ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

EFFECT OF SALT CONCENTRATION ON THE CHARACTERISTICS OF BIOMASS AND ORGANIC REMOVAL IN AN ANAEROBIC MEMBRANE BIOREACTOR

M.Sc. THESIS

BAŞAK DÖNMEZ

Department of Environmental Engineering

Environmental Sciences And Engineering Graduate Program

JUNE 2016

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

EFFECT OF SALT CONCENTRATION ON THE CHARACTERISTICS OF BIOMASS AND ORGANIC REMOVAL IN AN ANAEROBIC MEMBRANE BIOREACTOR

M.Sc. THESIS

BAŞAK DÖNMEZ
(501131737)

Department of Environmental Engineering

Environmental Sciences And Engineering Graduate Program

Thesis Advisor: Prof. Dr. Fatoş GERMİRLİ BABUNA
Thesis Co-Advisor: PDEng. Ir. Julian D. MUÑOZ SIERRA

JUNE 2016

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ANAEROBİK MEMBRAN BİYOREAKTÖRLERDE TUZ KONSANTRASYONUNUN ÇAMUR ÖZELLİKLERİ VE ORGANİK KİRLETİCİ GİDERİMİ ÜZERİNE ETKİSİ

YÜKSEK LİSANS TEZİ

BAŞAK DÖNMEZ
(501131737)

Çevre Mühendisliği Anabilim Dalı

Çevre Bilimleri ve Mühendisliği Yüksek Lisans Programı

Tez Danışmanı: Prof. Dr. Fatoş GERMİRLİ BABUNA
Eş Danışman: PDEng. Ir. Julian D. MUÑOZ SIERRA

HAZİRAN 2016

Başak DÖNMEZ, a M.Sc. student of ITU Graduate School of Science Engineering and Technology 501131737, successfully defended the thesis entitled "EFFECT OF SALT CONCENTRATION ON THE CHARACTERISTICS OF BIOMASS AND ORGANIC REMOVAL IN AN ANAEROBIC MEMBRANE BIOREACTOR", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Fatoş GERMİRLİ BABUNA** Istanbul Technical University	.....
Co-advisor :	**PDEng. Ir. Julian D. MUÑOZ SIERRA** Faculty of Civil Engineering and Geosciences, Department of Water Management, Section Sanitary Engineering, Delft University of Technology	.....
Jury Members :	**Doç. Dr. Nevin YAĞCI** Istanbul Technical University	.....
	Yrd. Doç. Dr. Mustafa Evren ERŞAHİN Istanbul Technical University	.....
	Prof. Dr. Neşe TÜFEKÇİ Istanbul University	.....

Date of Submission : 02 May 2016
Date of Defense : 10 June 2016

To my beloved family,

FOREWORD

First of all, I would like to thank to my supervisor Prof. Dr. Fatoş Germirli Babuna for giving me this great opportunity to go abroad and collaborate with TU Delft for my graduation project. During my master's studies both professional and personal in every situation she was a great advisor and supporter for me. Thank you so much for your precious advices and help.

Secondly, I would like to thank to my co-supervisor PDEng. Ir. Julian Munoz Sierra for accepting me to his project to work together. I had this great experience to work abroad with a broad research group in one of the best technical university in Europe. I had the chance to collaborate with so many experts in their fields and learn so many new things about my field. I have enhanced my knowledge, personal and professional perspective. The last one year was not easy for me all the time, since I was away from everything that I was used to. But in this period both for my work and the issues I have faced, he always helped and guided me.

As I said, it was one of the best experiences I had so far the last year when I was in Delft. I met with so many precious and nice people who always helped me and always with me when I needed. They made my days easier and fun in Delft. I have also learned so many things from different cultures and backgrounds. Therefore, I would like to thank to all my friends in Delft.

I would like to thank to my dear friends in Istanbul, who were always with me through thick and thin even if we were far away from each other for thousands of kilometers. They always supported me.

Last but not least, I would like to thank to my beloved family for supporting me in every decision that I made and making. I love you much and thank you for always being with me and making me a better person.

This research was carried out in the framework of the Dutch Technology Foundation (STW) Project No.13348.

May 2016

Başak DÖNMEZ
(Environmental Engineer)

TABLE OF CONTENTS

Page

ABBREVIATIONS

AnMBR : Anaerobic Membrane Bioreactor
ATP : Adenosine Triphosphate
BATH : Bacterial Adhesion to Hydrocarbons
BMP : Biomethane Potential Test
BOD : Biochemical Oxygen Demand
BSI : Biomass Stress Index
CER : Cation Exchange Resin
CFSTR : Continuous Flow Stirred Tank Reactor
COD : Chemical Oxygen Demand
CST : Capillary Suction Time
CSTR : Continuous Stirred Tank Reactor
dATP : Dissolved ATP
DCB : Divalent Cation Bridging
DOC : Dissolved Organic Carbon
EGSB : Expanded Granular Sludge Bed Reactor
EPS : Extracellular Polymeric Substances
FCM : Flow Cytometry
HRT : Hydraulic Retention Time
IE : Ion Exchange
LB-EPS : Loosely Bound EPS
MBR : Membrane Bioreactor
MEE : Multiple-Effect Evaporators
MLSS : Mixed Liquor Suspended Solids
MLVSS : Mixed Liquor Volatile Suspended Solids
OLR : Organic Loading Rate
PBS : Phosphate-Buffered Saline Solution
PI : Propidium Iodide
PN : Protein
PS : Polysaccharide
PSD : Particle Size Distribution
PVDF : Polyvinylidene Fluoride
RH : Relative Hydrophobicity
RLU : Relative Light Units
RO : Reverse Osmosis
SBR : Sequential Biological Reactor
SEP : Solar Evaporation Pan
SG : SYBR Green
SLR : Sludge Loading Rate
SMP : Soluble Microbial Products
SRF : Specific Resistance to Filtration
SRT : Sludge Retention Time
SS : Suspended Solids
tATP : Total ATP
TB-EPS : Tightly Bound EPS

TDS **:** Total Dissolved Solids
TMP **:** Transmembrane Pressure
TN **:** Total Nitrogen
TOC **:** Total Organic Carbon
TSS **:** The Total Suspended Solids
UASB **:** Upflow Anaerobic Sludge Blanket
UF **:** Ultrafiltration
VFA **:** Volatile Fatty Acids
VSS **:** Volatile Suspended Solids

SYMBOLS

A	**:** Filter Area
c	**:** Solids Concentration
P	**:** Pressure Drop
μ	**:** Filtrate Viscosity
τ	**:** Shear Stress
γ	**:** Shear Rate
r̂	**:** Spesific Resistance

LIST OF TABLES

Page

LIST OF FIGURES

Page

EFFECT OF SALT CONCENTRATION ON THE CHARACTERISTICS OF BIOMASS AND ORGANIC REMOVAL IN AN ANAEROBIC MEMBRANE BIOREACTOR

SUMMARY

Large quantities of saline wastewater are generated by many industrial sectors. Such wastewater containing high salinity and at the same time high organic substitutes, adversely influence the environment, when they discharged without prior treatment. Within the legislations, the removal of the organic compounds and the salinity is becoming stricter before discharge, in many countries. Saline effluents are mostly treated by the physico-chemical processes (e.g. evaporation, coagulation and flocculation, ion exchange and membrane techniques) since the biological treatment is strongly inhibited by salt concentrations. Due to sodium toxicity to sludge, after the treatment of the saline wastewater with conventional biological treatment systems are generating effluents with low quality and low removal efficiencies. Mainly, the performance obtains during a biological treatment depends on a proper adaptation of the microbial community in sludge or the use of halophilic organisms. Microorganisms use two different mechanisms for adaptation to the increases of osmotic pressure at the media. *Salt-in;* involves K^+ uptake and accumulation in the cell body to balance pressure differences between the cell membrane and the media. *Salt-out;* is a strategy uses compatible solutes, which are uncharged and highly water-soluble organic solutes that will be taken from the environment after an osmotic shock occurs. Both strategies are significant to increase the sludge activity and strength to prevent the microorganism against the hypersaline extracellular environment. High levels of salinity have detrimental impacts on physical and biochemical properties of the biomass. Cell plasmolysis, loss of microbial activity, disintegration and dehydration of bacteria cells, flocculation issues result within the exposure of cell to high salinity conditions. According to the disruptions of cell metabolic functions, low organic degradation capacities are attained. Therefore, biological systems are being considerably sensitive due to the changes of salinity level of the wastewater. Studies have been conducted to investigate the behavior of microorganisms during the biological treatment processes treating highly saline wastewaters however, to the best of our knowledge, they have not yet investigate the degradation of aromatic compounds under saline conditions. In this study, a laboratory scale anaerobic membrane bioreactor (AnMBR) treating saline synthetic wastewater under mesophilic conditions was used to examine the influence of sodium concentration on the biological and physical properties of the anaerobic biomass.

Priorly, to determine the robustness of the continuous system and to assess the K/Na ratios for the adaptation of the microorganisms during the sodium stress, two different biomethane potential test carried out using a batch assay by applying different K/Na ratios, with the inoculum sodium concentrations of 16, 20 and

24g$Na^{+}L^{-1}$. During both batch assays the media prepared with 16 g$Na^{+}L^{-1}$ exhibited the highest COD removal efficiencies.

The continuous study was carried out with four different salinity loading cycles, each have lasted for forty days. The sodium concentration range of the inlet solution was 6 to 46 g$Na^{+}L^{-1}$. In order to observe the sodium impact on bacterial activity, the amount and composition of the extracellular polymeric substances (EPS) and adenosine triphosphate (ATP) was examined. The destruction of sodium on cells after exposure was analyzed by determining the total and the damaged cell concentrations with flow cytometry measurements. After the sodium concentration 22 g$Na^{+}L^{-1}$, significant increase of damage on bacterial cells by 84%, a decrease in bacterial activity and less EPS production were obtained. Total suspended solids (TSS) and volatile suspended solids (VSS) analysis were used to detect the concentration of sludge. After the sodium exposure VSS concentration was decreased from 16.4 gL^{-1} to 7.6 gL^{-1}. TSS was found to increase with an increase in the sodium concentrations. Sludge dewaterability and filterability was assessed with capillary suction time (CST) and specific resistance to filtration (SRF) characteristics. Both parameters represented a similar trend to an increase in biomass sodium concentration up to 23 g$Na^{+}L^{-1}$ and decreased. The physical properties were characterized by relative hydrophobicity, particle size distribution (PSD) and viscosity analyses. EPS concentration shows a dominant impact on hydrophobicity and particle sizes due to the changes in sodium concentration. The treatment performance of the system was observed with chemical oxygen demand (COD) removal and phenol degradation capacity of bacteria and the effluent quality was observed with effluent turbidity. The removal efficiency of COD and phenol were decreased with an increase in salinity however, the system was able to overcome the salt fluctuations up to 30 g$Na^{+}L^{-1}$ with an average COD removal by 99%.

ANAEROBİK MEMBRAN BİYOREAKTÖRLERDE TUZ KONSANTRASYONUNUN ÇAMUR ÖZELLİKLERİ VE ORGANİK KİRLETİCİ GİDERİMİ ÜZERİNE ETKİSİ

ÖZET

Endüstriyel sektörler her yıl üretim prosesleri sonucu büyük miktarlarda atıksu üretmektedirler. Sodyum klorür birçok endüstride yaygın olarak kullanılmaktadır. Bu nedenle endüstrilerden kaynaklanan atıksuların yüzde beşinde yüksek tuz konsantrasyonuna rastlanmaktadır. Yüksek miktarda tuzluluk ve organik kirletici madde içeren bu atıksuların ön arıtım işlemlerinden geçirilmeden bertaraf edilmeleri alıcı ortamlar ve çevre açısından büyük tehdit oluşturmaktadır. Günümüzde birçok ülkede yasalar ile alıcı ortama deşarj kriterleri günden güne daha sıkı kontrollere maruz kalmakta, bu nedenle yüksek tuzluluk içeren atıksuların alıcı ortama deşarj öncesi arıtımları daha da önem kazanmaktadır. Tuzlu atıksular geleneksel biyolojik arıtma sistemleri ile arıtılırken yüksek tuz konsantrasyonunun bakterilerin çalışma verimi üzerinde oluşturduğu olumsuz etkiler nedeniyle arıtma verimi önemli ölçüde kısıtlanmaktadır. Arıtma sonrası düşük giderim verimli ve yüksek kirletici konsantrasyonu içeren çıkış suları oluşturulmaktadır. Bu nedenle yüksek tuzluluk içeren atıksuların arıtımında fiziko-kimyasal arıtma sistemleri yaygın olarak kullanılmaktadır (ör. Buharlaştırma, koagülasyon/flokülasyon, iyon değiştirme metodu ve membran ayırma prosesleri). Biyolojik arıtma sistemlerinde bulunan mevcut bakteri türlerinin zamanla tuzlu ortama adaptasyonları mümkün olsa da bu adaptasyon tuz miktarının aniden değişmesi/düşmesi sonucu kolayca kaybolmaktadır. Arıtma verimlerini arttırmak için genellikle tuzlu ortamlarda yaşayabilen (halofilik) mikroorganizmaların sistemde tercih edilmesi ve adaptasyonlarının sağlanması gerekmektedir. Ortamdaki tuz miktarına bağlı olarak hayatta kalabilen halofilik bakteriler üç sınıfa ayrılır; a*z halofilikler;* sodyum klorür konsantrasyonunun yüzde 1-5 olduğu durumlarda yaşayabilirler. *Orta halofilikler;* sodyum klorürün yüzde 5 ile 20 arasında olduğu durumlarda hayatta kalırlar. *Yüksek halofilikler*; ortamdaki sodyum varlığına karşı en çok dayanıklı olan türlerdir ve sodyum klorürün yüzde 20 ile 30 arasında bulunduğu ortamlarda rahatlıkla hayatta kalabilirler. Bu mikroorganizmalar adaptasyonları için iki farklı mekanizma kullanırlar. Bu mekanizmalardan ilki; mikroorganizların bünyelerinde potasyum iyonları (K^+) biriktirerek bakterilerin hücre duvarı ile ortam arasındaki osmotik basıncın ayarlanmasını sağlamalarına dayalıdır. Diğer bir mekanizma ise; ortama, tuza dayanıklı çözünmüş maddelerin eklenerek, mikroorganizmaların bunları bünyelerinde biriktirmesinin sağlanması ve kendilerini tuzun olumsuz etkilerine karşı korumalarını hedef alır. Bu iki mekanizmanın da önemi, ortamda osmotik basıncın yükseldiği durumlarda çamur aktivitesinin ve dayanıklılığının arttırılmasını ve mikroorganizmaların korunmasını sağlamalarıdır.

Ortamda bulunan yüksek miktardaki tuz konsantrasyonu sistemlerdeki çamurun fiziksel ve biyokimyasal özellikleri üzerinde birçok olumsuz ve yıkıcı etkiye sahiptir. Hücrelerin plazma bozulumu, mikroorganizma aktivitesinin kaybı, bakteri hücrelerinin su kaybı ve parçalanması ve flokülasyon oluşum problemleri yüksek tuzluluğa maruz kalmış mikroorganizmalarda sıkça karşılaşılan sonuçlardır. Metabolik aktivitenin bozulması genellikle arıtım sırasında düşük organik kirletici giderimlerine sebep olmaktadır. Bu nedenle tuzlu atık su girdisi olan biyolojik arıtma sistemleri diğer arıtma sistemlerine (fiziko-kimyasal prosesler) göre daha hassastırlar ve düşük giderim verimlerine sahip olmaktadırlar.

Bugüne kadar birçok bilimsel çalışma değişik sistemler ve farklı karakterde atıksular kullanarak mikroorganizmaların tuzlu ortamlarda biyolojik arıtma esnasındaki davranışlarını araştırmış fakat anaerobik arıtma sistemlerinde organik aromatik bileşenlerin giderimleri üzerine detaylı araştırmalar yapılmamıştır. Bu çalışmada mezofilik koşullar altında laboratuvar ölçekli Anaerobik Membran Biyoreaktör kullanılarak, tuzluluk içeren sentetik atıksu arıtımı araştırılmış ve bu arıtma sırasında atıksu içerisinde değişik konsantrasyonlardaki tuzun, anaerobik çamurun fiziksel ve biyokimyasal özellikleri üzerinde oluşturduğu etkiler çeşitli deneylerle incelenmiştir.

Sürekli çalışan sistem ile çalışmalara başlamadan önce, sistemin dayanıklılığını öngörmek ve kullanılacak anaerobik çamurun tuza adaptasyonunu en verimli şekilde sağlamak için hazırlanacak olan sentetik atıksu çözeltilerinde en uygun Potasyum/Sodyum oranının bulunması amacıyla iki farklı biyometan potansiyeli testi gerçekleştirilmiştir. Deneylerde kullanılacak besi ortamı 16, 20 ve 24 gL^{-1} sodyum konsantrasyonlarında çift kopya olarak hazırlanmıştır. İlk set deney düzeneğinde Potasyum/Sodyum oranları her bir besi ortamı için aynı değerde tutulmuştur (0.05). İkinci set deneylerde ise 16, 20 ve 24 gL^{-1} konsantrasyonları için Potasyum/Sodyum oranları sırasıyla 0.03, 0.024 ve 0.02 olarak hazırlanmıştır. Her bir besi ortamından (16, 20 ve 24 gL^{-1}) giderim verimlerini tespit etmek amacıyla belirli aralıklarla numuneler toplanmış ve kimyasal oksijen ihtiyacı (KOİ) ve fenol konsantrasyon analizleri yapılmıştır. Sistemde üretilen metan gazı miktarı, deney sonuna kadar otomatik metan potansiyeli test kontrol paneli tarafından saatlik olarak kaydedilmiştir. Deneyler sonucunda, en yüksek KOİ (yüzde 96) ve fenol (yüzde 99) giderim verimi, Potasyum/Sodyum oranının 0.024 olarak ayarlandığı test ortamında elde edilmiştir. İki farklı sette de en yüksek KOİ giderimi sodyum konsantrasyonun 16 gL^{-1} olduğu durumda gözlenmiştir.

Sürekli çalışan sistemde deneyler dört farklı sodyum yükleme serisi ile gerçekleştirilmiştir. Bütün çalışma boyunca sodyum konsantrasyonu 6 ile 46 gL^{-1} arasında değiştirilmiştir. Her bir yükleme serisi 40 gün sürmüş ve sodyum etkisinin incelenmesi için belirli aralıklarla çamur ve çıkış suyundan numuneler toplanıp, analizler yapılmıştır. İlk yükleme serisinde sodyum konsantrasyonu 16 ile 22 gL^{-1} arasında, 16 gL^{-1} besleme 24 saat ve 22 gL^{-1} besleme 48 saat sürecek şekilde değiştirilmiştir. İkinci yükleme serisinde her bir farklı tuz konsantrasyonu ile hazırlanan besiler 24 saat süresince reaktöre verilmiştir. Tuz içeriği 16 gL^{-1}'den başlanılarak 20, 22, 24 ve 26 gL^{-1} şeklinde arttırılmış ve daha sonrasında yine 24 saat aralıklarla kademeli olarak 16 gL^{-1} konsantrasyonuna düşürülmüştür. Üçüncü yükleme serisinde endüstrilerde oluşturulan atıksuların gerçek karakterlerini yansıtmak ve bu ani değişimlerin sistem üzerindeki etkilerini araştırmak amacıyla, geniş bir tuz konsantrasyonu aralığında hazırlanan çözeltiler (6 - 34 gL^{-1} konsantrasyon aralığında), 12, 24 ve 48 saat süre ile sisteme beslenmiştir. Her gün çözelti değiştirilmeden önce çıkış suyu numuneleri toplanmış ve özelliklerini

belirlemek amacıyla deneyler uygulanmıştır. Dördüncü ve son sodyum yükleme serisinde yüksek tuz konsantrasyonunun etkisini araştırmak amacıyla sentetik atıksu çözeltileri 34 ile 46 gL^{-1} sodyum konsantrasyonu aralığında hazırlanmış ve her biri 24 saat süre ile membran biyoreaktöre beslenmiştir. Sodyum konsantrasyonunun bakteriyel aktivite üzerine yaptığı etkileri gözlemlemek amacıyla mikroorranizmalar tarafından üretilen hücre dışı polimerik maddelerin bileşenleri ve miktarı ve hücre içerisinde depolanan adenozin trifosfat miktarları analiz edilmiştir. Tuza maruz kaldıktan sonra hücrelerde oluşan hasarı belirlemek için akışlı hücre sayımı yöntemi ile çamur içerisinde bulunan toplam hücre konsantrasyonu ve zarar görmüş hücre konsantrasyonu belirlenmiştir. Bu çalışmalar sonucu sodyum konsantrasyonunun 22 gL^{-l}'yi aştığı durumlarda hücre yapısında ciddi ölçüde hasar meydana geldiği ve bu değerden sonra hücrelerin ortalama olarak yüzde 84'ünün zarar görmüş olduğu gözlenmiştir. Ayrıca, hücre içi enerji depolanmasının düştüğü ve daha az hücre dışı polimerik madde üretiminin gerçekleştirildiği görülmüştür. Çalışmalar sırasında çamur konsantrasyonunun değişimini gözlemek amacıyla toplam katı madde ve askıda katı madde ölçümleri gerçekleştirilmiştir. Deney başlangıcından bitimine kadar ortamda artan tuz konsantrasyonu sonucu (16 gL^{-1}'den 36 gL^{-1}'ye) askıda katı madde miktarının başlangıç değeri olan 16.4 gL^{-1}'den deney sonucunda 7.6 gL^{-1}'ye düştüğü ve toplam katı madde miktarının sodyum konsantrasyonundaki değişimlere bağlı olarak pozitif bir şekilde değiştiği gözlenmiştir. Çamurun filtre edilebilirliği ve susuzlaştırılma kapasitesi üzerinde tuzun etkisinin araştırılması amacıyla çamur numuneleri ile kapiler emme süresi ve filtre edilebilirlik direnci deneyleri gerçekleştirilmiştir. Tuz konsantrasyonu 23 gL^{-1}'ye artana kadar iki parametrenin de sodyum değişimlerine karşı birbirleri ile benzer davranışlar sergilediği görülmüş ve tuz konsantrasyonu arttıkça her iki parametrenin de düştüğü fakat 23 gL^{-1} sonrasında tuz konsantrasyonu artmaya devam ettikçe her iki parametrenin de arttığı görülmüştür. Son yükleme serisinde her iki parametrenin de artışının sebebi olarak artan tuz konsantrasyonu etkisinde değişen diğer faktörler (partikül boyut değişimi ve katı madde miktarı) gösterilmiştir. Çamurun fiziksel özelliklerindeki değişimi gözlemlemek amacıyla partikül boyut analizleri, viskozite ve hidrofobisite analizleri yapılmıştır. Bu parametrelerin tuzun doğrudan etkisinden daha çok tuz konsantrasyonları değişimlerine bağlı olarak bakteriler tarafından üretilen hücre dışı polimerik madde konsantrasyonlarından etkilendiği gözlenmiştir. Sistemin her dört yükleme serisi süresince giderim veriminin bulunması amacıyla çıkış suyu numunelerinde KOİ ve fenol konsantrasyonları analiz edilmiştir. Tuz konsantrasyonu arttıkça her iki parametrenin konsantrasyonunun arttığı ve sistemin organik madde giderme veriminin düştüğü sonucu elde edilmiştir. Sistemin 30 gL^{-1} sodyum konsantrasyonuna kadar, tuzun olumsuz etkilerinin üstesinden gelebildiği ve ortalama olarak yüzde 99 KOİ giderim verimi ile çıkış suyu kalitesi elde ettiği görülmüştür.

1. INTRODUCTION

1.1 Importance of the Study

Large amount of wastewaters are been discharged from industries to environment all around the world and 5% of these effluents has saline or hypersaline characteristics, since sodium chloride is broadly used at various industries (Yang et al. 2013; Praveen et al. 2014). Saline conditions defines the water which contains substantial amount of salt (mostly NaCl) and hypersaline conditions defines the liquid with a salinity more than sea water, which is between 31-38 $gNaClL^{-1}$. Also in the municipal sewages salinity might increase due to the addition of water softeners containing sodium chloride or to the use of seawater as raw water for flushing lavatories (Pernetti & Palma 2005). According to the chemical, biological and physical properties of the surrounding environment, all living organisms are exposed to various parameters such as; pH, temperature and nutrient availability. To sense the condition changes in media is significant for microorganisms to be able to adapt and survive. Bacterial cells have two different mechanisms for adaption to the changes in the media (see section Adaptation Strategies) (Roeßler & Müller 2001). Moreover salt concentration is one of the most crucial and frequently changing parameter. Quantity of salts at the discharged water bodies, are mostly dependent on type of industry. Discharging wastewaters containing high salinity and high organic content without any pre-treatment application, is known to negatively affect the aquatic life and agriculture of receiving environment (Lefebvre & Moletta 2006). Since the sodium concentration could be two-three times more than sea water, it has to be controlled in order to prevent the negative effects not only to the surrounding media but also on the performance of biological treatment processes. Conventional treatment systems and cultures are sensitive to the sudden changes in ionic strength (Woolard & Irvine 1995). Moreover, they cannot adapt the environments with a salt concentrations more than 50 gL^{-1} (Lefebvre et al. 2004). They also stated a significant difficulty to operate the conventional systems correctly, when the level of effluent salt concentration (mainly NaCl and KCl) close to 120 gL^{-1}. Increasing salt

concentrations generates metabolic function disruptions and reduce degradation capacities of microbial cells. Elevated salt concentrations lead to cell lysis on microorganisms, which results in increases in effluent solid concentrations. Díaz et al. (2002) stated not only high concentrations but also a rapid reduction of salt concentration or variable salinity also denatures enzymes and disrupts cell membranes. Although conventional cultures could be acclimated to a certain amount of salt concentrations, the adaptation is quickly lost if salinity suddenly drops (Praveen et al. 2014). Therefore, many studies suggested the usage of halophilic bacteria for hypersaline wastewater treatments, since their higher adaptation capacity to saline environments (Lefebvre et al. 2005; Praveen et al. 2014). Yang et al. (2013) explained the resistance of halophilic bacteria by classifying them into three main groups due to their requirements to sodium concentrations (Table 1.1).

The endurance of the microorganisms to broad range of salinity enhances the opportunities for the biological treatment of wastewater, even under high saline conditions.

Table 1.1 : Classification of Halophilic Bacteria due to salinity requirements (Yang et al. 2013)

	Na^+ and NaCl Concentrations		
Classification	**Na^+ (g/L)**	**NaCl (g/L)**	**NaCl (%)**
Slight Halophiles	4.6–19.6	11.7–49.7	1–5
Moderate Halophiles	19.6–78.2	49.7–198.9	5–20
Extreme Halophiles	78.2–117.3	198.9–298.4	20–30

However, to the best of our knowledge, studies have not yet extensively demonstrated the behavior of microorganisms during the biological treatment processes, especially for the degradation of aromatic compounds under saline conditions. Therefore, it is important in this study to investigate the biomass properties and system organic removal performance under saline conditions.

1.2 Objective and the Scope of the Study

The objective of this study is to determine the influence of sodium concentrations on the biological and physical properties of an anaerobic sludge, in terms of bacterial

activity and biomass properties and to discuss the potential of anaerobic membrane bioreactor in saline wastewater treatment. The biological properties of the sludge were determined by assessing the amount and composition of the extracellular polymeric substances (EPS) and adenosine triphosphate (ATP). The physical properties were characterized by examining the sludge filterability, solids concentrations, viscosity, hydrophobicity and particle size distribution. Moreover, biodegradation performance of the system was determined by organic matter removal.

The second chapter represents the literature survey on saline conditions and more specifically the effect of saline conditions on microorganisms, microbial adaptation strategies, impact of sodium on treatment processes and the performance of membrane bioreactors under saline conditions.

The third chapter represents the materials and methods used during this study.

In fourth chapter the results obtained during the study from the experiments were demonstrated.

Finally, in the fifth chapter conclusions of the study were represented.

2. LITERATURE SURVEY – SALINE CONDITIONS

2.1 Industrial Waste Streams under Saline Conditions

Large quantities of saline wastewater, rich in salt and organic matter, are mostly generating by the industries due to usage of high amounts of salts during their production processes. Besides salts, the waste streams might also contain high concentrations of oil, organic acids and heavy metals (Woolard & Irvine 1995). Chemical industry, road de-icing, agricultural applications, food processing, textile and petroleum industry considers as the major source sectors producing saline effluents (Lefebvre & Moletta 2006). Production of soap, detergent, glass, paper, synthetic fibers, plastic, enamel and medicines also discharges saline wastewaters (Yang et al. 2013). Large volumes of brine are also generated during oil and gas recovery operations. In order to prevent the negative impact of saline wastewater on discharged area (e.g. soil, surface or ground water), prior treatment is required and in most of the countries this is tightened by the regulations.

Food Processing Industry. Salts are mainly used as nutrition or as food conservative during food processes. Meat canning/packing, curing, pickled vegetables, dairy products and the fish processing industries was stated as the most salt required industries in agro-food sectors (Lefebvre & Moletta 2006). Since the usage of dry salts and brine solutions to obtain final products, saline wastewaters are being generated in large quantities. In winemaking sectors, alkaline solutions, a mix of water, salt compounds, residual soda and organic matter from wine, are produced in high amounts after usage of calcium tartrate for cleaning the wine tanks (Lefebvre et al. 2004). During see food processing saline conditions are generally stem from seawater, which accompanies when unloading of fish. They also contain organic matter and proteinic nitrogen (Lefebvre & Moletta 2006). Also by using treated sea water during the processes of defrosting, butchering and washing, as a main water source, produces high organic-high salinity wastewater from sea food factories (Ng et al. 2005). Olive oil mills also generate large quantities of saline wastewaters; from

a medium sized olive oil mill around 10 m^3day^{-1} of wastewater discharge. Olive mill effluents contain very high COD values (up to 200 gL^{-1}) and a high concentration of phenols (more than 300 mgL^{-1}) (Stoller 2009). This wastewater is resistant to biological degradation because of its antimicrobial and phytotoxic properties and induces fouling problems on membrane due to the high concentration of pollutants. It threatens the environment by disposal. Olive mill effluents also contain high amounts of suspended and colloidal substances at concentrations about 190 gL^{-1} (Sarika et al. 2005). They stated a successful treatment is required to remove the suspended and colloidal matter, which typically contains proteins, oils and tannins.

Textile Industry. Tanning processes are one of the main sources of saline wastewater production in leather industries. These wastewaters with complex characteristics, due to addition variety of chemicals, are continuously generating in large volumes. Preservation of fresh skins are done by salts (mostly with sodium chloride) and they has to be removed in the tannery before further processing (Lefebvre et al. 2005). For this purpose large amount of water is using, which generates effluents with high salinity, high organic content and high suspended solids.

Petroleum Industry. Moderate, high or extreme saline conditions are induced by a significant number of oil polluted waste streams. Oily wastewater are often generated during the manufacturing processes, transport and refining of crude oil. These produced waters not only contains various kinds of complex chemical compounds but also have salinities in a wide range from fresh water up to three times the salinity of seawater and beyond (Díaz et al. 2002). They also found the increasing salinity, impacts inversely the biodegradation capacity of crude oil.

Chemical Industry. During the production of chemicals such as pesticides, herbicides, polyhydric compounds, organic peroxides and pharmaceuticals, are main sources of hypersaline wastewaters (Woolard & Irvine 1995). Masid et al. (2010) achieved COD removal by 73% and total dissolved solids (TDS) removal by 82%, treating high strength chemical industry water with a combination of ultrafiltration (UF) and reverse osmosis (RO) process. However, they also stated during the treatment studies, because of the high strength and organic concentration of chemical industry wastewater an irreversibly damage was observed with the membranes. In another study, high strength chemical industry wastewater (containing 44.1 gL^{-1} sodium) was assessed for its impact on anaerobic microbial community dynamics

and consequently mesophilic methane generation with biomethane potential tests (BMP) (Venkatakrishnan et al. 2014). Within the 30 days of experiment they obtained slight COD removal by 20% and production of volatile fatty acids (VFA) by 51%.

2.2 Impact of Sodium Chloride on Microorganisms

Detrimental impacts of high salt containing wastewater streams on microbiological activities were reported by many studies (Moon et al. 2003; Zhang et al. 2014; I Vyrides & Stuckey 2009). High levels of salinity is been known to induce high osmotic pressure on bacterial cells which results with plasmolysis, disintegration and dehydration of bacteria cell and loss of cell activity, therefore it leads to a low organic degradation capacities. It induces the production of the polymers and density difference between liquid stream and biomass, which is significant for floc formation and settling rate (Ng et al. 2005). Sodium is being toxic to bacterial cells at high intracellular concentrations since their electrochemical and osmotic interactions with nucleic acids and proteins (Valentine 2007). Moreover, salinity significantly influences the physical and biochemical properties of the biomass, leading to a change of surface charge, hydrophobicity, filterability and bioflocculation of biomass (Sun et al. 2010). Likewise the increases in sodium concentration, decreases of salinity also reported to be induce more serious impacts on microorganisms (Kargi & Dincer 1997). A decrease in BOD removal efficiency was found by 30% when the fresh water sludge was exposed to 30 gL^{-1}. However, after acclimation to high salinity, when the sludge dosed with fresh water the decrease of BOD removal efficiency was 75%. Moreover, the rapid changes were reported to have more adverse impact on microorganisms than gradual changes (Kincannon & Gaudy 1968). During the biological wastewater treatment presence of salts, mainly sodium chloride, has an adverse impact on the performance of processes since it reduces the treatment efficiency due to a direct toxic impact of the salts to disintegration of flocs and granules which leads biomass wash out (Yang et al. 2013).

2.3 Adaptation Strategies of the Microorganisms to Saline Environments

It is important for the microorganisms to adapt the saline conditions and changes in environment in order to avoid cell lysis under low osmolarity or dehydration under

high osmolarity conditions (Martin et al. 1999). There are two different adaptation strategies for bacteria and archaea to maintain a turgor pressure between inside of their cells and a hypersaline extracellular environment. These methods are known as salt-in and salt-out strategies (Roeßler & Müller 2001). Salt-in involves K^+ accumulating in the cell body to balance pressure differences across the cell membrane and salt-out uses compatible solutes which are uncharged and highly water-soluble organic solutes to prevent microorganisms from suffering damage due to osmotic pressure difference. In the salt-in strategy, extreme halophiles accumulate enormous quantities of potassium such as 4 to 7 $molL^{-1}$. Here, high levels of potassium are required for stabilize enzymes. For the salt-out strategy, compatible solutes are solutes that can present at high intracellular concentrations without disturbing metabolism. Both mechanisms are essential for increasing the sludge activity, the excretion of EPS and thus strength of anaerobic sludge particles (Yang et al. 2013).

Compatible Solutes. They will be taken from the environment after an osmotic shock occurs. This phase is been more efficient way because of allowing growth at the same time. Sugars, polyols, α-amino acids, β-amino acids and their derivatives could have been used as compatible solutes (Roeßler & Müller 2001). The most effective one is glycine betaine. Compatible solutes are not only used to balance the osmotic strength of cytoplasm but they are also compatible with macro molecular and cellular functions, influence protein structure and stability and increase the solubility of proteins (Welsh 2000). The main idea of the addition of compatible solutes is to counterbalance the positive charges. An anionic character has given to the solutes by addition of carboxylate, phosphate or sulphate group. The aim is that to equilibrate the high intracellular concentration of inorganic cations which is concentrate in cells. The efficiency of compatible solutes are related with their chemical structures. They can be accumulated up to molar concentrations without inhibiting the enzyme functions. In addition, they can stabilize proteins under unfavorable conditions in order to increase tolerance towards higher salinity, desiccation, freezing and elevated temperatures (Roeßler & Müller 2001). They do not only have a specific adaptation to salt stress but have a general stabilizing property as well. By the addition of compatible solutes, the osmophobic effect, which means an unfavorable interaction between the compatible solute and protein surface, forces the protein to stay in its

correctly folded state. Thus, protein would have less surface to expose to the solvent (Bolen & Baskakov 2001). The peptide backbones are responsible from these effects therefore it is not specific to salt adapted proteins. The adaptation mechanisms could have seen in different ways in different microorganisms (Yang et al. 2013).

K^+ Accumulation. This strategy is the most rapid response after an osmotic shock and its commonly used by extremely halophilic bacteria and halophilic archaea (Roeßler & Müller 2001). In most eubacteria, the accumulation of K^+ is an early response to an increase in external NaCl. K^+ uptake systems are defined to have ubiquity, high rates for ion transport and ability to gate ion flow (Martin et al. 1999). There are three mechanisms for K^+ uptake: Kup system, Trk system and Kdp system. Kup mediates low level K^+ uptake. Trk, which has induced after osmotic shock, mediates low affinity K^+ uptake with a high rate and could be activated at external K^+ concentrations above 1mM. Kdp induced after osmotic shock as well and this mediates high affinity K^+ uptake. Also it could be stated that the affinity values are differ from each other due to the different type of solutes (Roeßler & Müller 2001).

Although the adaptation of the sludge depends on various factors, it is proved to be possible (Lefebvre & Moletta 2006). Campos et al. (2002) found that high salt concentrations did not have long-term effects on the sludge physical properties, retaining high sludge concentrations of 20 $gVSSL^{-1}$ with a sludge volume index (SVI) of 11.4 $mlgVSS^{-1}$. However, Kargi et al. (2000) represented, performance of such salt-adapted systems is usually limited to less than 5% salt, with their study. In another study, during the anaerobic treatment of sludge from saline fish farm effluents, the system was found to strongly inhibited by salt even after more than 400 days of operation (Gebauer 2004). This contradiction indicates the adaptation to high sodium concentrations is more likely to happen if a selection of halotolerant species be present in the biomass, than the adaptation of every single microorganism (Gebauer 2004).

2.4 Impact of Sodium on Different Treatment Processes

Some studies have been reported operational difficulties of conventional wastewater treatment processes which were used to treat high salinity wastewater (Uygur & Kargı 2004; Ismail et al. 2008). During the biological treatment of the saline wastewaters, limited adaptation and sensitivity to changes in ionic strength and

diminished degradation capacity and high effluent suspended solids concentration were outlined as the major problems (Moon et al. 2003). Due to an osmotic difference across the cell wall, salt concentrations higher than 1% induce disintegration of cells because of the plasmolysis or recession of the cytoplasm. As a result, low removal of chemical and biological oxygen demands and increase of the effluent suspended solids obtain (Abou-Elela et al. 2010). Salt adaptation of microbial cultures are easily lost, when they exposed to salt free solutions, showing that they cannot be effectively used to treat saline waste streams with a salinity higher than 3-5% (Kargi & Dincer 1997). Furthermore, they explained increasing salt concentrations reduced the degradation kinetics. Since the hypersaline wastewaters are often persistent to biological treatment, physicochemical treatment processes are mainly used to remove the organic substances as well as the salts from such effluents. Evaporation, coagulation and flocculation, ion exchange and membrane techniques are the main technologies which have been broadly used (Lefebvre & Moletta 2006). Although wastewater can be desalinated effectively using these various techniques, the problem on treatment (besides the adverse impact of sodium on microbial metabolism) further becomes worse when the wastewater contains toxic organic compounds. Because the recovered salts will have high organic content and will be polluted with toxic compounds, they may not be suitable for reuse or disposal (Praveen et al. 2014). Therefore, the organic pollutants must be removed from the wastewater (the preferred strategy is biodegrading organic pollutants) prior to desalination.

Aerobic Treatment. Salinity with high percentage influences the operation of conventional aerobic systems adversely above chloride concentrations of 5 - 8 gL^{-1} (Lefebvre & Moletta 2006). A decrease of organic removal efficiency consequently, an increase in BOD and soluble COD concentration (due to the release of cellular material) is one of the major problems of aerobic systems under an increase of salinity (Lefebvre & Moletta 2006). Figueroa et al. (2008) was reported a complete removal of organic compounds in a sequential biological reactor (SBR) treating fish canning wastewater with a NaCl concentration of 30 gL^{-1}, however they obtained a reduced ammonia removal efficiency. Uygur & Kargı (2004) observed that the effluent COD removal efficiency dropped from 96 to 32%, NH_4-N removal efficiency decreased from 96 to 39% and PO_4-P removal decreased from 84 to 22%,

when salt content increased from 0 to 6%. However, they reported that acclimation is being possible depending on several factors such as; type and growth phase of microorganisms and also rapid or gradual increase of salt concentration during acclimation. Using halophilic microorganisms already demonstrated an advantage on treatment in which sodium chloride concentrations ranged from 10 to 150 gL^{-1} (Moon et al. 2003). By using activated sludge containing halotolerant bacteria, Kargi et al. (2000) achieved more than 95% of COD removal of an effluent generated by the pickling industry with a salt concentration by 3 - 6%. Similarly, Kubo et al. (2001) used sludge inoculated with halotolerant bacteria to treat agro-industrial hypersaline effluent (15% of NaCl) generated from pickled plum production plant and achieved 90% of COD removal efficiency in an SBR. Lefebvre et al. (2005) achieved 95%, 93%, 96% and 92% removal of COD, PO_4^{3-}, total nitrogen (TN) and suspended solids (SS), respectively with the aerobic treatment of a saline tannery effluent (34 $gNaClL^{-1}$) in a SBR.

In addition, high salinity also have a significant impact on respiration rates (Lefebvre & Moletta 2006). They indicated that the respiration rate of the microorganism decreased above 1% NaCl presence in the media; explaining that for the respiration more oxygen is used by cells grown in a media with high sodium concentration. In a study with activated sludge treating synthetic wastewater in bench-scale continuous flow stirred tank reactor (CFSTR), within a change of salt/biomass ratios between 0.37 and 30.7 gsalt $gVSS^{-1}$ respiration inhibition was obtained between 4% and 84% (Pernetti & Palma 2005).

Anaerobic Treatment. Anaerobic processes, found to be successful to treat wastewaters contains NaCl concentrations 10 to 70 gL^{-1}. Under high salinity and anaerobic conditions, it is possible to break the long chains of hydrocarbons and some of the aromatic compounds can cause inhibition in the process, which is important for the continuity of the process. Thus, all the possible effects of this compound should have been controlled by some mechanisms both, intracellular or extracellular (Yang et al. 2013). However, the degradation of propionic acid, is the main volatile fatty acid (VFA), might be problematic since it is found to be inhibited by the high salt concentrations (10.2 $gNa^{+}L^{-1}$) in a mesophilic anaerobic treatment of sludge from fish farm effluents (Gebauer 2004).

In addition, the significant role of the multiply-charged ions on sludge granulation process was reported by many studies. Pevere et al. (2007) reported the positive effect of Ca^{2+} on the aggregation of non-fed fine anaerobic granular sludge and the formation of bigger particles, which prevent the sludge washout from anaerobic bioreactors. In a recent study with a laboratory-scale expanded granular sludge bed reactor (EGSB), the addition of Ca^{2+} (2.5 mM $CaCl_2$) resulted with an increase of EPS content of the granule by 24.1%, which enhanced the bridging of the flocs and diminished the fouling potential of the membrane (Ding et al. 2015). However, when the presence of monovalent cations, a deterioration in the floc properties was obtained due to displacement of divalent cations from binding sites of flocs (Sobeck & Higgins 2002). Ismail et al. (2008) reported a sharp drop in granule strength as a result of high sodium concentrations (10 – 15 gL^{-1}) in a UASB reactor. Floc property deterioration was found to occur when the sum of the monovalent cation concentrations (Na^+, NH_4^+ and K^+) divided by the sum of the divalent cation concentrations (Ca^{2+} and Mg^{2+}) was greater than 2 (Higgins & Novak 1997).

Another reason of deflocculation might be related to the influence of high salinity on EPS content, excreted by microorganisms, since high salinity leads to a production of EPS with a small molecular weight distribution, which makes granular sludge less likely to form in reactors (Yang et al. 2013).

In addition, anaerobic microorganisms could synthesize more variety of enzymes than aerobic microorganisms that is significant for osmoadaptation of cells. Addition of compatible solutes or potassium could lessen the toxic effect as well by the chemicals can increase sludge activity effectively. Anaerobic processes do not have the problem of oxygen transfer under saline conditions. This is important for reducing the energy in these processes, which has been used for aeration and provides higher biomass concentrations. High sludge retention capacity has support the high potential of pollutant removal. Methane production could have been demonstrated a benefit on decreasing the operational costs of the system by reusing it (Yang et al. 2013).

As a result it can be stated that the efficiency of saline wastewater treatment in biological processes is dependent on various factors such as; presence of convenient microorganism culture, their adaptation to saline conditions, sodium chloride concentration, type of pollutant, addition of compatible solutes or divalent cations,

organic load and the degree of aeration (in case of aerobic treatment). Therefore, the influence of salinity on performance of treatment processes might be investigated profoundly due to all these variables in order to compare different systems.

2.5 Potential of Membrane Processes for the Treatment of Saline Wastewater

Membrane bioreactor (MBR) technology offers a promising and innovative option for wastewater treatment and reuse among the membrane processes. The process uses both a biological stage, where the biological degradation of pollutants carried out and a membrane module to separate the microorganisms from treated wastewater. Since the volumes of wastewater are increasing, the space availability is limiting and environmental standards are tightening, membrane bioreactors (MBRs) are being widely used for the treatment of various wastewater streams such as municipal wastewater, leachate, food production wastewater, synthetic wastewater, fish canning factory effluent and acidified wastewater (Yogalakshmi & Joseph 2010). Efficient and high retention capacity of biomass, by the help of the membrane, is one of the significant advantages of MBRs, providing a good opportunity for microorganisms to adapt the alterations in the media (Yang et al. 2013). Halophilic microorganisms, which can compete with the influent salinity fluctuations and can survive in environment with a wide range of sodium concentration, can easily grow in a membrane bioreactor. The membrane module provides a physical barrier for suspended solids and generates permeates free of suspended substances, bacteria and viruses (Yogalakshmi & Joseph 2010). There are two types of configurations for the membrane design; the membranes placed inside or outside of the bioreactor. Generally hollow fiber membranes are used in submerged MBR and tubular membranes are used in external MBR systems (Marrot et al. 2004). Membrane bioreactor process has demonstrated many advantages over the conventional activated sludge process. The main difference between these two treatment systems, is the separation of solids and treated wastewater by the use of membranes where in the conventional systems it is implemented by the final clarification step which is mainly depends on the activated sludge settling properties (Marrot et al. 2004). MBRs can retain all the biomass by easing the control of solid retention time. They allow higher biomass concentrations than traditional treatment systems, less need of the space and reactor size requirements, better effluent quality,

disinfection, increased volumetric loading rates, less sludge production, better operation reliability, stability and easy automatic control (Yogalakshmi & Joseph 2010).

Recently, the studies are been conducting with the saline wastewater to determine the adverse impacts of salts on the pollutant removal, biomass activity, microbial diversity, sludge filterability and membrane fouling, in order to increase the effectiveness and the robustness of MBR systems (Reid et al. 2006; Vyrides & Stuckey 2009; Johir et al. 2013; Jang et al. 2013). Reid et al. (2006) studied with the effluents which are subjected to salinity shocks in a range of 0.1 - 4 gL^{-1} with a pilot-scale immersed membrane bioreactor and obtained COD and ammonia removal by 98%. They reported that the concentration of EPS and SMP reverted back to the original levels when the sodium impact removed. Another study investigated the performance of a submerged anaerobic membrane reactor treating saline sewage under fluctuating concentrations of salinity (0 to 35 $gNaClL^{-1}$), attained a removal of dissolved organic carbon (DOC) by 99%. An increase in acclimation potential for salinity up to 40 $gNaClL^{-1}$ was found (Vyrides & Stuckey 2009). Johir et al. (2013) also studied with a wastewater containing salt concentration from 0 to 35 $gNaClL^{-1}$ and reported the decrease of DOC removal from 93% to 77% and ammonium removal 10% to 0% respectively, within the elevating salt concentrations. They stated the possible performance improvement of the system by sufficient time or by the acclimation of halophile microorganisms naturally. Artiga et al. (2008) studied the performance of a hybrid membrane bioreactor treating saline wastewater from a fish canning factory with salt concentrations up to 73 and 83 gL^{-1} and achieved COD removal by 77-92%. These studies demonstrate the higher COD removal capacity of the membrane bioreactors than the conventional technologies. Membranes, retain sludge effectively in their bioreactors and also prevent macromolecules from being washed out of the bioreactors, would improve the reduction of effluent COD (Yang et al. 2013). Anaerobic membrane bioreactors also demonstrated an advantage over aerobic membrane bioreactors for industrial wastewater treatment since the enzyme synthesize potential of anaerobic bacteria is greater than aerobic bacteria (Yang et al. 2013). Moreover, without the oxygen transfer problem, anaerobic membrane processes could save the energy needs for aeration and also allow much higher

biomass concentrations under saline conditions. Energy requirements during the process could be supported by the methane production of the system.

The main challenge in membrane bioreactors is membrane fouling problems. Fouling comprise of the accumulation of organic and inorganic particles in/on the membrane pores or on the surface. Membrane fouling could be affected from various parameters such as influent characteristics, reactor operation, membrane features and biomass properties (Dereli et al. 2012). Formation of a cake layer is described as the most significant fouling mechanism in anaerobic membrane bioreactors. The resistance of the cake generally consisted by microorganisms, inorganic and organic substances. Especially extracellular polymers are the main contributor to resistance (Marrot et al. 2004). Sodium ions are also reported as leading to the formation of a compact fouling layer on a membrane surface (Yang et al. 2013). The presence of high salinity in the wastewater may influence the physical and biochemical properties of sludge flocs as stated above. Moreover since the disruptive impacts of high salinity on biomass, increase of fine particles could be demonstrated in the bioreactor, which leads the fouling problems. Furthermore, microorganisms produce extracellular polymeric substances to prevent themselves from the negative impacts of salinity. The elevation of EPS presence in the reactor due to the increase of salinity, also induce the fouling problem of the membranes. Fouling or cake layer formation on the membrane surface, decrease the membrane fluxes. Decreases in the flux of an anaerobic membrane bioreactor diminishes its applicability to saline wastewater treatment (Yang et al. 2013).

3. MATERIALS AND METHODS

3.1 Experimental Setup and Operation

A laboratory scale Anaerobic Membrane Bioreactor (AnMBR), consisting of 6.5 L effective volume and using an ultra-filtration (UF) membrane module, was operated as continuous stirred tank reactor (CSTR) (Figure 3). The sludge in the AnMBR was continuously recirculated with a peristaltic pump and the cross-flow velocity was kept constant at 0.6 m s^{-1}. A tubular polyvinylidene fluoride (PVDF) membrane (Pentair, The Netherlands) was employed. To maintain the mesophilic conditions warm water was recirculated through double wall water jacket of the reactor and the temperature was kept constant at 35.0 ± 0.8 °C by a thermostatic water bath (Tamson Instruments, The Netherlands). The system was equipped with feed, recycle and effluent pumps (Watson Marlow 120U/DV, 220Du), pH and temperature sensors (Endress & Hauser, Memosens), and a gas meter (Ritter, Milligas Counter MGC-1 PMMA). The biogas was measured with wet tipping gas meters and daily biogas production was recorded online.

The operational conditions were shown in Table 3.1.

Table 3.1 : Operational Conditions of the lab-scale AnMBR.

Parameter	**AnMBR**	**Unit**
HRT	4.39	d
SRT	40.80	d
Flow rate	1.48	L/d
OLR	9.23	gCOD/L
SLR	1.132	gCOD/gVSSd
Flux	7.37	L/m^2h
TMP	80 – 250	Mbar
pH	8.10	pH
Temperature	35.2	ºC

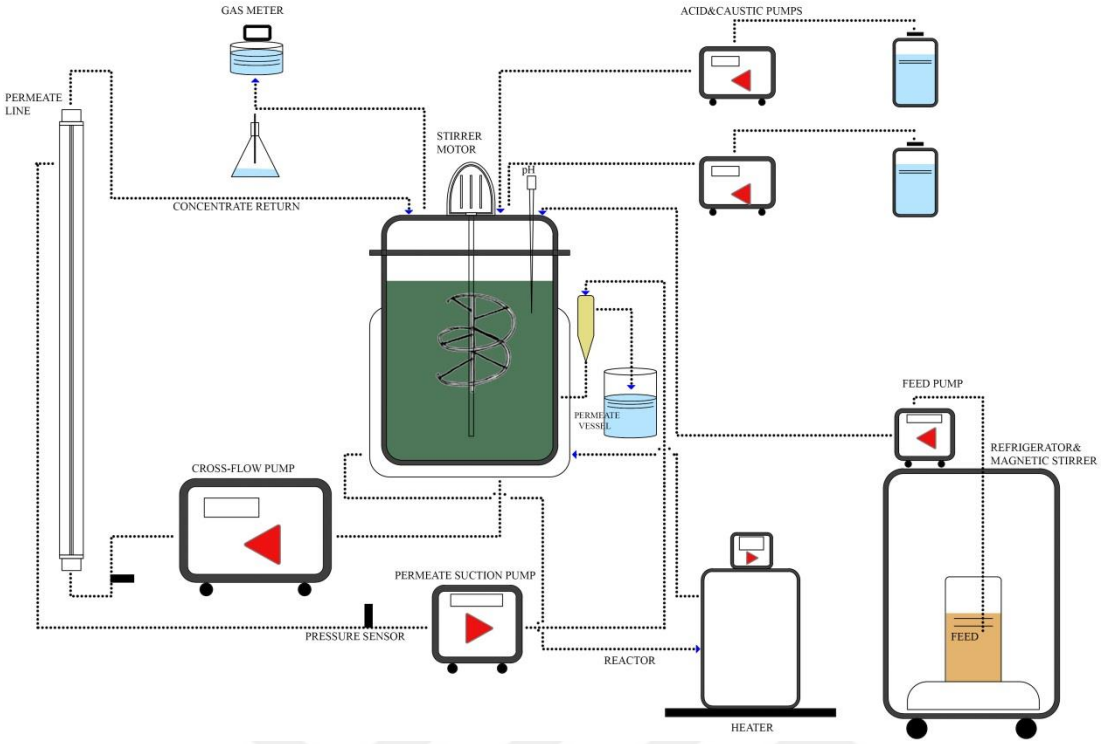

Figure 3.1 : Lab-scale Anaerobic Bioreactor Setup.

3.2 Sludge Characteristics

The reactor was seeded with concentrated anaerobic biomass obtained from a full-scale upflow anaerobic sludge blanket (UASB) reactor treating industrial wastewater (Shell, Moerdijk, The Netherlands). Initial characterization of the sludge before starting the experiments is shown in Table 3.2.

Table 3.2 : Initial Conditions of AnMBR sludge

	Quantity	**Unit**
COD	879	mgL^{-1}
Phenol	5.0	mgL^{-1}
TSS	29,3	gL^{-1}
VSS	16.5	gL^{-1}
Conductivity	43.8	$mScm^{-1}$
pH	8.3	pH

3.3 Waste Characterization

In this study, a synthetic feed solution was used. Solutions were prepared with different concentrations of $Na^{+}gL^{-1}$. K/Na ratio and Phenol proportion were kept at the same values of 0.05 and 500 mgL^{-1} each solution respectively. Inlet COD concentration was 33880 mgL^{-1}. Acetate was used as substrate and NaCl as sodium source. Macronutrient and micronutrient solutions were added in compositions as follows (in mgL^{-1} unless otherwise noted): NH_4Cl, 170000; $CaCl_2*2H_2O$, 8000; $MgSO_4*7H_2O$, 9000; $FeCl_3*6H_2O$, 2000; $CoCl_2*6H_2O$, 2000; $MnCl_2*4H_2O$, 500; $CuCl_2*2H_2O$, 30; $ZnCl_2$, 50; HBO_3, 50; $(NH_4)_6Mo_7O_2*4H_2O$, 90; Na_2SeO_3, 100; $NiCl_2*6H_2O$, 50; EDTA, 1000; Na_2WO_4, 80. The substrate solution was stored at 4ºC in order to avoid microbial degradation. The solution was always mixed thoroughly to avoid from precipitation of chemicals, before being fed into the bioreactor.

3.4 Salinity Fluctuation Studies

To assess the performance of AnMBR under short term salinity fluctuations for treating the phenolic wastewater, four different fluctuation cycles have implemented to reactor under mesophilic conditions. Short term fluctuations (12h - 24h - 48h) were applied to the reactor from 6 up to 34 $gNa^{+}L^{-1}$. Four different feeding modes were used. Each feeding mode has lasted forty days and in every cycle, sludge and permeate samples were collected periodically. The first cycle, called evenly distributed, was illustrated in Figure 3.2. Sodium concentration in the influent changed constantly from 16 $gNa^{+}L^{-}1$ for 24 hours to 22 $gNa^{+}L^{-l}$ for 48 hours and changed respectively. Effluent samples were collected every 48 hours. Figure 3.3 demonstrates the second cycle, stepwise increasing/decreasing, when the sodium concentration in the influent was gradually increased from 16 $gNa^{+}L^{-l}$ to 20, 22, 24 and 26 $gNa^{+}L^{-l}$ and decreased. During this cycle every concentration was lasted for 24 hours. Correspondingly, effluent samples were collected in every 24 hours. Third cycle, non-evenly distributed, was conducted to represent more actual concentration changes observed in highly dynamic industrial wastewater streams such as; chemical, petrochemical and petroleum refinery. Sodium concentration values are ranging from 6 to 34 $gNa^{+}L^{-l}$ with different exposure times of; 12, 24 and 48 hours were applied.

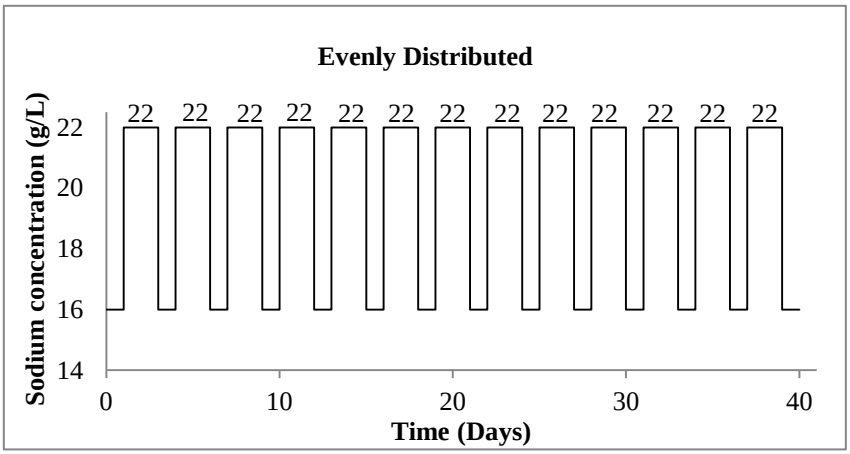

Figure 3.2 : Sodium concentration changes at first cycle; evenly distributed.

The changes in influent concentration were demonstrated in Figure 3.4. During this cycle effluent samples were collected in every 24 hours. During the first three cycles results showed the potentials of AnMBR in industrial wastewater treatment to overcome extreme conditions, such as the degradation of refractory organics (e.g., phenol) when fluctuations in high salt concentrations take place. It was decided to conduct another experimental run with higher concentration swings. During this fourth cycle, the concentrations were changed from 34 to 46 $gNa^{+}L^{-1}$ (Figure 3.5). Duration of each concentration was 24 hours and effluent samples were collected in every 24 hours. Influence of various salt peaks on treatability of the system, were examined with COD and phenol analyses. After salinity shocks, responses of microbial community were monitored with EPS, SMP and ATP; concentration of sludge with SS; filterability with CST and SRF; characteristics with Relative Hydrophobicity, Flow Cytometry, PSD and Viscosity analyses.

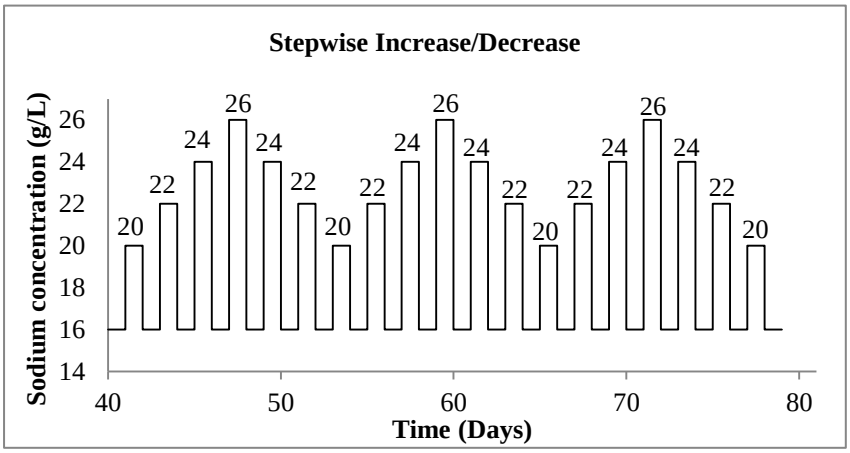

Figure 3.3 : Sodium concentration changes at second cycle; Stepwise Increase/Decrease.

Microbial analyses were conducted to detect the microbial species which were capable to overcome salinity changes.

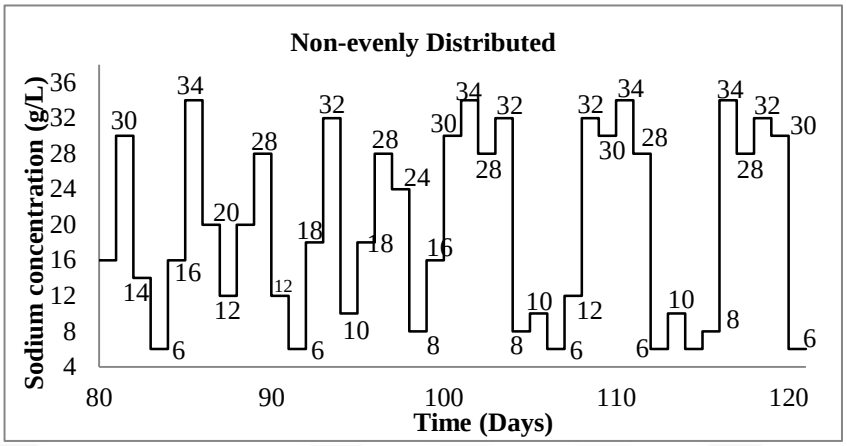

Figure 3.4 : Sodium concentration changes at third cycle; Non-evenly Distributed.

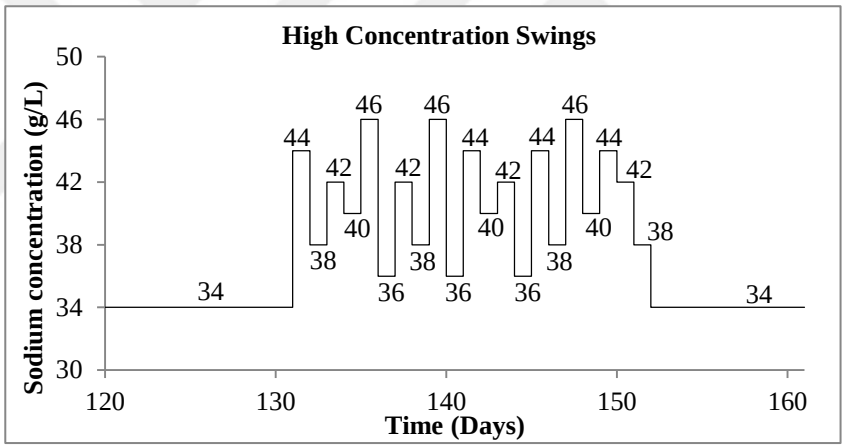

Figure 3.5 : Sodium concentration changes at fourth cycle; High Concentration Swings.

3.5 Biomethane Potential Test

Biomethane potential (BMP) test was applied to evaluate anaerobic biodegradability and methanogenic activity of sludge under various concentrations of salinity. To understand the osmoadaptive mechanism of microorganisms in our inoculum, two different approaches were examined in batch tests such as, keeping the ratio of K/Na similar and different in solutions. Test setup was prepared in 250 ml assay bottles with required amounts of substrate and inoculum as duplicate with a sodium acetate substrate contains 16, 20 and 24 gL^{-1} sodium concentration. Inoculum (based on VS)

to substrate ratio (I/S) was taken 1.5 gVS_I/gVS_s. The bottles were placed in a temperature controlled rotational shaker (New Brunswick™ Biological Shakers Innova® 44/44R, USA) at 35ºC and continuously stirred at 150 rpm for 30 days. In order to provide anaerobic conditions from the start of the experiment, the mediums were flushed with nitrogen gas. Stock solution contains resazurine, which is a redox indicator that turns pink at aerobic conditions and is blue or colorless at anaerobic conditions, was used to observe the change of conditions. One standard solution was added as a reference. Initial values of inoculum and substrate concentration were calculated by the software of assay control panel (Bioprocess Control, Lund, Sweden) due to our substrate (2 $gCODL^{-1}$) and inoculum (9.5 $gVSSL^{-1}$) concentration. For first batch, the ratio of K/Na kept similar, for the second batch the ratio implemented in various values of 0.03, 0.024 and 0.02. COD concentration was measured as $3500mgL^{-1}$.

During the BMP test, samples were taken every day to observe the anaerobic biological activity by standard tests such as COD and phenol. Methanogenic activity was recorded as hours by AMPTS (Automatic Methane Potential Test System, Bioprocess Control, Lund, Sweden) control panel.

3.6 Analytical Methods

To determine the effect of sodium fluctuations on permeate quality and examine the treatment capacity of AnMBR under high salinity conditions, during every four different cycles, effluent samples and sludge samples were collected in various hours. The mixed liquor samples were characterized for the following parameters: chemical oxygen demand (COD), phenol, total suspended solids (TSS), volatile suspended solids (VSS), Adenosine triphosphate (ATP), volatile fatty acids (VFA) concentration, pH and conductivity. Mixed liquor samples were centrifuged and filtered to remove the particles. For this purpose a filter with a 0.45 μm pore size was used. The effluent samples were analyzed for COD, phenol, turbidity, ATP, pH and conductivity. Analyses were implemented in duplicates. Moreover, methane content of biogas was examined.

COD. During the four different cycles, effluent and sludge samples were collected in different periods and analyzed as duplicates. Hach-Lange kits were used to determine chemical oxygen demand (COD). The extraction lasted 2 hours at 148ºC. Necessary

dilutions were applied, in total volume of 2 ml. COD quantity of sludge samples were analyzed with same procedure after centrifuging the samples for 10 minutes and then filtrating them through 0.45 μm disposable membrane filters.

Phenol. Merck – Spectroquant® Phenol cell kits, measuring a range between 0,1 – 2,5 mgL^{-1}, were used to determine phenol concentration of the samples. Samples were collected in different periods for different cycles and analyzed both for effluent and sludge. Necessary dilutions were applied, in total volume of 10 ml. Phenol and phenol derivatives in samples reacted with a thiazole derivative to form a red-violet azo dye and that is determined photometrically.

Solids. The total suspended solids (TSS) and volatile suspended solids (VSS) concentration were determined according to Standard Methods of the American Public Health Association (APHA 1985). Dried borosilicate glass fiber filters were weighed without sample and values recorded (for VSS measurements). Then under pressure filtration equipment, 3 ml of sludge samples were filtered. For drying purposes, samples were left for 3 hours in an oven for maintaining a temperature of 105ºC. At the end of drying samples were kept in desiccators for a few minutes and weighed again in balance (TSS concentration). Samples then transferred to 500ºC oven for approximately 3 hours to determine the VSS concentrations. Same procedure was applied for determining VS and TS but this time filtration was not used.

ATP. ATP, an excellent indicator of microbial activity as well as a direct indication of the living population, was analyzed with Aqua-Tools test kits (QG21W and QG21Wa) both for permeate and sludge samples. Samples were taken every 10 days, during the four different salinity loading cycles to observe the impact of sodium concentration on activity of microorganisms in biomass. Analyses were conducted in duplicates. Method explained by Aqua – Tools was followed (QG21W Test Kit Instructions, Aqua-tools, 2014). The total ATP (tATP) which measures ATP from both living and dead cells and the dissolved ATP (dATP) which determines ATP only from dead cells were implemented. After the dilution and extraction steps samples were read immediately by luminometer and values recorded manually in terms of relative light units (RLU). The cellular ATP (cATP) which represents ATP from living organisms was calculated with the equations given at the experiment protocol of Aqua-Tools (QG21W Test Kit Instructions, Aqua-tools, 2014). First,

conversion of RLU unit to ng ATP L^{-1} was made and cATP values were calculated in unit of ng ATP mL^{-1}. Results were represented as mg L^{-1} at Results and Discussion section.

$$\mathrm{tATP(ng\,ATP/mL)} = \frac{\mathrm{RLU_{tATP}}}{\mathrm{RLU_{ATP1}}} \text{ x } 11\ \mathrm{(ng\,ATP/ml)} \quad (1)$$

$$\mathrm{dATP(ng\,ATP/mL)} = \frac{\mathrm{RLU_{dATP}}}{\mathrm{RLU_{ATP1}}} \text{ x } 101\ \mathrm{(ng\,ATP/ml)} \quad (2)$$

$$\mathrm{cATP(ng\,ATP/mL)} = \mathrm{tATP(ng\,ATP/mL)} - \mathrm{dATP\ (ng\,ATP/mL)} \quad (3)$$

Biomass Stress Index (BSI %), representative of the stress level of the microbial community, was calculated with the equation given below.

$$\mathrm{BSI\ (\%)} = \frac{\mathrm{dATP(ng\,ATP/mL)}}{\mathrm{tATP(ng\,ATP/mL)}} \text{ x } 100 \quad (4)$$

BSI (%) calculations give us information about reactor operation due to stress observed with microbial cells. Interpretation Guideline, suggested by test protocol, is shown in Table 3.3 (QG21W Test Kit Instructions, Aqua-tools, 2014).

Table 3.3 : Interpretation Guideline for Bioreactors

Location	Parameter	Good Control	Preventive Action Required	Corrective Action Required
Bioreactors	cATP	Process Spesific		
	BSI	< 30	30 to 50	> 50
	ABR	> 25	10 to 25	< 10

Methane. The methane content in biogas was measured with a gas chromatograph equipped with a detector (Agilent Technologies, US). Variations of methane concentration due to sodium changes is shown in apendices chapter at Figure A. 3.

Turbidity. Determination of turbidity was conducted by a Hach 2100P Turbidmeter. Samples were read as duplicates and manually recorded.

pH and Conductivity. The production of methane will perform optimally at pH values between 6.5 – 7.5 thus it is important to monitor pH values both inside of the reactor and in at the permeate. pH, conductivity and temperature of both sludge and permeate were measured by probes connected with electrometers (Mettler-Toledo M200 system).

3.7 Sodium (Na^+) Concentration Measurement

In order to observe the real conditions and assess the impact of sodium concentrations inside the reactor as well as to determine the impact of sodium on effluent quality Na^+ (gL^{-1}) concentrations were measured by Metrohm Ion Chromatography. Essential dilutions were applied to samples and they were prepared in duplicates. Calibration is done with Sodium Standard for AAS solution in the range between 1 – 50 ppm. The final results were calculated by MagIC Net software at the end of sampling acording to the calibration curve.

3.8 Extracellular Polymeric Substances Assay

As the extraction method, cation exchange resin (CER) was used, proposed by (Frølund et al. 1996). This method removes cations from the sludge matrix by breaking up of the flocs and then lead them to release of EPS.

Sludge samples were collected every 10 days during the each fluctuation cycles. Prior to EPS extraction, VSS measurements were conducted to sludge samples in order to decide the volume of sample used in experiment. Analyses were conducted as duplicates by taking into account of the gVSS concentration of the sludge at same date. DOWEX Marathon C (20–50 mm mesh, sodium form, Fluka 91973), as cation exchange resin, was prepared 1 hour in advance. 14 gram of DOWEX per sample was weighed to wash it with Phosphate-Buffered Saline solution (PBS) at dark. The buffer solution consisted 2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl and 1 mM KCI at pH 7. Samples were centrifuged at 4°C with 12000rpm for 15 minutes to separate the soluble portions of proteins and polysaccharides which are present at bulk liquid of samples. After centrifugation, supernatants were filtered and kept to

make soluble microbial products (SMP) analysis (Frølund et al. 1996). Then the sludge pellet part was re-suspended to its original volume using the Phosphate Buffered Saline (PBS) solution for all samples. Centrifugation was repeated at same conditions, the obtained supernatant was discarded and biomass pellet was filled back to its original volume and mixed very well. Homogenized biomass was taken into extraction flask with resin and buffer solution. Extraction was carried out at 4°C for 4 hours while stirring the suspension at 800 rpm. At the end of extraction approximately 30 ml of samples (except the DOWEX beads at the bottom) was taken for centrifugation for 15 minutes. Supernatant was pass through 0.45 mm cellulose membranes and used as the EPS fraction for protein and carbohydrate analyses. The total EPS content was measured as the sum of these two substances (Ismail et al. 2010).

a. Protein Measurement

For the analysis of proteins the method of (Frølund et al. 1996), a modified method of (Lowry et al. 1951), was applied. For the calibration standard Bovine Serum Albumin, BSA, in a concentration range between 0 – 500 mgL^{-1} was used. Reagents: A: 143mM NaOH and 270mM Na_2CO_3. B: 57mM $CuSO_4$. C: 124mM Na-tatrate. D: Mixture of reagents A, B and C in the proportion 100:1:1 which has prepared freshly before measurements. E: Folin reagent diluted 1:2 with distilled water. Procedure: 1.5 ml sample was rapidly mixed with Vortex-Mixer 2,1 ml reagent D. Left 10 minutes for room temperature Reagent E was added rapidly (0.3 ml) and the solution whirly mixed. After 45 min at room temperature the absorbance at 750 nm was read with an UV-VIS spectrophotometer. Protein concentrations were determined from a calibration curve obtained with protein standards, and normalized against VS concentration (Ismail et al. 2010).

b. Carbonhydrates Measurement

To analyze polysaccharide quantity in our samples, modified method of (Dubois et al. 1956) was used. Calibration is done with standard D-Glucose monohydrate, in two different concentration ranges between 0 – 200 mgL^{-1} and 0- 500 mgL^{-1} was used. Reagents: A: 5 % Phenol solution and B: 95 – 97 % sulfuric acid. Measurements were carried out as duplicates.1 ml of A was added to 1 ml of sample and mixed thoroughly. Reagent B added rapidly and left for 5 minutes to cool down

in room temperature. Test tubes was mixed once more and left 25 minutes at room temperature. Then the adsorption is measured at a wavelength of 490nm against blank with a UV-VIS spectrophotometer. The concentration was calculated from the measured absorbance by the use of calibration curve and amount of polysaccharides was expressed in mgL^{-1}. This concentration was normalized against the VS concentration (Ismail et al. 2010).

3.9 Sludge Relative Hydrophobicity Measurement

To determine the relative hydrophobicity (RH) quantity of sludge samples a method of Bacterial Adhesion to Hydrocarbons (BATH) (Rosenberg et al. 1980) was used with Dodacane as carbon phase. Analyses were conducted in triplicates. 4 ml of samples were used for measurements. To achieve 1 g/L TSS concentration in sludge samples, they were diluted with permeate. Absorbance readings were held at 600 nm in a UV spectrophotometer (Jenway 7315, Bibby Scientific, UK). Permeate was read as blank and the diluted sample was measured as the initial value (Abs_i). As hydrocarbon treatment, 4 ml of dodacane added to each sample, vigorously mixed for 1 minute and left for 10 minutes for phase separation (at room temperature). The water phase of the sample was taken carefully and its absorbance was measured as (Abs_f) (Dereli, Loverdou, et al. 2015). Relative hydrophobicity was calculated according to the equation (5) given below.

$$\mathrm{RH}(\%) = \left(1 - \frac{\mathrm{Abs_f}}{\mathrm{Abs_i}}\right) * 100 \qquad (5)$$

3.10 Filterability of the Sludge

Capillary Suction Time (CST) and Specific Resistance to Filtration (SRF) parameters of sludge samples were analyzed at the beginning and the end of every salinity load cycle since they are good indicators of determining the sludge filterability and dewaterability. Due to the filtration capacity of samples, essential dilutions were made up to 100 ml and filtrated with a batch filtration process in a metal cylinder by using Whatman #1 filter papers under pressure of 1 bar, given from top of the system. The weight of the filtrate was recorded manually due to the speed of filtration (usually as minutes, in some cases as seconds). Measurements were

conducted in duplicates. The equation (6) given below is used to determine the specific resistance, r̂, from a SRF filtration test by plotting t/ V versus V.

$$\hat{r} = \frac{2PA^2 b}{\mu C} \quad (6)$$

Where;

A = filter area (cm^2)

P = pressure drop (1 bar = 10^5Pa)

μ = filtrate viscosity ($1.1x10^{-3}$Pa.s)

c = gTSS/mL (were measured for every different sample)

The required parameters for calculating the specific resistance are given in Table 3.4.

Table 3.4 : Required Parameters for SRF analyze

Parameters	**Value**	**Unit**	**Value**	**Unit**
Pressure drop (P)	1019,72	g/cm²	1	bar
Viscosity (μ)	1,1E-07	N*s/cm²	0,011	poise
Solids content (C)	Differs for each sample	g/mL		
Filter surface Area (A)	38,465	cm²		

Until the cake layer would form fully on the filter, the filtrate volume increases much more rapidly for the first seconds. Due to the filtration capacity of sludge this might take even minutes.

Therefore, since the linear relationship has not obtained yet, initial measurements were discarded for the calculation of the average specific resistance, while assessing the results.

For determination of capillary suction time of the samples, a CST-meter equipment by Triton Electronics, Model 304M, was used. Samples were read as triplicates and 6.4 ml of sample were used per reading. Filtration was conducted with Whatman #17 filter papers. Observed values were recorded manually.

3.11 Particle Size Distribution

Particle size distribution (PSD) is a crucial parameter since the impact of sodium concentrations on sludge properties could be observed. Therefore, the sludge suspensions were obtained using a EyeTech^{TM} with a detection range of 0.1-2,000 μm. The particle size distribution of the sludge samples were expressed as volume and the average size is quoted as the median based on volume equivalent diameter (D50). D_{10} and D_{90} values were also represented at appendices chapter in Figure A. 1 and Figure A. 2 respectively.

3.12 Rheological Tests

Rheology describes the deformation of sludge under the impact of stresses (Dentel 1997). It enhances the mixing in the reactor systems and the interaction between microbial community and its substrate (Ghasimi et al. 2015). To characterize the rheological behavior of the matter, shear stress (τ) was determined as a function of shear rate (γ) (A universal dynamic rheometer (Paar Physica UDS 200, Studdgart, Germany) equipped with a water bath for temperature adjustment, was used to conduct the viscosity analysis and determine the rheological characteristics of sludge samples. The software US200/32 (V2.30) was used for programming and data logging (Ghasimi et al. 2015).

3.13 Flow Cytometry Assay

Flow cytometry (FCM) experiments were conducted as a rapid, cultivation-independent tool to determine the bacteriological quality and biological stability of our sludge samples after sodium loads. The objective of microbial physiological analysis is to differentiate the living and dead cells in the sludge samples (Wang et al. 2010). FCM was used together with fluorescent satins to label for specific detection of targeted cells since it provides significant information about total cell concentration, viability, characteristics or identity of microorganisms (Prest et al. 2013). Combination of two stains used for microbial quantification. SYBR Green (SG), one of the nucleic acid stain, which measures all microbial cells, regardless of their physiological state and Propidium iodide (PI), one of the most commonly used

stain to examine membrane integrity (Wang et al. 2010). By using both stains together, viability of microbial community in our sludge samples was determined.

Staining protocol. Sludge samples were diluted (to obtain bacterial concentration less than 2 x 10^5 cells mL^{-1}) and stained according to the protocol defined by Prest et al. (2013) for determination of total bacterial cells. 500µl of diluted sludge samples were preheated at 35ºC for 5 minutes and they have stained with 10 µl SYBR® Green I (1:100 dilution in DMSO; Molecular Probes) and incubated at 35ºC for 10 minutes. Evaluation of the intact cells was conducted by staining the diluted sludge samples with a solution containing SYBR® Green I and propidium iodide (0.3 mM) (Prest et al. 2014). The same steps were followed as described above; samples preheated for 5 minutes at 35ºC, stained with solution and incubated at 35ºC for 10 minutes.

Flow Cytometric Measurement. For the analysis, BD Accuri C6® flow cytometer (BD Accuri cytometers, Belgium) was used, with a 50 mW laser emitting at a fixed wavelength of 488 nm. The FCM was equipped with a volumetric counting hardware, calibrated to measure the number of particles in 50 µL of a 500 µL sample. Measurements were performed at pre-set flow rate of 35 µL min^{-1}. For data processing with the BD Accuri CFlow® software was used.

4. RESULTS AND DISCUSSION

4.1 The Effect of Sodium on the Removal of Organics

In order to examine the impact of sodium concentration variations on the organic matter removal efficiencies, experimental studies were performed. Organic content of the effluent samples were measured in terms of COD (mgL^{-1}) and phenol (mgL^{-1}). The variations of COD concentration at the effluent were shown in Figure 4.1 and the effluent phenol concentrations were demonstrated in Figure 4.2. During the first, evenly distributed, cycle the average COD concentration of the effluent was 273 mgL^{-1}. The highest concentration of 392 mgL^{-1} and lowest concentration of 101 mgL^{-1} were observed when the reactor was exposed to an inlet sodium concentration of 22 and 16 $gNa^{+}L^{-1}$ respectively (Figure 4.1 a). During the second cycle, namely stepwise increase/decrease, as inlet sodium concentrations were gradually increased and decreased, elevations and drops in effluent sodium concentrations and fluctuations in COD concentration profile was observed (Figure 4.1 b). Between day 40 and 43, COD concentration decreased from 322 to 116 mgL^{-1} when a 3% decrease in sodium concentration was obtained. Until day 48, an increase in COD concentration from 116 to 139 mgL^{-1} was measured while effluent sodium concentrations were increased from 18.2 to 20 $gNa^{+}L^{-1}$. However, from day 58 to 62, when sodium concentration increased from 17.8 gL^{-1} to 21 $gL^{-}1$, COD concentration found to drop from 148 to 97 mgL^{-1}. From day 70 to 72, an increase in COD concentration from 112 to 442 mgL^{-1} was obtained, while the sodium concentration of effluent increased by 14.8%. Figure 4.1 c demonstrates the changes of COD concentration at the third cycle, when biomass was exposed to non-evenly distributed concentrations of sodium. Between the days 80 and 84 COD concentration was decreased from 428 to 168 mgL^{-1}, when a decrease of sodium concentration observed from 21 to 18.3 gL^{-1}. The maximum COD concentration of 1200 mgL^{-1} was observed at day 90, when the sodium concentration of the reactor was about 20 gL^{-1}. After day 100, especially between days 102 - 106 there was a decrease of COD by 35.5%, when there was a decrease of sodium concentration by 16%.

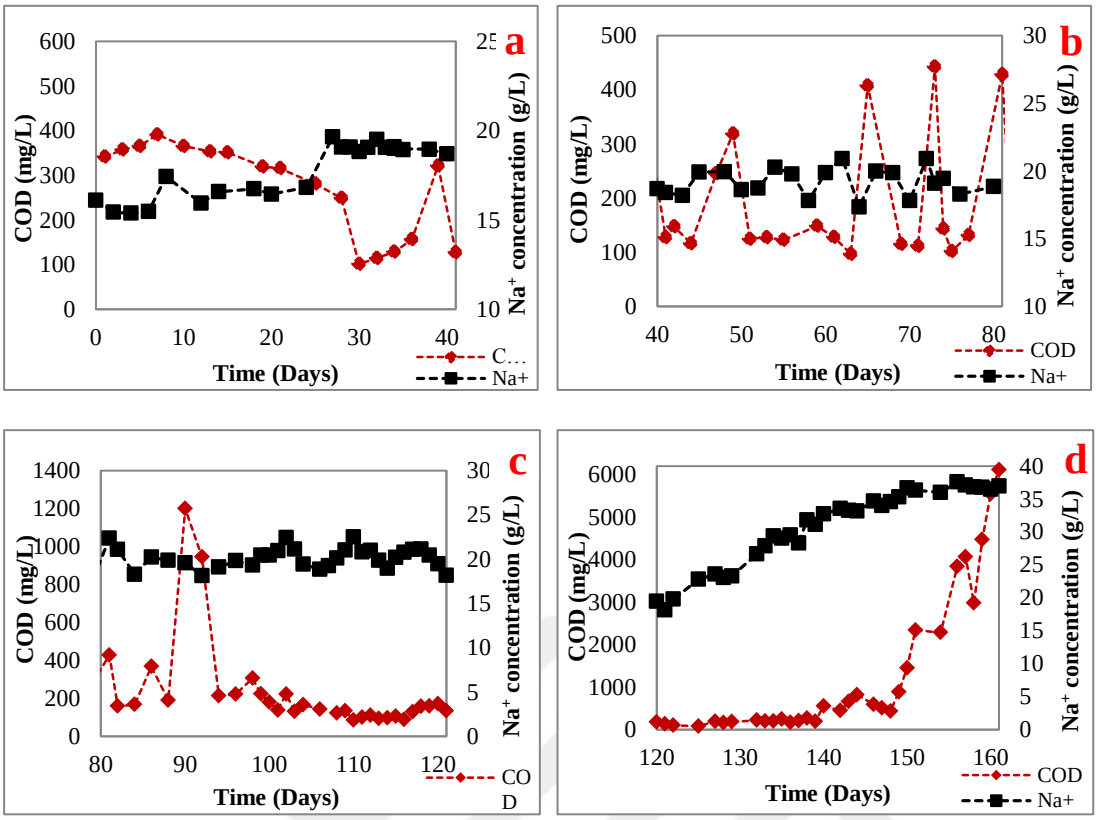

Figure 4.1 : Variation of COD concentration in the MBR effluent due to salinity (a) Evenly Distributed; (b)Stepwise Increase/Decrease; (c) Non-evenly Distributed; (d) High Concentration Swings.

Also from day 114 to 118, sodium concentration was found to increase gradually from 19 to 21 gL^{-1} meanwhile an increase in COD concentration obtained from 97 to 158 mgL^{-1}. However, the biomass was found to be able to compete with those sodium fluctuations at the media with an average outlet COD concentration of 143 mgL^{-1} and an average COD removal of 99%. Figure 4.1 d indicates the variation at COD at fourth cycle, high concentration swings, when the effluent sodium concentration was gradually increased from 19 gL^{-1} to a maximum sodium concentration of 37.6 gL^{-1}. Especially from sodium concentration of 32 gL^{-1} and onwards the COD removal efficiency dropped from 98% to 82%. From day 140, higher COD concentrations than in previous cycles were obtained with the effluent COD varying between 500 and 6000 mgL^{-1}. Very significant negative impact of sodium on organic removal was found at this cycle, when effluent COD concentration was increased 12 times from the beginning to the end of the 40 days cycle. During the all cycles COD degradation was found to negatively impacted from the increases at sodium concentration. The inhibitory impact of salinity on microbial activities was also observed with phenol degradation. Increasing sodium

concentration resulted in a decrease of organic removal efficiency and an increase in effluent phenol concentration (Uygur & Kargı 2004; Yogalakshmi & Joseph 2010). At the evenly distributed cycle maximum concentration of 5.5 mgL^{-1} was measured (Figure 4.2 a). The average phenol concentration of effluent was 3 mgL^{-1} with an average phenol removal efficiency of 99%. Within the stepwise increase and decreases cycle, effluent phenol concentration was found to be slightly affected by sodium changes, as shown in Figure 4.2 b. From day 43 to 48, an increase in sodium concentration from 18 to 20 gL^{-1}, induced an increase in phenol concentration by 48%. Afterwards, until day 51 a decrease of phenol concentration by 59% was obtained, while sodium concentration decreased to 18.6 gL^{-1}. Also a significant increase of effluent phenol concentration by 78% was obtained from day 71 to 73 while 15% increase of effluent sodium concentration was observed. A significant increase at the phenol concentration of effluent samples was observed after day 80 until day 90 within the third cycle, non-evenly distributed (Figure 4.2 c). The maximum effluent phenol concentration of 157 mgL^{-1} was observed with a phenol removal efficiency of 68% at day 90.

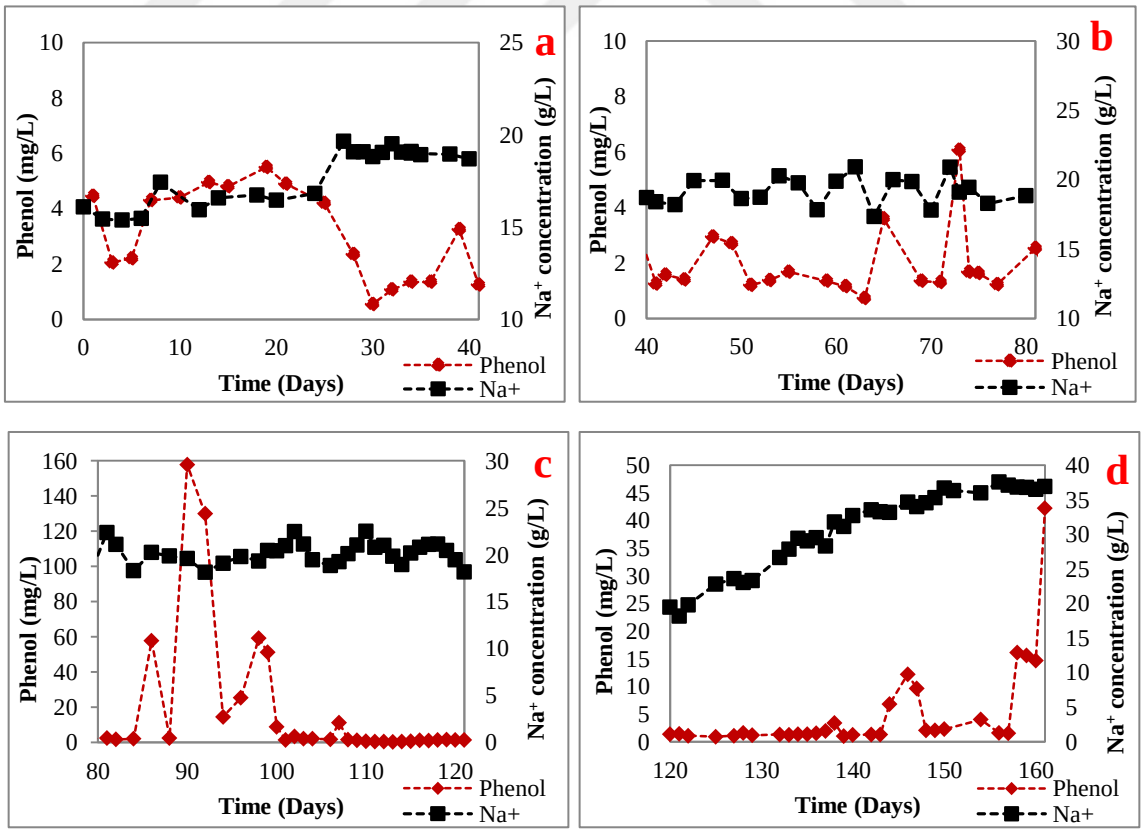

Figure 4.2 : Variation of Phenol concentration in the MBR effluent due to salinity (a) Evenly Distributed; (b) Stepwise Increase/Decrease; (c) Non-evenly Distributed; (d) High Concentration Swings.

However, between day 98 and 101, when the biomass was exposed to an increasing sodium concentration from 16 gL^{-1} to 21 gL^{-1}, a great decrease at the outlet phenol concentration from 59.2 to 1.45 gL^{-1} was obtained. From day 100 to 120, biomass was found to overcome the sodium impact in terms of degradability of phenol. The average effluent phenol concentration was 2.2 mgL^{-1} with an average phenol removal of 99%. This might be explained by an acclimation of our sludge to the sodium changes in the media since it was exposed to sodium for 100 days and could cope with that in terms of organic matter degradation. Figure 4.2 d shows the variation of phenol concentration during the fourth, high concentration swings, cycle. Until day 142 results demonstrate that phenol degradation was successful within an average removal of 99%. Between the days 142 and 146 a great increase of 89.5% outlet phenol concentration was obtained when sodium concentration increased from 33.5 to 34.7 gL^{-1}. This sudden increase might be also explained by the cumulative impact of sodium since the biomass was exposed to high sodium concentrations since the beginning of the fourth cycle. From day 146 to 148 a decrease of 83.2% phenol concentration determined. Until the end of cycle, phenol concentration found to increase in the effluent after the system exposed to a sodium concentration about 34 gL^{-1}. A maximum concentration of 42.2 mgL^{-1} phenol was measured at the end of the cycle at 36.9 gL^{-1} sodium concentration.

4.2 The Effect of Sodium on Effluent Turbidity

Effluent turbidity was measured in order to see the effect of sodium concentration variations on effluent quality, since turbidity give an indication of the level of colloid material and residual suspended matter of samples, even though it is after ultrafiltration. Moreover, supernatant turbidity can be a measure of the population of dispersed bacteria (Abdollahzadeh Sharghi et al. 2014). Figure 4.3 demonstrates the changes in the effluent turbidity during the complete study due to the changes in effluent sodium concentration. Maximum value of 135 NTU and minimum value of 6 NTU was measured. At evenly distributed cycle, during first 30 days of operation a positive relationship between effluent turbidity and sodium concentration was observed. For instance between the days 27 and 30, turbidity was found to decrease from 28.6 to 18 NTU while there was a decrease of sodium concentration from 19.6 to 19 gL^{-1}. Also from day 30 to 33, turbidity increased from 18 NTU to 21 NTU

while sodium concentration increased from 19 to 19.5 gL^{-1}. However, during the stepwise increase/decrease cycle, at several points, a negative relationship between effluent turbidity and sodium concentration was observed. For instance, from day 45 to 50 when the outlet sodium concentration was decreased from 20 to 18.6 gL^{-1}, effluent turbidity found to increase 72 NTU. Also between the days 62 and 64 effluent sodium concentration was decreased from 21 gL^{-1} to 17 gL^{-1} and the effluent turbidity was increased from 10 NTU to 65 NTU respectively.

During the third cycle when non-evenly distributed concentrations of sodium were applied, the results demonstrated an opposite relationship of effluent turbidity and sodium concentration.

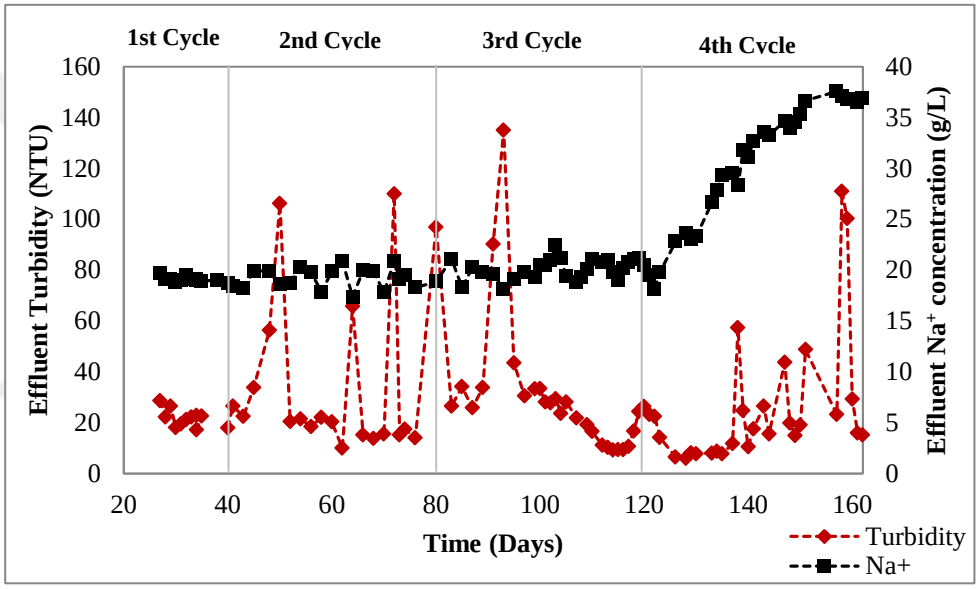

Figure 4.3 : Variation of the effluent turbidity due to the changes in effluent salinity (all cycles).

From day 83 to 93, an increase in effluent turbidity from 26.5 to 135 NTU was obtained while the sodium concentration decreased from 21 to 18 gL^{-1}. Also between the days 93 and 103, turbidity found to decrease from 135 to 29 NTU while an increase in effluent sodium concentration from 18 to 22 gL^{-1} was found. However, between the days 110 and 115, while a decrease of effluent sodium concentration observed from 21 to 19 gL^{-1}, a decrease of 43% at the effluent turbidity was obtained. The highest turbidity of 135 NTU was determined during this cycle when sodium concentration was 18.1 gL^{-1}. Abdollahzadeh Sharghi et al. (2014) stated an inverse relationship between sodium and effluent turbidity in their study. During the fourth cycle various effluent turbidity fluctuations were observed after being exposed

to higher sodium concentration swings. The lowest turbidity of 6 NTU was measured at this cycle. From day 122 to 128, effluent turbidity was decreased from 22 NTU to 6 NTU while an increase at sodium concentration from 18 to 23.6 gL^{-1} was observed. Between the days 138 and 140 a decrease of effluent turbidity from 57 NTU to 10 NTU was measured while sodium concentration was increased from 28 to 31 gL^{-1}. Moreover, between the days 151 and 157, when an increase at sodium concentration from 36 gL^{-1} to 37 gL^{-1} observed, the effluent turbidity was found to decrease from 48 NTU to 23 NTU. However, at some points a positive relationship was observed such as between day 144 and 147 where the turbidity was increased from 15 to 44 NTU within an increase of sodium from 33 to 35 gL^{-1}. Also between the days 149 and 151, effluent turbidity increased from 15 to 49 NTU. During the last 5 days of the cycle a decrease at the effluent turbidity was observed within a decrease at sodium concentration. Ng et al. (2005) reported a slight decrease in effluent turbidity when NaCl concentration was increased from 0 to 10 gL^{-1} however, after 10 $gNa^{+}L^{-1}$ they found an increase in effluent turbidity based on increased NaCl concentration. They explained the reason of increase in turbidity beyond 10 $gNa^{+}L^{-1}$ by collapse of some microorganism species due to the hypertonic conditions, which induced a release of cellular components such as protein, polysaccharide, nucleic acid and lipid in the effluent. They also explained the improvement of effluent turbidity, while sodium concentration was increasing up to 10 gL^{-1} by the impact of electrostatic interactions. Since the increase in ionic strength allows an immediate affinity between microbial surfaces and cations which enhances flocculation and reduces turbidity. Therefore, in our study the increase of turbidity with an increase of salinity, especially during the fourth cycle, might be explained by the release of non-dissolved cellular components due to microorganism plasmolysis above 26 $gNa^{+}L^{-1}$. However, Lefebvre & Moletta (2006) explained the increase of effluent turbidity by the quantity reduction of the filamentous bacteria under hypersaline conditions. They stated the protozoans are significant in the mechanical integrity and structure of the flocs and they could reduce effluent turbidity. However their resistance to salinity shocks is limited therefore, they cannot survive more than 24 hours to salinity shocks higher than 40 gL^{-1} (Salvadó et al. 2001; Lefebvre & Moletta 2006). $EPS_{protein}$ (EPS_p) content was also determined to elucidate influence on turbidity, since it has a significant impact on flocculation. Reid et al. (2006) stated a positive relation between $EPS_{protein}$ concentration and the turbidity of effluent in their study with

activated sludge samples. Sheng et al. (2010) also stated the importance of interactions between EPS and cells in terms of flocculation ability of microbial aggregates, to achieve a low turbidity and a high quality of effluent. Turbidity was increased with an increase of EPS_p and was decreased with a decrease in EPS_p content. Figure 4.4 demonstrates the changes of turbidity with changes in $EPS_{protein}$ concentration. A similar trend between $EPS_{protein}$ and turbidity was found in our results. Between the days 51 and 71, 85% decrease of turbidity was obtained when the EPS_p concentration was decreased 79%. Especially during the last cycle a clear interaction between turbidity and EPS_p was obtained. A decrease at EPS_p concentration from 42.5 to 22.8 $mg.g^{-1}$VSS was led to a drop at effluent turbidity for 33 NTU. Therefore, if we look back to Figure 4, to understand the decreases at turbidity during the last 10 days of the study (due to the changes at sodium concentrations), we could determine a cumulative impact of sodium by the parameters, which were influenced by sodium concentration changes (such as EPS_p).

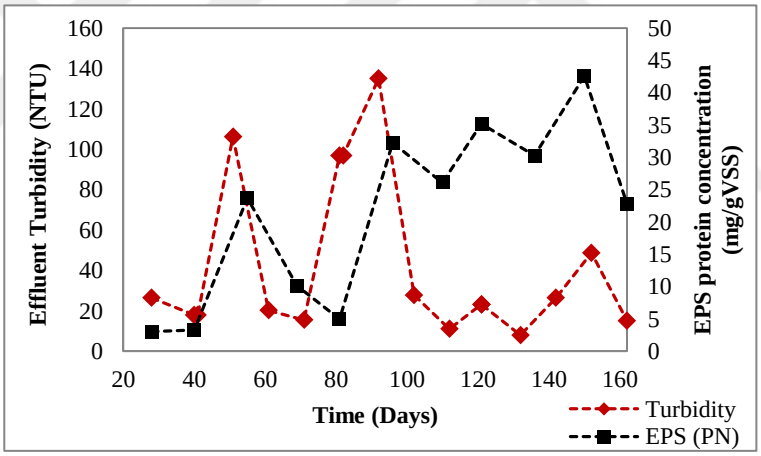

Figure 4.4 : Variation of effluent turbidity due to changes at EPSprotein.

4.3 The Effect of Sodium on Solids Concentration

The variation of Total suspended solids (TSS) and Volatile suspended solids (VSS) concentration in the sludge samples during the salinity studies at different sodium concentrations are shown in Figure 4.5. The TSS and the VSS concentrations at the beginning of the study were 29 gL^{-1} and 16.4 gL^{-1}, respectively. During the first cycle, within 40 days, sodium concentration of sludge was increased from 16 to 18.7 gL^{-1} and a decrease by 36% in TSS concentration was obtained concurrently (Figure 4.5 a). During the second cycle, sodium concentration was slightly increased from

18.7 to 21 gL^{-1} while also an increase in TSS concentration was observed from 18.7 to 24 gL^{-1}. A positive relation was obtained during the second cycle, where the TSS was increased within an increase in sodium concentration. Moreover, a significant decrease at TSS concentration from 24 to 14 gL^{-1} was observed during the first 15 days of the third cycle, while there was a decrease of sodium concentration from 21 to 19 gL^{-1}. From day 95 to 101, an increase in sodium concentration by 9% was exhibited which resulted in a 43% increase at TSS concentration. Last 20 days of the third cycle demonstrated a drop at TSS concentration of 24.5, 11.5 and 8.2 gL^{-1} at 21, 20 and 19.4 $gNa^{+1}L^{-1}$ respectively.

During the fourth cycle, a great increase by 47% of sodium concentration between the beginning and the end of the cycle was obtained, when an increase in TSS concentration from 8.2 to 12.4 gL^{-1} was observed. The positive relationship observed during second, third and fourth cycle, agree with the study of Yogalakshmi & Joseph (2010).

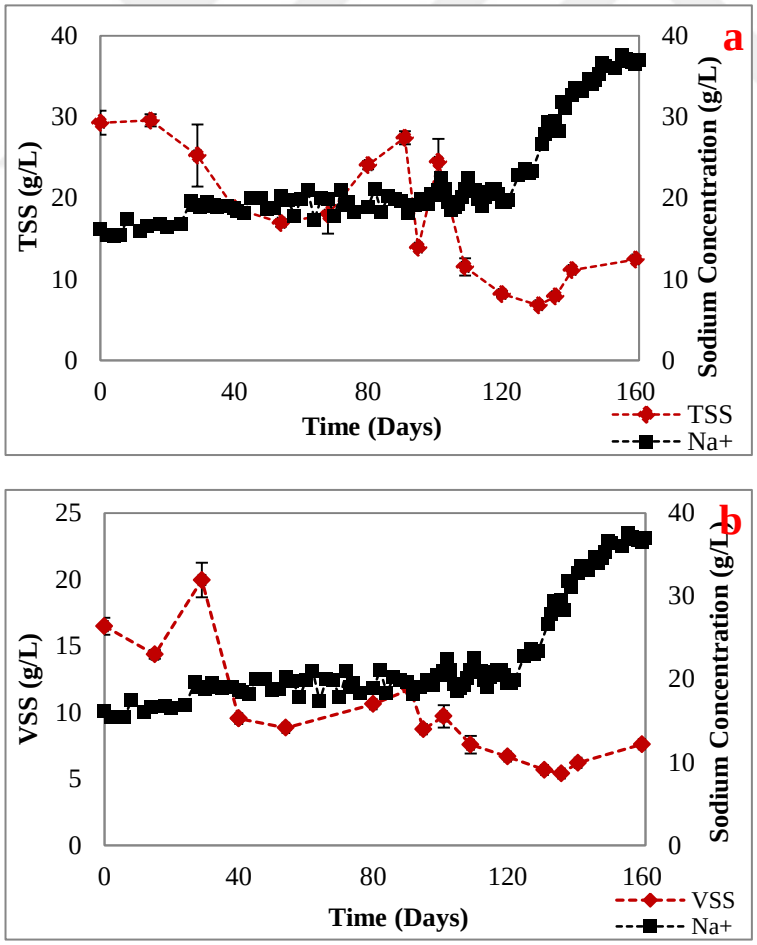

Figure 4.5 : Variation of solids in the MBR sludge due to salinity changes - all cycles (a) Total Suspended Solids (TSS) (b) Volatile suspended solids (VSS).

They stated an increase in MLSS concentration by 4% at sodium chloride shock load of 5 gL^{-1}. Likewise, MLSS concentration was increased by 8-10% and 13-16%, when they applied the sodium shock loads of 10-30 gL^{-1} and 50-60 gL^{-1} respectively.

A significant increase at sodium concentration leads to hypertonic conditions, where biomass expels water because of the osmotic pressure and results with death of biomass, which induces decreases at VSS concentration (Yogalakshmi & Joseph 2010). The variations of VSS concentration of biomass, due to the variations in sodium concentration of sludge, represented at Figure 4.5 b. During the first 15 days of first cycle, VSS concentration was decreased from 16.4 to 14.3 gL^{-1}, while sodium concentration was found to increase from 16 to 17 gL^{-1}. However, from day 15 to 29, an increase of VSS concentration from 14.3 to 20 gL^{-1} was obtained within a slight increase in sodium concentration. During the second cycle (from day 40 to 80) VSS concentration increased gradually from 9.5 to 10.6 gL^{-1}, within an increase in sodium concentration from 18.7 to 21 gL^{-1}. During the third cycle, from day 80 to 91, VSS concentration increased from 10.6 to 11.7 gL^{-1}, while a decrease of sodium observed by 7%. Between the days 91 and 101, VSS concentration decreased from 11.7 to 9.7 gL^{-1} while the sodium concentration increased by 6.5%. However after day 101 until the end of third cycle, VSS concentration decreased 31% with a decrease of sodium concentration by 13%. The last cycle demonstrated a decrease in VSS values while there was an increase in sodium concentration until day 136. From day 120 to 136, VSS decreased by 19% (from 6.7 to 5.7 gL^{-1}) when an increase in sludge sodium obtained by 34% (from 19.4 to 29.5 gL^{-1}). After day 136 and onwards VSS concentration increased with in an increase in sodium concentration respectively, showing a negative relation with sodium changes.

The results presented in Figure 4.6 shows the VSS/TSS ratio in the reactor during the complete study with four different cycles. Yogalakshmi & Joseph (2010) stated a drop in MLVSS/MLSS ratio to 0.7 to 0.6 during their study, when they increase the sodium concentration loads from 5 to 30 gL^{-1} and from 50 to 60 gL^{-1}, respectively. The decrease of ratio demonstrates more quantity of inorganic constituents mainly sodium chloride in the sludge (Yogalakshmi & Joseph 2010). The ratio changes determined in a range of 0.4 – 0.83. During the first cycle from day 0 to 15 a reverse relation between the ratio and sodium was observed and ratio dropped from 0.56 to 0.48 within an increase in sodium by 6%.

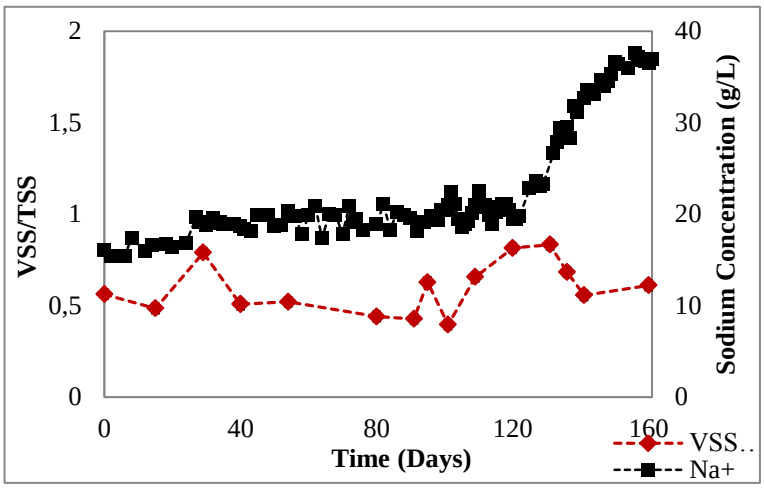

Figure 4.6 : Variation of VSS/TSS ratios due to salinity changes (all cycles).

However, from day 15 to 30 when the sodium concentration slightly increased to 19 gL^{-1} a significant increase by 38% VSS/TSS ratio was obtained. Second cycle represented a similar behavior with the results of Yogalakshmi & Joseph (2010) and VSS/TSS ratio was found to decrease from 0.5 to 0.4 within an increase in sodium concentration by 11%. During the third cycle from day 80 to 95 ratio increased from 0.4 to 0.6 with a reduce of sodium from 21 to 19 gL^{-1}. Likewise, from day 95 to 101, sodium was increased from 19 to 21 gL^{-1} and a drop in ratio was observed from 0.6 to 0.4. Until the end of third cycle sodium concentration reduced by 7% and ratio was found to increase from 0.4 to 0.8. A positive relation of sodium and VSS/TSS ratio was observed at third cycle. Also, the last cycle represented a decrease of ratio from 0.8 to 0.55 while sodium concentration continuously increased from 19.4 to 33 gL^{-1}. However, last 20 days of cycle demonstrated an increase in ratio by 9% within an increase in sodium concentration by 10.6%.

4.4 Extracellular Polymeric Substances and Soluble Microbial Products Formation

Extracellular polymeric substances (EPS) are metabolic compounds which are secreted by cells and accumulating on cell surfaces (Wang et al. 2013; More et al. 2014). They could also comprised of cell lysis, cell surface material shedding and sorption from environment (Liu & Fang 2002). These substances found in two forms; bound EPS (e.g. attached organic materials) and soluble EPS in other words, soluble microbial products (SMP) (e.g. soluble macromolecules or colloids) (Sponza 2003).

EPS contains a variety of organic substances such as; proteins, polysaccharides, nucleic acids, humic-like substances, lipids and heteropolymers such as glycoproteins. However, proteins (PN) and polysaccharides (PS) assumed as the most dominant components of extracted EPS thus, they conceived as the representative of EPS and SMP (Liu & Fang 2003). Table 4.1 and Table 4.2 summarize the amounts of EPS and SMP extracted, during the complete study with four different cycles at different sodium concentrations respectively. At the first cycle, during the first 14 days of operation, a great decrease in protein content of EPS from 19.6 to 0.2 mg $gVSS^{-1}$ was observed, while sodium concentration increased from 16.1 to 17 gL^{-1}, within the first sodium shock load after being acclimated to 16 gL^{-1}. Also an increase in protein content of SMP by 23% was found as a result of the first sodium shock load (between the days 0 and 14). This increase in SMP, might be induced by protein dissolution of EPS by bacterial enzymes and converted to SMP (Yogalakshmi & Joseph 2010). Moreover, polysaccharide contents of EPS and SMP were found to increase by 13% and 63% respectively. A maximum PS concentration of 3.6 mg g^{-1}VSS at 17 $gNa^{+1}L^{-1}$ was obtained in SMP at this cycle. After day 14 until day 40, while there was an increase in sodium concentration from 17 to 18.7 gL^{-1}, an increase of PN and PS contents of EPS by 95% and 6% was obtained. Vyrides & Stuckey (2009) stated an increase of EPS over time as a response of the biomass to overcome the high sodium toxicity, when the biomass exposed to 40 g NaCl L^{-1}. Reid et al. (2006) applied a salt shock and the results demonstrated an increase in both carbohydrate and protein levels of SMP and EPS concentrations, with increased salinity. Wang et al. (2015) found that the increase in salinity could cause bacteria to produce more exopolysaccharides due to the variation of osmotic pressure between bacteria and the media. Abbasi & Amiri (2008) demonstrated that the osmotic pressure created by salinity, may stimulate the microorganisms to produce exopolysaccharides to avoid salinity, which lead to an increase of PN in the EPS and SMP. However, during second cycle, both proteins and polysaccharides of EPS indicated a decrease from day 54 to 80 with an increase of sodium concentration from 20.3 to 21 gL^{-1}. In SMP, protein concentration increased from 17.6 to 28.3 mg $gVSS^{-1}$ and then decreased again from 28.3 to 24.2 mg $gVSS^{-1}$ likewise, PS concentration first increased from 1.5 to 3.4 mg $gVSS^{-1}$ and then decreased again from 3.4 to mg $gVSS^{-1}$ within the increase in sodium.

Table 4.1 : Variation of protein and polysaccharide concentration in EPS due to changes at sodium concentration (all cycles)

	Time (Days)	Na^+(g/L)	EPS [mg $gVSS^{-1}$]	
			Polysaccharides	**Protein**
1st cycle - Evenly Distributed	0	16.1	2.7	19.6
	14	17.0	3.1	0.2
	28	19.1	3.5	3.0
	40	18.7	3.3	3.3
2nd cycle - Stepwise Increase/Decrease	54	20.3	1.9	23.7
	68	20.0	1.7	10.1
	80	21.0	1.1	5.0
3rd cycle - Non-evenly Distributed	94	19.0	0.4	32.3
	108	21.0	0.2	26.1
	120	19.4	2.1	35.2
4th cycle - High concentration swings	134	29.4	1.9	30.3
	148	34.6	2.4	42.5
	160	36.9	0.9	22.8

Laspidou & Rittmann (2002) explained the decrease in bound EPS and the increase in SMP with increasing sodium chloride shock loads by the plasmolysis and release of intracellular constituents. They also stated, when un-metabolised products accumulate because of incomplete degradation of organic substances and microbially produced polymers, an increase in SMP could be observed. During the third cycle from day 94 to 108, while sodium concentration was increased from 19 to 21 gL^{-1}, a decrease of PN and PS in EPS by 19% and 50% was obtained respectively. From day 108 to 120 an inverse relationship was observed between sodium concentration and protein and polysaccharide contents. Both were found to increase by 26% and 90%, while the sodium concentration was decreased from 21 to 19.4 gL^{-1}. PS content of SMP responded to the changes of sodium positively and first increased by 72% within an increase in sodium concentration (from day 94 to 108) and decreased by 86% within a decrease in sodium concentration (from day 108 to 120). A decrease of 89% was observed in protein contents during this cycle. Abdollahzadeh Sharghi et al. (2014) found a significant drop in the total EPS concentration when salt concentration is increased from 200 to 250 g L^{-1}, however without an increase in SMP concentration at the same time.

Table 4.2 : Variation of protein and polysaccharide concentration in SMP due to changes at sodium concentration (all cycles)

	Time (Days)	Na^+(g/L)	SMP [mg $gVSS^{-1}$]	
			Polysaccharides	**Protein**
1st cycle - Evenly Distributed	0	16.1	1.3	49.5
	14	17.0	3.6	64.1
	28	19.1	2.7	44.5
	40	18.7	3.0	26.2
2nd cycle - Stepwise Increase/Decrease	54	20.3	1.5	17.6
	68	20.0	3.4	28.3
	80	21.0	0.7	24.2
3rd cycle - Non-evenly Distributed	94	19.0	0.1	127.8
	108	21.0	0.3	51.0
	120	19.4	0.04	14.0
4th cycle - High concentration swings	134	29.4	1.7	62.9
	148	34.6	1.6	72.5
	160	36.9	2.3	136.6

They explained the decrease in EPS concentration by a corresponding drop in the bacterial growths with an increase in salt concentration to 250 gL^{-1}. Another study explained the decrease in the bound protein content by ion-exchange reactions; when the sodium concentration increased, high concentrations of monovalent cations displaced the divalent cations from the floc and led to a decrease in the bound protein content (Higgins et al. 1997). This might also explain the protein decreases in EPS during our experiments within an increase in sodium concentration without an increase in SMP protein concentration (between the days 68-80 and 94-108). Maximum sodium concentrations were applied during the fourth cycle and sodium concentrations were increased gradually from 19.4 to 36.9 gL^{-1}. Accordingly, PN and PS contents of EPS were increased until day 148. However, last results (day 160) showed decrease by 46.4% and 60.1% in PN and PS of EPS respectively, even though there was 6% increase in sodium concentration. SMP results demonstrated an increase at protein contents from day 120 to 160, 14.0, 62.9, 72.5 and 136.6 mg $gVSS^{-1}$ were determined at elevated sodium concentrations of 19.4, 29.4, 34.6 and 36.9 gL^{-1} respectively. Here, we could observe the impact of solubilized protein in EPS induced an increase in SMP as stated above. Another reason of increase in SMP might be the production of polymers by some certain microorganisms, to protect themselves against the sodium chloride shock loads (Yogalakshmi & Joseph 2010).

The maximum protein concentration of 136.6 mg $gVSS^{-1}$ in SMP determined during the fourth cycle, when the maximum concentration 36.9 gL^{-1} of sodium was obtained. Meanwhile, protein and polysaccharide of EPS was found to decrease, demonstrating the increase in salinity could increase the solubility of protein and carbohydrate of EPS, which might be another reason for increasing the SMP (Zhang et al. 2014). However, PS contents demonstrated a decrease from 1.7 to 1.6 mg $gVSS^{-1}$ and again an increase to 2.3 mg $gVSS^{-1}$, while sodium concentration was increasing.

PN/PS ratios in EPS and SMP were depicted to make a comparison of the responses obtained from all different cycles (Table 4.3). Wang et al. (2015) stated the reverse relationship between salinity and PN/PS ratio, which indicates a higher increase in polysaccharide content than protein, when the biomass was exposed to increasing sodium concentrations. The reason for the increase of PS amounts in the EPS might be induced by the death of some bacteria (Sponza 2002). Similar to results of Wang et al. (2015), during the first cycle, the PN/PS ratio of SMP was decreased with an increase in sodium concentration. When the sodium concentration were increased from 16.1 to 18.7 gL^{-1} a decrease of 38, 18, 16.8 and 8.8 was determined in PN/PS ratio of SMP. However, during the first 14 days of operation, the ratio of EPS demonstrated a decrease from 7.2 to 0.1. After day 14, an increase in ratio of EPS from 0.1 to 1.0 found, when sodium concentration increased from 17 to 18.7 gL^{-1}. During the second cycle, between the days 40 and 54 sodium concentration increased and it resulted with an increase by 23% and 92% at ratios of SMP and EPS respectively. However, the decrease of sodium concentration between the days 54 and 68 caused a decrease of ratio by 26% and 51% at SMP and EPS respectively.

Moreover, at the end of second cycle PN/PS ratio was decreased 26% in EPS and increased 76% in SMP with an increase at sodium concentration (from 20 to 21 gL^{-1}). During the third cycle, within the swing at sodium concentration which increased from 19 to 21 gL^{-1} and decreased to 19.4 gL^{-1} respectively, PN/PS ratio of EPS responded with a similar behavior i.e. an increase from 88 to 124.4 and a decrease to 16.7. However, the PN/PS ratio of SMP responded reversely and first decreased from 1521.2 to 168.5 and increased to 324 between the days 94 and 120. Last cycle with high sodium fluctuations, demonstrated more consistent behavior.

Table 4.3 : Variations of PN/PS ratio of EPS and SMP due to changes at sodium concentration (all cycles)

	Time (Days)	$Na^{+}(gL^{-1})$	PN/PS ratio	
			EPS	SMP
1st cycle - Evenly Distributed	0	16.1	7.2	38
	14	17.0	0.1	18
	28	19.1	0.9	16.8
	40	18.7	1.0	8.8
2nd cycle - Stepwise Increase/Decrease	54	20.3	12.5	11.4
	68	20.0	6.1	8.4
	80	21.0	4.3	35.3
3rd cycle - Non-evenly Distributed	94	19.0	88	1521.2
	108	21.0	124.4	168.5
	120	19.4	16.7	324
4th cycle - High concentration swings	134	29.4	16	36.7
	148	34.6	18.1	45.4
	160	36.9	24.3	59.7

After day 134 and when sodium concentration continuously increased, PN/PS ratio of EPS and SMP also found to increase gradually. During the study, determination of an increase in PN/PS ratio, while there was an increase at sodium concentration, demonstrates the change of protein concentration were higher than the change of polysaccharides under elevated salinity. For instance when we consider the last cycle when sodium concentration increased from 29.4 to 36.9 gL^{-1} protein contents of SMP was increased 54% however polysaccharide contents were increased by 25%.

During the complete study, PN/PS ratio of SMP was found to be greater than the ratio of EPS. This was explained by Wang et al. (2015) and Zhang et al. (2014) that protein contents in the SMP was more sensitive to salinity variation than the proteins in the EPS, since proteins could be found in the extracellular environment due to the secretion of intracellular proteins or enzymes or cell lysis, but carbohydrates are extracellular components, more synthesized for specific functions. Moreover, both in EPS and SMP, protein concentration was higher than polysaccharides. Ismail et al. (2010) indicated the protein fraction of EPS was much higher, around 87-94%, than the polysaccharide fraction in their study. Sponza (2003) stated within EPS analysis, 60 and 80% of extracellular substances was attributed to protein and the remaining percentage consisted of the DNA and carbohydrate. This study also explains the predominance of proteins in sludge EPS. The high level exoenzymes in EPS matrix

might be observed, when there were an uptake of readily biodegradable organic substrate, such as glucose and acetate (Sponza 2003). This might be another indirect impact of increasing SMP concentration because of the conversion of proteins in EPS by exoenzymes.

4.5 Relative Hydrophobicity

Relative Hydrophobicity (RH) is a physical property which represents the tendency of water to repel the non-polar molecules. Therefore, measuring the hydrophobicity of bacterial cell surfaces is significant to understand the mechanisms by which component can bind to the surfaces (Lindahl et al. 1981). During the four different cycles, responses of microorganism biomass due to varying salt concentrations were observed. Variation of RH with changing effluent sodium concentrations are as illustrated in Figure 4.7. After the first 10 days of sodium exposure at the evenly distributed cycle, sodium concentration was increased from 16 to 17.5 gL^{-1} resulting in a drop of RH from 50.6% to 48% respectively (Figure 4.7 a). Until day 20, while sodium concentration decreased to 16.4 gL^{-1} a simultaneous increase in RH from 48% to 51.1% was observed. From day 20 to 40, while sodium concentration increased about 12%, RH was dropped gradually from 51.1% to 41% and to 35.1%. Results obtained during the first cycle demonstrated a reverse relationship of RH and sodium concentration; an increase in hydrophobicity with a drop in sodium concentration and vice versa. Similarly, Wang et al. (2015) demonstrated a rapid decrease of RH of granular sludge (from 73% to 46.6%) with the increase in salinity from 0% to 5% and a slow decrease of RH when the salinity increased from 5% to 8%. During the first 10 days of second, stepwise increase/decrease - cycle when the sodium concentration were fluctuated from 18.2 to 20 gL^{-1} and back again to 18.6 gL^{-1}, RH was increased from 35.1% to 39.2% (Figure 4.7 b). Between the days 50-60 and 60-70 small swings were obtained at sodium concentration. An average of 6.3% increase in sodium concentration resulted in a decrease of RH to 36% (day 50-60). However from day 60 to 70, 10% decrease at sodium concentration was resulted in a decrease of RH to 29.4%. Moreover, during the last 10 days of second cycle, a significant raise from 29.4% to 52.1% at RH was obtained with an increase of 5.5% at sodium concentration.

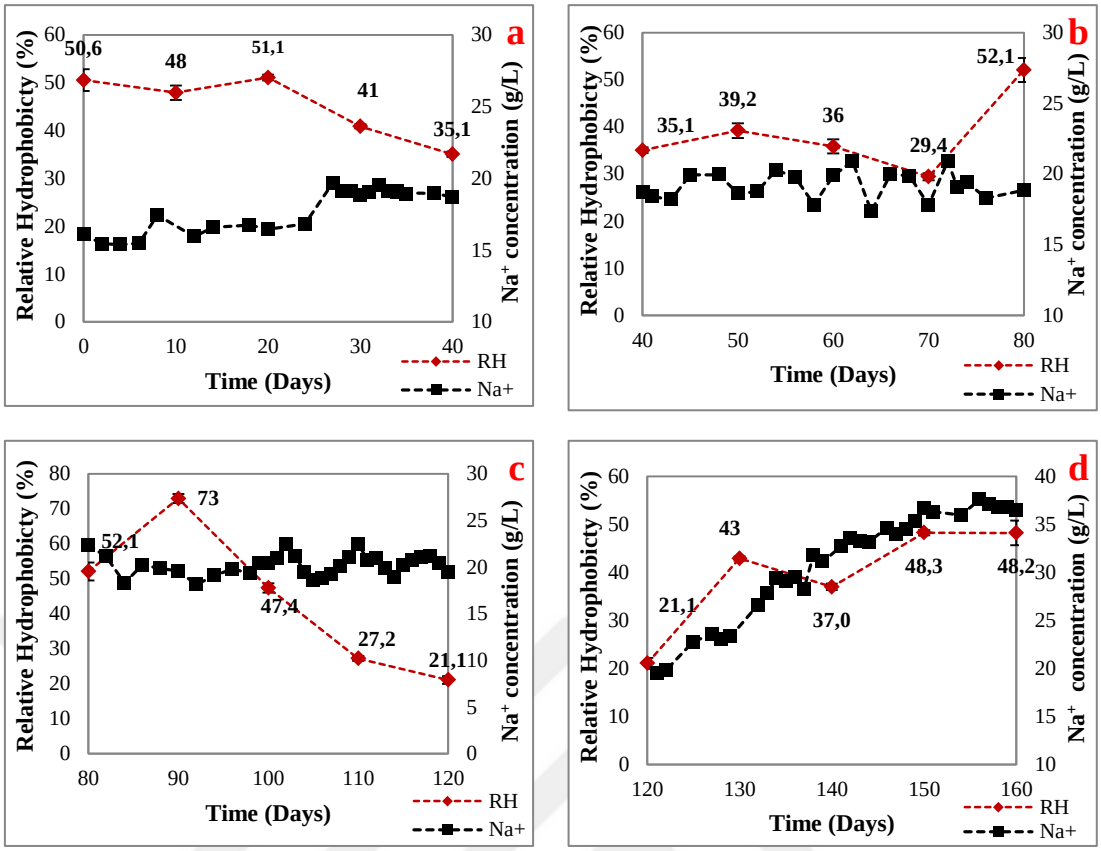

Figure 4.7 : Variation of Relative Hydrophobicity due to salinity changes (a) Evenly Distributed; (b) Stepwise Increase/Decrease; (c) Non-evenly Distributed; (d) High Concentration Swings.

These positive relations might be explained by the influence of other operational parameters, since the hydrophobicity of the microorganisms cell surface found to be variable due to substrate, temperature, pH, EPS or bacterial culture composition (Liu & Fang 2003; Dereli, Heffernan, et al. 2015). They indicated that bound proteins are often correlated with sludge hydrophobicity. Since proteins in EPS are regarded as hydrophobic and the polysaccharides as hydrophilic components, it is possible to make a positive correlation between Protein/Polysaccharide ratio in EPS and hydrophobicity of sludge surface (Wang et al. 2015). Ersahin et al. (2014) indicated the PN/PS ratio as a factor that influence the hydrophobicity and surface charge of the sludge and stated high ratios resulted in high hydrophobicity. Zhang et al. (2014) stated the decreases of EPS by salt shock load, induced surface negative charges of sludge as well as hydrophobic properties. As the EPS and SMP results demonstrated above, we could observe a decrease of bound protein as well as the ratio (both in EPS and SMP) between the days 60-70, which are in accordance with findings of previous studies. More et al. (2014) stated the influence of EPS on hydrophobicity of

microbial aggregates by claiming that when the EPS particles or molecules are not able to bound or interact electrostatically with water molecules, induce hydrophobic properties into the EPS matrix. Also, when an increase of RH was found between days 70-80, an increase in PN/PS ratio of SMP was observed. During the third cycle, between the days 80 and 90, sodium concentration was decreased from 21 to 20 gL^{-1} and induced an increase in RH from 52.1 to 73% (Figure 4.7 c). Until day 100, RH dropped to 47.4% while sodium concentration was increased by 4%. Between the days 100 and 120, some fluctuations were observed at sodium concentration, where the increasing concentrations were resulted in a decrease of RH to 21.1%. Figure 4.7 d exhibits the variation of RH during the fourth cycle. During this cycle, an increase at RH was found while an increase at sodium concentration was observed, especially between the days 120-130 and 140-150. An increase of proteins by 77% and 13% was obtained between days 120-130 and 140-150, respectively. As well as the PN/PS ratio was found to increase from day 140 to 150. Therefore while considering the impact of sodium on hydrophobicity properties of microbial cells, it is significant to examine the behavior of other parameters (such as EPS) due to the changes of sodium concentration, since they might have an indirect influence on hydrophobicity.

4.6 Particle Size Distribution

Small sized solid particles such as; microbial cells, extracellular organic particles and inorganic precipitates generally accumulate on the membrane surface during the membrane filtration processes (Ersahin et al. 2014). These particles become denser over time and produce a cake layer. It might cause fouling problems and flux limitation impacting the performance and the filtration capacity of the membrane therefore, particle size is being crucial to determine. Figure 4.8 shows the changes in particle size distribution (PSD) of the sludge samples in terms of median particle diameter (D50 μm) during the four different cycles, ran under different sodium concentrations. Figure 4.8 a represents the results obtained during the first cycle; evenly distributed. During the first 10 days of the first cycle, 45% decrease in particle size of sludge was determined, while the sodium concentration demonstrated an increase from 16 to 17.5 gL^{-1}. After day 10 particle sizes of sludge were increased from 19.1 to 28.8 μm, while sodium concentration decreased down to 16.4 gL^{-1}. High concentrations of monovalent cations induce a displacement of the divalent

cations from cell surfaces and cause weaker bridges between polymers, which results in a decomposition of the floc structure into small individual flocs (Zhang et al. 2014). This might explain the decrease in particle sizes while there was an increase in sodium concentration. Findings of Abdollahzadeh Sharghi et al. (2014) demonstrated a decrease of D_{50} floc sizes (from 15.9 µm to 11.2 µm) with an increase in salt concentration 100 to 250 gL^{-1}. However from day 20 to 30, particle sizes increased with 12.6% increase in sodium concentration.

Between the days 30 and 40 when a slight decrease of sodium concentration by 2% was obtained, a decrease in particle sizes from 34.1 to 28 µm was measured. PSD is closely related to EPS concentration, especially with protein content, since it could strengthen bonds between flocs and induce flocculation. Ersahin et al. (2014) found finer particles in their bulk sludge, when EPS amount reduced due to reduced flocculation at low EPS concentrations. Similarly, Sponza (2002) indicated that flocs formed under anaerobic conditions, while treating effluents from a chemical industry, a reduction of protein level resulted in decreases of floc diameter from 154 to 70 µm.

The decreases in proteins could induce weakening of flocs and result in

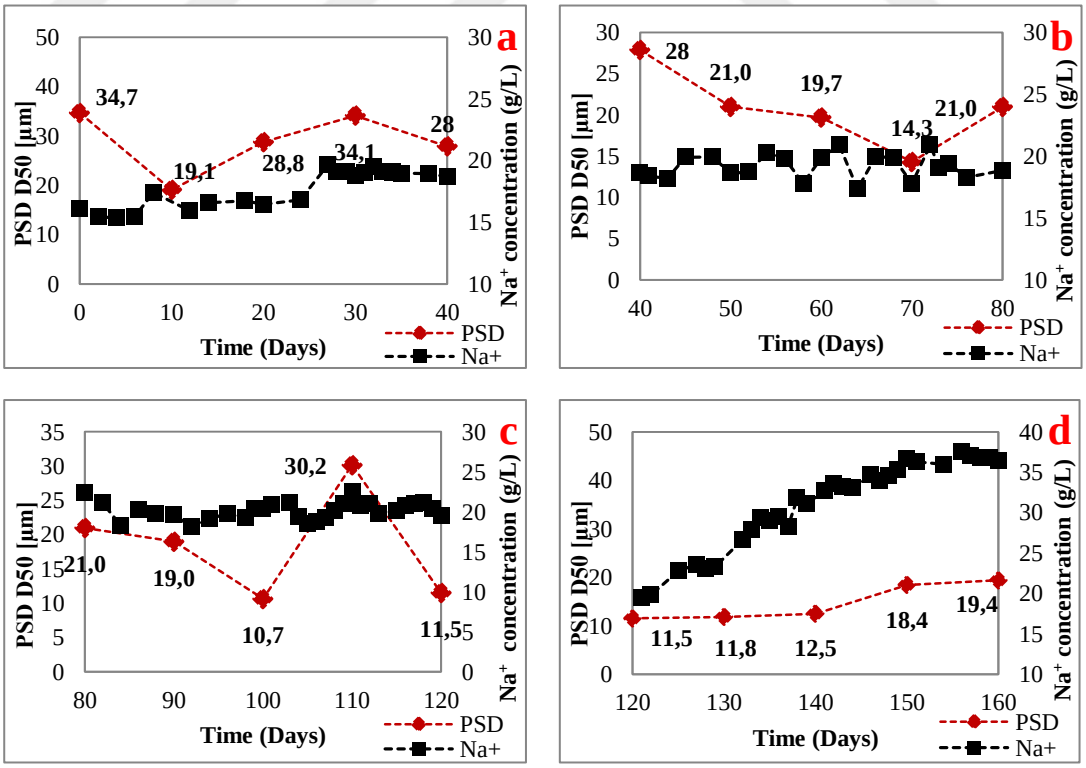

Figure 4.8 : Variations of sludge particle size distribution due to salinity changes (a) Evenly Distributed; (b) Stepwise Increase/Decrease; (c) Non-evenly Distributed; (d) High Concentration Swings.

deflocculation (Frølund et al. 1996). Between the days 20 and 30 we also observed an increase of protein in EPS by 93%, might be the reason of increase in particle size. Figure 4.8 b exhibits the results obtained during the second cycle; stepwise increase and decrease. Between the days 50 and 60, 8.2% decrease of particle size was determined, while there was an increase of sodium concentration by 6.3%. From day 60 to 70, a decrease of particle sizes were determined, while an average decrease of sodium was obtained, from 19.8 to 17.8 gL^{-1}. However, meanwhile a decrease of protein concentration of EPS by 57% was obtained, which could be the reason of particle size decrease. Between the days 70 and 80 particle sizes represented an increase of 32%, when a decrease in sodium concentration was measured. Figure 4.8 c shows the variation of particle sizes during the third cycle; non-evenly distributed. During the first 10 days of operation, when an average decrease of sodium concentration from 21 to 19.6 gL^{-1} obtained, particle sizes found to decrease from 21 to 19 μm. Between the days 90 and 100, an increase of sodium concentration resulted with a significant decrease at particle sizes from 19 to 10.7 μm. Between the days 100 and 110, an increase in sodium concentration of 17.5% obtained, especially between the days 105 and 110, while an increase at particle sizes from 10.7 to 30.2 μm measured. Ismail et al. (2010) found in their study that the granules grown at 20 $gNa^{+}L^{-1}$ were clearly bigger than the granules grown at 10 $gNa^{+}L^{-1}$; explaining by the presence of excessive amounts of sodium compounds at 20 gL^{-1} might loosen the binding of EPS and induce enlarged granules, whereas at the same time weaker granule structure was observed because of repulsive electrostatic forces. Jang et al. (2013) indicated a significant increase of floc sizes when the NaCl concentrations increased from 0 to 20 gL^{-1}. Until day 120, a decrease of 13.5% in sodium concentration resulted in a decrease by 39% in particle sizes. Figure 4.8 d represents the results determined during the fourth cycle; high concentration swings. During 40 days of operation, sodium concentration was increased continuously until 36.9 $gNa^{+}L^{-1}$, while particle sizes gradually increased from 11.5 to 19.4 μm. At the same time during the fourth cycle, protein concentration of SMP in sludge was increased from the beginning until the end of the cycle as stated on previous sections, might be the reason of increase at particle size. Since protein content is significant in sludge flocculation, a decrease in PN/PS ratio is also correlated to a decrease of median particle sizes (Dereli, Heffernan, et al. 2015). Similarly, during the last cycle, PN/PS ratios of both EPS and SMP were found to increase. Such a finding could explain the

increases of particle sizes between the days 120 and 160. Variations of particle sizes D_{10} and D_{90} due to the variations of sodium concentrations applied during all cycles, were also demonstrated in Figure A. 1 and Figure A. 2.

4.7 Capillary Suction Time and Spesific Resistance to Filtration

Capillary Suction Time (CST) and Specific Resistance to Filtration (SRF) values were analyzed and used as an indicator to determine the sludge filterability and dewaterability since they represent the release of water from the sludge matrix (Gray 2015). Figure 4.9 and Figure 4.10 shows the variations of SRF and CST values due to changes in sodium concentration, respectively. They were determined at the beginning and the end of every mode of influent salinity fluctuation. During the first cycle, a great decrease of 89% in SRF was determined, while sodium concentration was increased from 16 to 18.7 gL^{-1} within 40 days of operation (Figure 4.9). During second cycle, biomass was exposed to stepwise increasing and decreasing concentrations and between the days 40 and 80, the average sodium concentration was increased from 18.7 to 21 gL^{-1}. Accordingly, SRF values were found to decrease by 74%. Between the days 80 and 120, a decrease at sodium concentration from 21 to 19.4 gL^{-1} observed, while SRF value was determined to increase by 40%. In the light of results obtained during the first three cycles, a reverse relationship between sodium concentration and SRF values could be derived. Jin et al. (2004) also stated that the sludges capable to readily release their water have a low SRF or CST. Therefore, low values indicate better dewatering properties. However, during the fourth cycle an opposite situation observed. From day 120 to 160, while sodium concentration continuously increased from 19.4 to 36.9 gL^{-1}, also an increase in SRF value by 38% was found. Dewaterability of sludges were stated to be influenced by many characteristics such as; particle size distribution, bound water content, viscosity, structure and composition of the flocs and extracellular polymeric substances (EPS) (Cetin & Erdincler 2004). Especially some studies indicated the significant influence of EPS and SMP concentrations on filterability and dewaterability of sludge (Sponza 2002; Cetin & Erdincler 2004; More et al. 2014).

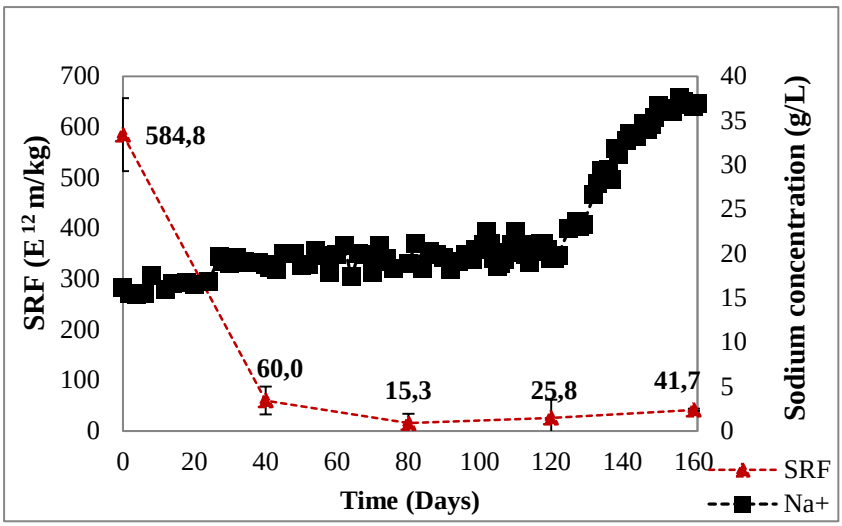

Figure 4.9 : Variation of SRF values of the sludge due to in salinity changes - all cycles.

Sponza (2002) reported a reduced protein fraction in sludge EPS improved the sludge dewaterability, since the proteins had a high water-holding capacity. Likewise, Cetin & Erdincler (2004) stated an improvement on sludge filterability and compactibility, when a decrease of protein contents determined. More et al. (2014) stated a negative effect on sludge dewatering, while higher concentrations of SMP were presented. Therefore the increase of SRF values during the fourth cycle, while sodium concentration was elevated, could be induced by the increase of protein contents. Especially a great increase of 54% at PN contents in SMP determined during the fourth cycle could be the explanation of increase in SRF values. Moreover, Cetin & Erdincler (2004) showed an increase of SRF, with an increase in total solids (TS) concentration from 2.38 to 5.66 kg m^3. An increase of TS concentration by 34% was determined during the fourth cycle, which might be the reason of the increase in SRF by 38%. SRF defined as an independent parameter from TSS concentration by Dereli et al. (2014). However, they found higher SRF of the sludge with an increase in TSS concentration in their study. Moreover, a significant relationship between SRF and particle size of the sludge flocs stated by many researches (More et al. 2012a; Dereli et al. 2014; Ersahin et al. 2014). A reverse relationship was determined between these two parameters, indicating that smaller size of particles might induce higher values of SRF since they can easily attach on the membrane surface and/or fill cavities in the membrane layer and therefore may contribute cake layer formation and compaction (Ersahin et al. 2014). When we consider the relation of particle size and SRF, during the decrease between

day 90 and 100, meanwhile an increase in SRF value was determined. However, during the fourth cycle, an increase at particle sizes with an increase at sodium concentration was determined and SRF values were found to elevate during this period as well. When we consider the variation of small sized particles (D_{10} μm) in our sludge, since they have a significant role in filterability, however an increase was obtained from day 120 to 160 (Figure 4.11). Results obtained during the fourth cycle, are in contradiction with the results of aforementioned studies. This positive relation might be explained by the increase of particle sizes because of excessive amount of sodium in the media as stated in previous section. However, granules become weaker at the same time and during filtration they might induce an increase in resistance to filtration. Figure 4.10 demonstrates the variations of CST values.

During the first cycle, a decrease from 419 to 122.3 seconds in CST was observed, while there was an increase in sodium concentration from 16 to 18.7 gL^{-1} during 40 days of operation. Second cycle was found to behave similarly to an increase in sodium concentration. Between the days 40 and 80 sodium concentration was increased from 18.7 to 21 gL^{-1} accordingly, CST results were found to decrease from 122.3 to 12.8 seconds. During the third cycle, between the days 80 and 120, an average decrease at sodium concentration demonstrated from 21 to 19.4 gL^{-1}, while 25% increase at CST value was determined. However, during the fourth cycle CST was increased from 17.1 to 37.6 seconds, when there was a great increase in sodium concentration from 19.4 to 36.9 gL^{-1} during 40 days of operation. The results obtained during the first three cycles (evenly distributed, stepwise increase/decrease and non-evenly distributed) also represented an inverse relationship between sodium concentration and CST (sec). However, the increase between the days 120 and 160 could be explained by the effect of other parameters especially, the increase of protein content of extracellular polymeric substances while the sodium concentration increased, as stated above. Close relation between TSS concentration and CST were also stated by other studies; claiming that sludges with high solids content and a low CST value, could be easily dewatered (Scholz 2005; Ersahin et al. 2014). However, during the fourth cycle, we obtained an increase in CST values with an increase of TSS concentrations by 45%. The results seem to be in contradiction with the previously-mentioned studies.

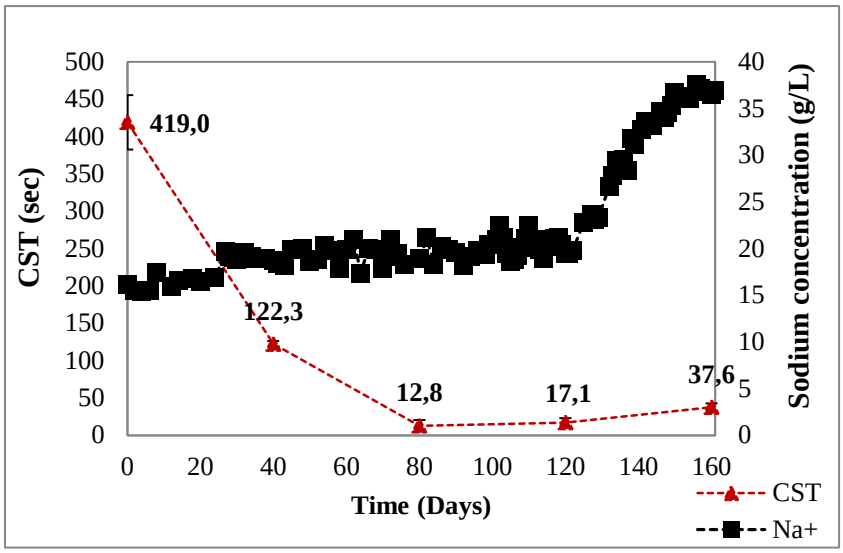

Figure 4.10 : Variation of CST values of the sludge due to salinity changes – all cycles.

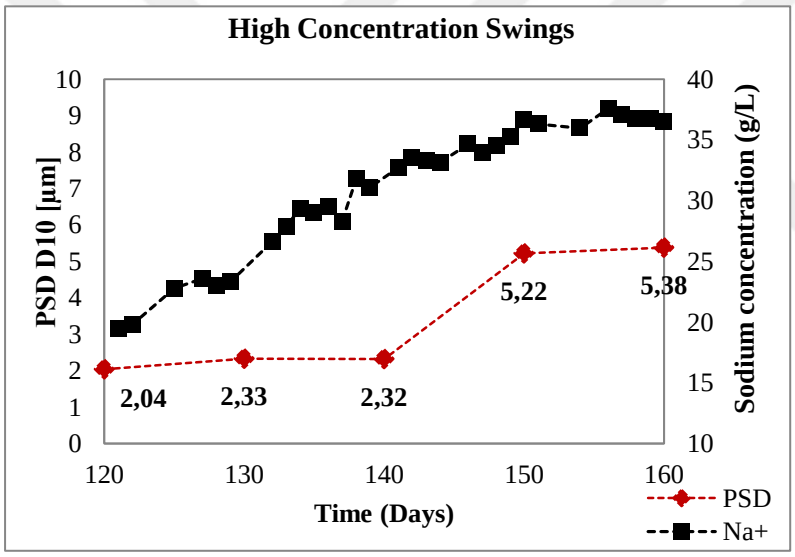

Figure 4.11 : Variations of sludge particle size distribution (D_{10}) due to salinity changes at high concentration swings cycle.

Moreover, higher dosage of EPS found to increase smaller size flocs which were blocked the filter surface on CST tests and resulted in an increase in CST values (More et al. 2012b). During the last cycle, total EPS was found to increase by 17%. This fact might be the reason of obtaining an increase in CST.

From Figure 4.9 and Figure 4.10, a similar trend in SRF and CST could be observed against the variations of sodium concentrations in the influent media. Some researches mentioned that these two parameters cannot be consider as substitutions for each other since they have inadequate quantitative relationship (Smollen 1986;

Jin et al. 2004) and some reported similar behavior of the CST and the SRF, suggesting that these parameters may be correlated (Pollice et al. 2007). CST might be a faster indicator to measure for evaluating dewaterability due to simple equipment requirement and fast outputs (Gray 2015).

4.8 Viscosity

Rheological character of the sludge was examined with a rheometer, as the representative of the deformation of a matter under shear stress effect and the results were shown in Figure 4.12 in terms of viscosity parameter (mPa.s). Since the wastewater sludges have a non-Newtonian fluid behavior (Seyssiecq et al. 2003), only apparent viscosity was determined with sludge samples (Pevere et al. 2006). First cycle, evenly distributed, demonstrates constant decrease of viscosity from results during forty days of operation, while sodium concentration was increased from 16 to 18.7 gL^{-1} (Figure 4.12 a). At second cycle, stepwise increase and decrease, during first 14 days viscosity was decreased by 10.2% within an increase by 8% in sodium concentration (Figure 4.12 b). However, between the days 54 and 68, viscosity continued to decrease from 1.4 to 1.35 mPa.s while sodium concentration was decreased from 20.3 to 19.8 gL^{-1}. From day 68 to 80 an increase in viscosity from 1.35 to 1.45 mPa.s was obtained when a decrease in sodium concentration was observed from 19.9 to 18.9 gL^{-1}. Figure 4.12 c represents the results obtained at third cycle, non-evenly distributed. During the first 14 days of operation a slight increase from 1.45 to 1.5 mPa.s was determined in viscosity when an average decrease of 11% in sodium concentration was measured. From day 110 to 120, a decrease of 11.4% was measured in viscosity while sodium concentration had an average decrease of 13.5%.

During the fourth cycle from day 130 and onwards sodium concentration increased constantly until the day 160 and an increase in viscosity from 1.3 to 1.7 mPa.s was determined (Figure 4.12 d). Abdollahzadeh Sharghi et al. (2014) recently reported a decrease of the apparent viscosity from 3.2 mPa.s to 2.1 mPa.s (at shear rate of 100 s^{-1}) when they increased the NaCl concentration of influent from 50 gL^{-1} to 100 gL^{-1}. However, they also found an increase in viscosity to 2.9 mPa.s, with a further increase in salinity to 250 $gNaClL^{-1}$ claiming that the mixed liquor became less non-

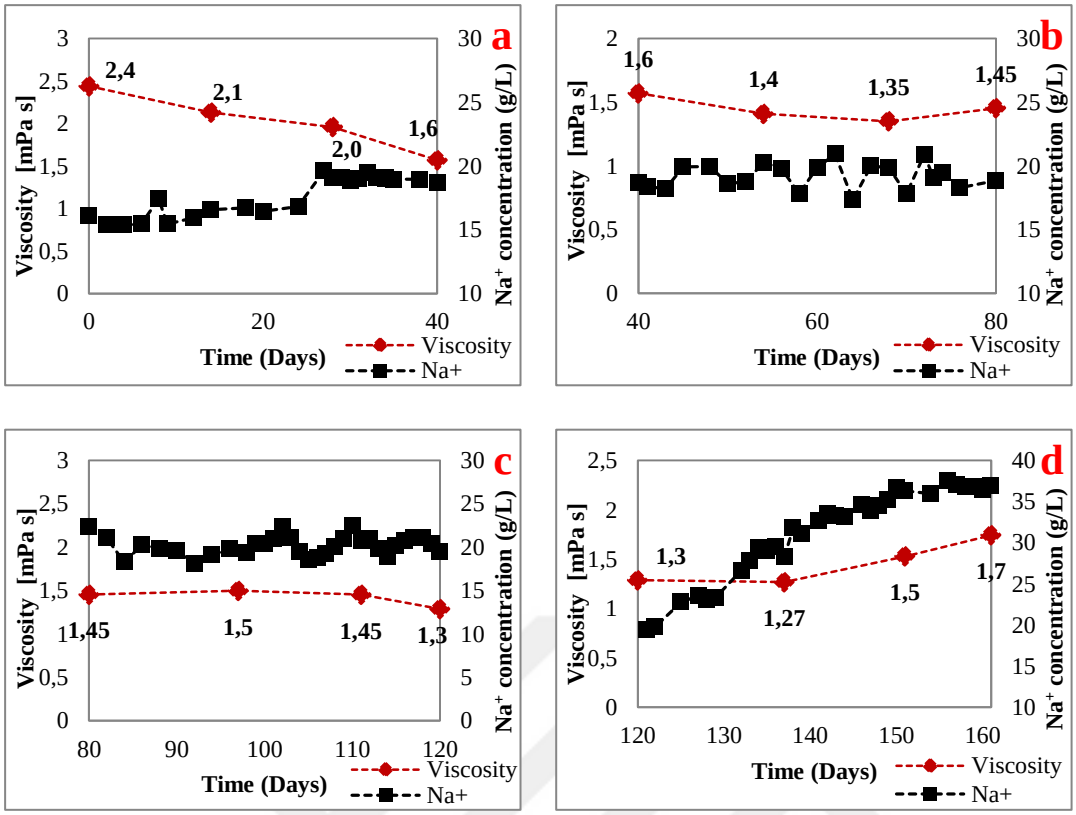

Figure 4.12 : Evolution of sludge viscosity due to salinity (a) Evenly Distributed; (b) Stepwise Increase/Decrease; (c) Non-evenly Distributed; (d) High Concentration Swings.

Newtonian, when salt concentration is increased above 100 gNaClL^{-1}. Therefore, the increase of viscosity between the days 137 and 160, might be explained by the change of sludge behavior due to great increase in sludge sodium concentration over 30 gL^{-1}. However, the experimental data obtained during the study regarding the impacts of sodium concentrations on sludge viscosity, is not enough yet to make a clear correlation between these two parameters.

Some studies demonstrated that sludge viscosity is dependent mainly on TSS content and reactor temperature, in membrane bioreactors (Hasar et al. 2004; Pollice et al. 2007; Ozgun et al. 2013). They stated a positive relationship between viscosity and TSS concentration of sludge, for instance, Hasar et al. (2004) reported an increase of sludge viscosity from 0.02 Pa.s to 0.16 Pa.s while an increase in MLSS concentration from 2870 mgL^{-1} to 12285 mgL^{-1} was determined.

Figure 4.13 represents the variation of viscosity against the TSS concentration of sludge. During the complete study viscosity and TSS exhibits a similar behavior, where viscosity was decreased with a decreasing TSS concentration and vice versa. From day 0 to 40 (first cycle) a decrease of viscosity by 35% was obtained with a

drop of TSS concentration by 36%. Similarly, during the second cycle, from day 40 to 68 viscosity and TSS were found to decrease by 14% and 4% respectively.

Between the days 68 and 97, viscosity of sludge found to increase by 9% with an increase in TSS concentration from 18 to 24.5 gL^{-1}. Until the day 130, both parameters were found to decrease simultaneously. From day 136 and until the end of the study, while an increase of TSS by 36% was obtained, viscosity of sludge was also found to increase by 27%. The results obtained from all cycles, indicates a positive relationship between viscosity and TSS concentration of sludge, which supports the previous studies.

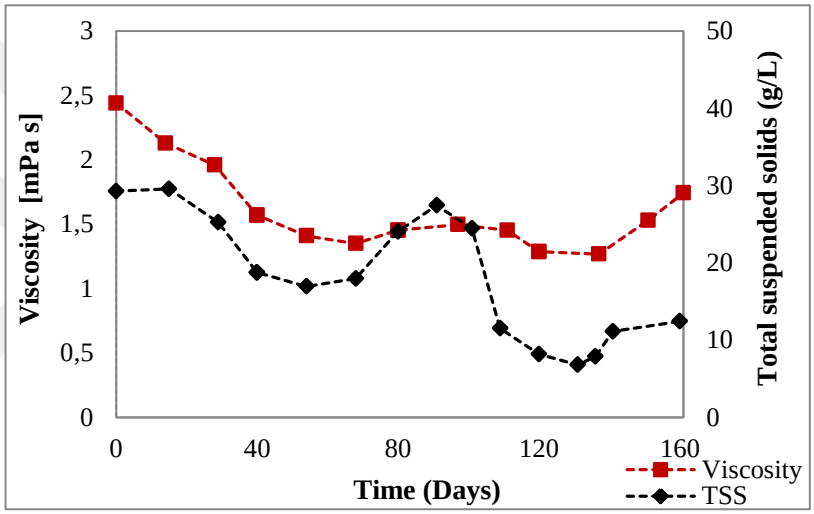

Figure 4.13 : Evolution of sludge viscosity due to variations in TSS concentration (all cycles).

4.9 Effect of Sodium on Adenosine Triphosphate Concentration

Adenosine triphosphate concentration (ATP) (mgL^{-1}), as an excellent indicator of both the cell viability and the metabolic status of a microorganism, was measured in sludge. ATP concentration also has been used to predict biomass levels in anaerobic digestion (Shanmugam & Horan 2009). Figure 4.14 represents the variation determined in biomass ATP content due to the changes of sodium concentrations. The higher results of ATP content represent the higher bioactivity of the biomass, since the ATP exists only in viable cells as energy-storing compound and disappears quickly, when cell dies (Yu et al. 2000; Chen 2004). During the first cycle, from day 0 to 20, ATP concentration was decreased by 55% while there was an increase in sodium concentration by 5%. From day 20 to 50, ATP concentration was increased

greatly from 0.24 to 1.06 mgL^{-1}, while there was a slight decrease in sodium concentration. The maximum concentration of ATP, 1.06 mgL^{-1} was determined during the second cycle, while the sodium concentration was measured as 19 gL^{-1}. This increase in ATP, i.e. the increase of microbial activity, might indicate the adaptation of microorganisms to the media with sodium concentration about 19 gL^{-1}, by time. During the third cycle, from day 80 to 102, decrease of ATP concentration by 32% obtained while sodium concentration was increased by 6.4%. Between the days 102 and 112, an increase of ATP concentration was determined from 0.1 to 0.5 mgL^{-1}, while sodium concentration decreased by 6.5%.

During the fourth cycle from day 120 to 160, sludge sodium concentration was continuously increased from 19.4 to 36.9 gL^{-1} and induced a significant decrease in ATP concentration, until the end of cycle. Especially, after the sodium concentration of 26 gL^{-1} very significant decrese of ATP concentration demonstrates the detrimental impact of sodium on microorganism activity. When we consider the results obtained during the four different influent loading cycles, they represent a negative correlation between sodium concentration and ATP of biomass, thus the increase in sodium concentration induce a decrease of microbial activity. Gu et al. (2015) recently reported variations of specific ATP content, based on total volatile solids (mg $gVSS^{-1}$), in an anaerobic osmotic membrane bioreactor. When they changed the conductivity from 1.1 to 20 mS cm^{-1} every 14-days cycles, values varied between 0.1 to 0.4 mg $gVSS^{-1}$, showing that the elevated salt concentration has slightly affected bioactivity of sludge.

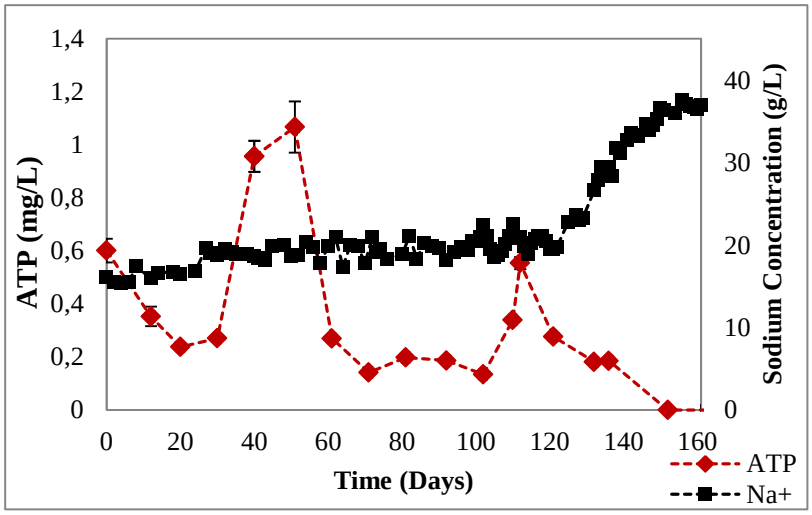

Figure 4.14 : Variation of ATP concentration due to changes of salinity (all cycles).

Biomass stress index (BSI %), which provides a measure of the stress level (quality) of the microbial community, was examined as supplementary data, in order to determine the impact of salinity on ATP concentration of biomass samples. BSI (%) was calculated with the equation (7) given below.

$$BSI\ (\%) = \frac{dATP\ (ng\ ATP/mL)}{tATP\ (ng\ ATP/mL)}\ x\ 100\ \% \qquad (7)$$

Figure 4.15 represents the results obtained for BSI (%), by varying the sodium concentrations. Higher results demonstrate negative effect of the sodium concentration on the activity of microbial community.

Results below 30%, indicates health of living biomass is in an acceptable range in the bioreactor. Results ranged between 30% and 50% indicate that the living biomass health has deviated outside of the acceptable range and preventive actions should be taken, in order to eliminate the cell stress and improve the biomass activity and health. BSI values higher than 50% indicate that the living biomass health has deviated significantly outside of acceptable range and immediate corrective actions should be taken to improve health of the biomass by eliminating stressors (QG21W Interpretation Guidelines, Aqua-tools,2014). During the first cycle, BSI (%) was determined over 30% when sodium concentration was increased from 16 to 18.7 gL^{-1}. Until day 20 an increase of 18% in sodium concentration induced a stress on biomass and the BSI was moved from 47.5% to 52.5%, which indicates the negative impact of sodium concentration on biomass activity. From day 20 to 40 BSI was

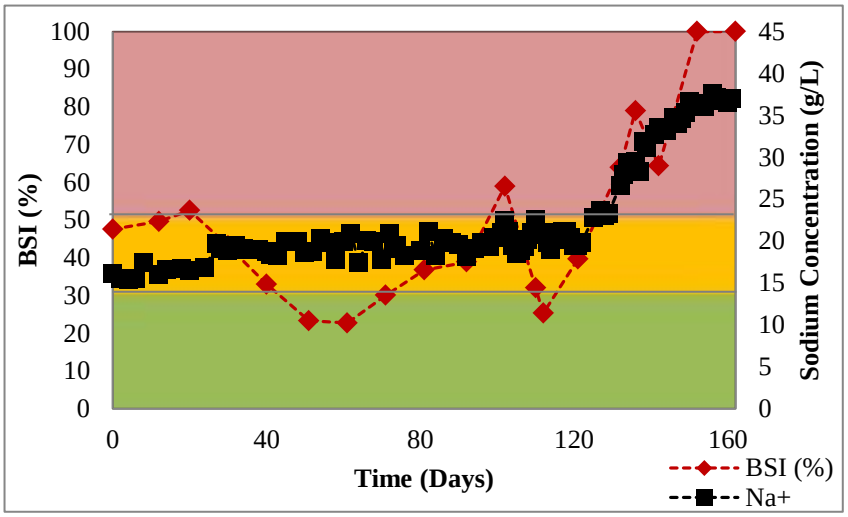

Figure 4.15 : Variations of BSI (%) of sludge due to salinity changes.

decreased from 52.5 to 33%, while sodium concentration increased by 5%, such a finding could be attributed to the acclimation of biomass to this new range of sodium concentration (about 19 $gNa^{+}L^{-1}$) after being at 16 g $Na^{+}L^{-1}$ for long term. During second cycle, from day 40 to 60 a decrease of BSI was determined, from 33 to 22.7%. Especially between day 50 and 60, results demonstrated an acceptable microbial activity in our biomass. This might also indicates the acclimation of microorganisms to an increased sodium concentration from 16 to 20 gL^{-1} within 60 days of operation. However after day 60 until day 102, an increase in sodium concentration up to 22.4 gL^{-1} induced an increase in BSI results up to 59%. This represents that microbial activity of biomass is significantly impacted by a higher sodium concentration than 22 gL^{-1}. Until the end of third cycle, sodium concentrations were gradually decreased from 22.4 to 19.4 gL^{-1} accordingly, a decrease in BSI was determined from 59% to 25.3%. During the fourth cycle, with a great increase in sodium concentration from 19.4 to 36.9 gL^{-1}, BSI was increased from 39.5% to 100%. Especially after the sodium concentration of 26 gL^{-1}, the biomass was found over 50% stress indicating the significant desructive impact of sodium on microorganisms and living biomass health is significantly outside of acceptable range. BSI 100% represents that dissolved ATP (dATP) exceeds the amount of total ATP (tATP) values. This might be explained by the disruption of cells under high salinity. 40 days of operation with high sodium concentrations demonstrated a significant negative effect of sodium on microbial activity. The highest stress indexes were determined during the last cycle. According to the results obtained, a serious damage on microbial community could be stated under sodium stress, especially in concentrations higher than 26 $gNa^{+}L^{-1}$.

4.10 Flow Cytometry Analysis

Cell concentration was measured by flow cytometry analysis to determine the impact of salinity studies on bacterial viability. Experiments were conducted to have supportive information together with ATP and BSI results about influences on bacterial cells after sodium exposure. At the beginning of the study total cell and damaged cell concentration was 144 and 36 $10^{3}cellsmL^{-1}$ respectively. From the first cycle until the end of third cycle an increase in the concentration of damaged cells were obtained (Figure 4.16). During the first 20 days of first cycle, a decrease by

34% in total cell concentration and an increase in damaged cells were obtained, while the sodium concentrations were increased by 18%. Until the end of the first cycle total cells were found to increase however, damaged cells were also increased by 78%. During the second cycle an average of 81% of the cells were found to be damaged cells, while sodium concentration increased up to 21 gL^{-1}. During the third cycle, within the fluctuations in salinity between 18 and 24 gL^{-1} significant damage in cells were observed with an increase of damaged cell concentration by 17%. During the all cycles an adverse influence of sodium on cell concentration was observed.

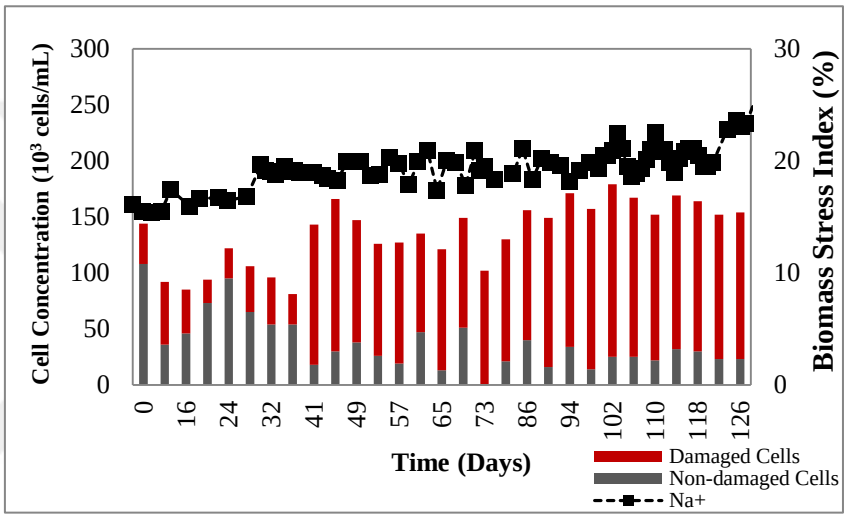

Figure 4.16 : Total and damaged cell concentration changes during the first three salinity cycles due to sodium changes.

4.11 Biomethane Potential Test

Before the continuous salinity studies batch tests were conducted at assay bottles with the concentrations of 16, 20 and 24 gL^{-1} sodium to predict the robustness of the reactor during the continuous studies, to determine minimum and maximum sodium concentrations that could be applied to the biomass and to assess the K/Na ratio for the microorganism adaptation to sodium changes. To understand the adaptation strategies of microbial community due to osmotic changes, two different tests were developed with two different K/Na ratio; keeping the same ratio of 0,05 and applying different ratios for each sodium concentration. Figure 4.17, Figure 4.18 and Figure 4.19 shows the results of COD and phenol concentrations under 16 gL^{-1}, 20 gL^{-1} and 24 gL^{-1} sodium concentration respectively.

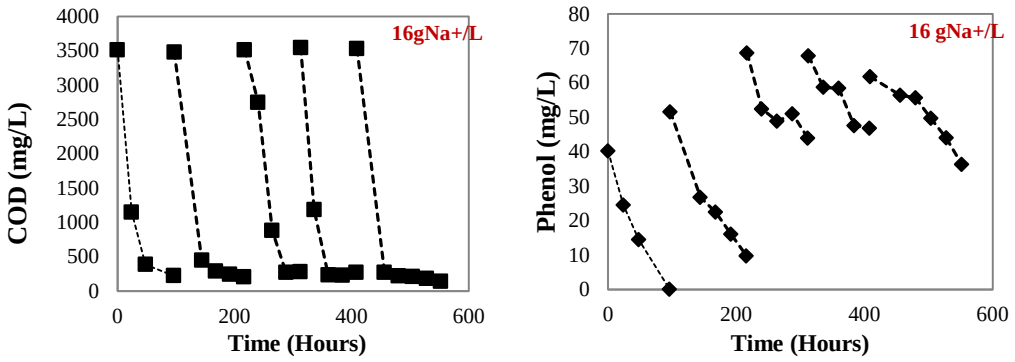

Figure 4.17 : Variations of COD and phenol degradation under 16gL^{-1} sodium concentration while the K/Na ratio is 0.05.

Bottles were fed with new feed averagely in every 96 hours. Initial COD was 3500 mgL^{-1} and phenol concentration was 50 mgL^{-1} for first two feeds and 70 mgL^{-1} after the second feed. Under 16 gNa^{+}L^{-1}, an average 93.6% COD removal and 58% phenol removal determined. Lowest COD concentration of 140 mgL^{-1} was achieved at the end of fifth feeding, phenol found to be degraded successfully at the end of first feed and after the third feed, a clear decrease of phenol removal and increase of phenol concentration was obtained. Under 20 gNa^{+}L^{-1}, an average 91.6% COD removal and 39% phenol removal was determined. Lowest concentration of COD achieved at the end of fifth cycle was 248.7 mgL^{-1} and the lowest phenol concentration was determined as 9 mgL^{-1} at the end of first feed. Under 24 gNa^{+}L^{-1} average COD removal was 89.6% and phenol removal was 25%. Lowest COD and phenol concentrations were 319 mgL^{-1} and 21 mgL^{-1} respectively. During the experiments at each bottle after the third feed, a clear decrease on phenol removal was observed. This might be explained by the accumulation of phenol at the media after the third feed, which induced a toxic effect on bacterial activities.

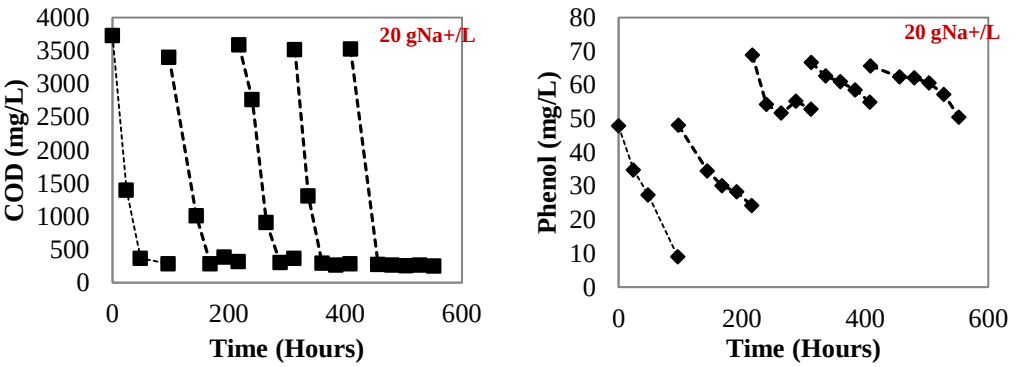

Figure 4.18 : Variations of COD and phenol degradation under 20gL^{-1} sodium concentration while the K/Na ratio is 0.05.

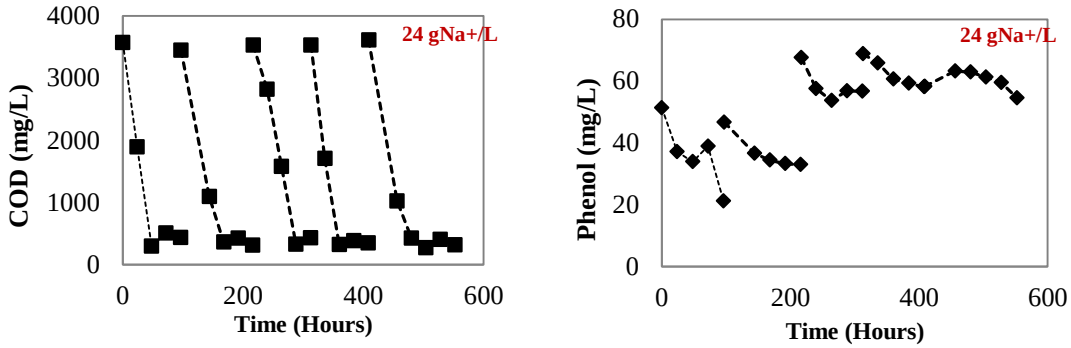

Figure 4.19 : Variations of COD and phenol degradation under 24gL^{-1} sodium concentration when the K/Na ratio is 0.05.

According to the removal efficiencies achieved, the negative impact of sodium on microorganisms could be observed in terms of biodegradability, when sodium concentrations were increased, COD and phenol concentrations were also found to increase and removals were decreased.

Lowest removal of COD and phenol were determined under 24 $gNa^{+}L^{-1}$. As the BSI (%) results were indicated in previous section that after the threshold concentration of sodium (about 22 $gNa^{+}L^{-1}$) significant decrease of microbial activity, therefore a decrease of degradation capacity is expected. Methane production of the biomass was recorded by control panel. For the microorganisms, which were exposed to sodium concentration 16, 20 and 24 gL^{-1}, the total methane productions were found as 0.81, 0.88 and 0,77 LCH_4/h respectively. Lowest production was determined at 24 gL^{-1} sodium conditions. Total volume of 0,92 LCH_4/h was obtained for the control media, which was prepared without NaCl addition. A decrease in methane production was determined with an increase in sodium concentration.

Figure 4.20, Figure 4.21 and Figure 4.22 exhibits the variations of COD and phenol degradation under 16, 20 and 24 gL^{-1} sodium concentrations respectively, when the ratio of K/Na was applied different for each media; 0.03, 0.024 and 0.02 respectively. Bottles were fed with new feed in every 168 hours. Initial COD was 3500 mgL^{-1} and phenol concentration was 50 mgL^{-1}. During this batch, feeds were changed four times. COD and phenol removal under 16 $gNa^{+}L^{-1}$ were 96.6% and 96.8% respectively. Lowest COD concentration was 56 mgL^{-1}. Phenol was degraded completely at the end of first three feedings. Under 20 $gNa^{+}L^{-1}$, an average 96% COD removal and 99% phenol removal were determined. Lowest COD concentration of 118 mgL^{-1} was obtained.

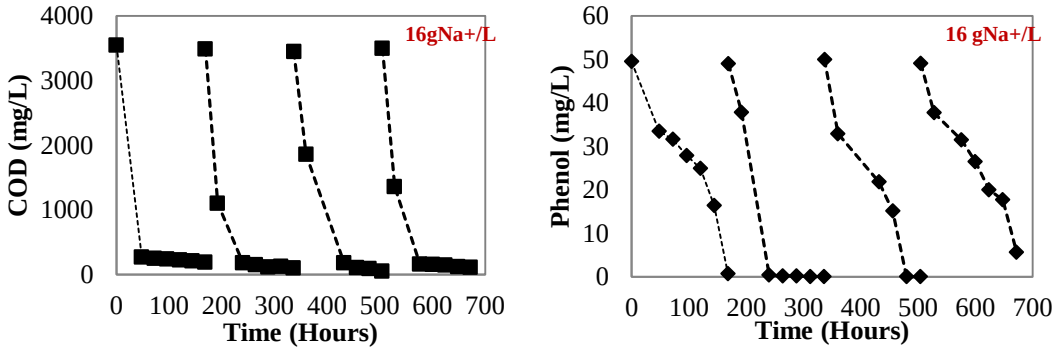

Figure 4.20 : Variations of COD and phenol degradation under 16 gL^{-1} sodium concentration (K/Na ratio: 0.03).

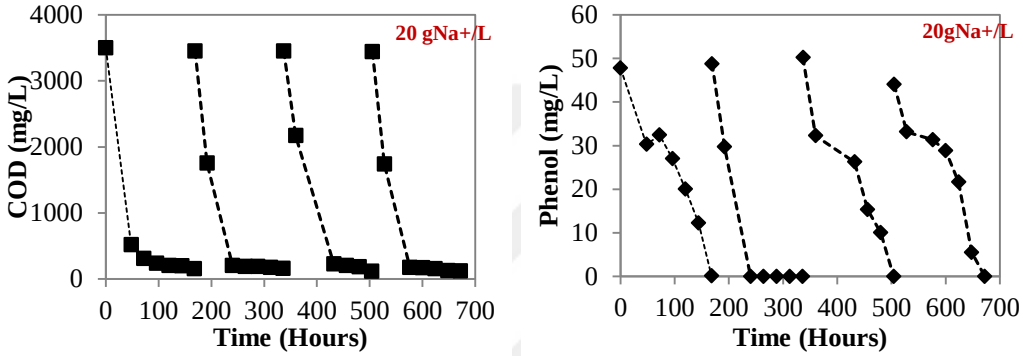

Figure 4.21 : Variations of COD and phenol degradation under 20 gL^{-1} sodium concentration (K/Na ratio: 0.024).

During all the feedings, complete phenol degradation by microorganisms was observed. Under 24 $gNa^{+}L^{-1}$, an average 82.7% COD removal and 94.4% phenol removal were determined. Lowest concentration of COD was 179 mgL^{-1}. According to the results, a better degradation could be determined during the second batch test, while the K/Na ratios applied differently to each media with different sodium concentration. Even though, with 24 $gNa^{+}L^{-1}$ a better conversion of organic material than the first batch test was obtained. Although, the bacteria in the biomass was found to be more successful to overcome the sodium impact, the total methane production for every media was found to be lower than the study where the ratio kept similar for each concentration. Total volume of 0,71 LCH_4/h was obtained for the control media. Total methane production was 0.66, 0.68 and 0,65 LCH_4/h under 16, 20 and 24 $gNa^{+}L^{-1}$ respectively. Again the lowest COD and phenol removal and total methane volume were determined on the media exposed to 24 $gNa^{+}L^{-1}$. These results demonstrated the adverse impact of sodium on biomass activities especially after 22 $gNa^{+}L^{-1}$.

When the bacterial cells were exposed to sodium concentrations at the surrounding area, they tend to uptake inorganic ions such as K^+ and Cl^- from the media, in order to balance the osmotic pressure between internal and external medium. With this uptake strategy, microorganisms are raising the water activity of their cells by accumulation of K^+ (Roeßler & Müller 2001).

Different transport systems are present for various microbial communities to carry out the conservation of the cells, during harsh external conditions (Roeßler & Müller 2001), which obtains osmotic equilibrium by maintaining a cytoplasmic salt concentration similar to that of the surrounding environment. The ionic composition of the cytoplasm is characterized by the presence of molar concentrations of KCl (Oren 1999). Therefore, the quantity of K^+ present at the surrounding media to overcome the osmotic pressure induced by sodium is being crucial for the microorganisms' uptake strategies.

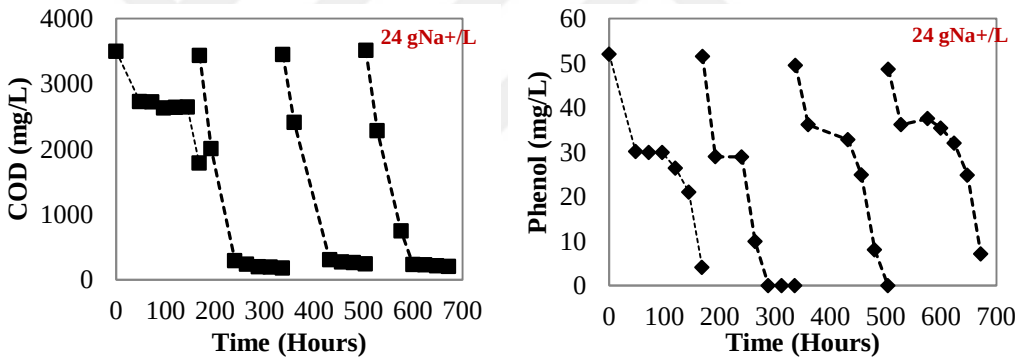

Figure 4.22 : Variations of COD and phenol degradation under 24gL^{-1} sodium concentration (K/Na ratio: 0.02).

5. CONCLUSIONS

This study investigated the influence of sodium concentration on anaerobic sludge characteristics and the organic removal efficiency of the anaerobic membrane bioreactor system, exposed to rapid sodium changes. The study demonstrated a significant impact of sodium on physical and biochemical properties of anaerobic sludge. The following specific conclusions can be drawn:

- High salinity has an initially negative influence both on COD and phenol removals. However, the system was able to overcome the salt fluctuations up to 30 $gNa^{+}L^{-1}$ with an average COD removal by 99%. This demonstrates an adaptation of our sludge to the sodium changes in the media since it was exposed to sodium for about 140 days and could cope with that in terms of organic matter degradation. When the sodium concentration is decreased, COD and phenol removal efficiencies are restored.
- $EPS_{protein}$ concentration was significantly influenced the effluent turbidity and a clear positive relation was obtained between these two parameters. During the first cycle within the first sodium expose to the biomass (after being acclimated to 16 $gNa^{+}L^{-1}$) up to 20 $gNa^{+}L^{-1}$ was negatively affected the turbidity, an increase in effluent turbidity was observed. After 30 $gNa^{+}L^{-1}$, due to release of non-dissolved cellular components (because of microorganism plasmolysis) effluent turbidity found to increase.
- Total suspended solids concentration demonstrated a positive relationship with the sodium concentration. An increase in sodium induced an increase in TSS concentration and vice versa. Since, the presence of high sodium concentration leads to hypertonic conditions which results with death of biomass, a negative relation between VSS concentration and sodium was obtained.
- ATP concentration was used as an indicator of cell viability due to sodium impact. ATP represented a negative correlation between salinity. The elevated salt concentration has affected microbial activity of sludge and decreased the

ATP concentrations. Biomass stress indexes demonstrated quality of the microbial community due to sodium exposures. During the first cycle (within the first sodium shock, after being acclimated to 16 $gNa^{+}L^{-1}$ for a long term) BSI increased due to the disruption of cells under high salinity. However, until day 60 an acclimation of biomass to this new range of sodium concentration was found with decreasing BSI results. A serious damage on microbial community was obtained concentrations higher than 26 $gNa^{+}L^{-1}$.

- Flow cytometry results supported the ATP and BSI findings. Increasing sodium concentrations induced an increase in damaged cell concentration of sludge. During the first cycle, the first sodium shock resulted with decrease of total cell concentration by 44%. Until the end of third cycle, an average by 84% of the total cells was found to be damaged by sodium increases over 26 $gNa^{+}L^{-1}$.
- An increase of EPS and SMP was exhibited as a response to the variation of osmotic pressure between bacteria and the media due to increasing salinity conditions. However, great increases of sodium induced the solubility of $EPS_{protein}$ contents and resulted in an increase at $SMP_{protein}$ concentration. After the sodium concentration of 26 $gNa^{+}L^{-1}$ because of the negative sodium impact on microbial activity, resulted with less EPS production. When the effect of sodium was removed EPS and SMP was decreased again.
- Between relative hydrophobicity and salinity, a negative relation was obtained until the sodium concentration of 22 $gNa^{+}L^{-1}$. However, when the sodium concentration continued to increase, after 26 $gNa^{+}L^{-1}$, the indirect impact of EPS concentration was found to influence relative hydrophobicity positively since the protein content of EPS regarded as hydrophobic and the polysaccharides as hydrophilic components. Especially during the last cycle due to an increase of sodium concentration, PN/PS ratio increased by 34% and consequently RH was found to increase by 23%.
- Particle sizes of the sludge were found closely related to EPS concentration of sludge, especially to the increasing protein concentrations, which strengthen bonds between flocs and induce flocculation. A positive relation between particle sizes and PN/PS ratios of EPS and SMP was obtained. During the first

three cycles increase in sodium concentration up to 22 $gNa^{+}L^{-1}$, resulted in deterioration of sludge particles and reduced the PSD values.

- CST and SRF were measured to determine the sludge filterability and dewaterability changes after sodium exposures. Until the last cycle SRF and CST demonstrated a decrease with in an increase of sodium up to 21 $gNa^{+}L^{-1}$. After that concentration both parameters increased consequently with an increase in sodium concentration. The impact of TSS concentration on filterability was observed. An increase of TSS concentration by 34% as well as an increase in SRF by 38% was determined during the fourth cycle. Moreover, a similar trend in SRF and CST was observed during the complete study.
- Sludge viscosity represented variations due to the changes in TSS concentration of the sludge. During the complete study, viscosity and TSS exhibited a similar behavior, where viscosity was decreased with a decreasing TSS concentration and vice versa.
- Biomethane potential test was conducted to assess the K/Na ratio for the microorganism for adaptation to sodium changes and predict the robustness of the reactor prior to the continuous studies. The higher COD and phenol removals by 96% and 99% respectively were found in batch when the K/Na ratio of 0.024 was implemented. Total volume of methane produced under every sodium concentration (16, 20 and 24 $gNa^{+}L^{-1}$) was higher when the ratio kept 0.05. In both cases (when the ratio kept similar and applied differently) the media with 16 gL^{-1} sodium showed higher COD removal efficiencies.

REFERENCES

Abbasi, A. & Amiri, S. (2008). Emulsifying behavior of an exopolysaccharide produced by Enterobacter cloacae. *African Journal of Biotechnology*, 7(10), pp.1574–1576. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-44649095265&partnerID=tZOtx3y1.

Abdollahzadeh Sharghi, E., Bonakdarpour, B. & Pakzadeh, M. (2014). Treatment of hypersaline produced water employing a moderately halophilic bacterial consortium in a membrane bioreactor: Effect of salt concentration on organic removal performance, mixed liquor characteristics and membrane fouling. *Bioresource Technology*, 164, pp.203–213. Available at: http://dx.doi.org/10.1016/j.biortech.2014.04.099.

Abou-Elela, S.I., Kamel, M.M. & Fawzy, M.E. (2010). Biological treatment of saline wastewater using a salt-tolerant microorganism. *Desalination*, 250(1), pp.1–5. Available at: http://dx.doi.org/10.1016/j.desal.2009.03.022.

APHA. (1985). Standard Methods for Examination of Water and Wastewater. *American Public Health Association*, 16th Editi(American Public Health Association). Available at: http://www.mwa.co.th/download/file_upload/SMWW_10900end.pdf [Accessed February 20, 2016].

Artiga, P. et al. (2008). Use of a hybrid membrane bioreactor for the treatment of saline wastewater from a fish canning factory. *Desalination*, 221(1-3), pp.518–525.

Bolen, D.. & Baskakov, I. V. (2001). The osmophobic effect: natural selection of a thermodynamic force in protein folding. *Journal of Molecular Biology*, 310(5), pp.955–963. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11502004.

Campos, J.L. et al. (2002). Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. *Water research*, 36(10), pp.2555–60. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0043135401004675.

Cetin, S. & Erdincler, A. (2004). The role of carbohydrate and protein parts of extracellular polymeric substances on the dewaterability of biological sludges. *Water Science and Technology*, 50(9), pp.49–56.

Chen, H. (2004). ATP content and biomass activity in sequential anaerobic/aerobic reactors. *Journal of Zhejiang University Science*, 5(6), pp.727–732. Available at: http://link.springer.com/article/10.1007/BF02840988 [Accessed March 5, 2016].

Dentel, S. (1997). Evaluation and role of rheological properties in sludge management. *Water Science and Technology*, 36(11), pp.1–8. Available at: http://www.sciencedirect.com/science/article/pii/S0273122397006628.

Dereli, R.K., Loverdou, L., et al. (2015). A systematic study on the effect of substrate acidification degree and acidogenic biomass on sludge filterability. *Water Research*, 82, pp.1–10. Available at: http://dx.doi.org/10.1016/j.watres.2015.04.044.

Dereli, R.K. et al. (2014). Implications of changes in solids retention time on long term evolution of sludge filterability in anaerobic membrane bioreactors treating high strength industrial wastewater. *Water Research*, 59, pp.11–22. Available at: http://dx.doi.org/10.1016/j.watres.2014.03.073.

Dereli, R.K., Heffernan, B., et al. (2015). Influence of high lipid containing wastewater on filtration performance and fouling in AnMBRs operated at different solids retention times. *Separation and Purification Technology*, 139, pp.43–52. Available at: http://dx.doi.org/10.1016/j.seppur.2014.10.029.

Dereli, R.K. et al. (2012). Potentials of anaerobic membrane bioreactors to overcome treatment limitations induced by industrial wastewaters. *Bioresource Technology*, 122, pp.160–170. Available at: http://dx.doi.org/10.1016/j.biortech.2012.05.139.

Díaz, M.P. et al. (2002). Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. *Biotechnology and Bioengineering*, 79(2), pp.145–153.

Ding, A. et al. (2015). Effect of calcium addition on sludge properties and membrane fouling potential of the membrane-coupled expanded granular sludge bed process. *Journal of Membrane Science*, 489, pp.55–63. Available at: http://dx.doi.org/10.1016/j.memsci.2015.04.001.

Dubois, M. et al. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, 28(3), pp.350–356. Available at: http://pubs.acs.org/cgi-bin/doilookup/?10.1021/ac60111a017 [Accessed July 11, 2014].

Ersahin, M.E. et al. (2014). Applicability of dynamic membrane technology in anaerobic membrane bioreactors. *Water Research*, 48(1), pp.420–429. Available at: http://dx.doi.org/10.1016/j.watres.2013.09.054.

Figueroa, M. et al. (2008). Treatment of saline wastewater in SBR aerobic granular reactors. *Water Science & Technology*, 58(2), p.479. Available at: http://wst.iwaponline.com/cgi/doi/10.2166/wst.2008.406.

Frølund, B. et al. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research*, 30(8), pp.1749–1758.

Gebauer, R. (2004). Mesophilic anaerobic treatment of sludge from saline fish farm effluents with biogas production. *Bioresource Technology*, 93(2),

pp.155–167. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960852403003225.

Ghasimi, D.S.M. et al. (2015). Microbial population dynamics during long-term sludge adaptation of thermophilic and mesophilic sequencing batch digesters treating sewage fine sieved fraction at varying organic loading rates. *Biotechnology for Biofuels*, 8(1), p.171. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84944459057&partnerID=tZOtx3y1.

Gray, N.F. (2015). Capillary Suction Time (CST). In *Progress in Filtration and Separation*. Elsevier, pp. 659–670. Available at: http://linkinghub.elsevier.com/retrieve/pii/B9780123847461000173.

Gu, Y. et al. (2015). Development of anaerobic osmotic membrane bioreactor for low-strength wastewater treatment at mesophilic condition. *Journal of Membrane Science*, 490, pp.197–208. Available at: http://dx.doi.org/10.1016/j.memsci.2015.04.032.

Hasar, H. et al. (2004). Rheological properties of activated sludge in a sMBR. *Biochemical Engineering Journal*, 20(1), pp.1–6. Available at: http://www.sciencedirect.com/science/article/pii/S1369703X04000567 [Accessed February 27, 2016].

Higgins, B.M.J., Member, A. & Novak, J.T. (1997). Characterization of extracellular protein and it's role in bioflocculation. *Journal of Environmental Engineering*, 123(May), pp.479–485. Available at: http://ascelibrary.org/doi/abs/10.1061/(ASCE)0733-9372(1997)123:5(479).

Higgins, M.J. & Novak, J.T. (1997). The Effect of Cations on the Settling and Dewatering of Activated Sludges: Laboratory Results. *Water Environment Research*, 69(2), pp.215–224. Available at: http://www.jstor.org/stable/25044865.

Ismail, S.B. et al. (2008). Effects of high salinity wastewater on methanogenic sludge bed systems. *Water Science and Technology*, 58(10), pp.1963–1970.

Ismail, S.B. et al. (2010). Extracellular polymeric substances (EPS) in upflow anaerobic sludge blanket (UASB) reactors operated under high salinity conditions. *Water Research*, 44(6), pp.1909–1917. Available at: http://dx.doi.org/10.1016/j.watres.2009.11.039.

Jang, D. et al. (2013). Effects of salinity on the characteristics of biomass and membrane fouling in membrane bioreactors. *Bioresource technology*, 141, pp.50–6. Available at: http://www.sciencedirect.com/science/article/pii/S0960852413002976 [Accessed February 26, 2016].

Jin, B., Wilén, B.-M. & Lant, P. (2004). Impacts of morphological, physical and chemical properties of sludge flocs on dewaterability of activated sludge. *Chemical Engineering Journal*, 98(1-2), pp.115–126. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1385894703002183.

Johir, M.A.H. et al. (2013). Effect of salt concentration on membrane bioreactor (MBR) performances: Detailed organic characterization. *Desalination*, 322, pp.13–20. Available at: http://dx.doi.org/10.1016/j.desal.2013.04.025.

Kargi, F. & Dincer, A.R. (1997). Biological Treatment of Saline Wastewater by Fed-Batch Operation. *Journal of Chemical Technology & Biotechnology*, 69(2), pp.167–172. Available at: http://doi.wiley.com/10.1002/(SICI)1097-4660(199706)69:2<167::AID-JCTB696>3.0.CO;2-C.

Kargi, F., Dinçer, A.R. & Pala, A. (2000). Characterization and biological treatment of pickling industry wastewater. *Bioprocess Engineering*, 23(4), pp.371–374. Available at: http://link.springer.com/article/10.1007/s004499900174.

Kincannon, D.F. & Gaudy, a. F. (1968). Response of biological waste treatment systems to changes in salt concentrations. *Biotechnology and Bioengineering*, 10, pp.483–496. Available at: \\Byzance\Doclbe\Documentation\BibliographieLBE-ArticlesPDF\Kincannon-BB1968.pdf.

Kubo, M. et al. (2001). Treatment of hypersaline-containing wastewater with salt-tolerant microorganisms. *Journal of Bioscience and Bioengineering*, 91(2), pp.222–224.

Laspidou, C.S. & Rittmann, B.E. (2002). Non-steady state modeling of extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Research*, 36(8), pp.1983–1992.

Lefebvre, O. et al. (2005). Halophilic biological treatment of tannery soak liquor in a sequencing batch reactor. *Water Research*, 39(8), pp.1471–1480.

Lefebvre, O. et al. (2004). Treatment of hypersaline industrial wastewater by a microbial consortium in a sequencing batch reactor. *Environmental technology*, 25(5), pp.543–53.

Lefebvre, O. & Moletta, R. (2006). Treatment of organic pollution in industrial saline wastewater: A literature review. *Water Research*, 40(20), pp.3671–3682. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0043135406005082.

Lindahl, M. et al. (1981). A new test based on "salting out" to measure relative hydrophobicity of bacterial cells. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 677(3-4), pp.471–476. Available at: http://linkinghub.elsevier.com/retrieve/pii/0304416581902610.

Liu, H. & Fang, H.H.P. (2002). Extraction of extracellular polymeric substances (EPS) of sludges. *Journal of Biotechnology*, 95(3), pp.249–256.

Liu, Y. & Fang, H.H.P. (2003). Influences of Extracellular Polymeric Substances (EPS) on Flocculation, Settling, and Dewatering of Activated Sludge. *Critical Reviews in Environmental Science and Technology*, 33(3), pp.237–273.

Lowry, O.H. et al. (1951). Article : Protein Measurement With the Folin Phenol Reagent. *Journal of Biological Chemistry*, 193, pp.265–275.

Marrot, B. et al. (2004). Industrial wastewater treatment in a membrane bioreactor: A review. *Environmental Progress*, 23(1), pp.59–68.

Martin, D.D., Ciulla, R. a & Roberts, M.F. (1999). Minireview Osmoadaptation in Archaea. *Appied and Enviromental Microbiology*, 65(5), pp.1815–1825.

Masid, S. et al. (2010). Impact of electrooxidation on combined physicochemical and membrane treatment processes: Treatment of high strength chemical industry wastewater. *Desalination*, 259(1-3), pp.192–196. Available at: http://dx.doi.org/10.1016/j.desal.2010.04.007.

Moon, B.H. et al. (2003). Effects of salt concentration on floc characteristics and pollutants removal efficiencies in treatment of seafood wastewater by SBR. *Water science and technology : a journal of the International Association on Water Pollution Research*, 47(1), pp.65–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12578175.

More, T.T. et al. (2012a). Bacterial polymer production using pre-treated sludge as raw material and its flocculation and dewatering potential. *Bioresource Technology*, 121, pp.425–431. Available at: http://dx.doi.org/10.1016/j.biortech.2012.06.075.

More, T.T. et al. (2012b). Bacterial polymer production using pre-treated sludge as raw material and its flocculation and dewatering potential. *Bioresource Technology*, 121, pp.425–431. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960852412009893.

More, T.T. et al. (2014). Extracellular polymeric substances of bacteria and their potential environmental applications. *Journal of Environmental Management*, 144, pp.1–25. Available at: http://dx.doi.org/10.1016/j.jenvman.2014.05.010.

Ng, H.Y. Ong, S.L. & Ng, W.J. (2005). Effects of Sodium Chloride on the Performance of a Sequencing Batch Reactor. *Journal of Environmental Engineering*, 131(11), pp.1557–1564. Available at: http://ascelibrary.org/doi/10.1061/(ASCE)0733-9372(2005)131:11(1557).

Oren, A. (1999). Bioenergetic aspects of halophilism. *Microbiology and molecular biology reviews : MMBR*, 63(2), pp.334–48. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=98969&tool=pmcentrez&rendertype=abstract.

Ozgun, H. et al. (2013). A review of anaerobic membrane bioreactors for municipal wastewater treatment: Integration options, limitations and expectations. *Separation and Purification Technology*, 118, pp.89–104. Available at: http://dx.doi.org/10.1016/j.seppur.2013.06.036.

Pernetti, M. & Palma, L. Di. (2005). Experimental Evaluation of Inhibition Effects of Saline Wastewater on Activated Sludge. *Environmental Technology*, 26(6), pp.695–704. Available at: http://www.tandfonline.com/doi/abs/10.1080/09593330.2001.9619509.

Pevere, A. et al. (2007). Effect of Na+ and Ca2+ on the aggregation properties of

sieved anaerobic granular sludge. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 306(1-3 SPEC. ISS.), pp.142–149.

Pevere, A. et al. (2006). Viscosity evolution of anaerobic granular sludge. *Biochemical Engineering Journal*, 27(3), pp.315–322.

Pollice, A. et al. (2007). Physical characteristics of the sludge in a complete retention membrane bioreactor. *Water Research*, 41(8), pp.1832–1840. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0043135407000395.

Praveen, P. et al. (2014). Biodegradation of Phenol from Saline Wastewater using Forward Osmotic Hollow Fiber Membrane Bioreactor coupled Chemostat. *Elsevier B.V.*, 94, pp.125–133. Available at: http://dx.doi.org/10.1016/j.bej.2014.11.014.

Prest, E.I. et al. (2014). Combining flow cytometry and 16S rRNA gene pyrosequencing: A promising approach for drinking water monitoring and characterization. *Water Research*, 63(0), pp.179–189. Available at: http://dx.doi.org/10.1016/j.watres.2014.06.020.

Prest, E.I. et al. (2013). Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. *Water Research*, 47(19), pp.7131–7142. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0043135413008361.

Reid, E., Liu, X. & Judd, S.J. (2006). Effect of high salinity on activated sludge characteristics and membrane permeability in an immersed membrane bioreactor. *Journal of Membrane Science*, 283(1-2), pp.164–171.

Roeßler, M. & Müller, V. (2001). Osmoadaptation in bacteria and archaea: Common principles and differences. *Environmental Microbiology*, 3(12), pp.743–754.

Rosenberg, M., Gutnick, D. & Rosenberg, E. (1980). Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters*, 9(1), pp.29–33. Available at: http://femsle.oxfordjournals.org/content/9/1/29-1.abstract [Accessed January 8, 2016].

Salvadó, H. et al. (2001). Effects of shock loads of salt on protozoan communities of activated sludge. *Acta Protozoologica*, 40(3), pp.177–185.

Sarika, R., Kalogerakis, N. & Mantzavinos, D. (2005). Treatment of olive mill effluents: Part II. Complete removal of solids by direct flocculation with poly-electrolytes. *Environment International*, 31(2), pp.297–304.

Scholz, M. (2005). Review of Recent Trends in Capillary Suction Time (CST) Dewaterability Testing Research. *Industrial & Engineering Chemistry Research*, 44(22), pp.8157–8163. Available at: http://pubs.acs.org/doi/abs/10.1021/ie058011u.

Seyssiecq, I., Ferrasse, J.-H. & Roche, N. (2003). State-of-the-art: rheological characterisation of wastewater treatment sludge. *Biochemical Engineering Journal*, 16(1), pp.41–56. Available at: http://www.sciencedirect.com/science/article/pii/S1369703X0300021

4 [Accessed October 28, 2015].

Shanmugam, P. & Horan, N.J. (2009). Simple and rapid methods to evaluate methane potential and biomass yield for a range of mixed solid wastes. *Bioresource Technology*, 100(1), pp.471–474. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960852408005270.

Sheng, G., Yu, H. & Li, X. (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnology Advances*, 28(6), pp.882–894. Available at: http://dx.doi.org/10.1016/j.biotechadv.2010.08.001.

Smollen, M. (1986). Dewaterability of municipal sludges 2: Sludge characterization and behaviour in terms of SRF and CST parameters. *National Institute for Water Research*. Available at: http://www.wrc.org.za/Lists/Knowledge Hub Items/Attachments/6466/WaterSA_1986_12_0402_abstract.pdf [Accessed February 21, 2016].

Sobeck, D.C. & Higgins, M.J. (2002). Examination of three theories for mechanisms of cation-induced bioflocculation. *Water Research*, 36(3), pp.527–538.

Sponza, D.T. (2002). Extracellular polymer substances and physicochemical properties of flocs in steady- and unsteady-state activated sludge systems. *Process Biochemistry*, 37(9), pp.983–998.

Sponza, D.T. (2003). Investigation of extracellular polymer substances (EPS) and physicochemical properties of different activated sludge flocs under steady-state conditions. *Enzyme and Microbial Technology*, 32(3-4), pp.375–385. Available at: http://www.sciencedirect.com/science/article/pii/S0141022902003095 [Accessed February 24, 2016].

Stoller, M. (2009). On the effect of flocculation as pretreatment process and particle size distribution for membrane fouling reduction. *Desalination*, 240(1-3), pp.209–217. Available at: http://dx.doi.org/10.1016/j.desal.2007.12.042.

Sun, C. et al. (2010). Salinity effect on a biofilm-MBR process for shipboard wastewater treatment. *Separation and Purification Technology*, 72(3), pp.380–387.

Uygur, A. & Kargı, F. (2004). Salt inhibition on biological nutrient removal from saline wastewater in a sequencing batch reactor. *Enzyme and Microbial Technology*, 34(3-4), pp.313–318. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0141022903003661.

Valentine, D.L. (2007). Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nature reviews. Microbiology*, 5(4), pp.316–323.

Venkatakrishnan, H. et al. (2014). Effect of a high strength chemical industry wastewater on microbial community dynamics and mesophilic methane generation. *Journal of Environmental Sciences (China)*, 26(4), pp.875–884. Available at: http://dx.doi.org/10.1016/S1001-

0742(13)60515-X.

Vyrides, I. & Stuckey, D.C. (2009a). Adaptation of anaerobic biomass to saline conditions: Role of compatible solutes and extracellular polysaccharides. *Enzyme and Microbial Technology*, 44(1), pp.46–51.

Vyrides, I. & Stuckey, D.C. (2009). Effect of fluctuations in salinity on anaerobic biomass and production of soluble microbial products (SMP(s)). *Biodegradation*, 20(2), pp.165–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18677440 [Accessed February 26, 2016].

Vyrides, I. & Stuckey, D.C. (2009b). Saline sewage treatment using a submerged anaerobic membrane reactor (SAMBR): Effects of activated carbon addition and biogas-sparging time. *Water Research*, 43(4), pp.933–942. Available at: http://dx.doi.org/10.1016/j.watres.2008.11.054.

Wang, Y. et al. (2010). Past, present and future applications of flow cytometry in aquatic microbiology. *Trends in Biotechnology*, 28(8), pp.416–424. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0167779910000739.

Wang, Z. et al. (2013). Effect of salinity on extracellular polymeric substances of activated sludge from an anoxic-aerobic sequencing batch reactor. *Chemosphere*, 93(11), pp.2789–2795. Available at: http://dx.doi.org/10.1016/j.chemosphere.2013.09.038.

Wang, Z. et al. (2015). Effects of salinity on performance, extracellular polymeric substances and microbial community of an aerobic granular sequencing batch reactor. *Separation and Purification Technology*, 144, pp.223–231. Available at: http://www.sciencedirect.com/science/article/pii/S1383586615001410 [Accessed February 21, 2016].

Welsh, D.T. (2000). Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. *FEMS Microbiology Reviews*, 24(3), pp.263–290. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1574-6976.2000.tb00542.x/abstract\ninternal-pdf://276/full.html\n<Go to ISI>://000087800400003.

Woolard, C.R. & Irvine, R.L. (1995). Treatment of hypersaline wastewater in the sequencing batch reactor. *Water Research*, 29(4), pp.1159–1168.

Yang, J. et al. (2013). Impact of Na + on Biological Wastewater Treatment and the Potential of Anaerobic Membrane Bioreactors: A Review. *Critical Reviews in Environmental Science and Technology*, 43(24), pp.2722–2746. Available at: http://www.tandfonline.com/doi/abs/10.1080/10643389.2012.694335.

Yogalakshmi, K.N. & Joseph, K. (2010). Effect of transient sodium chloride shock loads on the performance of submerged membrane bioreactor. *Bioresource Technology*, 101(18), pp.7054–7061. Available at: http://dx.doi.org/10.1016/j.biortech.2010.03.135.

Yu, J. et al. (2000). Distribution and change of microbial activity in combined

UASB and AFB reactors for wastewater treatment. , 315.

Zhang, H. et al. (2014). Performance enhancement and fouling mitigation by organic flocculant addition in membrane bioreactor at high salt shock. *Bioresource Technology*, 164, pp.34–40.

APPENDICES

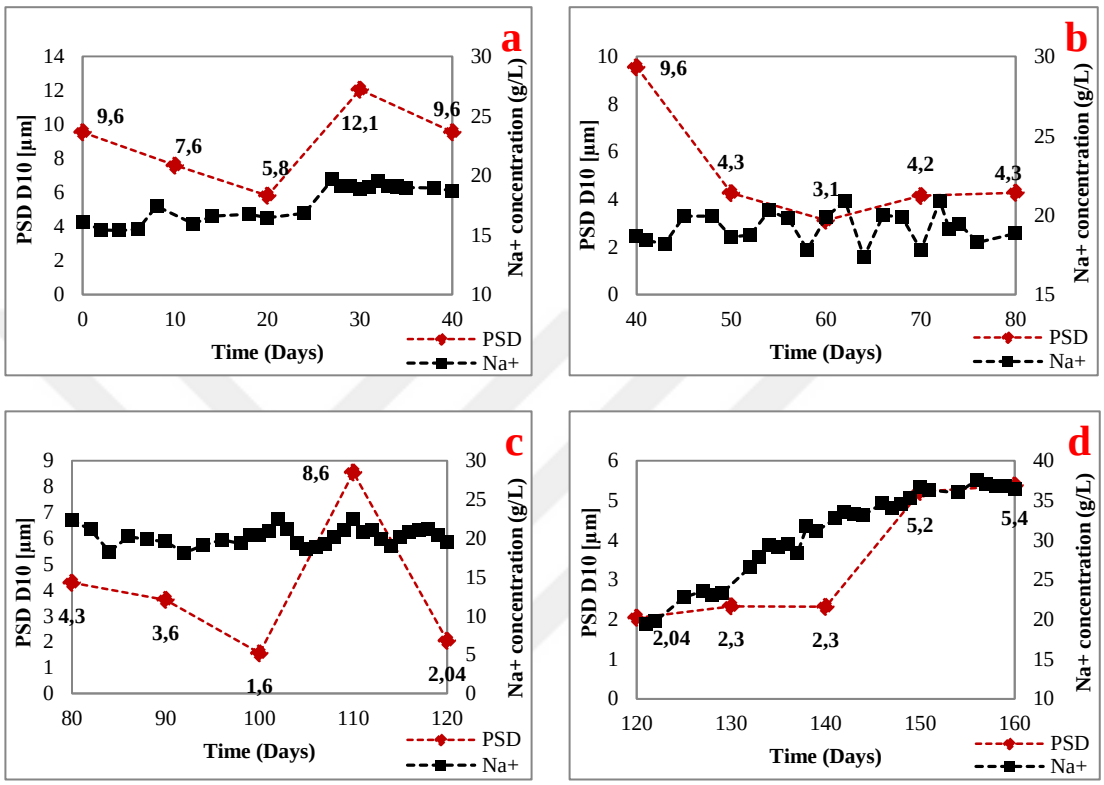

Figure A. 1 : Variations of sludge particle size distribution (D10) due to salinity changes (a) Evenly Distributed; (b) Stepwise Increase/Decrease; (c) Non-evenly Distributed; (d) High Concentration Swings.

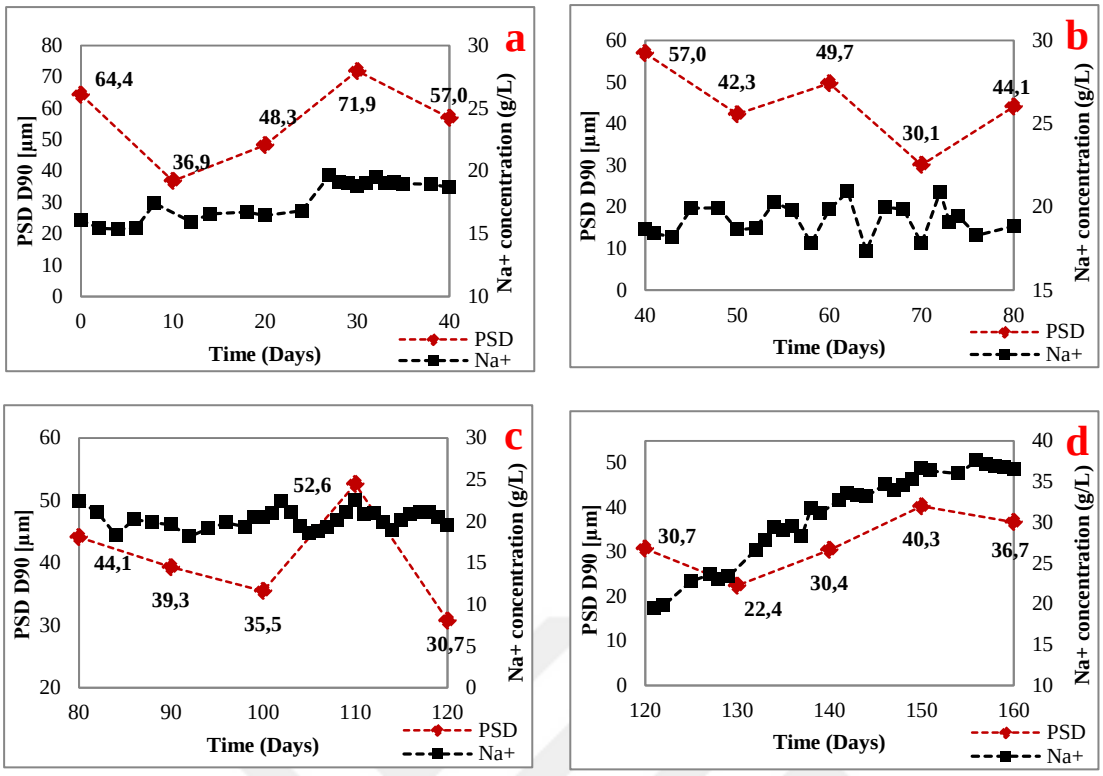

Figure A. 2 : Variations of sludge particle size distribution (D90) due to salinity changes (a) Evenly Distributed; (b) Stepwise Increase/Decrease; (c) Non-evenly Distributed; (d) High Concentration Swings.

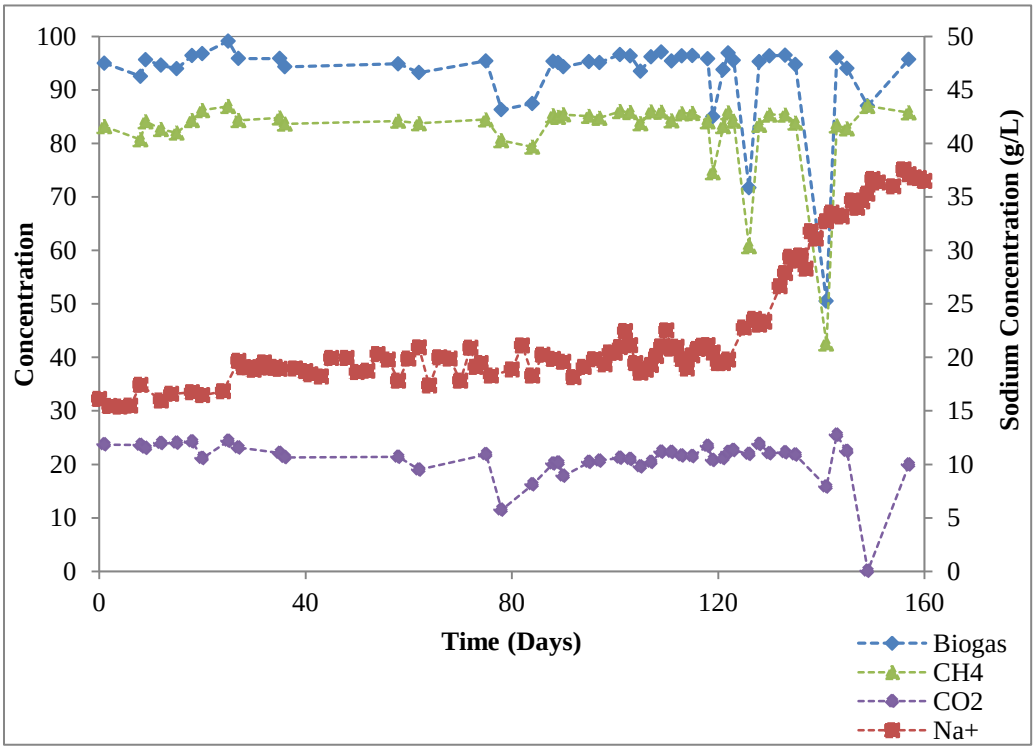

Figure A. 3 : Biogas concentration variations due to the changes in sodium concentration

CURRICULUM VITAE

Name Surname **: Başak DÖNMEZ**

Place and Date of Birth **: 27/06/1991 Istanbul**

E-Mail **: basakdonmez@windowslive.com**

EDUCATION **:**

- **B.Sc.** **: Yıldız Technical University, Environmental Engineering**

PROFESSIONAL EXPERIENCE AND REWARDS:

- 05.2015 – 05.2016 Delft University of Technology Department of Water Management, *Research Intern / Graduation Project*
- 06.2013 – 07. 2013 TEKSAN Generator and Power Systems, *Health, Safety & Environment Intern*
- 03.2013 – 06.2013 YTU Environmental Engineering Laboratory, *BSc. Graduation Project*
- 08.2012 – 09.2012 Doluca Wine Company, *Engineering Intern*

TRAININGS and CERTIFICATES:

- *Athens Programme* – Safety Engineering Course
  University of Leuven – 03.2015
- *Athens Programme* - Environmental Biotechnology Course
  Czech Technical University in Prague – 11. 2014
- *Certificate of Graduation*- AtıkMatık: Packing Wastes Recycling Machine Project
  GEMIM Entrepreneurship Training and Incubation Center – 06. 2014
- *Environmental Technician Sertificate*
  Turkish Ministry of Environment and Urbanisation – 01.2014
- *Occupational Health and Safety Specialist Certificate*
  Turkish Ministry of Labor and Social Security – 12.2013