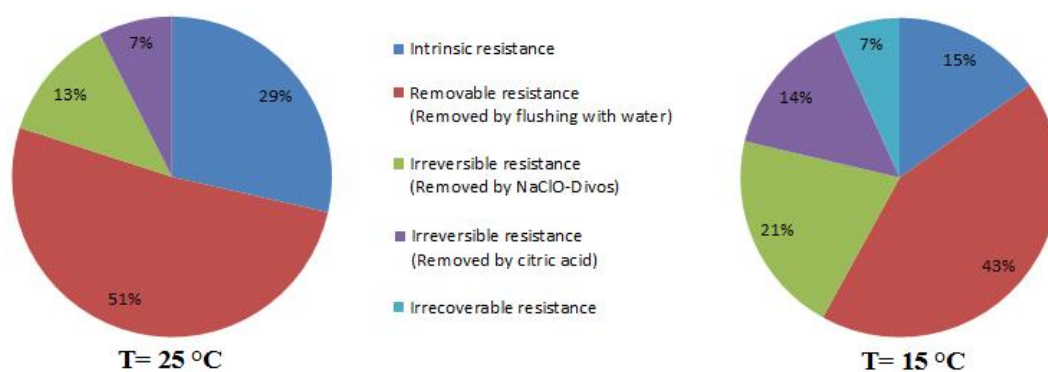


Figure 5.13 : Contribution of different resistances to R_{Ts} at the two operational temperatures.

5.4 Conclusion

Temperature affected the filtration performance of the membrane coupled UASB reactor. Decreasing the temperature from 25 °C to 15 °C greatly influenced the characteristics of the UASB effluent, resulting in an increased turbidity, COD and SMP concentrations and a decreased D50 as a result of colloids accumulation at lower temperatures. As a result, the R_T increased significantly. The overall microbial diversity also changed throughout the experimental periods, possibly invoked by the drop in temperature. The applicable flux in the AnMBR at 15 °C was mainly limited by higher SMP production and the decrease in D50. However, the overall treatment performance of the AnMBR was not affected significantly, because the physical removal by the membrane compensated for the decreased biological removal rate at 15 °C. The higher load of SMP and submicron particles to the membrane possibly led to the formation of a compact gel layer on the membrane surface at the low temperature. The results revealed more severe fouling at 15 °C in comparison to 25 °C, with cake layer representing the main fouling resistance during both experimental periods. Results of critical flux tests also coincide with the R_T tests of the AnMBR. However, severe fouling at 15 °C despite the operation at sub-critical flux conditions confirmed that critical flux is not a useful indicator to determine the long-term filterability in the UASB–UF coupled AnMBR setup. Based on the observed results, although the overall treatment performance is high, membrane coupled UASB reactors are presently not yet considered a technically feasible alternative in the treatment of municipal wastewater at 15 °C in terms of filtration performance. Therefore, further studies should be done and other configurations,

including a UASB reactor combined with an additional sludge digester, need to be evaluated in order to reduce non-stabilized particulate matter accumulation in the sludge bed and mitigate rapid membrane fouling for the low temperature applications of membrane coupled UASB reactors.

6. COMPARATIVE EVALUATION OF THE SLUDGE CHARACTERISTICS ALONG THE HEIGHT OF UPFLOW ANAEROBIC SLUDGE BLANKET COUPLED ULTRAFILTRATION SYSTEMS⁽⁵⁾

6.1 Introduction

Membrane coupled UASB reactors have been considered as an alternative of interest for the treatment of municipal wastewater in AnMBR systems (Gao et al., 2010; Liu et al., 2012; Ozgun et al., 2013a; Ozgun et al., 2015a). UASB reactors can be used upfront as biofilters before membrane treatment, which prevents the membrane from excessive exposure to high SS concentration since the sludge bed would entrap most of the particulate matter by adsorption and biodegradation (Kataoka et al., 1992; An et al., 2009a; Wu et al., 2009; Zhang et al., 2011; Ozgun et al., 2013b). Enhanced interception of solids in the sludge bed limits the solids and colloidal load to the membrane and thus, membrane flux may become less dependent on the reactor MLSS concentration, leading to high membrane fluxes (van Voorthuizen et al., 2008; Ozgun et al., 2013a; Martin Garcia et al., 2013).

Results of the study in Chapter 4 elucidated the potentials of membrane coupled UASB reactors for the treatment of municipal wastewater under mesophilic conditions. However, under sub-mesophilic conditions, hydrolysis of the retained particulates likely becomes the rate-limiting step and particulate matter accumulation in the sludge bed will occur leading to activity loss (Lettinga et al., 2001; Ozgun et al., 2015b). Low temperature based limitations in the growth of methanogens will lead to further sludge bed deterioration resulting in a poor soluble COD removal, which will lead to more severe pore clogging problems. The latter was confirmed

⁽⁵⁾ This chapter is based on:

Ozgun, H., Ersahin, M.E., Zhou, Z, Tao, Y., Spanjers, H., van Lier, J.B. Comparative evaluation of the sludge characteristics along the height of upflow anaerobic sludge blanket coupled ultrafiltration systems. *under review*.

with the results of the study in Chapter 5. Therefore, for municipal wastewater treatment at low and/or fluctuating temperatures, membrane integration to a UASB-Digester system (Mahmoud et al., 2004) can be an attractive option in order to overcome the hydrolysis limitation induced by low temperature and to reduce non-stabilised particulate matter accumulation in the sludge bed.

In the UASB-Digester system, the non-degraded particulate COD entrapped in the sludge bed of the UASB reactor is further stabilized in a separate CSTR type digester operated under optimal mesophilic conditions (30-35 °C) (Mahmoud et al., 2004). By recirculating the digested solids to the UASB reactor, the sludge bed SMA is increased resulting in enhanced removal of soluble organics. System performance is affected by several factors related to the sludge recirculation such as sludge characteristics, recirculation rate and sludge volume. Therefore, careful optimization of the sludge recirculation in UASB-Digester systems is required in order to maintain complete conversion of biodegradable dissolved COD.

Sludge characteristics generally change over the height of UASB reactors. Variation of sludge concentration along the UASB reactor depends on several factors such as gas production or COD load per unit area; upflow velocity, HRT and sludge settling characteristics (Narnoli and Mehrotra, 1997). As a consequence, a number of studies and research activities dealing with the assessment of the change in sludge characteristics over the height of UASB reactors due to applied upflow velocity and/or entrapped or rising gas bubbles have been carried out on bench, pilot and full-scale UASB installations (Agrawal et al., 1997; Uemura and Harada, 2000; Sponza, 2001; Mahmoud et al., 2004; Mahadevaswamy et al. 2004; Elmitwalli and Otterpohl, 2007; Vadlani and Ramachandran, 2008). Agrawal et al. (1997) investigated the SS and VSS concentrations along the reactor height and maximum SS concentration in the sludge bed was found at 30 to 70 cm height from the bottom of the reactor having a height of 180 cm. SS concentration was lower at the bottom of the reactor due to the floating mode of sludge related with the occlusion of gas in the bed, especially during the winter time. Operating a bench-scale UASB reactor treating raw sewage at relatively low temperatures, Mahmoud et al. (2004) reported a declining trend in TS and VS values with the increase in sludge bed height and observed a clear stratification around 40% height. Reactor scale effects might have played a role in the obtained results. However, VS/TS ratio was almost constant through the sludge

bed height indicating the equal stabilization of sludge along the bed height. Uemura and Harada (2000), operating a UASB treating sewage, observed an increase in soluble COD at the bottom levels of the sludge bed due to the liquefaction of entrapped solid organics. However, the values decreased afterwards due to the further progress of methanization.

All these studies about the sludge characteristics were investigated on solely UASB reactors. However, following the results of the study in Chapter 4, significant changes were observed in the sludge bed due to elimination of hydraulic shear as bacterial selection pressure factor when membranes are coupled to UASB reactors. Membrane incorporation induced an accumulation of fine particles and a decrease in EPS concentration in the sludge, both partly responsible for decreasing the PSD and thus, affecting sludge settleability. Therefore, a significant change in sludge stratification along the UASB reactor height is expected when membrane units are coupled to the effluent. However, so far, there is no information in the literature available concerning this possible impact.

The main aim of this study is to identify the best location in the sludge bed, i.e. agreeing with sludge having the lowest stability and/or highest concentration, for conveying the sludge from the UASB reactor to the digester. Within this concept, the sludge profile was investigated by collecting sludge samples along the different heights of the UASB reactor in an AnMBR treating municipal wastewater. The sludge characteristics were comparatively evaluated in terms of solids content, PSD, sludge morphology, SMA and stability. Besides, pyrosequencing was carried out for the samples from each location in order to compare the microbial community composition including both archaeal and bacterial communities and the relative abundance of microbial species.

6.2 Materials and Methods

6.2.1 Wastewater source

Synthetic municipal wastewater was used as feed, having the same composition as the one described in Chapter 3. The composition of the concentrated substrate solution and the characterization of the synthetic municipal wastewater at the UASB inlet are presented in Chapter 3 in Table 3.1 and Table 3.2, respectively.

6.2.2 Seed sludge

The reactor was seeded with flocculent anaerobic sludge obtained from a pilot-scale UASB reactor treating black water (Sneek, the Netherlands). The characteristics of the seed sludge are presented in Table 3.3 in Chapter 3.

6.2.3 Experimental setup

The sludge samples were derived from a laboratory-scale AnMBR consisting of a UASB reactor with an effective volume of 7 L coupled to an external membrane module. A schematic diagram of the experimental setup is illustrated in Figure 5.1 of Chapter 5.

Peristaltic pumps (Watson Marlow 120U/DV) were used for circulating the flow between the UASB reactor and the membrane module, and obtaining permeate through the membrane. Furthermore, recirculation over the membrane surface was performed by a peristaltic pump (Watson Marlow 620UN/R) in order to maintain a cross-flow velocity of 1 m/s across the membrane surface independently of the circulation flow between the UASB reactor and the membrane module. This concentrate circulation flow-rate was adjusted enabling a stable upflow velocity in the UASB reactor. The biogas was collected by means of a three-phase separator installed at the top part of the UASB reactor. Biogas flow rate was measured with a gas meter (Ritter, Milligas Counter MGC-1 PMMA). Temperature was controlled by means of a water bath (Tamson Instruments, the Netherlands). Temperature and pH inside the bioreactor were monitored on-line by a probe combined with a transmitter (Elscolab, M300 ISM). A computer accessible via LabView software was used to control the pumps and collect the data.

The external cross-flow tubular membrane module contained 28 membrane fibers with an internal diameter of 1.5 mm and a length of 80 cm. Each membrane fiber had 0.0038 m² of total membrane surface area. Pentair X-Flow (Enschede, the Netherlands) PES UF membranes with a pore size of 30 nm were used in the AnMBR. The cross-sectional image and the detailed characteristics of the membrane are presented in Figure 5.2 and Table 5.1 of Chapter 5. Pressure sensors (AE Sensors, ATM -800/+600 mbar, the Netherlands) were installed in the membrane feed, concentrate and permeate lines in order to measure the TMP.

6.2.4 Experimental procedure

Sludge samples were taken from different heights of the UASB reactor on Day 47 and on Day 63 after an operational period of 63 days, in order to study the degree of sludge stratification over the reactor height. Four sampling ports were selected for sludge sampling; the first one at 9 cm above the base of the UASB and the others at 16 cm to 24 cm intervals along the height of the reactor (Figure 6.1). Sludge samples are denoted as S1, S2, S3 and S4, respectively.

The AnMBR system was operated in a continuous mode at an average HRT of about 6 h and an average OLR of 2 kg COD/m³.day. The upflow velocity of the UASB reactor was set at 0.6 m/h based on results discussed in Chapter 3 and kept constant during the whole experimental period. The average pH and temperature of the system remained stable around 6.9 and 15 °C, respectively. Membrane operation consisted of alternating between 3 minutes filtration and 20 seconds backwash, at a membrane flux of 12.3 L/m².h. The cross-flow velocity of the membrane was 1 m/s.

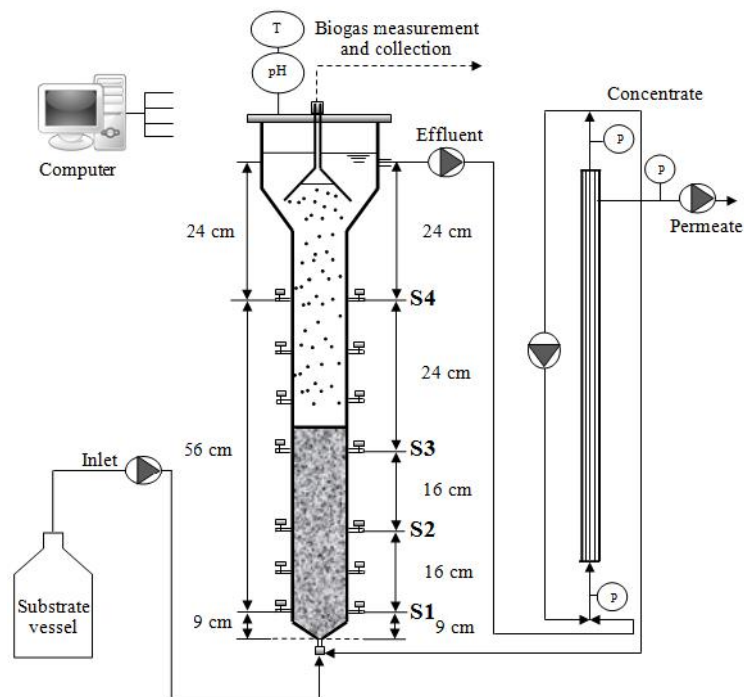


Figure 6.1 : Schematic overview of the membrane coupled-UASB AnMBR and the sludge sampling ports S1-S4 over the height of the UASB reactor.

6.2.5 Experimental methods

COD, TN, TSS, VSS, TS and VS were measured following Standard Methods (APHA, 2005). Each analysis was performed in triplicate. The samples for soluble COD measurement were filtered through a 0.45 µm filter.

The PSD of the sludge samples was assessed by using direct image analysis. Image analysis of particles with a size range of 50~5000 μm was obtained by fluorescence stereo microscope (Leica M205 FA, Germany) with QWin image analyzing software (QG2-32, version V 3.5.1). For assessing the SMP content of sludge samples, a volume of 5 ml sludge was washed by PBS (pH=7.2) and centrifuged at 7000g for 7 minutes at 4 °C. The supernatant was filtered using a 0.45 μm filter and the filtrate was collected for SMP analysis. The pellet was re-suspended and vigorously washed with 10 ml PBS and then ultrasonicated at 40 kHz (Cole-Parmer Ultrasonic, the Netherlands) for 3 minutes. A high speed centrifuge (17000g) was applied for 20 minutes under 4 °C and the supernatant obtained was filtered using a 0.45 μm filter for EPS assessment. The phenol-sulfuric acid method was used to quantify polysaccharides (Dubois et al., 1956). The concentration of protein was determined using the Bradford method (Bradford, 1976).

SMA of the sludge samples was determined by using AMPTSII (Bioprocess Control, Sweden) (Li et al., 2011a). Stability of sludge samples was tested by obtaining the specific ultimate methane production. Detailed explanations about SMA and stability tests can be found in Chapter 4.

Microbial community structure was investigated using 454-pyrosequencing. Details about sample preparation, DNA extraction, pyrosequencing and post analysis of pyrosequencing data can be found in Chapter 4.

6.3 Results and Discussion

Figure 6.2 present the profiles of sludge characteristics along the height of UASB reactor in terms of TS and VS. VS/TS increased from 0.70 ± 0.01 for S1 to 0.88 ± 0.01 for S3, followed by a slight decrease to 0.87 ± 0.02 for S4. The relatively low VS/TS ratio observed at the bottom of the UASB reactor might be attributed to an inorganic sedimentation layer being present there. The maximum concentration of TS in the sludge bed was found to be 41 g/L at the bottom of the reactor, whereas a decrease was observed at the higher part of the reactor, which is consistent with the results of Mahmoud et al. (2004) treating sewage in a UASB and a UASB-Digester system. The TS concentration decreased to 8 g/L in S4. The VS-TS as well as VSS-TSS showed a declining trend in concentration from bottom to top of the UASB with clear stratification at about 46% height of the bottom of the reactor. Highest TSS and

VSS concentrations were obtained at the bottom part of UASB reactor followed by a decrease with height. Besides, a significant increase in the VSS/TSS ratio from 0.73 ± 0.02 for S1 to 0.97 ± 0.004 for S3 was observed, followed by a slight decrease to 0.93 ± 0.01 for S4. The total COD concentrations decreased from 46 g/L for S1 to 9 g/L for S4, in agreement with the TS(S) and VS(S) concentrations.

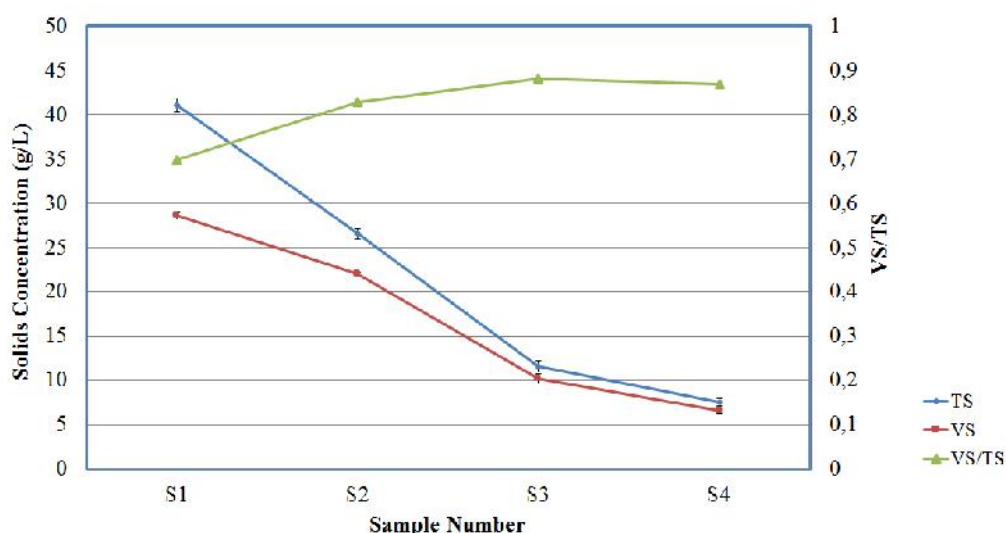


Figure 6.2 : TS and VS concentrations and VS/TS along the height of the membrane coupled UASB reactor.

The number and volume based PSD of each sludge sample are depicted in Figure 6.3(a) and Figure 6.3(b), respectively. A different size distribution was obtained for each of the sludge samples from different heights. Figure 6.3(b) shows that, 85% to 90% of the total particle volume for S1 are bigger than 2 mm. The smaller particles with diameters below 100 μm represented only 1% of the total volume of particles for S1, but made up a large fraction of the total number in the sludge. An increase in the height led to a shift in the size distribution, with an increase in the volume of smaller sized particles. Fraction of smaller particles with diameters below 100 μm increased to 24% of the total volume of particles for S4 and particles bigger than 1.6 mm are not present for S4. Overall, the general trend in size distributions was very similar in both types of PSD graphs with the majority of bigger particles contributing to 85-90% of the total volume at lower levels in comparison to upper levels. The results of PSD analysis along the UASB height are in agreement with the results of Sponza (2001), which also confirmed the decrease in particle size in the upper part of the UASB reactor.

Morphological changes of the sludge were also visualized along the height of membrane coupled UASB reactor (Figure 6.4). More circular flocs with smoother surface and a larger diameter were observed at the lower levels, whereas smaller flocs with rough surface existed at the upper levels, which supported the PSD results.

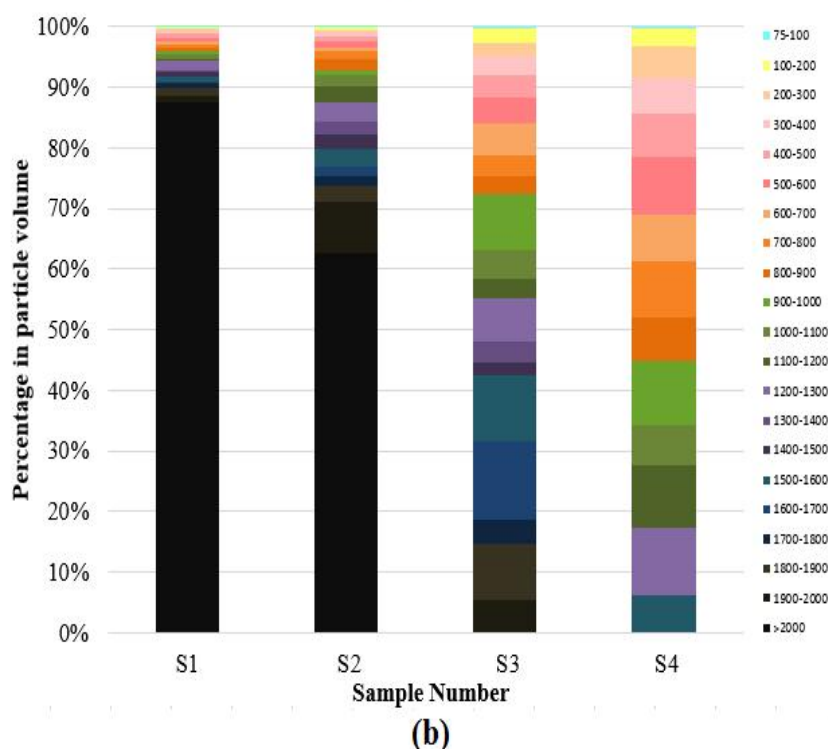
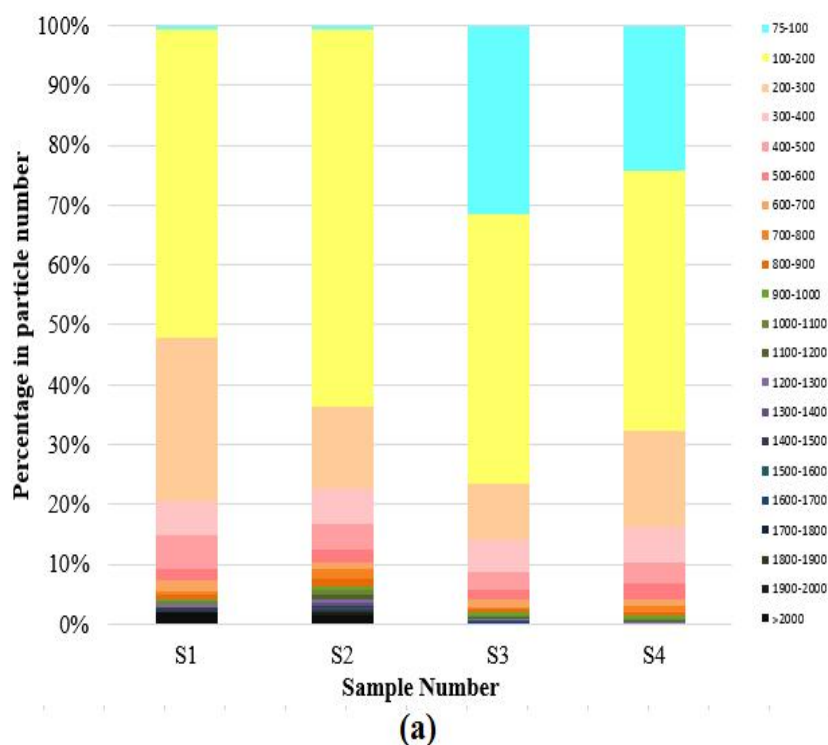
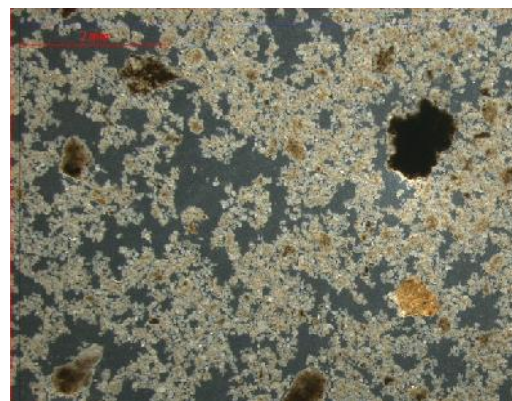
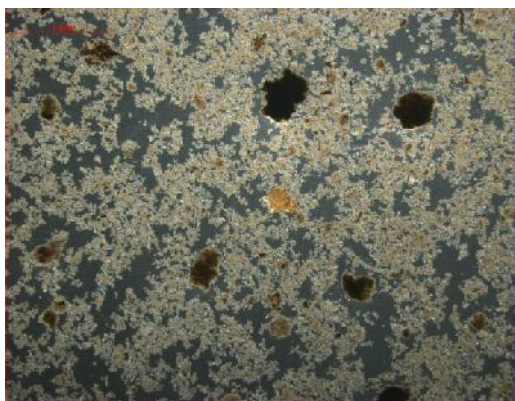
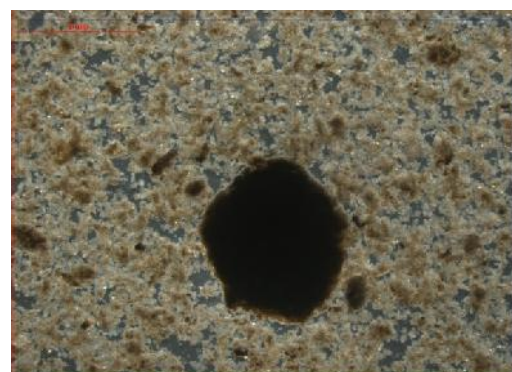
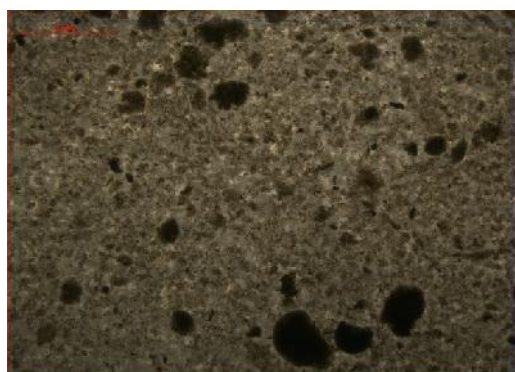


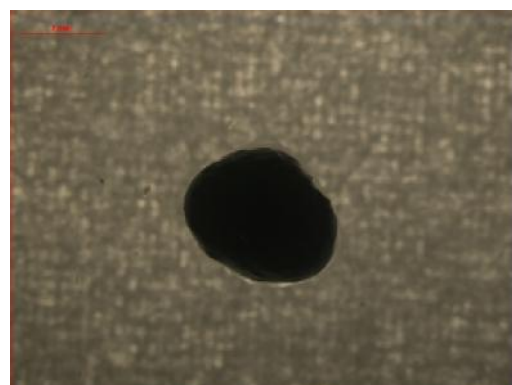
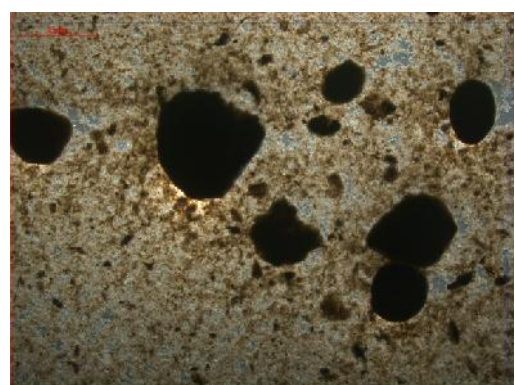
Figure 6.3 : PSD of the sludge samples in terms of (a) particle number and (b) particle volume along the membrane coupled UASB reactor.



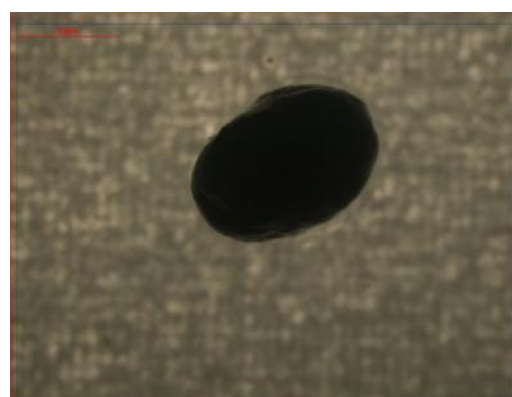
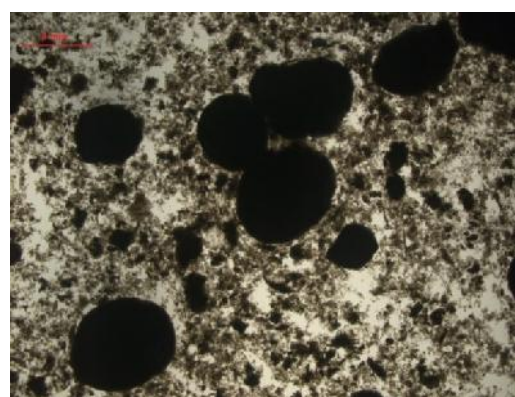
S4



S3



S2



S1

Figure 6.4 : Morphological changes of sludge samples along the UASB reactor.

SMP and EPS concentrations of the sludge samples along the UASB reactor height are presented in Table 6.1. These samples contained cells, cell flocs bound by EPS, SMP, and un-degraded or partially degraded particulate matter. The protein contents of SMP and EPS were found higher than their polysaccharide contents for all of the sludge samples. Higher protein content may be attributed to the presence of exo-enzymes in the sludge flocs and cell lysis compounds (Neyens et al., 2004). An increasing trend was observed for SMP concentration with the increase in reactor height. Drews et al. (2006) indicated that a drop in SMP content can occur due to biodegradation in a MBR. Therefore, SMP increase at higher levels might result from the lower degradation rates observed with the increase in height. Protein and polysaccharide content in the SMP were 1.89 mg/g VSS and 1.01 mg/g VSS for S1, which increased to 3.30 mg/g VSS and 2.62 mg/g VSS for S4, respectively. However, a decreasing trend was observed for EPS compositions, with the highest concentrations at the down part of the sludge bed, coinciding with the dominance of large particles (Figure 6.3).

Table 6.1 : SMP and EPS contents of the sludge samples along the membrane coupled UASB reactor height (Average±Standard Deviation).

Time	SMP		EPS	
	Protein (mg/g VSS)	Polysaccharide (mg/g VSS)	Protein (mg/g VSS)	Polysaccharide (mg/g VSS)
S1	1.89±0.05	1.01±0.09	3.79±0.10	2.10±0.14
S2	2.10±0.03	1.38±0.04	2.48±0.03	0.90±0.05
S3	3.28±0.19	1.52±0.25	2.07±0.07	0.80±0.11
S4	3.30±0.08	2.62±0.11	1.77±0.08	0.80±0.13

Figure 6.5 shows the SMA and stability along different heights of the UASB reactor. SMA values of S1 and S2 are higher than those of S3 and S4, which indicates that the biomass in the lower levels is more active than that in the upper level. Highest SMA value of 0.64 g COD/g VSS.d was observed for the sludge collected from the bottom. Following that, SMA decreased gradually to 0.44 g COD/g VSS.d for S2 and then remained constant thereafter for S3 and S4. Large particle sizes may reduce the SMA due to enhanced mass transfer limitations (Bhunja and Ghangrekar, 2007). However, SMA values were found to be higher at the low part of the sludge bed that was characterised by a large particle size. The highest value at 9 cm may be ascribed to the contribution due to the effect of properly controlled wastewater flow, growth

of hydrolytic microorganisms capable of decomposing the solid organics accumulated in the reactor bottom part and population shift. Stability tests were also performed in order to determine whether the sludge was equally stabilized over the bed or not. According to the results, the sludge stability decreased from 0.53 g CH₄-COD/g COD for S1 to 0.64 g CH₄-COD/g COD for S2. Following that decrease, an increase in stability was observed for S3 and S4. So according to the sludge stability tests, S2 contains more biodegradable solids accumulated in the sludge bed, which makes it favourable as an extraction point of sludge for the coupled digester.

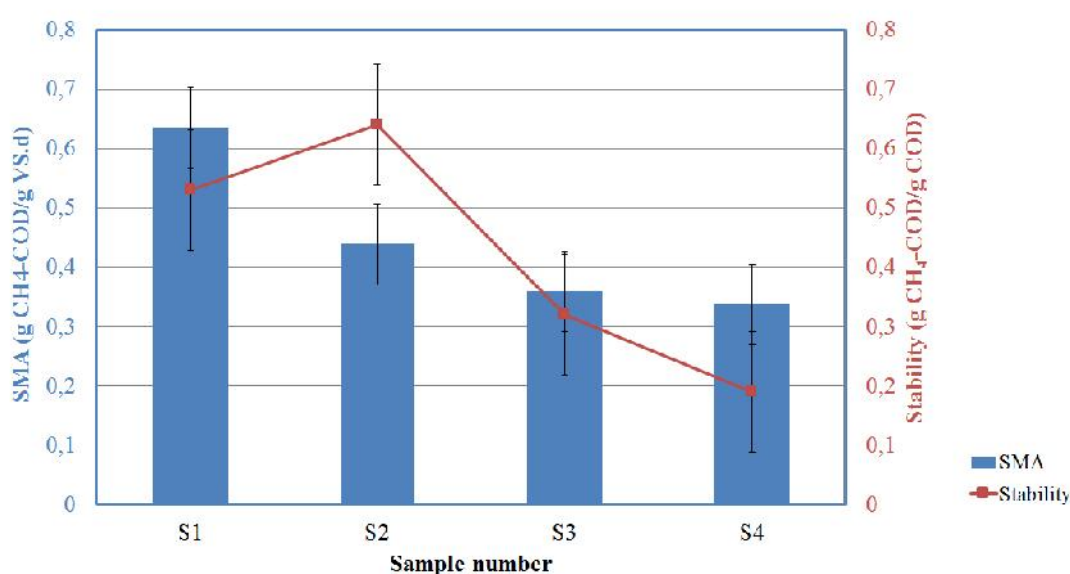


Figure 6.5 : SMA and stability of sludge samples along the height of the membrane coupled UASB reactor.

16S rDNA was extracted from sludge taken from the four sampling points. A total of 10078 sequences were retrieved by 454-tag Pyrosequencing. The clone libraries showed marked differences in microbial community composition at different reactor heights. Remarkable differences in microbial community structure were found between S1-S3 and S4, while such differences between S1, S2 and S3 were small. Details of bacterial and archaeal abundance can be seen in Appendix C.

The peaks of alpha-diversity index (Shannon, Chao1 and OSN) appeared for S2 (Figure 6.6), indicating the most diverse microbial community at the second bottom sampling port of the UASB. The index of S1 and S3 were slightly lower than S2. The alpha-diversity index of S4 was lowest compared to the other three sludge samples, and decreased by 42% (Chao1), 38% (OSN) and 20% (Shannon) compared to the

corresponding maximum. This means that the UASB biomass from the top part of the sludge bed had a much less diversity than the middle and bottom biomass. Figure 6.7 presents the Lorenz distribution curves based on the abundance of each operational taxonomic unit (OTU) that originated from pyrosequencing of 16S rDNA of the communities of different heights of UASB. The 45 degree diagonal is the theoretical perfect evenness line representing an absolutely even microbial community. A curve further away from the diagonal has a lower evenness or higher dominance since evenness quantifies how relative abundance is distributed among species (Wilsey and Stirling, 2007). The Lorenz distribution curves showed that the biomass community of S4 was less even than the other three positions, meaning that several species greatly dominated the upper part of UASB microbial community. The biomass community of S4, dominated by less species, can be considered less diverse in comparison to the ones in which several different species have a similar abundance.

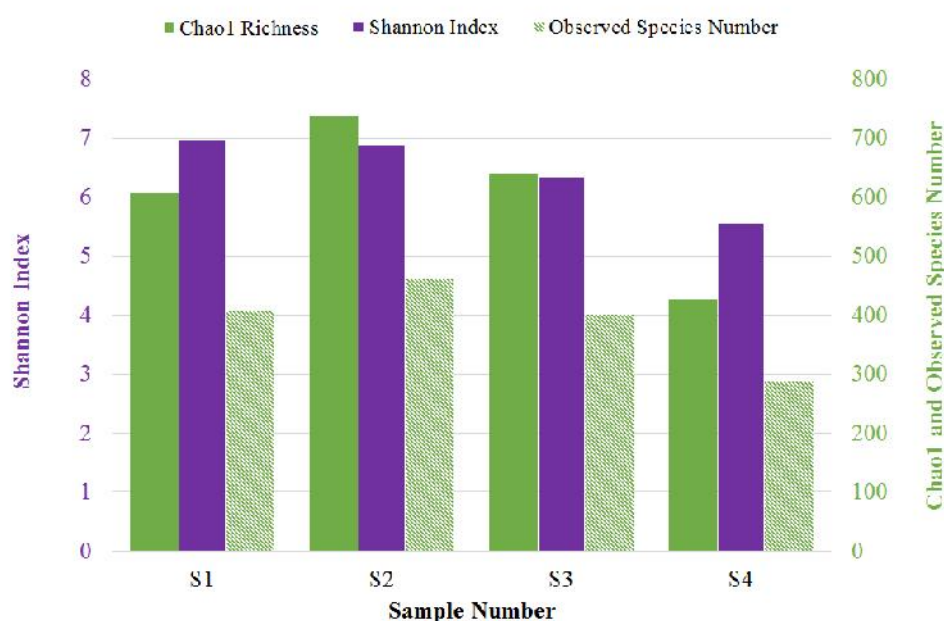


Figure 6.6 : Alpha-diversity index of each sludge sample along the UASB reactor.

The abundances of Bacteria and Archaea were quantified along the height of the reactor. Remarkable increasing/decreasing trends of several bacterial and archaeal species were discovered along the height of UASB from bottom to top. However, the compositions of the microbial community for S1, S2 and S3 were markedly different from that of S4. For example, the number of *Cytophaga* clones decreased dramatically from 19.9% for S1 to 2% for S4. The most abundant *Clostridium*-

related OTU obtained for S1, S2 and S3 was also observed for S4, but the number of clones was significantly less. Instead, OTUs similar to *Ferrimonas marina* (28%), as well as *Novispirillum itersonii* (14.3%) and *Cloacibacterium normanense* (6.4%) appeared to be dominant for S4 (Figure 6.8, Table C.1, Appendix C).

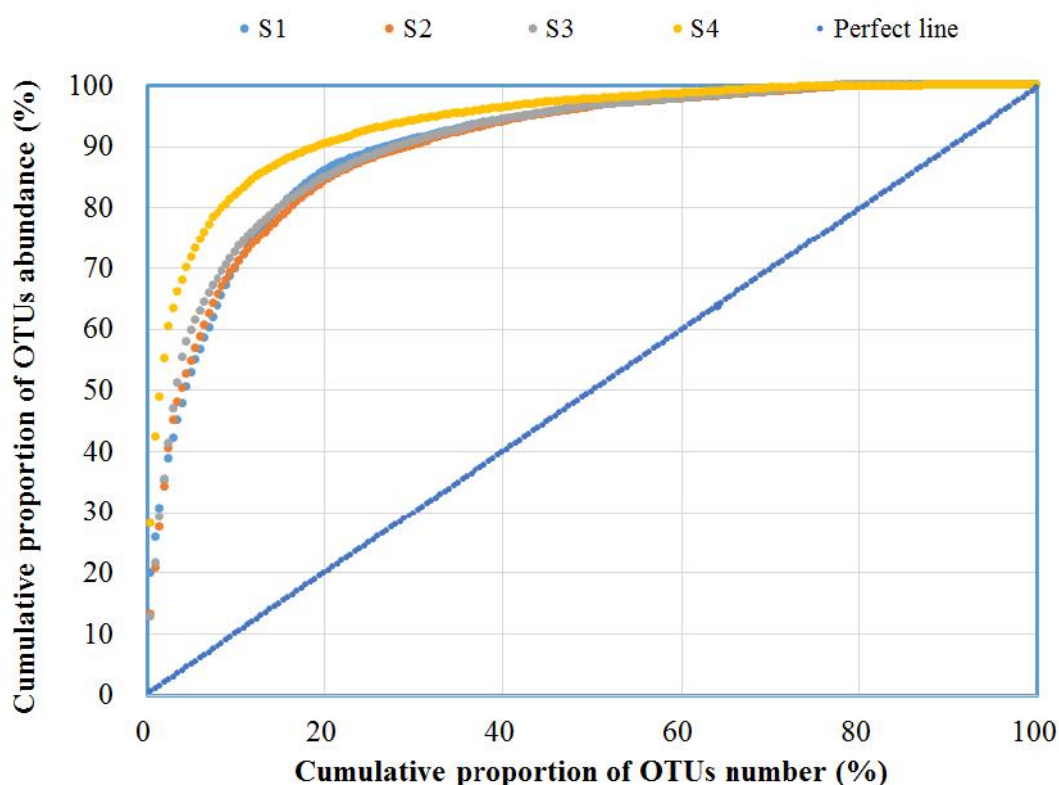


Figure 6.7 : Lorenz distribution curves of each sludge sample along the UASB reactor.

Likely, availability of specific substrates and/or physical selection had contributed to the varied microbial communities along the UASB height. For instance, as typical primary fermenting bacteria, *Cytophaga sp.* can grow competitively under nutrient rich environments (Pankratov et al., 2011; Panichnumsin et al., 2012). The relative abundance of *Cytophaga sp.* decreased by 90% from bottom to top. This can be related to the gradient decrease of substrate bioavailability (i.e. COD, Figure 6.9). The drop in *Cytophaga sp.* abundance in the upper part of the UASB might be related to the lack of granules there. PSD results (Figure 6.3) and microscopic pictures (Figure 6.4) showed much more granular sludge in the bottom part of the UASB and nearly no granules were found in the middle part. Abundant growth of acetogenic bacteria such as *Cytophaga sp.* is often related to granular biofilms and its abundance decreased when the granules are deteriorated (McKeown et al., 2009).

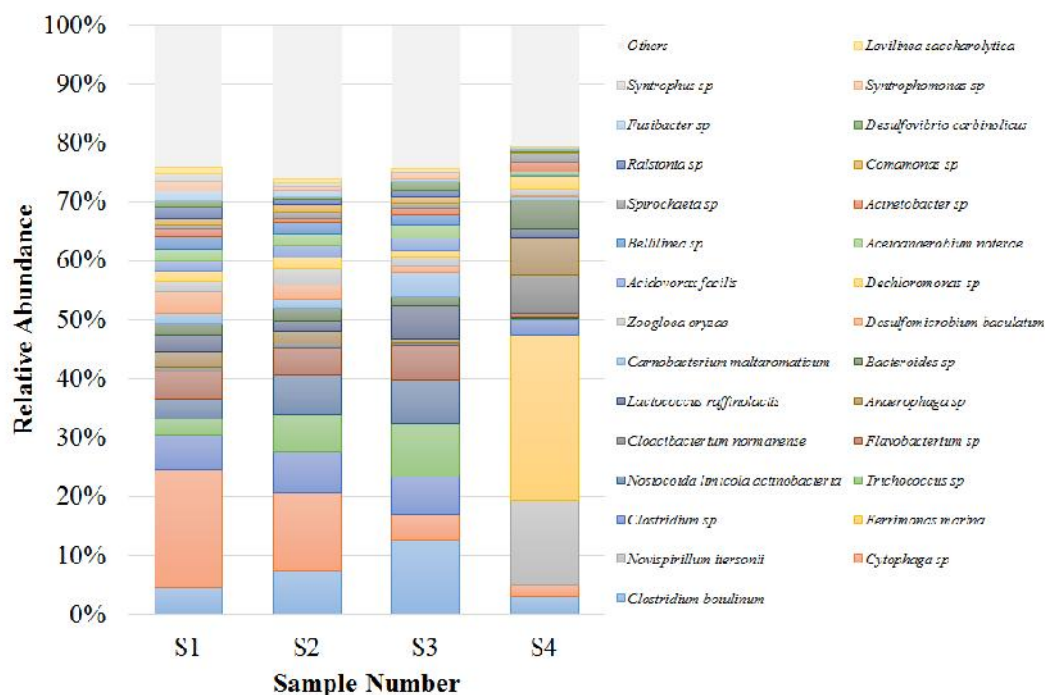


Figure 6.8 : Relative abundance of major bacterial genera.

Water quality profile for soluble COD is shown in Figure 6.9. Influent has an average soluble COD concentration of 159 mg/L. This increased to 385 mg/L at a height of 10 cm from the bottom, likely due to hydrolysis of entrapped solid organics that accumulated in the lower portion of the reactor and then decreased along the reactor height due to further progress of methanization. As suggested by the water quality profiles, most of the organic matter in the wastewater was degraded in the bottom part of the reactor, implying that the dominant phylotypes in the bottom part of the UASB could be responsible for removing organic matter. Higher soluble COD removal in the lower granular sludge bed zone was also observed in the study of Ahn et al. (2001). The same authors also measured higher specific uptake rates near the inlet compared to other volume fractions, indicating accumulation of active biomass near the reactor inlet (Ahn et al., 2001). The most abundant OTU in the S1 belonged to the genus *Cytophaga* (Figure 6.8). This OTU, that represented approximately 20% of all of the clones from S1 (344/1721 clones), decreased with reactor height, i.e. 236/1798, 70/1662 and 43/2034 clones in S2, S3, S4, respectively. This drop coincided with the observed soluble COD decrease along the height of the reactor (Figure 6.9).

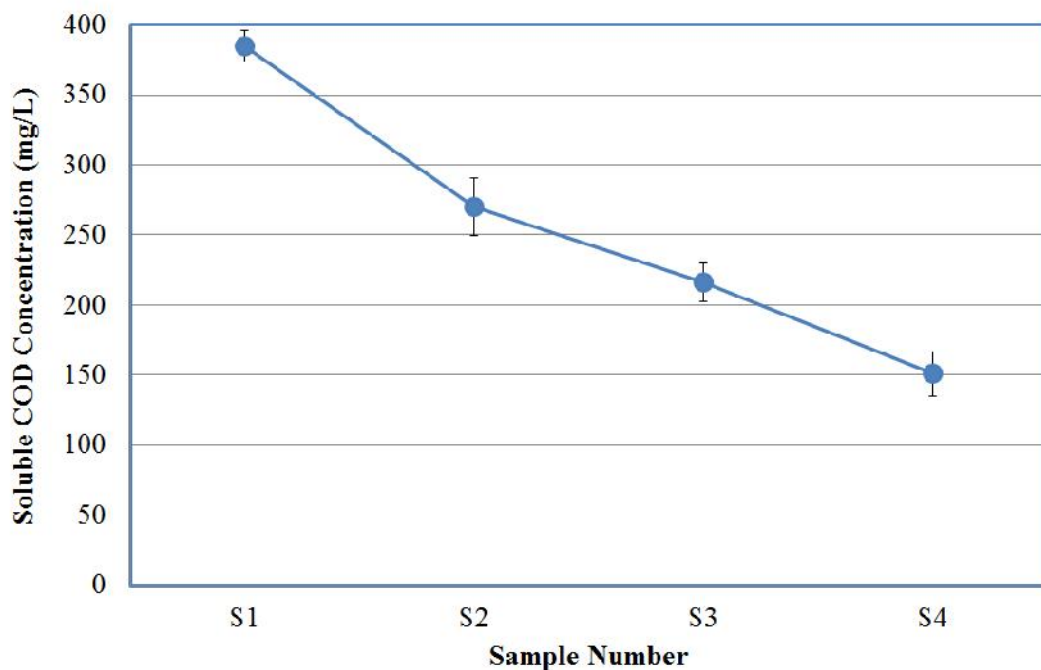


Figure 6.9 : Soluble COD concentration along the height of the membrane coupled UASB reactor.

The variation in sludge composition is also illustrated by the variation in archaeal communities over the height of the sludge bed (Figure 6.10, Table C.2, Appendix C). Species belonging to genus *Methanosaeta* were the pre-dominant archaeal species in the lower parts (S1 and S2) of the UASB, but their abundance decreased to 1/2 for S3 and even down to 1/3 for S4. As a typical acetoclastic methanogen, the growth of *Methanosaeta sp.* is highly related to the presence of acetate and its availability to biomass (Penning et al., 2006; Ince et al., 2011). High abundance appeared in the bottom part, coinciding with the likely presence of the methanogenic precursor acetate that is generated by acidogens and acetogens converting influent organic matter. In addition, *Methanosaeta sp.* are commonly identified as the predominant acetate consuming methanogen in granular sludge, which was particularly present at the bottom of the UASB reactor (Figure 6.3 and Figure 6.4) (El-Mamouni et al., 1997; McKeown et al., 2009; Kovacik et al., 2010; Kobayashi et al., 2011; Khemkhao et al., 2012).

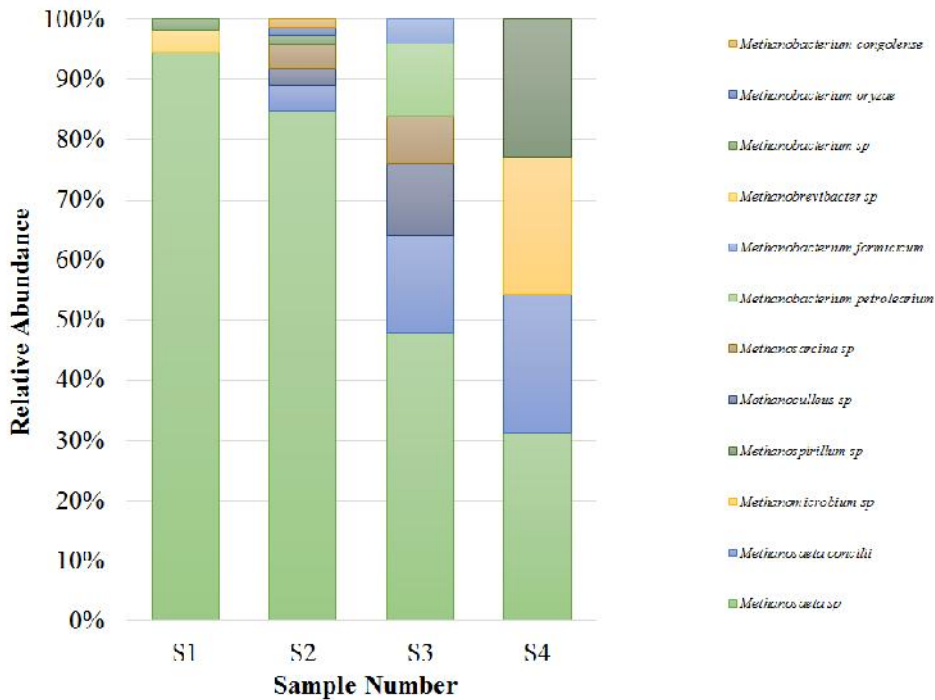


Figure 6.10 : Relative abundance of all archaeal species.

6.4 Conclusion

Irrespective the AnMBR operation mode, sludge bed stratification manifested in a UASB reactor coupled to a UF membrane filtration unit. Results showed striking differences in sludge characteristics at different reactor heights along the UASB reactor. Even though membrane incorporation led to a deterioration in sludge bed settleability, the reactor biomass became segregated into several zones. In the lower zones, there was variation in biomass concentration with height; and in the upper zones, the concentration of biomass was more or less uniform. The lower zone had dense granulated biomass, whereas the upper zone had loose active and nonactive biomass. Analysis over the height of the reactor showed that the TS, VS, TSS and VSS concentrations in the reactor decreased with increased height, and highest COD concentration of 46 g/L was found at the bottom of the reactor. Image analysis showed significant differences in particle size between each sampling port. Results clearly showed that the highest SMA was obtained with the sludge taken from the bottom parts. The most active biomass remained near the inlet of the reactor; whereas, non-active biomass consisting of loose, suspended particles and flocculents was present at the top. Comparative sequence analysis at the phylum level revealed changes in microbial community composition with reactor height. A high diversity of

microbial species were present in the lower part of the reactor, whereas less diversity was observed in the middle and upper parts. The observed sludge bed stratification contradicts the pre-assumption that the UASB sludge bed is fully mixed when coupled to a membrane unit in an AnMBR setup. Our findings indicate that sludge conveyance from the low temperature UASB reactor to the parallel operating digester for sludge stabilisation is location specific. Considering the low sludge stability and solids content stratification, the sludge to be recirculated from UASB reactor to digester is recommended to be taken from 40-50% of the sludge bed height.

7. AN INTEGRATED ANAEROBIC MEMBRANE BIOREACTOR-DIGESTER SYSTEM TO SUPPORT LOW TEMPERATURE ANAEROBIC TREATMENT OF MUNICIPAL WASTEWATER⁽⁶⁾

7.1 Introduction

Anaerobic processes are generally operated under mesophilic (35 °C) and sometimes thermophilic (55 °C) temperature conditions. However, for wastewaters with a low organic content and relatively low temperature (e.g., municipal wastewater), methane production is by far insufficient to cover the heating requirement for distinctly increasing the temperature. Therefore, anaerobic reactor systems treating low concentrated wastewaters are generally operated under ambient temperature conditions (An et al., 2009a). This also explains why full-scale anaerobic treatment of municipal wastewater has been restricted to tropical areas, where the temperature of municipal wastewater allows for sufficiently fast hydrolysis of complex organics and SS (Zhang et al., 2013). Laboratory-scale research has shown the feasibility of low temperature (6–15 °C) application of anaerobic processes with easily biodegradable substrates (such as VFAs, semi skimmed milk or nonfat dry milk) for both high and low strength wastewaters (Rebac et al., 1998; Rebac et al., 1999; Zhang et al., 2013). Although operation at low ambient temperatures appears technically feasible, SRTs need to be lengthened compared to mesophilic operation since hydrolysis of solids is restrained due to the lower temperature, leading to accumulation of non-stabilised matter in the sludge bed (van Lier et al., 2008). Thus, municipal wastewater treatment by anaerobic systems in more temperate climates is still considered a challenge since municipal wastewater belongs to the complex

⁽⁶⁾ This chapter is based on:

Ozgun, H., Zhou, Z, Ersahin, M.E., Tao, Y., Spanjers, H., van Lier, J.B. An integrated anaerobic membrane bioreactor-digester system to support low temperature anaerobic treatment of municipal wastewater. *under review*.

wastewater category due to the high fraction of particulate organic material, moderate biodegradability and its low strength. Under low temperature ($<20\text{ }^{\circ}\text{C}$) conditions, the growth of methanogens is very slow and hydrolysis of particulate matter into dissolved molecules becomes the rate-limiting step, which results in the accumulation of SS in the reactor (Ozgun et al., 2013a). The solids accumulation will impose a more frequent sludge discharge. Consequently, at lower temperatures the amount of excess sludge produced will increase leading to a shorter SRTs, which might then limit the net yield of methanogens resulting in a poor organic matter conversion efficiency and thus deterioration of sludge stability with a low SMA (Mahmoud et al., 2004). The latter will result in a poor soluble COD removal and an overall deterioration of the digestion process (Mahmoud, 2008). Therefore, the anaerobic treatment efficiency is largely dependent on local ambient temperatures (Lew et al., 2011). Due to solids accumulation, the SRT becomes too short, furthermore, the excess sludge of the UASB reactor still requires stabilization before appropriate use or final disposal (Zhang et al., 2012), and the liquid effluent needs further treatment.

The current challenge in anaerobic sewage treatment technology development is to amend the system to temperate climate conditions and/or to regions that are periodically subjected to low temperatures. Various investigations have been carried out to overcome the process constraints related to the low temperatures and it has been stated that anaerobic sewage treatment is certainly not limited to regions of hot climates (Mahmoud, 2002). Besides operational aspects such as sludge characteristics and upflow velocities, the technical design of the overall process influences the performance (Halalsheh, 2002; Mahmoud, 2002; Seghezzo, 2004). One of the current solutions for the treatment of raw domestic sewage at low temperature is a two-step UASB system where in the first step mainly the removal of SS and partial hydrolysis and acidification occurs, while in the second step the hydrolyzed organic matter is converted to biogas (Zeeman et al., 1997; Elmitwalli et al., 2002; Halalsheh, 2002; Seghezzo, 2004). However, results show similar performances of the two-step reactor in comparison to a one step system, due to low removal efficiencies and low SRT in the second stage. Zeeman et al. (1997) and Elmitwalli et al. (2002) proposed two-stage systems consisting of a high loaded first stage entrapping the SS, followed by a methanogenic stage for soluble COD

removal. The produced sludge from the high loaded first stage is, by definition, not stabilised and needs further stabilization in a separate digester. Typical problems of high loaded UASB reactors, such as sludge flotation and washout of active biomass were observed. Applications of membranes for enhanced sludge retention may alleviate some of those challenges. For example, application of an AnMBR for sewage treatment resulted in lower effluent COD values (<100 mg/L) and SS concentrations (<1 mg/L) compared to conventional UASB reactors at 18–25 °C (An et al., 2009a). However, based on the results of Chapter 5, although overall treatment performance is high, membrane coupled UASB configuration is not found technically feasible for the treatment of municipal wastewater at 15 °C considering the deterioration of the filtration performance, which would be a bottleneck to the practical engineering application of AnMBRs.

Lettinga and Hulshoff Pol (1991) proposed a novel technology consisting of an integrated high loaded UASB reactor and digester for treating sewage at low temperature. In the UASB-Digester system, sludge circulation occurs between a UASB and a digester. Municipal sewage was treated in a UASB at low temperature. The solids, which are entrapped in the UASB sludge bed, are conveyed to a digester operated at optimal conditions where they can be stabilised and the digested sludge is re-circulated to the UASB reactor, thereby providing additional methanogenic biomass to convert soluble COD at low temperatures. In this system, only the fraction of the municipal sewage that is transferred from a UASB reactor to a digester needs to be heated. The UASB reactor of this system is operated under cold conditions (8–20 °C), while designed for summer conditions. As the loading rate is too high to allow for complete stabilization of entrapped SS in the low temperature UASB reactor, these solids are transferred and stabilized in the sludge digester that operates at higher temperature.

Several studies (Álvarez et al., 2004; Mahmoud et al., 2004; Mahmoud, 2008; Mahmoud et al., 2008; Lew et al., 2011; Zhang et al, 2012) have focused on the impact of digester coupling on the performance of UASB reactors. A previous laboratory experiment indicated that the removal efficiencies are increased by about 5% applying a UASB-Digester system compared to single stage UASB reactor (Ruiz et al., 1998). Inhibition of methanogenic bacteria appeared in the external digester, limiting the performance of the system, but this problem could be solved by means of

an adequate start-up and feeding strategy (Álvarez et al., 2004). Álvarez et al. (2004) investigated the performance of a UASB-Digester system for the treatment of low temperature raw municipal wastewater and found stable anaerobic treatment and a slight SMA increase. Mahmoud et al. (2004) also compared the one-stage UASB reactor with a combined UASB-Digester, where the accumulated sludge from the UASB reactor was directed to a CSTR-Digester, operating at 35 °C, for further treatment. The obtained results were promising as compared with the one-stage UASB reactor. The UASB-Digester had a better performance than a single stage UASB reactor at 15 °C, with a COD removal of 66% versus 44%. This system was investigated in the Netherlands under controlled temperature conditions. Following that study, Mahmoud (2008) investigated the system in Palestine, where climate and sewage characteristics are quite different in order to assess the system response to the Mediterranean climate seasonal temperature fluctuations. UASB performance had remarkably improved after incorporating a digester to the system and anaerobic treatment of high strength sewage during a hot period in the UASB-Digester system was found very promising. Mahmoud et al. (2004) achieved an average COD removal efficiency of 66% at 15 °C with municipal sewage of a low soluble COD fraction (19–24% of total COD). However, several authors have shown that the average COD removal efficiency of the system decreases to only 37–46% when treating municipal sewage with a considerably higher soluble COD fraction (33–44%), mainly caused by insufficient methanogenic activity in the UASB reactor (Álvarez et al., 2004; Zhang et al., 2012). Zhang et al. (2013) improved the performance of UASB-Digester system for these types of municipal sewage by adding co-substrate to the sludge digester. A pilot-scale UASB-Digester system was applied to treat real municipal sewage, and glucose was added in the sludge digester as a model co-substrate in order to produce additional methanogenic biomass, which was continuously recycled to inoculate the UASB reactor. An increase was observed in soluble sewage COD removal efficiency, SMA and methane production and co-digestion is proposed as a suitable approach to support a UASB-Digester for pretreatment of low temperature municipal sewage.

Optimization of sludge recirculation should be done carefully in UASB-Digester systems in order to maintain complete conversion of biodegradable dissolved COD. System performance is affected by several factors in sludge recirculation such as

sludge characteristics, recirculation rate and sludge volume. In previous studies of Álvarez et al. (2004), Mahmoud et al. (2004) and Mahmoud (2008), the sludge recirculation rate was regulated to maintain the sludge bed height at a certain level. Besides, the location for sludge removal in the sludge bed of the UASB reactor was not optimized. The amount of sludge that needs to be recirculated is crucial to the viability of the UASB-Digester system, as it determines the required energy input to heat the transferred sludge from 15 to 35 °C. Lew et al. (2011) optimized the sludge recirculation process by determining the best location in the sludge bed for removing sludge. During cooler periods below 20 °C, influent wastewater particulate matter accumulates in the top part of the UASB reactor sludge and can be conveyed to the digester. The digester can be designed with an operational volume 33% smaller than the UASB. Zhang et al. (2012) also improved the sludge recirculation process of a UASB-Digester system treating domestic sewage at low temperature by investigating the effect of sludge recirculation rate on the performance. In this work, the effect of the sludge recirculation rate in the UASB-Digester system on COD removal efficiency, biogas production, the stability of the UASB sludge and the digester sludge, and methanogenic activity of the UASB sludge at 15 °C, was investigated. The results showed that the total COD removal efficiency increases with increasing sludge recirculation rate. Based on the energy available in the wastewater, a maximum sludge recirculation flow of 2-2.5% of the influent flow rate still allows energy self-sufficiency. The results present practical information for the operation and improvement of the UASB-Digester system treating domestic sewage at low temperature.

All of these studies about the digester incorporation were investigated on solely UASB reactors. However, with growing interest in cost-effective water reclamation techniques, AnMBRs are becoming increasingly popular for the treatment of municipal wastewater when high effluent quality and/or agricultural use of treated effluents is required (Ho and Sung, 2010; Ozgun et al., 2013a; Prieto et al., 2013; Wei et al., 2014). Membrane fouling, which causes a reduction in the flux throughout the operation, remains an unavoidable drawback of the AnMBR and limits its widespread application in wastewater treatment (Wang et al., 2013). Fouling mechanisms depend on many factors such as membrane characteristics, sludge characteristics, environmental conditions, reactor design and the operation strategy

(An et al., 2009a). Membrane coupled UASB reactors may be a promising approach to reduce problems related to membrane fouling (Ozgun et al., 2013b). High fluxes are expected in membrane coupled UASB configurations since the membrane is now only challenged by the supernatant but not by the bulk sludge. The results presented in Chapter 4 clearly supported the applicability of a membrane-coupled UASB reactor for municipal wastewater treatment at 25 °C, in spite of the sludge bed deterioration. A pathogen free but nutrient rich permeate quality was achieved during the AnMBR stage with quite stable TMP values (Ozgun et al., 2015a). However, at 15 °C the system performance deteriorated (Chapter 5; Ozgun et al., 2015b). Further studies should be done and other configurations need to be evaluated in order to mitigate rapid membrane fouling for the low temperature applications of membrane-coupled UASB reactors. Digester coupling can be an attractive alternative for producing high quality and nutrient-rich effluents for reuse purposes under moderate climate conditions. So far, there is no study in the literature about the impact of digester incorporation to the membrane-coupled UASB systems treating low temperature sewage.

The present work investigated an AnMBR-Digester system treating municipal wastewater at a temperature of 15 °C. The AnMBR was firstly operated without incorporating the digester aiming at monitoring of the overall AnMBR performance, i.e. (i) treatment performance (ii) filtration performance and (iii) sludge quality. This was done to obtain a reference to assess the achievements obtained from incorporating a digester. Afterwards, the one-stage AnMBR was modified to an AnMBR-Digester system by incorporating a digester operated at 35 °C in order to assess the performance of the AnMBR-Digester system on the same indicators. The characteristics of the sludge that will be circulated to the digester determines the viability of the AnMBR-Digester system. Results described in Chapter 6 showed differences in sludge stability and a clear sludge stratification over the reactor height, and indicated that the best location for sludge recirculation is at about 40-50% of the sludge bed height. Additionally, the sampling location at about 75% of the sludge bed height was comparatively evaluated for removing sludge. The overall information obtained from this study will be valuable for the operation and improvement of the AnMBR-Digester system treating municipal wastewater at low temperatures.

7.2 Materials and Methods

7.2.1 Wastewater source

Synthetic municipal wastewater as described in Chapter 3 (Table 3.1 and Table 3.2) was used as feed.

7.2.2 Seed sludge

The UASB reactor was seeded with flocculent anaerobic sludge obtained from a pilot-scale UASB reactor treating black water (Sneek, the Netherlands). The characteristics of the seed sludge are presented in Table 3.3 in Chapter 3. The excess sludge withdrawn from the AnMBR at different intervals during the operation at 15 °C was used as inoculum for the digester.

7.2.3 Experimental setup

The experimental setup consisted of a laboratory-scale CSTR type digester with an effective volume of 4 L and UASB type reactor with an effective volume of 7 L coupled with an external membrane module. The experimental period was divided into three stages: (i) before digester incorporation, when the system was operated as an AnMBR, (ii) after digester incorporation, when the system was operated as an AnMBR-Digester system with sludge recirculation from the sludge transfer point of S3 (Figure 7.1) and (iii) after digester incorporation, when the system was operated as an AnMBR-Digester system with sludge recirculation from the sludge transfer point of S2 (Figure 7.1).

A schematic diagram of the experimental setup is depicted in Figure 7.1. The AnMBR was equipped with feed, circulation pump and effluent pump (Watson Marlow 120U/DV). The circulation pump (Watson Marlow 120U/DV) was used for circulating the flow between the UASB reactor and the membrane module, and obtaining permeate through the membrane. Furthermore, recirculation over the membrane surface was applied by a peristaltic pump (Watson Marlow 620UN/R) in order to maintain a cross-flow velocity of 1 m/s across the membrane surface independently of the circulation flow between the UASB reactor and the membrane module. This concentrate circulation flow rate was adjusted enabling a constant upflow velocity in the UASB reactor.

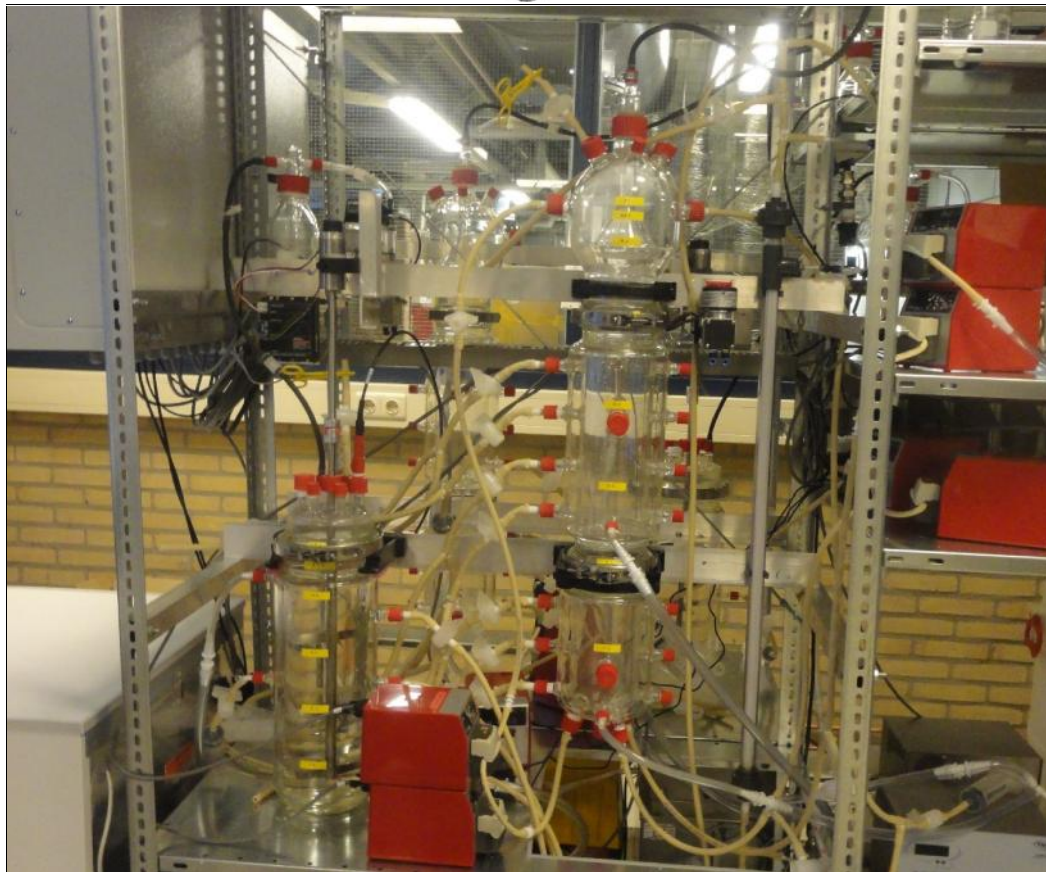
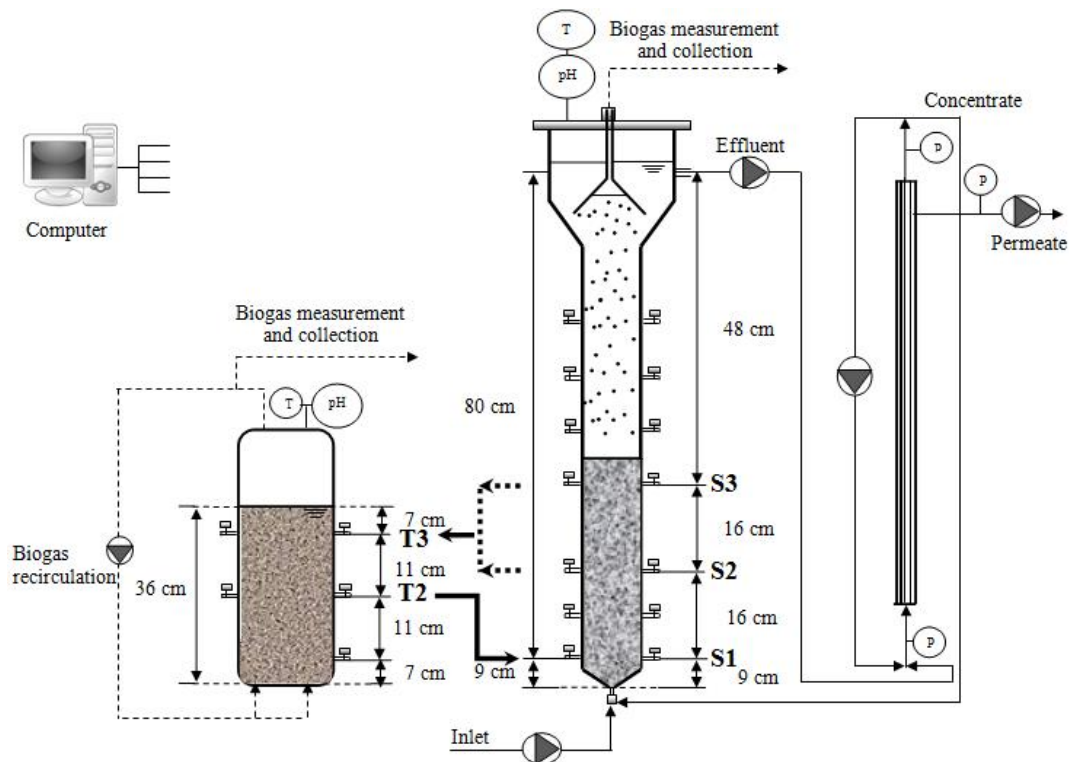


Figure 7.1 : Schematic diagram of the AnMBR-Digester system.

UASB effluent was directed through an external cross-flow tubular module containing 28 UF (0.03 μm pore size) membrane fibers (Pentair X-Flow), and the concentrate was returned to the UASB reactor. Each membrane fiber had an internal

diameter of 1.5 mm and a length of 80 cm, with an effective filtration area of 0.0038 m². The characteristics of the membrane are presented in Table 5.1 in Chapter 5. Pressure sensors (AE Sensors, ATM -800/+600 mbar, the Netherlands) were installed in the membrane feed, concentrate and permeate lines in order to measure the TMP.

Taps were installed over the whole UASB reactor height at about 8 cm apart for sludge recirculation and analysis. The biogas was collected by means of a three-phase separator installed at the top part of the UASB reactor. Hereafter, the AnMBR setup was modified to the AnMBR-Digester system by incorporating a CSTR type digester. Biogas flow rates of the AnMBR and digester were measured separately with a gas meter (Ritter, Milligas Counter MGC-1 PMMA). Produced biogas was recycled by a diaphragm pump (KNF, N86 KTDCB) via a diffuser at the bottom of the reactor to provide mixing inside the digester. Temperature was controlled by means of a water bath (Tamson Instruments, the Netherlands). Temperature and pH inside the reactors were monitored on-line with a probe combined with a transmitter (Elscolab, M300 ISM). A computer running LabView software (LabVIEW 10.0.1, National Instruments) was used to control the pumps and collect the data.

7.2.4 Experimental procedure

The experimental work was carried out over three successive periods. In the first period, the AnMBR was operated for 63 days at 15 °C without incorporating the digester. Afterwards, the AnMBR was modified to the AnMBR-Digester system by incorporating a CSTR digester operated at 35 °C. Two sampling points at different heights of the UASB sludge bed, represented by S2 (located at 25 cm height about 40-50% of the sludge bed height) and S3 (located at 41 cm height about 75% of the sludge bed height), were comparatively investigated for optimal sludge recirculation location. In the second and third stage, the AnMBR-Digester system was operated for 126 days, of which during the first 63 days S3 was used for sludge circulation and S2 during the remaining days. The operational and design details of the AnMBR-Digester system are given in Table 7.1. As can be seen in Figure 7.1, the sludge was transferred from one reactor to another once a day by recirculating the sludge from the sludge transfer points of S2 and S3 in UASB reactor to the sludge transfer point of T3 in digester and then from the sludge transfer point of T2 in the Digester to the

sludge transfer point of S1 in UASB reactor, ca. 9 cm from the bottom representing the lowest part of the AnMBR. Sludge transfer of S1 to digester was not investigated in order to avoid short-circuiting of the UASB influent. This sludge recirculation rate between digester and AnMBR was 200 mL/d, which corresponded to 0.8% of the influent flow rate of 25 L/d. During each stage, the AnMBR was operated with cycles consisting of 180 seconds of filtration, followed by 20 seconds of backwash. Backwash was applied by reversing the flow direction, keeping the flux at the same value as during filtration.

Table 7.1 : Operational conditions of the AMBR-Digester system.

Parameters	Unit	AnMBR	Digester
HRT	day	0.25	20
OLR	kg/m ³ .day	2	-
Temperature	°C	15	35
Upflow Velocity	m/h	0.6	-
Cross-flow Velocity	m/s	1	-
Flux	L/m ² .h	12.3	-

7.2.5 Experimental methods

7.2.5.1 Analysis

COD, NH₄⁺-N, TN, TP and TSS were measured in triplicate following Standard Methods (APHA, 2005). The samples for soluble COD measurement were filtered through a 0.45 µm filter. The turbidity and pH were measured by HACH 2100N Turbidimeter and by a WTW multi720 pH meter, respectively. The PSD of the effluent was assessed by a Mastersizer 2000 (Malvern Instruments, Hydro 2000 MU) that has a detection range of 0.02-2000 µm. Laser diffraction technique was used to measure the size of the particles. The methane content in the biogas was measured by a Varian 3800 gas chromatograph equipped with a flame ionization detector (Varian, Palo Alto, CA). Statistical calculations were carried out by using Minitab 16 software (Minitab Inc., USA).

SMA of the sludge samples was assessed by an AMPTSII (Bioprocess Control, Sweden) (Li et al., 2011a). Stability of sludge samples was assessed by obtaining the specific ultimate methane production. Detailed explanations about SMA and stability tests can be found in Chapter 4.

7.2.5.2 Filtration resistance

After long-term reactor operation, the fouled membranes were cleaned with a sequential cleaning procedure to calculate the various fouling resistances and evaluate the characteristics of membrane fouling. Details about the assessment of the various resistances can be found in Chapter 5.

7.2.5.3 Microbial community analysis

Microbial community structure was investigated using 454-pyrosequencing. Details about sample preparation, DNA extraction, pyrosequencing and post analysis of pyrosequencing data can be found in Chapter 4.

7.3 Results and Discussion

7.3.1 Treatment performance

Figure 7.2 shows the total and soluble COD in the UASB effluent and final permeate of the AnMBR system during all experimental stages. Stable operation performance was considered to be achieved once stable effluent COD and biogas production were obtained. During the single stage operation of the AnMBR, average total and soluble UASB effluent COD were 1759 ± 92 mg/L and 153 ± 3 mg/L, respectively, under stabilised conditions. Following this period, sludge was started to be recirculated from the sludge transfer point S3 from the UASB reactor, starting at the beginning of stage 2. During the second stage, a gradual increase was observed in average total COD to the value of 1966 ± 32 mg/L. However, the average soluble COD concentration of 149 ± 6 mg/L was found to be similar to the values obtained during the first stage. Therefore, the observed increase in total COD concentration may be attributed to the release of colloidal and suspended particles from the sludge bed resulting from the recirculation. Following the second stage, the sludge to be recirculated from UASB reactor to digester was taken from S2 where the sludge had the lowest stability, as recommended based on the results of Chapter 6. The effect of the utilization of a certain sludge transfer point on the system performance was investigated. The lowest average total COD concentration of 1405 ± 51 mg/L was achieved at this stage also with an additional decrease in average soluble COD concentration to 133 ± 3 mg/L. Compared to the second stage results, the observed

decrease in total and soluble COD concentration during the third stage confirmed the effect of sludge transfer point on the performance of AnMBR-Digester systems.

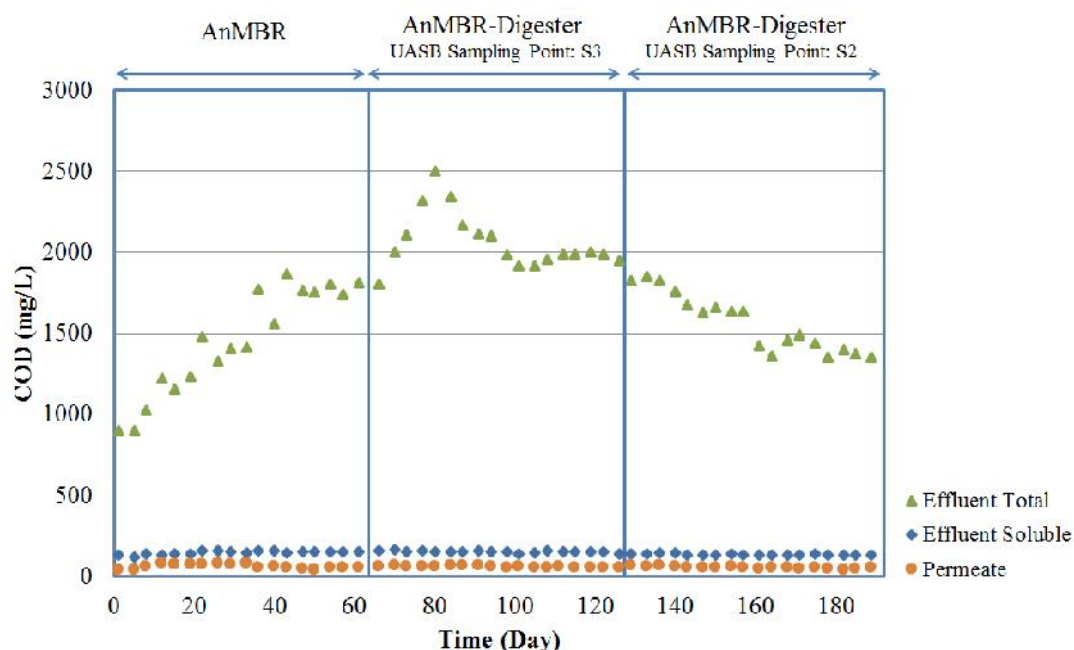


Figure 7.2 : Total and soluble COD concentrations in the UASB effluent and final permeate during three experimental stages.

The lowest average total and soluble COD concentration in the UASB effluent was achieved during the third stage. Possibly improved methanogenic and hydrolytic conditions in the combined system were established in the third stage. The improvement in soluble COD removal might be either attributed to the soluble COD transfer to the digester which is subsequently converted to methane in the digester, or to the increased methanogenic capacity coming from the digester to the UASB reactor. Higher colloidal and suspended COD removal may result from a better solids entrapment (physical process), which is caused by improved digestion conditions based on the existence of the capturing-de-capturing mechanism for solids removal in the sludge bed. Elmitwalli et al. (2001) found that the colloidal particles in the sewage are highly biodegradable and attributed the poor colloidal removal during anaerobic sewage treatment at low temperatures to poor physical removal. Similarly, the improvement of suspended COD removal may also have resulted from the improved digestion conditions, which on its turn enhanced colloidal COD removal, since colloids may be generated from the SS (Elmitwalli et al., 2000). Mahmoud et al. (2004) also suggested the existence of a capturing-de-capturing mechanism for improved colloidal and suspended COD removal efficiency in the UASB-Digester

system. They hypothesized that a particle to be digested, should firstly be captured and once captured, it would be degraded in case the digestion conditions are appropriate. Otherwise, it might detach from the sludge bed. Therefore, the physical removal of solids might not only be affected by the characteristics of the influent particles and the sludge but also by the prevailing digestion conditions (Mahmoud et al., 2004).

Average total COD removal efficiencies, based on influent and permeate COD, were about 90% with average COD concentrations of 51 ± 5 mg/L, 55 ± 2 mg/L and 49 ± 4 mg/L in the permeate. A slight difference in the overall treatment performance of the AnMBR during each experimental stage was due to the physical retention capacity of the membrane. Higher overall suspended and colloidal COD removal efficiencies in spite of the higher total COD in the UASB effluent during the first and second stages were mainly due to the accumulation in the sludge bed.

SMA and stability measurement results of the UASB and the digester sludge are shown in Table 7.2. The highest SMA of the UASB sludge was obtained during the third stage indicating the improved conversion of sewage solids to methane and biomass, and an increased supply of methanogens to the UASB sludge. SMA of the sludge in the UASB reactor was about 90% of that in the digester during the third stage. The sludge digested in the digester had also substantially higher stability than the excess sludge from the UASB reactor of the UASB-Digester system.

The stability of the UASB sludge remained constant during the first and second stages, showing that the sludge is relatively unstable, i.e. it still contains considerable amounts of biodegradable solids and accumulation of such solids in the sludge bed. This may be explained by a limited hydrolysis of the entrapped solids, which is in agreement with the results presented by de Man (1990). However, the stability of the UASB sludge drastically improved from 0.65 ± 0.02 to 0.54 ± 0.02 g CH₄-COD/g COD during the third stage. The sludge accumulation during the first and second stages was accompanied with UASB effluent quality deterioration (Figure 7.2). However, average total COD removal efficiencies, based on influent and permeate COD, were similar during all experimental stages. Thus, although the total COD removal efficiencies were similar, lower stability in the first and second stages compared with the third stage was actually attributed to the organic solids accumulation during the first and second stages. The incorporation of the digester and selection of the sludge

transfer point of S2 for sludge transfer resulted in improved stabilization of the accumulated solids. Higher stability of the anaerobic sludge through the biodegradation and removal of e.g. fats and other inactive substances coincided with an observed increased SMA.

Table 7.2 : SMA and stability of the AnMBR and the digester sludge.

Experimental Period	SMA (g CH ₄ -COD/g VS.d)		Stability (g CH ₄ -COD/g COD)	
	UASB	Digester	UASB	Digester
AnMBR	0.38±0.01	-	0.64±0.03	-
AnMBR-Digester (Sampling Point: S3)	0.35±0.02	0.37±0.01	0.65±0.02	0.11±0.01
AnMBR-Digester (Sampling Point: S2)	0.44±0.01	0.49±0.01	0.54±0.02	0.09±0.01

Table 7.3 shows the average biogas production rate of the UASB and the digester at different experimental stages. Average methane contents of the biogas were 69±4%, 70±3% and 73±2% during the three experimental stages. The highest total methane production was achieved during the third stage when particulate and colloidal COD transfer to the digester occurred, which could be then converted to methane. The biogas production in the digester significantly increased during the third stage resulting from the increase in the captured suspended and colloidal COD. Besides that, the increase can be explained by the large amounts of methanogens supplied from the digester to the UASB reactor that enhanced the conversion of soluble COD to methane in the UASB reactor. This explanation is consistent with the results in Figure 7.2 showing a decrease in the soluble COD concentration in the UASB effluent during the third stage.

Lower biogas production rate was obtained from the UASB reactor in comparison to the biogas from the digester during all the experimental stages. The recovered methane in the gas amounted to 0.12±0.01, 0.13±0.01 and 0.23±0.01 L CH₄/g COD_{removed} for the 3 stages, respectively. During the single stage operation of the AnMBR, an average methane yield of 0.12±0.01 L CH₄/g COD_{removed} was obtained, which represented 33% of the maximum theoretical value, i.e. 0.369 L CH₄/g COD_{removed} at 15 °C. A slight increase in the methane yield to 0.13±0.01 L CH₄/g COD_{removed}, which was 35% of the maximum theoretical value, was observed during

the second stage. COD difference between influent and permeate of the AnMBR was represented as the removed COD in the calculation of methane yield but apparently the actually degraded COD that was converted to methane was lower than the removed COD. Besides, a large part of the methane might be dissolved in the permeate. Smith et al. (2013) found that up to 40-50% of total methane generated in an AnMBR can be dissolved in the permeate. Methane yields below theoretical values are therefore commonly observed in AnMBR studies. Martinez-Sosa et al. (2011a) and Huang et al. (2011) reported methane yields ranging from 0.124 to 0.27 L CH₄/g COD_{removed} in AnMBR studies. The highest methane yield was achieved during the third stage confirming the higher biodegradability of COD removed.

Table 7.3 : Average biogas production rate in the UASB and the digester.

Experimental Period	Biogas production in the UASB reactor (L/ d)	Biogas production in the digester (L/d)	Total biogas production (L/d)
AnMBR	2.0±0.1	-	2.0±0.1
AnMBR-Digester (UASB Sampling Point: S3)	0.9±0.1	1.3±0.1	2.2±0.2
AnMBR-Digester (UASB Sampling Point: S2)	1.1±0.1	2.4±0.2	3.5±0.3

Table 7.4 presents the overall removal efficiencies in terms of COD, TSS, TN, NH₄⁺ and TP during each experimental stage. As a general comparative evaluation of the system performance during all stages, regardless of the increases in COD and TSS concentrations in the UASB effluent, SS-free (non-detected) AnMBR permeate was obtained with average COD values between 49 and 55 mg/L, standing for overall COD removal efficiencies of about 90% in the AnMBR. As expected nitrogen and phosphorus concentrations in the permeate were higher compared to the influent values, underlining the fertilising value of the treated effluent. However, when nutrient removal is required, a separate post-treatment step needs to be combined with the proposed AnMBR system.

Table 7.4 : Influent and permeate characteristics in terms of COD, TSS, TN, NH_4^+ and TP during anaerobic municipal wastewater treatment in the single stage AnMBR and the AnMBR-Digester system.

Parameter	Influent mg/L	Single stage AnMBR		AnMBR-Digester system (UASB Sampling Point: S3)		AnMBR-Digester system (UASB Sampling Point: S2)	
		Permeate mg/L	Removal %	Permeate mg/L	Removal %	Permeate mg/L	Removal %
COD	530±30	51±5	90±0.9	55±2	90±0.4	49±4	91±0.7
TSS	230±25	< 10	>99	< 10	>99	< 10	>99
TN	54±5.2	65±7	~0	75±11	~0	63±3	~0
NH_4^+	36±5.5	64±9	~0	73±9	~0	59±3	~0
TP	12±0.8	14±4	~0	15±3	~0	14±2	~0

7.3.2 Filtration performance

7.3.2.1 Flux and TMP

Figure 7.3 presents the applied flux and resulting TMP during each experimental stage. The membrane flux was maintained constant at $12.3 \text{ L/m}^2\cdot\text{h}$ throughout the experimental study. During each run, membrane cleaning including physical, base and acid cleaning steps was carried out nearly every 30 days to recover the P, followed by another operational period. After stable reactor conditions were achieved, gradual increase in the TMP was observed over time during operation of the single stage AnMBR showing evidence of gradual membrane fouling. The highest point in the curve corresponded to the TMP of 437 mbar measured at the end of the first stage. Operation of the AnMBR at low temperature resulted in abrupt membrane fouling evidenced by a substantial increase in TMP over the course of 28 days. Similar TMP increase profiles were developed during the second stage. TMP also increased rapidly within each filtration cycle, reaching values of 418 and 426 mbar, respectively. However, TMP increased slowly with operation time and low TMP values were maintained during the third stage, which is in agreement with the results of UASB effluent characteristics (Figure 7.2). Single stage operation of the AnMBR resulted in the fastest membrane fouling, followed by second and third stage. The differences between first, second and third stage suggested an increased filtration performance after digester incorporation and sludge transfer from the sludge transfer point S2. The slow increase in TMP in the third stage is most likely related to the low solids content in the UASB effluent and presence of bigger particles as evidenced by the PSD measurements.

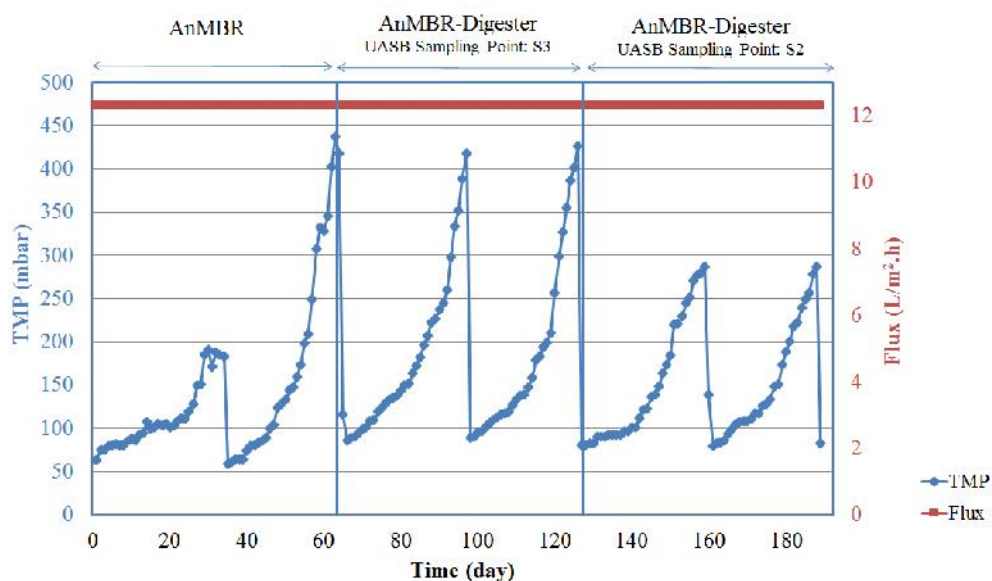


Figure 7.3 : Filtration performance of a single stage AnMBR and an AnMBR-Digester system.

7.3.2.2 TSS and turbidity

Figure 7.4 presents TSS and turbidity in the effluent of the UASB reactor. Average TSS concentrations were 505 ± 34 mg/L, 741 ± 73 mg/L and 476 ± 30 mg/L during the experimental stages. A considerable increase from the range of 440-552 mg/L to 611-820 mg/L was observed in the TSS concentration during the second stage confirming the release of suspended particles from the sludge bed. This increase was followed by a decrease during the third stage. Consequently, there was no significant change in TSS concentrations in the effluent between the first and third stage. However, different average turbidities in the UASB effluent were observed during each stage. The average turbidity in the effluent was 732 ± 46 NTU during the first stage, whereas it increased to 889 ± 122 NTU during the second stage. Subsequently, considerable decrease to 363 ± 36 NTU was observed during the third stage. Variations observed in effluent turbidity between the first and third stage seem contradictory to TSS results which do not show significant differences. Observed results are attributed to the decrease in colloidal particles washout from the system instead of suspended particles washout after digester incorporation as turbidity is an indicator of both suspended and/or colloidal particles. Since TSS concentrations were very similar at first and third stages, the higher fouling rate at the first stage was probably not associated with TSS concentration. Smaller colloidal particles measured during the single stage operation of AnMBR could be partly responsible for the higher fouling rate, related with their fouling tendency due to the low back-transport.

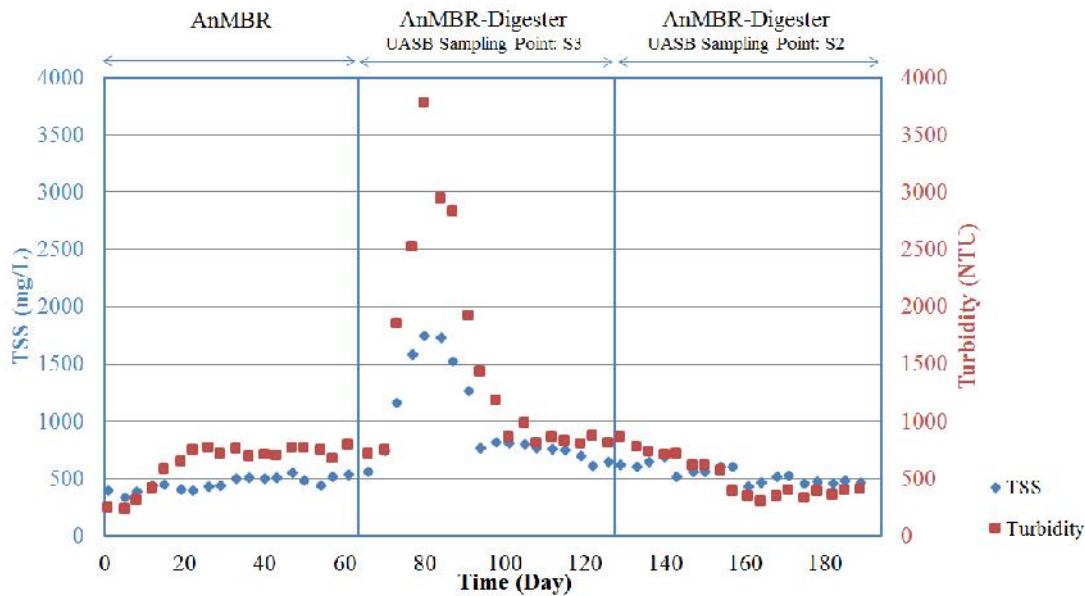


Figure 7.4 : TSS concentration and turbidity in the UASB effluent of a single stage AnMBR and an AnMBR-Digester system.

7.3.2.3 Particle size distribution

Figure 7.5 presents PSD curves of the UASB effluent. During the single stage operation of the AnMBR, there was a shift to smaller sizes of the distribution pattern and an apparent decrease in the D50 was observed, resulting in an effluent having a D50 of 25 μm . Likewise, the range of D50 that was measured in the UASB effluent at the second stage, was between 27 μm to 37 μm , whereas an apparent increase in the D50 was observed at the third stage, from 30 μm on Day 129 to 55 μm on Day 189, resulting in an extinction of a distribution curve at lower ranges. Finer particles observed in the UASB effluent could explain the accelerated fouling development (Figure 7.3) during the first and second stage since hydrodynamic back transport of particles is positively related with particle size (Tardieu et al., 1998). From these results, it was concluded that at the single stage operation of AnMBR, small sized particles were washed out more vigorously from the system. Increase in the D50 after digester incorporation and sludge transfer from S2 is likely related to the enhancement in the biodegradation of influent solids retained in the UASB reactor.

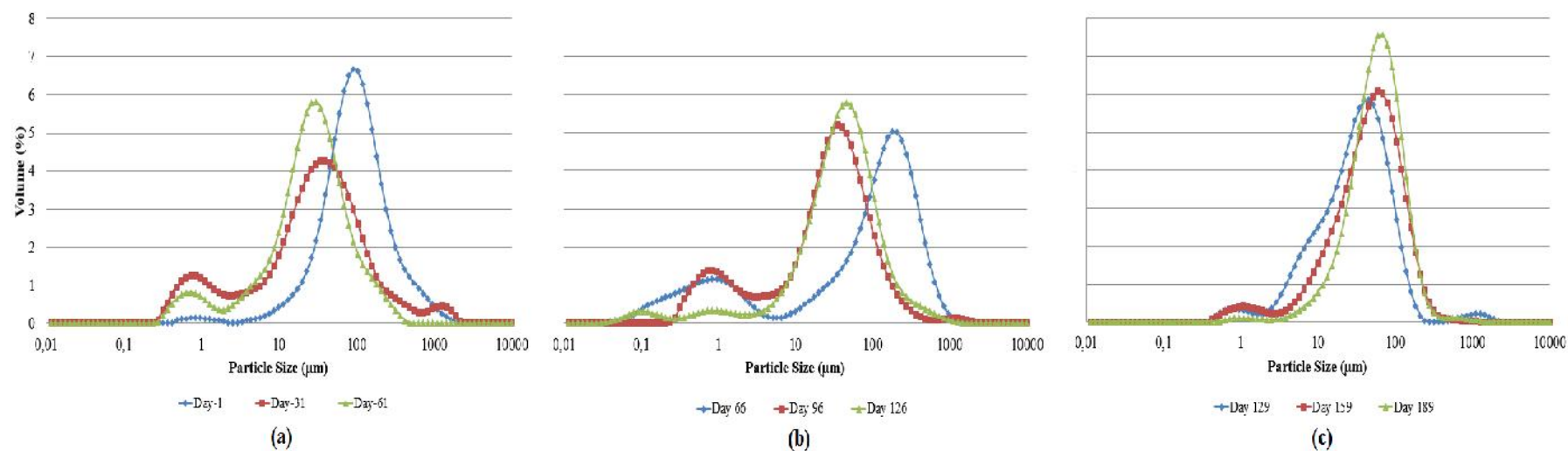


Figure 7.5 : PSD of the UASB effluent during anaerobic municipal wastewater treatment at different operational stages (a) AnMBR, (b) AnMBR-Digester (UASB Sampling Point: S3), (c) AnMBR-Digester (UASB Sampling Point: S2).

7.3.2.4 Filtration resistances and cleaning tests

The filtration resistances are summarized in Table 7.5. The results clearly show that the filtration resistances at the third stage were significantly lower than those at the first and second stage. Higher contents of foulants in the UASB effluent and finer flocs (Figure 7.5) tended to form a denser and nonporous cake layer, giving rise to increased filtration resistance before digester incorporation. Massé et al. (2006) also reported that a reduction in the particle size results in a more compact cake layer structure and hence higher cake resistance. Membrane resistance after the cleaning procedure was similar to the resistance of the new membrane at the third stage, whereas membrane resistances after the cleaning procedure were higher in comparison to the virgin ones during first and second stage.

Table 7.5 : Total filtration resistances.

Procedure	Parameter	Unit	Single stage AnMBR		AnMBR- Digester system (UASB Sampling Point: S3)		AnMBR- Digester system (UASB Sampling Point: S2)	
			Day- 31	Day- 61	Day- 96	Day- 126	Day- 159	Day- 189
Virgin membrane	Permeability	L/(m ² .h.bar)	779	679	438	392	377	344
	Resistance	10 ¹² m ⁻¹	0.48	0.56	0.86	0.96	1.07	1.1
Before cleaning	Permeability	L/(m ² .h.bar)	119	100	95	83	139	136
	Resistance	10 ¹² m ⁻¹	3.16	3.69	4.27	4.57	2.84	2.8
After physical cleaning	Permeability	L/(m ² .h.bar)	194	184	169	164	274	270
	Resistance	10 ¹² m ⁻¹	1.9	2	2.18	2.46	1.47	1.4
After NaOCl-Divos solution cleaning	Permeability	L/(m ² .h.bar)	339	264	254	256	345	313
	Resistance	10 ¹² m ⁻¹	1.11	1.4	1.5	1.58	1.17	1.2
After citric acid cleaning	Permeability	L/(m ² .h.bar)	640	395	372	348	371	327
	Resistance	10 ¹² m ⁻¹	0.59	0.93	1.09	1.06	1.1	1.15

For all the runs, the $R_{\text{intrinsic}}$ of the membrane and the resistance caused by pore-clogging were found smaller compared to the cake layer resistance (Figure 7.6). Cake layer resistances accounted for most of the total resistance, representing 43%, 47% and 52% of the R_T for the 3 stages, respectively, which is consistent with the results obtained by Chu et al. (2005). Normally, the sludge is retained in the sludge bed of the UASB. However, some dispersed solids in the reactor reached the membrane module with the upward liquid flow and adhered to the membrane surface due to permeate extraction. It was clear that a foulant layer had built up on the surface, while the fouling of the membrane pores was insignificant. Irreversible fouling was marginal since it only contributed to about 35%, 28% and 11% of the

total resistance for the 3 stages, respectively, indicating the feasibility and importance of the fouling control by minimizing the cake resistance in AnMBRs. However, the importance of $R_{\text{irreversible}}$ increased during the first and second stage. As shown in Figure 7.6, a decrease was observed in the contribution of irreversible fouling resistance to R_T of the system at the third stage.

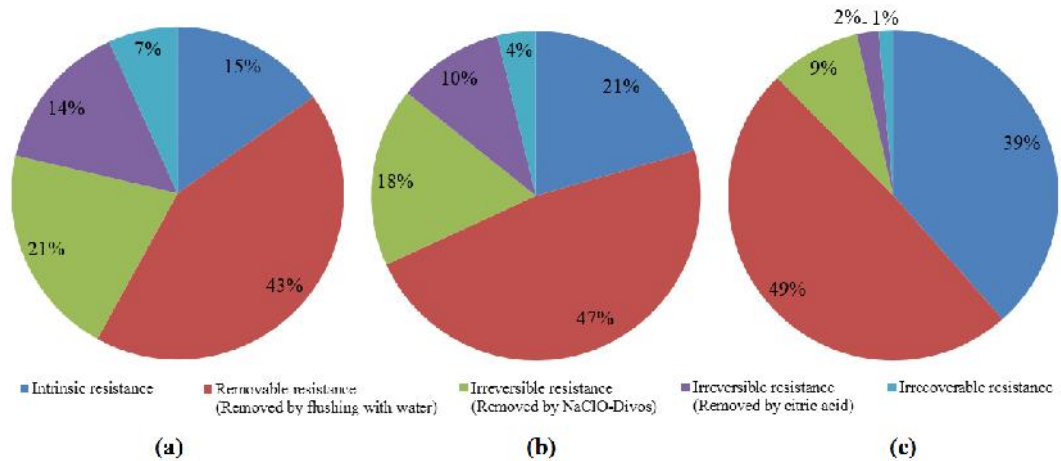


Figure 7.6 : Contribution of different resistances to R_T at different operational stages (a) AnMBR, (b) AnMBR-Digester (UASB Sampling Point: S3), (c) AnMBR-Digester (UASB Sampling Point: S2).

7.3.3 Microbial community

The performance of the AnMBR was slightly negatively affected when the sludge was recirculated from the sludge transfer point S3, but recovered and even enhanced when the sludge transfer was done from the sampling point S2. It is expected that the microbial community could have changed. In order to gain insights into the relationship between microbial-ecological characteristics and changes in reactor performance in response to the incorporation of the digester and the transfer from different points, biomass samples were collected from both the digester and UASB at different operational stages including the seeding sludge for the digester and UASB for further analysis using 454-pyrosequencing.

From an ecological point of view, a repeated temperature shock caused by the biomass exchange between the digester (under 35 °C) and UASB (15 °C) is an environmental stress to both communities, and under such stress some species can outgrow and some may be suppressed (Zhou et al., 2013; Li et al., 2014). A direct result of such blending of biomass between the digester and UASB was that the UASB community became more and more different to the initial or seed community,

but became more and more similar to the digester's community. Such trend can be clearly seen from the change of alpha-diversity (Figure 7.7), which is a comprehensive assessment over the richness and evenness of each single biomass. The alpha-diversity includes four indices: Shannon-Wiener index, Chao1 richness, phylogenetic diversity and OSN. The alpha-diversity of the UASB significantly decreased ($p < 0.001$ for each index) from the first stage to the third stage.

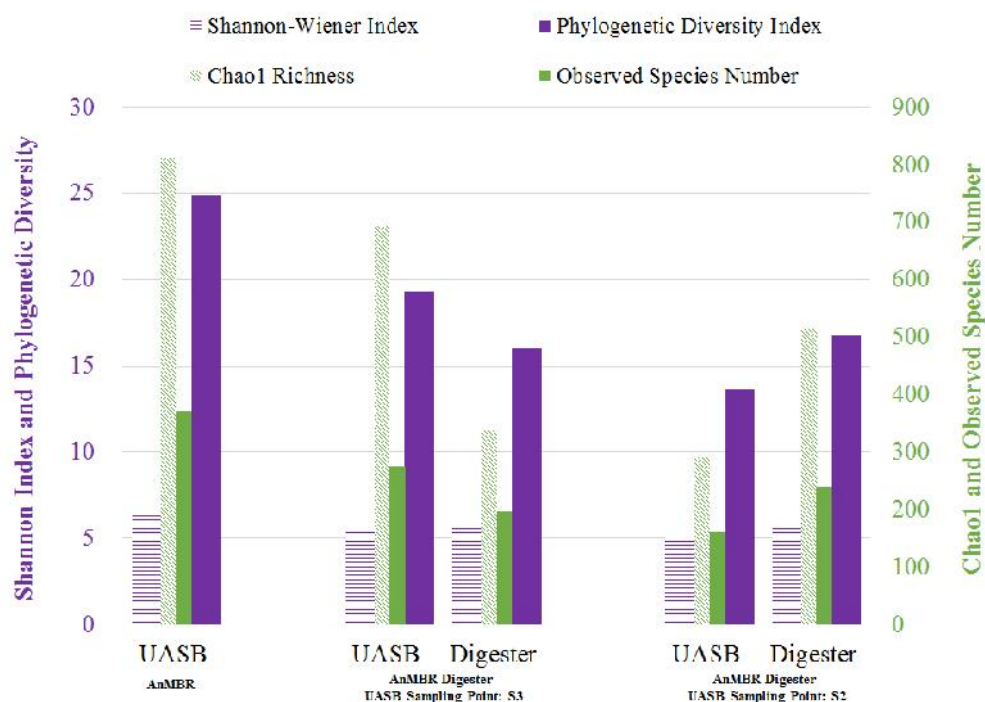


Figure 7.7 : Alpha-diversity of the biomass samples collected from the UASB and digester during AnMBR, AnMBR-Digester (UASB Sampling Point: S3), AnMBR-Digester (UASB Sampling Point: S2) operation.

The transition from the second stage to the third was induced by lowering the transfer point from S3 down to S2. S2 is closer to the bottom of the UASB, where more and larger particles exist. Hence, the change in the microbial communities in both the digester and UASB, along with the variation of reactor performance, can be attributed to the difference in sludge samples that were transferred from S3 (smaller size) or S2 (larger size). It is well known that large granules comprise abundant methanogens (Lens et al., 1993), which were found at S2 more near the bottom of the UASB reactor, close to the feed entrance point. Under the prevailing low temperature conditions in the UASB, the in-reactor methanogenic activity of the granules is relatively low, however, the activity will significantly increase when transferred to the

digester under mesophilic temperature. The observed results, such as an enhanced methane production in the digester (Table 7.3), agree well with this assumption.

Clear variation in bacterial assemblies was observed in the UASB before and after the incorporation of the digester (Figure 7.8, Table D.1, Appendix D). Some bacterial species that were originally predominant in the first stage decreased by a great percentage in the second stage, such as *Lactococcus raffinolactis* (from 12.8% to 2.0%) and *Cytophaga sp.* (from 7.1% to 2.4%). *Lactococcus raffinolactis* is prevalent in many environments and like many species belonging to the genus *Lactococcus*, due to their commercial values, they have been deeply investigated both physiologically and genetically (Meslier et al., 2012). *Lactococcus raffinolactis* cells are ovoid, always in pairs or short chains. They have peculiar property of fermentation and are found in both industrial dairy processing reactors (Boucher et al., 2003) and AD reactors (Nelson et al., 2011; Espana-Gamboa et al., 2012). *Lactococcus raffinolactis* were the most abundant species of the UASB in the first stage. However, their relative abundance sharply decreased when the digester biomass was introduced. Another previously dominant species, *Cytophaga sp.*, are typical fermenting bacteria that prefer mesophilic conditions in AD reactors (Weissbrodt et al., 2014). Their relative abundance was high (7.1 %) in the first stage but gradually decreased. The explanation of that is possibly a combination of the unfavourable temperature (15 °C) and the interruption by the digester biomass.

There are also some bacterial species that were not dominant in the first stage but became dominant in the second stage, including *Flavobacterium sp.* (from 3.2% to 18.5%), *Acinetobacter sp.* (from 4.8% to 18.9%), *Bacteroides sp.* (from 2.9% to 9.8%), *Clostridium botulinum* (from 3.2% to 11.8%). *Flavobacterium sp.*, *Acinetobacter sp.* and *Bacteroides sp.* are all common anaerobes that are able to ferment hydrocarbons under stressed conditions (Álvarez et al., 2006b; Ueki et al., 2011; Mesle et al., 2013). They are prevalent in soils, sewage and ADs (Madigan et al., 2011; Gao et al., 2012; Ke et al., 2014; Li et al., 2013). It is intriguing to see an increased abundance of *Clostridium botulinum*. *Clostridium botulinum* is a typical spore-forming species, generally widespread in soils, sediments, feces and biogas systems (Bagge et al., 2010; Vidal et al., 2013). A common characteristic shared among these four species is that they are able to grow under stressed conditions, and such ability favours them

to possibly outcompete other bacteria during the exchange between the digester and UASB.

During the third stage, most of the key species had lower abundance except for *Lactococcus raffinolactis* and *Cloacibacterium normanense*, whose abundance increased from 2.0% to 6.0% and from 1.6% to 15.8%, respectively. *Lactococcus raffinolactis* apparently recovered in abundance at the third stage, following a possible adaptation to the temperature shock. *Cloacibacterium normanense* is a facultative anaerobic, rod-shaped microorganism that was originally isolated from raw sewage (Allen et al., 2006). This species is able to degrade complex organic matter and hence is frequently discovered in anaerobic wastewater treatment systems (Suihko and Skytta, 2009; Maspolim et al., 2015). The significant increase in the abundance of *Cloacibacterium normanense* might be related to the performance enhancement during the third stage. It is reported that *Cloacibacterium normanense* is able to grow in a broad range of temperatures with distinct growth between 18 and 36 °C (Allen et al., 2006).

There were also some substantial changes in the digester community. First, two very dominant species, *Trichococcus* sp. and *Nostocoida limicola actinobacteria* were gradually eliminated. Their abundance decreased from over 30% to less than 1%. In addition, the relative abundance of *Bellilinea* sp. greatly increased when the sludge transfer point of S3 changed to S2, from 24.6% to 31.2%. Members of *Bellilinea* are obligate anaerobic fermenters that produce VFAs (Ma et al., 2013). The mesophilic strains of *Bellilinea* sp. were originally isolated from rice paddy soil (Ma et al., 2013) and have been found in UASB reactor (Yamada et al., 2007). It has been reported that *Bellilinea* sp. are specialized in propionate-degradation and can cooperate with hydrogenotrophic methanogens under mesophilic conditions (Yamada et al., 2007). Similar to *Bellilinea* sp., another typical syntrophic bacteria, *Carboxydibrachium* sp., also showed an increased abundance from 1.2% at the second stage to 9.8% at the third stage. *Carboxydibrachium* sp. are very versatile (Subbotina et al., 2003; Fardeau et al., 2004). Such versatility can not only guarantee them a wide niche under stressed conditions such as the temperature shock of the digester-UASB exchange, but also enable cooperation with acetoclastic and hydrogenotrophic methanogens. The presence of a stable methanogenic community agrees with the improved biogas production in the third stage.

The archaeal community of the UASB was also affected by the inclusion of the digester (Figure 7.9). The acetoclastic methanogen *Methanosaeta sp.* was the most dominant archaea in the UASB before incorporation of the digester, but its relative abundance decreased from 83.7% at the first stage to 64.3% at the second stage after digester coupling (Figure 7.9, Table D.2, Appendix D). The UASB community was again dominated by acetoclastic *Methanosaeta sp.* at the third stage, with an abundance as high as 86.9%. A hydrogenotrophic methanogen, *Methanospirillum sp.*, had a high abundance (28.6%) at the second stage, whereas this species was not detectable before the digester coupling. The increase in hydrogenotrophic methanogens indicates the presence of syntrophic associations in the UASB (Stams and Plugge, 2009). *Methanosaeta* prevailed in all the digester samples with high abundance over 80%. It has been reported that *Methanosaeta sp.* have high substrate affinity, which makes them dominant in the low residual acetate conditions like the current system (Bialek et al., 2013).

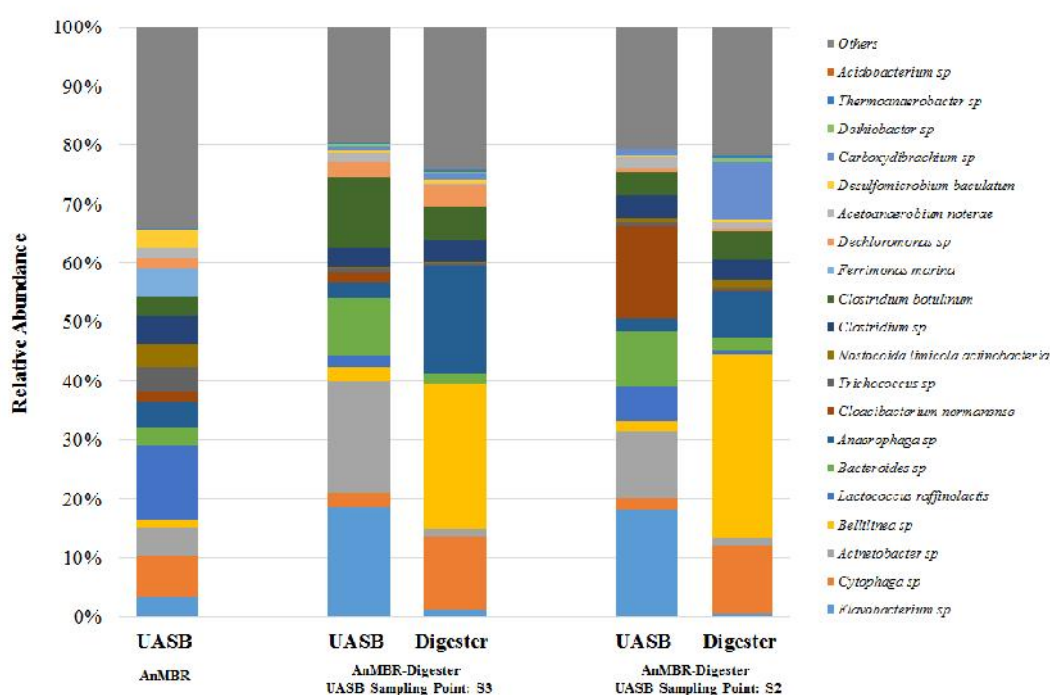


Figure 7.8 : Relative abundance of major bacterial species in the digester and UASB during AnMBR, AnMBR-Digester (UASB Sampling Point: S3), AnMBR-Digester (UASB Sampling Point: S2) operation.

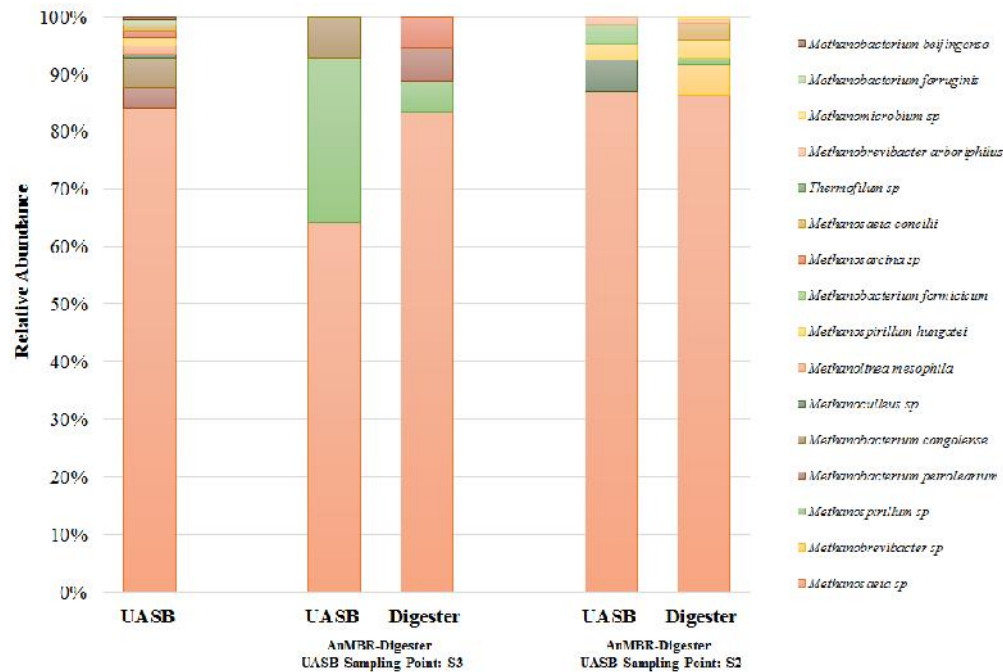


Figure 7.9 : Relative abundance of all archaeal species in the digester and UASB during AnMBR, AnMBR-Digester (UASB Sampling Point: S3), AnMBR-Digester (UASB Sampling Point: S2) operation.

7.4 Conclusion

The results of these investigations reveal the high potential of the new configuration AnMBR-Digester system for treating sewage at 15 °C as it combines wastewater treatment and sludge stabilization. The filtration performance of the investigated single stage AnMBR was limited by the low temperature. Single stage AnMBR operation at 15 °C led to organic solids accumulation in the UASB reactor and thus, greatly influenced the characteristics of UASB effluent, resulting in an increase in COD concentration and turbidity and a decrease in D50, which led to a significant increase in the R_T . Similar TMP increase profiles were observed during the operational period after digester incorporation with sludge transfer from S3, which coincided with the slight changes in PSD, turbidity and COD in the UASB effluent. However, change in sludge transfer location from about 75% of the sludge bed height to about 40-50% of the sludge bed height after digester incorporation had remarkably influenced the characteristics of UASB effluent, resulting in a decrease in turbidity, soluble and total COD and an increase in D50, which led to a significant decrease in the R_T . Improved stability and SMA of the sludge were achieved in the UASB reactor with an increase in total biogas production. The sludge recirculation

from S2 in the AnMBR-Digester system improved both the solids physical removal and the conversion, confirming the importance of selecting the proper sludge transfer point, especially for the filtration performance of the AnMBR-Digester systems. Therefore, under optimized sludge recirculation conditions, the integrated AnMBR-Digester system represents an efficient technology for anaerobic sewage treatment in order to mitigate rapid membrane fouling for the low temperature applications of membrane coupled UASB reactors.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions from the Various Research Steps

Upgrading of existing anaerobic treatment processes with membranes can be of vital importance, especially when high effluent quality and/or use of treated effluents are considered. In recent years, the AnMBR has been gaining attention as a means to treat low strength wastewater due to its inherent benefits including shorter start-up periods, smaller footprint, superior effluent quality leading to reuse and recycling possibility, complete biomass retention without any concern of sludge washout, and energy recovery through methane production (Ho and Sung, 2010; Ozgun et al., 2013a; Wei et al., 2014). AnMBR effluents are characterised by a very high reduction in pathogen content, which makes them suitable for agricultural use (Saddoud et al., 2006; Ellouze et al., 2009). Therefore, an AnMBR can be used not only for on-site wastewater treatment, but also for the generation of nutrient-rich irrigation water for agricultural applications (Prieto et al., 2013). However, the wide application of the AnMBRs in domestic utilities, especially in full-scale systems, has not yet become a reality, which is partly because of system novelty and reluctance owing to membrane fouling problems (Chu et al., 2005). Therefore, AnMBR systems will likely benefit from the development of efficient technologies to prevent fouling.

Treatment of municipal wastewater by different types of anaerobic bioreactors in AnMBRs has drawn considerable attention of many researchers. Different types of anaerobic bioreactors, including CSTR, UASB, EGSB, etc., have been investigated in combination with various types of membranes in different configurations (Ozgun et al., 2013a). In high-rate anaerobic reactors such as sludge bed systems and anaerobic filters, biomass is retained either by the formation of granular and/or thick flocculent sludge or by attachment to a support material. Because in membrane coupled anaerobic high-rate reactors biomass is not directly subjected to membrane filtration, dense cake layer formation and consolidation will be less apparent in comparison with membrane coupled CSTRs. Therefore, UASB reactors may offer a good opportunity for being combined with membranes. However, the impact of

membrane application on sludge immobilization and sludge bed stability is not yet well understood.

Despite the expected more efficient membrane filtration step when applying sludge bed reactors, for the treatment of municipal wastewater under sub-mesophilic conditions, hydrolysis of the retained particulates likely becomes rate-limiting and particulate matter accumulation in the sludge bed will occur, subsequently resulting in activity loss (Lettinga et al., 2001). A significant reduction in the hydrolysis rate of solids is typical at low temperatures due to the low activity of anaerobic microorganisms (Lettinga et al., 2001; Zhang et al., 2010). Methane loss is another important issue of concern especially at low temperature, since methane solubility in the liquid phase increases with decreasing temperature. Gimenez et al. (2012) proposed biogas-assisted mixing as a limitation for super-saturation and a guarantee for a minimum concentration of dissolved methane in the effluent of AnMBR systems. Furthermore, a thinner and less porous cake layer on the membrane surface can be expected, possibly leading to more severe pore clogging in UASB coupled AnMBRs, because the membrane would not be protected by a cake layer, but exposed directly to only fine particles instead of a range of particle sizes. Therefore, the degree of small particle retention by the sludge bed will be of prime importance for the feasibility of coupled membrane filtration. Further studies should be done and new configurations need to be evaluated in order to mitigate rapid membrane fouling in membrane coupled UASB reactor systems under low temperature conditions.

An integrated AnMBR-Digester system may offer a solution for these challenges. The combination of a UASB and a digester, the so-called UASB-Digester system, has been shown to be successful for mutual sewage treatment with a high concentration of suspended organic solids at low temperature and sludge stabilization (Álvarez et al., 2004; Mahmoud et al., 2004; Mahmoud, 2008; Mahmoud et al., 2008; Lew et al., 2011; Zhang et al., 2012).

This thesis investigated the development and optimization of AnMBR technology for municipal wastewater treatment enabling energy recovery and effluent reuse in irrigation. A membrane coupled UASB reactor was used to produce high quality and nutrient-rich effluent for reuse purposes. Moreover, a digester was incorporated with the AnMBR system to solve the bottlenecks of AnMBRs under moderate climate conditions.

Based on the various studies and results presented in this dissertation, the following conclusions can be drawn.

In Chapter 3, an optimum upflow velocity was identified that will result in an effluent with good filterability for the case of membrane coupled UASB systems. Despite the fact that the functioning of UASB systems depends on both physical parameters and biological processes, the physical parameters have been barely reported in literature. The reason is that the underlying mechanisms are complex and depend on various interrelated parameters. In addition, the lack of a serious attempt to gather the entire physical theme in one picture, have resulted in just a superficial understanding of this field of science. Better understanding of the interaction and role of these parameters is essential for the development of anaerobic treatment technologies (Mahmoud, 2002). One of the most important parameters that might affect the solids-liquid separation process by filtration through the sludge bed of a UASB reactor is upflow velocity. Thus, upflow velocity plays a major role in controlling the fouling potential that is one of the most important challenges for stable operational process performance of membrane coupled UASB reactors. A high upflow velocity in a UASB coupled membrane system resulted in an increased load of small particles to the membrane. PSD of the effluent varied significantly with variation in upflow velocity. Effluent characterization results coincided with filterability tests. The increased effluent particle size at the lower upflow velocity was associated with a better filterability. Better filterability was obtained with an upflow velocity of 0.6 m/h in comparison to 1.2 m/h. Results show that upflow velocity is an important factor affecting the effluent filterability in UASB reactors.

In Chapter 4, the impact of membrane integration on the performance of the UASB reactor was assessed based on both biological removal efficiency and physical aspects. An upflow velocity of 0.6 m/h was selected based on the results of Chapter 3. Coupling a membrane to a UASB reactor resulted in significant changes in both physical and biological aspects, mainly due to the elimination of hydraulic dilution as bacterial selection pressure factor. EPS content decreased and a shift in PSD of the sludge to smaller particles was observed in the UASB reactor after membrane addition. Decrease in the sludge particle size deteriorated the sludge bed settleability, which led to a higher sludge washout, resulting in an increase in COD, TSS and SMP concentrations of the UASB effluent. A decrease in the sludge activity was observed

after membrane incorporation. Stability tests confirmed that the decrease in SMA was related to the particulate matter accumulation. However, SS-free permeate with an average COD of 42 mg/L was obtained and despite the sludge bed deterioration, the average TMP value was 85 mbar during the entire operation period indicating that no severe membrane fouling occurred in the AnMBR. Overall system performance increased in terms of COD removal efficiency and methane production after membrane incorporation. Considering all responses of the UASB reactor to membrane incorporation, the overall results put UASB reactor forward as a suitable alternative for coupling membranes in AnMBRs at 25 °C.

In Chapter 5, the effect of low temperature on the removal efficiency and filterability of the AnMBR has been investigated. Temperature affected the filtration performance of the membrane coupled UASB reactor. Decreasing temperature from 25 °C to 15 °C greatly influenced the characteristics of UASB effluent, which led to a significant increase in the R_T . However, overall treatment performance of the AnMBR was not affected significantly because of the physical removal capacity of the membrane. More severe fouling occurred at 15 °C in comparison to 25 °C. Higher SMP production at lower temperature induced more membrane pore blocking and enhanced cake layer development on the membrane surface. Based on the overall results, it is concluded that membrane coupled UASB configuration is not found technically feasible for the treatment of municipal wastewater at 15 °C considering the deterioration of the filtration performance, which would be a bottleneck to the practical engineering application of AnMBRs.

In Chapter 6, the sludge characteristics along the height of the UASB reactor were investigated in terms of sludge morphology, activity and stability in order to identify the most appropriate sludge transfer point between the UASB reactor and digester. Differences were observed in sludge characteristics at different reactor heights along the UASB reactor. Membrane incorporation led to a deterioration in the sludge bed settleability due to the accumulation of particles (Chapter 4 and Chapter 5); however, stratification was still pronounced in sludge characteristics along the UASB reactor height and the reactor biomass became stratified into several zones. The lower zone of the UASB reactor had dense granulated biomass, whereas the upper zone had loose active and non-active biomass. The highest SMA was obtained in the sludge taken from the bottom of the UASB reactor. The active biomass remained near the

inlet of the reactor; whereas, non-active biomass consisting of loose, suspended particles and flocs accumulated at the top part of the sludge bed. Treatment was achieved by a stratified microbial habitats responsible for removing organic matter in the membrane coupled UASB reactor. Because of sludge stability and solid content stratification, the sludge to be recirculated from UASB reactor to digester is recommended to be taken from about 40-50% of the sludge bed height where the sludge has the lowest stability.

In Chapter 7, the impact of digester coupling on treatment and filterability performance of the AnMBR was evaluated by transferring the digested sludge to the UASB reactor to improve its methanogenic capacity. Sludge recirculation from the sludge transfer point S2, located at 40-50% of the sludge bed height in the AnMBR-Digester system, improved both the solids physical removal and conversion, confirming the importance of sludge transfer point location. The AnMBR-Digester system with the proper sludge transfer point provided substantially better removal efficiencies than the single stage AnMBR. Permeates with a very low or maybe negligible pathogen content which is nutrient rich can be obtained with the AnMBR-Digester system at stable TMP enabling compliance with the regulations for water reuse. The results show that the sludge in the UASB of the AnMBR-Digester system was substantially more stable compared to the sludge in the single stage AnMBR. Also, the digester sludge has substantially higher stability than the excess sludge from the UASB of the AnMBR-Digester system. Under optimized sludge recirculation conditions, the integrated AnMBR-Digester system represents an efficient technology for anaerobic sewage treatment in order to mitigate rapid membrane fouling for low temperature applications of membrane coupled UASB reactors.

8.2 Main Outcomes and Evaluation

SUR test proved to be capable of providing information about the filterability of the UASB effluent since effluent characterization results coincided with the filterability and reversibility tests. The reversibility and SUR values of the UASB effluent were reciprocally correlated.

The colloidal and soluble fractions ($<1\ \mu\text{m}$) play an important role in the filterability of the UASB effluent. The effluent filterability goes hand in hand with the amount of

submicron particles, which in turn is influenced by the upflow velocity. Therefore, the amount of submicron particles is very likely an important factor determining effluent filterability.

The upflow velocity (Chapter 3) does not have a strong influence on the overall AnMBR functioning since incorporation of the membrane to the UASB reactor results in significant differences in the UASB effluent (Chapter 4).

The prevailing selection pressure proved to be capable of providing sludge with good settling properties (Chapter 4). Under the applied experimental conditions when the UASB reactor was operated as the sole process before membrane incorporation, light and dispersed sludge was washed out while heavier particles were retained in the reactor. After membrane incorporation, more dispersed biomass was clearly visible in the UASB reactor, indicating the presence of a bulking type of sludge with very poor settling characteristics. We therefore postulate that the addition of the membrane barrier eliminates the hydraulic selection pressure required for a sludge bed with good settling properties in the UASB reactor.

The metabolic capacity of the sludge sampled under the prevailing conditions were assessed by using SMA tests. Addition of the membrane resulted in a significant drop in the SMA, which can likely be attributed to the changes in both physical and biological properties of the sludge. After membrane addition, influent particulate matter accumulated within the system, due to the presence of a physical barrier, thus lowering the SMA. Stability tests may be performed in order to test the extent of non- or slowly biodegradable matter accumulation in the sludge bed.

Temperature is a dominating parameter influencing the filtration performance of the AnMBR, which was shown to be linked to the UASB effluent characteristics. Therefore, seasonal temperature changes which are apparent under full-scale conditions, will likely impact both operation and performance of the AnMBR similar to full-scale aerobic MBR reactors (Krzeminski et al., 2012). However, membrane separation allows to achieve a relatively stable quality of permeate despite the changes in temperature. The quality of permeate is most likely not affected by the changes in filterability.

Flux-step determination of the critical flux should not be used to predict long-term TMP behaviour in real AnMBR systems (Chapter 5), which supported the former

hypothesis of Le Clech et al. (2003). This method is based on a short-term run, but nonetheless provides useful data on comparative fouling propensity.

Cake layer composition is directly related to the D50 and PSD. The presence of particles $<1\ \mu\text{m}$ instead of TSS concentration, determined the cake layer resistance and thereby the achievable operational fluxes. The cake layer resulting only from small particles showed a compact structure, characterised by a higher resistance and lower fluxes. In contrast, the cake built up with bigger particles was more porous and therefore allowed a higher permeate flow (Chapter 5), which agrees with previous findings (Jeison et al., 2009b). The foulant layer had been built up on the surface, whereas the fouling of the membrane pores was insignificant. However, an increase in the contribution of the irreversible fouling resistance to the R_T of the system indicated more pore blocking, which is a drawback of the extension of the membrane filtration period, applying fluxes exceeding the critical flux.

Even though membrane incorporation led to a deterioration in sludge bed settleability, a.o. due to the accumulation of particles, sludge bed stratification was still pronounced, as evidenced by the sludge characteristics along the UASB reactor height. After prolonged periods of operation, the reactor biomass became segregated into several zones (Chapter 6).

The selection of the type of anaerobic reactor apparently has a strong influence on the overall AnMBR functioning. Compared to AnMBRs consisting of a CSTR, membrane fluxes may become less dependent on the reactor MLSS concentration, possibly leading to high membrane fluxes in AnMBRs consisting of UASB reactors at ambient temperatures. Depending on the operational temperature, the digester can be by-passed. However, for low temperature applications, the incorporation of a sludge digester in an AnMBR system is indispensable to improve the filtration performance of the system.

Optimization of sludge recirculation should be done carefully in AnMBR-Digester systems in order to maintain complete conversion of biodegradable dissolved COD. For instance, selection of the optimum sludge transfer point resulted in significant changes especially for the filtration performance of AnMBR-Digester systems (Chapter 7).

8.3 Future Perspectives and Recommendations for Further Research

Based on the conducted research described in this dissertation several aspects that deserve attention and require further focus are proposed.

- ✓ The excellent performance of the AnMBR-Digester system requires confirmation by long-term bench-scale studies under defined conditions, along with relevant research at pilot-scale. However, the technology requires optimization in terms of digestion and compactness. When scaled-up, biogas utilization for heating the digester should be considered.
- ✓ More research is needed into the interaction between the sludge physicochemical characteristics, digestion conditions and sludge physical behavior. The integrated AnMBR-Digester system requires further fine tuning, especially with respect to the size of the digester and recirculation rate between the digester and the AnMBR.
- ✓ The research presented in this thesis has been conducted in order to assess the feasibility of anaerobic treatment of municipal wastewater under low temperature conditions. Considering the wide variety of sewage influent characteristics, the AnMBR-Digester system should be studied under field conditions, which so far is only limitedly described in literature. Recently, Zhang et al. (2012) reported the feasibility of the UASB-Digester system for the treatment of domestic sewage at low temperature with a low influent dissolved COD to total COD ratio.
- ✓ Inorganic fouling by the precipitation of struvite, potassium ammonium phosphate and/or calcium carbonate may be one of the major concerns in fouling due to the release of ammonia and PO_4^{3-} from organic nitrogen and phosphorus during AD and the pH increase as a result of changes in CO_2 partial pressure and alkalinity generation in AnMBRs (Liao et al., 2006). Salazar-Pelaez et al. (2011b) estimated that the chemical equilibrium for struvite precipitation is not reached in municipal wastewaters with lower concentrations of $\text{NH}_4\text{-N}$, PO_4^{3-} and magnesium compared to industrial wastewaters. Therefore, struvite precipitation would unlikely occur. However, not only the concentration of these ions but also the membrane properties can play a significant role in struvite precipitation (Kang et al.,

2002). It is therefore recommended, to further investigate the possible occurrence of struvite in AnMBRs treating municipal wastewaters and its impact on irreversible fouling.

- ✓ One of the major concerns about using treated municipal wastewater in agriculture is related with the removal of toxicity and endocrine disrupting chemicals. Industrial discharges to municipal sewers can cause serious problems such as toxicity and organic shock loads in the end-of-pipe wastewater treatment plants. Various studies address the removal of endocrine disrupting chemicals in municipal wastewater treatment by MBR systems (Cases et al., 2011; Tadkaew et al., 2011; Boonyaroj et al., 2012). In aerobic MBRs, phenolic compounds, phthalates and estrogens can be more effectively removed in comparison to conventional activated sludge systems through biodegradation, adsorption and membrane rejection mechanisms (Cases et al., 2011). However, there exists quite limited information about their fate and biodegradation in AnMBRs (Ho et al., 2007). Obviously, more research should be conducted on the fate of these priority pollutants in AnMBR systems.
- ✓ Salinity can be another problem for the treatment of municipal wastewater, especially for coastal residential areas with improper infrastructure which allows the infiltration of sea water into sewer systems. In this situation, more attention should be given to fouling control in order to have a feasible treatment technology.
- ✓ When agricultural use of the treated effluent is not considered, a nutrient removal step is generally required to comply with discharge standards. Innovative solutions as a nitrogen polishing step with Anammox processes and autotrophic nitrogen removal through post-treatment are currently investigated in the water line of wastewater treatment and can be integrated. These processes are disturbed by presence of solids, organic compounds and COD in the water. AnMBR-Digester systems will produce more clean effluents and are therefore better compatible with these systems.
- ✓ A synthetic municipal wastewater recipe that aims to mimic a real municipal wastewater was used in the study. However, real wastewater may contain

antibiotics and other organic pollutants from domestic and industrial discharges, which seldom appears in the synthetic wastewater. Therefore, further experiments should be performed with real wastewater in order to confirm the comparability of the synthetic wastewater composition to real wastewater.

- ✓ Alternative membrane materials, reactor and membrane types, and membrane integration possibilities for the AnMBR technology in municipal wastewater treatment schemes are of interest for further development (Ozgun et al., 2013a). More research should be conducted to understand the impact of different membrane materials and membrane integration possibilities. Based on our results we speculate that membrane coupled UASB reactor may result in the first applications of nanofiltration coupled to bioreactors. Besides, different membrane integration possibilities, including the one that employs the membrane unit as a polishing step after the UASB reactor without the concentrate stream return to the bioreactor, need to be investigated.

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APPENDICES

APPENDIX A: Table A.1 and Table A.2

APPENDIX B: Table B.1 and Table B.2

APPENDIX C: Table C.1 and Table C.2

APPENDIX D: Table D.1 and Table D.2

APPENDIX A

Table A.1 : The dominant bacterial species detected by pyrosequencing in the inoculum and sludge samples before and after membrane addition.

Species	Relative Abundance (%) ^a		
	Seed Sludge	Before membrane addition	After membrane addition
<i>Cytophaga sp</i>	16.5	18.6	28.6
<i>Bacteroides sp</i>	13.1	8.2	1.8
<i>Anaerophaga sp</i>	6.0	2.6	3.1
<i>Flavobacterium sp</i>	0.0	4.1	10.1
<i>Mucilaginibacter rigui</i>	0.0	1.7	0.0
<i>Desulfomicrobium baculatum</i>	0.0	11.8	1.2
<i>Syntrophus sp</i>	2.6	0.6	0.6
<i>Desulfovibrio carbinolicus</i>	0.0	0.0	0.6
<i>Geobacter sp</i>	0.6	0.2	0.7
<i>Aeromonas sharmana</i>	0.0	4.5	4.0
<i>Aeromonas sp</i>	0.0	6.1	1.3
<i>Aeromonas schubertii</i>	0.0	3.0	0.1
<i>Pseudoalteromonas sp</i>	3.0	0.3	0.1
<i>Acinetobacter sp</i>	0.0	1.6	2.7
<i>Dechloromonas sp</i>	0.0	1.4	0.1
<i>Ralstonia sp</i>	0.3	0.0	2.4
<i>Acidovorax sp</i>	0.0	1.4	2.0
<i>Hydrogenophaga sp</i>	0.0	0.2	0.8
<i>Imtechium assamiensis</i>	0.0	0.2	0.8
<i>Comamonas sp</i>	0.0	0.6	0.8
<i>Variovorax sp</i>	0.0	0.0	0.7
<i>Bellilinea sp</i>	5.1	5.0	2.1
<i>Levilinea saccharolytica</i>	0.4	3.4	2.0
<i>Chloroflexus sp</i>	0.5	0.2	1.7
<i>Clostridium sp</i>	6.2	3.1	6.1
<i>Thermoanaerobacter sp</i>	8.2	0.3	0.1
<i>Dethiobacter sp</i>	8.5	0.1	0.2
<i>Acetoanaerobium noterae</i>	0.0	1.3	1.0
<i>Acidaminococcus sp</i>	2.3	0.1	0.0
<i>Fusibacter sp</i>	0.0	0.0	1.8
<i>Syntrophomonas sp</i>	0.4	0.3	0.6
<i>Trichococcus sp</i>	0.0	1.4	1.1
<i>Bacillus sp</i>	0.5	1.1	0.5
<i>Carnobacterium maltaromaticum</i>	0.0	1.1	0.4
<i>Enterococcus sp</i>	0.0	1.0	0.5
<i>Leadbetterella sp</i>	0.0	0.0	0.7
<i>Acidobacterium sp</i>	5.3	0.1	0.1
<i>Synergistes sp</i>	1.3	0.8	0.4
<i>Spirochaeta sp</i>	0.3	0.7	1.5
<i>Leptospira sp</i>	0.9	0.4	1.1

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

Table A.2 : The all archaea detected by pyrosequencing in the inoculum and sludge samples before and after membrane addition.

Species	Relative Abundance (%) ^a		
	Seed Sludge	Before membrane addition	After membrane addition
<i>Methanobacterium beijingense</i>	2.1	0.0	2.7
<i>Methanobacterium ferruginis</i>	0.0	29.3	0.0
<i>Methanobacterium oryzae</i>	0.0	2.4	1.4
<i>Methanobacterium petrolearium</i>	14.6	24.4	15.1
<i>Methanobacterium sp</i>	0.0	24.4	16.4
<i>Methanobrevibacter sp</i>	43.8	4.9	8.2
<i>Methanolinea mesophila</i>	14.6	0.0	0.0
<i>Methanomicrobium sp</i>	2.1	0.0	0.0
<i>Methanosaeta sp</i>	14.6	14.6	56.2
<i>Candidatus Nitrosocaldus yellowstonii</i>	2.1	0.0	0.0
<i>Thermofilum sp</i>	6.3	0.0	0.0

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

APPENDIX B

Table B.1 : The dominant bacterial species detected by pyrosequencing in the inoculum and sludge samples at different operational temperatures.

Species	Relative Abundance (%) ^a		
	Seed Sludge	T= 25 °C	T= 15 °C
<i>Cytophaga sp</i>	16.5	28.6	7.1
<i>Bacteroides sp</i>	13.1	1.8	2.9
<i>Desulfomicrobium baculatum</i>	0.0	1.2	3.1
<i>Bellilinea sp</i>	5.1	2.1	1.2
<i>Aeromonas sp</i>	0.0	1.3	0.6
<i>Clostridium sp</i>	6.2	6.1	4.7
<i>Anaerophaga sp</i>	6.0	3.1	4.5
<i>Aeromonas sharmana</i>	0.0	4.0	0.2
<i>Thermoanaerobacter sp</i>	8.2	0.1	0.1
<i>Dethiobacter sp</i>	8.5	0.2	0.1
<i>Flavobacterium sp</i>	0.0	10.1	3.2
<i>Levilinea saccharolytica</i>	0.4	2.0	0.7
<i>Syntrophus sp</i>	2.6	0.6	0.6
<i>Pseudoalteromonas sp</i>	3.0	0.1	0.0
<i>Acinetobacter sp</i>	0.0	2.7	4.8
<i>Trichococcus sp</i>	0.0	1.1	4.1
<i>Synergistes sp</i>	1.3	0.4	0.5
<i>Dechloromonas sp</i>	0.0	0.1	2.0
<i>Acetoanaerobium noterae</i>	0.0	1.0	1.6
<i>Carnobacterium maltaromaticum</i>	0.0	0.4	1.1
<i>Ralstonia sp</i>	0.3	2.4	0.7
<i>Fusibacter sp</i>	0.0	1.8	0.6
<i>Chloroflexus sp</i>	0.5	1.7	0.4
<i>Spirochaeta sp</i>	0.3	1.5	1.1
<i>Desulfovibrio carbinolicus</i>	0.0	0.6	1.2
<i>Lactococcus raffinolactis</i>	0.0	0.0	12.8
<i>Acidovorax sp</i>	0.0	2.0	0.3
<i>Acidovorax facilis</i>	0.0	0.0	1.7
<i>Leptospira sp</i>	0.9	1.1	0.4
<i>Comamonas sp</i>	0.0	0.8	0.8
<i>Nostocoida limicola actinobacteria</i>	0.0	0.0	3.9
<i>Ferrimonas marina</i>	0.0	0.0	4.8
<i>Clostridium botulinum</i>	0.0	0.0	3.2
<i>Cloacibacterium normanense</i>	0.0	0.0	1.7
<i>Acidobacterium sp</i>	5.3	0.1	0.1
<i>Syntrophorhabdus aromaticivorans</i>	1.3	0.0	0.4
<i>Acidaminococcus sp</i>	2.3	0.0	0.0
<i>Fervidobacterium sp</i>	1.5	0.0	0.0
<i>Eubacterium sp</i>	1.6	0.0	0.0
<i>Hydrogenophaga sp</i>	0.0	0.8	0.6
<i>Imtechium assamiensis</i>	0.0	0.8	0.3
<i>Leadbetterella sp</i>	0.0	0.7	0.0
<i>Variovorax sp</i>	0.0	0.7	0.0

Table B.1 (continued) : The dominant bacterial species detected by pyrosequencing in the inoculum and sludge samples at different operational temperatures.

Species	Relative Abundance (%) ^a		
	Seed Sludge	T= 25 °C	T= 15 °C
<i>Geobacter sp</i>	0.6	0.7	0.2
<i>Syntrophomonas sp</i>	0.4	0.6	0.6
<i>Enterococcus sp</i>	0.0	0.5	0.0
<i>Aeromonas schubertii</i>	0.0	0.1	0.0

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

Table B.2 : The all archaea detected by pyrosequencing in the inoculum and sludge samples at different operational temperatures.

Species	Relative Abundance (%) ^a		
	Seed Sludge	T= 25 °C	T= 15 °C
<i>Methanobacterium beijingense</i>	2.1	2.7	0.5
<i>Methanobacterium ferruginis</i>	0.0	0.0	1.0
<i>Methanobacterium oryzae</i>	0.0	1.4	0.0
<i>Methanobacterium petrolearium</i>	14.6	15.1	3.6
<i>Methanobacterium sp</i>	0.0	16.4	0.0
<i>Methanobrevibacter sp</i>	43.8	8.2	0.0
<i>Methanobacterium congelense</i>	0.0	0.0	5.1
<i>Methanolinea mesophila</i>	14.6	0.0	1.5
<i>Methanomicrobium sp</i>	2.1	0.0	0.5
<i>Methanosaeta sp</i>	14.6	56.2	83.7
<i>Methanosaeta concilii</i>	0.0	0.0	1.0
<i>Candidatus Nitrosocaldus yellowstonii</i>	2.1	0.0	0.0
<i>Thermofilum sp</i>	6.3	0.0	0.0
<i>Methanosarcina sp</i>	0.0	0.0	1.0
<i>Methanoculleus sp</i>	0.0	0.0	0.5
<i>Methanospirillum hungatei</i>	0.0	0.0	1.5

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

APPENDIX C

Table C.1 : The dominant bacterial species detected by pyrosequencing in the sludge samples along the UASB reactor.

Species	Relative Abundance (%) ^a			
	S1	S2	S3	S4
<i>Clostridium botulinum</i>	4.7	7.5	12.7	3.0
<i>Cytophaga sp</i>	20.0	13.1	4.2	2.0
<i>Trichococcus sp</i>	2.7	6.3	8.9	0.2
<i>Nostocoida limicola</i>				
<i>actinobacteria</i>	3.3	6.7	7.5	0.1
<i>Flavobacterium sp</i>	4.7	4.6	5.7	0.8
<i>Anaerophaga sp</i>	2.8	2.1	0.5	6.5
<i>Lactococcus raffinolactis</i>	2.7	1.7	5.7	1.3
<i>Bacteroides sp</i>	2.0	2.2	1.4	5.1
<i>Zoogloea oryzae</i>	1.7	2.9	1.4	1.1
<i>Desulfomicrobium baculatum</i>	3.7	2.4	1.1	0.2
<i>Acidovorax facilis</i>	1.8	2.1	2.1	0.4
<i>Carnobacterium maltaromaticum</i>	1.7	1.5	4.3	0.6
<i>Comamonas sp</i>	1.2	1.3	1.3	0.3
<i>Dechloromonas sp</i>	1.7	1.9	1.1	2.0
<i>Acetoanaerobium noterae</i>	1.8	1.9	2.4	0.4
<i>Bellilinea sp</i>	2.4	2.0	1.7	0.0
<i>Geobacter sp</i>	0.8	1.0	0.2	0.2
<i>Syntrophomonas sp</i>	1.7	0.7	0.8	0.1
<i>Novispirillum itersonii</i>	0.0	0.2	0.1	14.3
<i>Ferrimonas marina</i>	0.0	0.0	0.0	28.0
<i>Cloacibacterium normanense</i>	0.7	0.7	0.7	6.4
<i>Acinetobacter sp</i>	1.0	0.7	1.1	1.5
<i>Aeromonas sp</i>	0.6	0.8	0.5	0.7
<i>Spirochaeta sp</i>	0.9	1.0	0.7	1.7
<i>Desulfovibrio carbinolicus</i>	1.1	0.4	1.4	0.2
<i>Fusibacter sp</i>	1.6	1.1	0.5	0.5
<i>Ralstonia sp</i>	1.7	1.0	1.1	0.1
<i>Synergistes sp</i>	0.4	0.7	0.2	0.1
<i>Dehalobacter sp</i>	0.6	0.6	0.2	0.0
<i>Rhodocyclus sp</i>	0.8	0.7	1.1	0.4
<i>Clostridium sp</i>	5.9	6.8	6.4	2.7
<i>Obesumbacterium proteus</i>	0.1	0.1	0.6	0.2
<i>Levilinea saccharolytica</i>	1.1	0.8	0.5	0.0
<i>Clostridium sporogenes</i>	0.6	0.7	0.8	0.1
<i>Zoogloea caeni</i>	0.8	0.7	0.4	0.2
<i>Syntrophus</i>	1.3	0.5	0.3	0.0
<i>Fusibacter sp</i>	1.6	1.1	0.5	0.5
<i>Rahnella aquatilis</i>	0.0	0.8	0.7	0.7
<i>Leucobacter sp</i>	0.2	0.7	0.6	0.4
<i>Comamonas terrigena</i>	0.8	1.1	0.4	0.0

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

Table C.2 : The all archaea detected by pyrosequencing in the sludge samples along the UASB reactor.

Species	Relative Abundance (%) ^a			
	S1	S2	S3	S4
<i>Methanosaeta sp</i>	94.5	84.7	48.0	31.2
<i>Methanosaeta concilii</i>	0.0	4.2	16.0	22.9
<i>Methanomicrobium sp</i>	0.0	0.0	0.0	22.9
<i>Methanospirillum sp</i>	0.0	0.0	0.0	22.9
<i>Methanoculleus sp</i>	0.0	2.8	12.0	0.0
<i>Methanosarcina sp</i>	0.0	4.2	8.0	0.0
<i>Methanobacterium petrolearium</i>	0.0	0.0	12.0	0.0
<i>Methanobacterium formicicum</i>	0.0	0.0	4.0	0.0
<i>Methanobacterium sp</i>	1.8	1.4	0.0	0.0
<i>Methanobacterium oryzae</i>	0.0	1.4	0.0	0.0
<i>Methanobacterium congolense</i>	0.0	1.4	0.0	0.0
<i>Methanobrevibacter sp</i>	3.6	0.0	0.0	0.0

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

APPENDIX D

Table D.1 : The dominant bacterial species detected by pyrosequencing in the sludge samples from the UASB and digester during AnMBR, AnMBR-Digester (UASB Sampling Point: S3), AnMBR-Digester (UASB Sampling Point: S2) operation.

Species	Relative Abundance (%) ^a				
	AnMBR	AnMBR-Digester		AnMBR-Digester	
	UASB	UASB Sampling Point: S3	Digester	UASB Sampling Point: S2	Digester
<i>Flavobacterium sp</i>	3.2	18.5	1.2	18.1	0.5
<i>Cytophaga sp</i>	7.1	2.4	12.3	1.9	11.6
<i>Acinetobacter sp</i>	4.8	18.9	1.4	11.4	1.3
<i>Bellilinea sp</i>	1.2	2.4	24.6	1.8	31.2
<i>Lactococcus raffinolactis</i>	12.8	2.0	0.0	6.0	0.8
<i>Bacteroides sp</i>	2.9	9.8	1.8	9.3	2.1
<i>Anaerophaga sp</i>	4.5	2.7	18.2	2.0	7.8
<i>Cloacibacterium normanense</i>	1.7	1.6	0.0	15.8	0.2
<i>Trichococcus sp</i>	4.1	0.7	0.4	0.5	0.4
<i>Nostocoida limicola actinobacteria</i>	3.9	0.2	0.1	0.8	1.2
<i>Clostridium sp</i>	4.7	3.2	3.9	3.8	3.5
<i>Clostridium botulinum</i>	3.2	11.8	5.5	4.0	4.7
<i>Ferrimonas marina</i>	4.8	0.0	0.0	0.0	0.2
<i>Dechloromonas sp</i>	2.0	2.8	3.9	0.8	0.5
<i>Acetoanaerobium noterae</i>	1.6	1.5	0.3	1.9	1.2
<i>Desulfomicrobium baculatum</i>	3.1	0.3	0.4	0.2	0.4
<i>Carboxydibrachium sp</i>	0.0	0.8	1.2	1.1	9.8
<i>Dethiobacter sp</i>	0.1	0.5	0.1	0.0	0.5
<i>Thermoanaerobacter sp</i>	0.1	0.1	0.4	0.0	0.6
<i>Acidobacterium sp</i>	0.1	0.0	0.0	0.0	0.0

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

Table D.2 : The all archaea detected by pyrosequencing in the sludge samples from the UASB and digester during AnMBR, AnMBR-Digester (UASB Sampling Point: S3), AnMBR-Digester (UASB Sampling Point: S2) operation.

Species	Relative Abundance (%) ^a				
	AnMBR UASB	AnMBR-Digester UASB Sampling Point: S3		AnMBR-Digester UASB Sampling Point: S2	
		UASB	Digester	UASB	Digester
<i>Methanosaeta sp</i>	83.7	64.3	83.3	86.9	85.8
<i>Methanobrevibacter sp</i>	0.0	0.0	0.0	0.0	5.3
<i>Methanospirillum sp</i>	0.0	28.6	5.6	0.0	1.2
<i>Methanobacterium petrolearium</i>	3.6	0.0	5.6	0.0	0.0
<i>Methanobacterium congolense</i>	5.1	7.1	0.0	0.0	0.0
<i>Methanoculleus sp</i>	0.5	0.0	0.0	5.6	0.0
<i>Methanolinea mesophila</i>	1.5	0.0	0.0	0.0	0.0
<i>Methanospirillum hungatei</i>	1.5	0.0	0.0	2.8	3.0
<i>Methanobacterium formicicum</i>	0.0	0.0	0.0	3.3	0.0
<i>Methanosarcina sp</i>	1.0	0.0	5.6	0.0	0.0
<i>Methanosaeta concilii</i>	1.0	0.0	0.0	0.0	3.0
<i>Thermofilum sp</i>	0.0	0.0	0.0	0.0	0.0
<i>Methanobrevibacter arboriphilus</i>	0.0	0.0	0.0	1.4	0.6
<i>Methanobacterium ferruginis</i>	1.0	0.0	0.0	0.0	0.0
<i>Methanomicrobium sp</i>	0.5	0.0	0.0	0.0	0.6
<i>Methanobacterium beijingense</i>	0.5	0.0	0.0	0.0	0.0

^a The relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

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- Her main research areas are membrane processes, anaerobic biotechnology, wastewater and water treatment technologies, industrial pollution control and environmental economics.
- She was selected as a participant for EC funded Marie Curie Training Course “EF2: Nanostructured materials and membrane modelling and simulation” Actions in 2008.
- In November 2010, she also has joined the TU Delft, Sanitary Engineering Section as a PhD researcher to conduct her PhD studies on anaerobic membrane bioreactors under the supervision of Prof. Cumali Kınacı, Prof. Jules B. van Lier and Assoc. Prof. Henri Spanjers.
- She got the PhD Fellowship Award provided by Turkish Academy of Sciences (TUBA) in 2010.
- She received German Water Partnership-Award Turkey 2015 for young water professionals, specialising in the issue of water and wastewater from her PhD topic.

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