TREATMENT AND VALORIZATION OF ANAEROBIC DIGESTATE

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

NİLÜFER ÜLGÜDÜR

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
ENVIRONMENTAL ENGINEERING

MARCH 2019

Approval of the thesis:

TREATMENT AND VALORIZATION OF ANAEROBIC DIGESTATE

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ABSTRACT

TREATMENT AND VALORIZATION OF ANAEROBIC DIGESTATE

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March 2019, 288 pages

Anaerobic digestion is a widely applied process for the stabilization and treatment of high-strength wastes. The process has two outputs, biogas and digestate. Even though biogas produced during the treatment is a renewable energy source and has positive impacts on improving the economics of the plant, the treatment and disposal of the digestates present a challenge. The treatment methods offered so far are either costly or low yielded which drives off these uneconomic and non-viable treatment processes from being solutions for digestate management. Digestates are still commonly applied on land as a fertilizer or soil conditioner. However, the land application of the digestate is exposed to several concerns regarding pollution, limited applicability and regulatory aspects. In this thesis study, a residual biogas potential (RBP) test was initially conducted on six digestate samples with the aim of developing a new approach for digestate management. The test indicated that significant biogas yields (0.111-0.326 L_{biogas}/g VS) and total chemical oxygen demand (COD_t) removal (21-84%) could be obtained from digestates in 70 days. The high-rate anaerobic treatment was then applied on the digestate having the highest residual biogas yield to reduce the time required to digest the residual organics. COD_t was removed by 56-63% in 1.3-1.4 days of hydraulic retention time (HRT). A subsequent process of microalgal nutrient removal resulted in 92.7-93.7% of ammonium nitrogen and 95.6-97.8% of dissolved

phosphorus reduction. The results were promising and verified the applicability of the high-rate anaerobic and microalgal nutrient removal processes for the digestate management.

Keywords: Digestate, High-Rate, Anaerobic Treatment, Residual Biogas, Microalgae, Nutrient Removal.

ÖZ

ANAEROBIK ÇÜRÜTÜCÜ ÇIKIŞ SUYUNUN ARITILMASI VE DEĞERLENDİRİLMESİ

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Anaerobik çürütme, kirlilik yükü yüksek atıkların stabilizasyonu ve arıtılması için yaygın olarak uygulanan bir işlemdir. Bu işlemin biyogaz ve çürütücü çıkış suyu olmak üzere iki çıktısı vardır. Arıtım sırasında üretilen biyogazın yenilenebilir enerji kaynağı olmasına ve tesisin ekonomisini iyileştirme yönünde pozitif etkileri olmasına rağmen, çürütücü çıkış suyunun arıtılması ve bertarafı bu endüstri için bir engeldir. Şimdiye kadar önerilen artıma yöntemlerinin maliyetli veya düşük verimli olması bu ekonomik ve uygulanabilir olmayan işlemleri çürütücü çıkış suyunun yönetimi için çözüm oluşturmaktan uzaklaştırır. Çürütücü çıkış suyu hâlâ toprağa gübre veya düzenleyici olarak uygulanmaktadır. Ancak, toprak uygulaması kirlilik, sınırlı uygulanabilirlik ve yasal mevzuat ile ilgili bazı kaygılara sebep olmaktadır. Çürütücü çıkış suyunun yönetimine yeni bir yaklaşım getirmek amacı ile ilk olarak 6 çürütücü çıkış suyu numunesine kalan biyogaz potansiyeli (KBP) testi uygulanmıştır. Test, çürütücü çıkış sularından 70 gün içinde önemli miktarlarda biyogaz verimlerinin (0,111-0,326 $L_{biyogaz}$/g VS) ve toplam kimyasal oksijen ihtiyacı (COD_t) gideriminin (%21-84) elde edilebileceğini göstermiştir. Daha sonra kalan organik maddelerin çürütülmesi için gereken süreyi azaltmak üzere kalan biyogaz potansiyeli en fazla olan çürütücü çıkış suyu için yüksek hızlı anaerobik arıtım uygulanmıştır. 1,3-1,4 günlük

hidrolik tutma süresinde oranında %56-63 COD_t giderilmiştir. Takibindeki süreçte uygulanan mikroalg işlemi ile %92,7-93,7 amonyum azotu ve %95,6-97,8 çözünmüş fosfor giderimi sağlanmıştır. Sonuçlar ümit verici olup çürütücü çıkış suyunun yüksek hızlı anaerobik ve mikroalgal besiyer madde giderme işlemlerinin uygulanabilirliğini ortaya koymuştur.

Anahtar Kelimeler: Çürütücü Çıkış Suyu, Yüksek Hızlı, Anaerobik Arıtım, Kalan Biyogaz, Mikroalg, Besiyer Madde Giderimi.

To the Life

ACKNOWLEDGMENTS

First of all, I would like to record my deepest gratitude to my supervisors Assoc. Prof. Dr. Tuba Hande Ergüder Bayramoğlu and Prof. Dr. Göksel N. Demirer for their valuable advices, guidance and encouragement.

I wish to express my sincere thanks to my committee members, Prof. Dr. Filiz B. Dilek, Prof. Dr. Meryem Beklioğlu, Prof. Dr. Anatoli Dimoglo and Doç. Dr. Selim Sanin. I'm very grateful for their feedbacks and valuable recommendations.

I am also grateful to Nur Filiz and Duygu Tolunay, the members of Limnology Laboratory of the Department of Biological Sciences of Middle East Technical University, for their valuable support and help. I would also like to thank to the undergraduate student in my department, Duygu Keskin, for her pro bono help in the laboratory. I am very lucky to have so lovely friends; Elif Küçük, Ebru Koçak, İlker Balcılar and Selcen Ak. Thank you all for your encouragement and support all the time I need. I also appreciate the support held by my roommates in METU: Mert Erkanlı and Tercan Çataklı.

I want exclusively to thank my son, Oğuz Ege Ülgüdür, for being a motivation during my studies. I really appreciate his patience whenever I needed. It is impossible to express how I am thankful to my husband Yavuz Selçuk Ülgüdür, my mother Hanife Demirci and my father Cemal Demirci. Thank you all for the trust and the belief in me. This Thesis was supported by Office of Scientific Research Projects Coordination of Middle East Technical University [BAP- 03-11-2016-002].

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ABBREVIATIONS

AAF	Anaerobic attached film
ABR	Anaerobic baffled reactor
ACT	Anaerobic contact reactor
AD	Anaerobic digestion
AF	Anaerobic filter
AFBR	Anaerobic fluidized bed reactor
AFFBR	Anaerobic fixed film bed reactor
AFFEBR	Anaerobic fixed film expanded bed
AFFFBR	Anaerobic fixed film fixed bed reactor
AFFR	Anaerobic fixed film reactor
AMBR	Anaerobic mitigating blanket reactor
APBR	Anaerobic packed bed reactor
ASBR	Anaerobic sequencing batch reactors
ASTBR	Anaerobic structured bed reactor
ATS	Attached total solids
AVS	Attached volatile solids
BMP	Biochemical methane potential
BSI	British Standards Institution
BTL	Biomass to liquid fuel
C:N	Carbon to nitrogen
CH_4	Methane
CM	Completely mixed
CO_2	Carbon dioxide
COD	Chemical oxygen demand (mg/L)
COD_a	Chemical oxygen demand-added (mg/L)
COD_{eff}	Chemical oxygen demand-effluent (mg/L)
COD_{inf}	Chemical oxygen demand-influent (mg/L)

COD_r	Chemical oxygen demand-removed (mg/L)
COD_s	Chemical oxygen demand-soluble (mg/L)
COD_t	Chemical oxygen demand-total (mg/L)
CSAR	Continuously stirred anaerobic reactor
CSTR	Continuously stirred tank reactor
DLD	Diluted liquid digestate
DOP	Dissolved organic phosphorus
DRP	Dissolved reactive phosphorus (mg/L)
DSFLD	1/10 diluted, sequentially filtered liquid digestate from 600, 425, 175, 100, 63 and 53 μm pore-sized filters
DUP	Dissolved unreactive phosphorus
DWW	Dilution wastewater
EEAD	Electrolysis enhanced anaerobic digestion
EGSB	Expanded bed granular sludge blanket
FBR	Anaerobic fixed bed reactor
GLS	Gas-liquid-solid
GWP	Global warming potential
HRT	Hydraulic retention time
IA	Intermediate alkalinity (mg/L as $CaCO_3$)
IC	Internal circulation reactor
LD	Liquid digestate
MBBR	Moving bed biofilm reactor
MFDLD	Diluted and mesh filtered liquid digestate
N_2	Nitrogen gas
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NH_4^+-N	Ammonium nitrogen (mg/L)
NO_2^--N	Nitrite nitrogen (mg/L)
NO_3^--N	Nitrate nitrogen (mg/L)

OLR	Organic loading rate
PA	Partial alkalinity (mg/L as $CaCO_3$)
PAS	Publicly Available Specification
PBR	Photobioreactor
PO_4^{3-}	Phosphate
PolyP	Polyphosphate
PP	Particulate phosphorus
R	Correlation coefficient
R^2	Coefficient of determination
RBP	Residual biogas potential
RBY	Residual biogas yield ($L_{biogas/}g\ VS_{added}$, m^3 biogas /kg VS_{added}, m^3 biogas/kg COD_t removed, m^3 biogas/kg COD_r)
RWW	Raw wastewater
SB	Sludge bed
SRT	Solids retention time
TA	Total alkalinity (mg/L as $CaCO_3$)
TAN	Total ammonia nitrogen (mg/L)
TDP	Total dissolved phosphorus (mg/L)
ThOD	Theoretical oxygen demand
TKN	Total kjeldahl nitrogen (mg/L)
TP	Total phosphorus (mg/L)
TS	Total solids (mg/L)
TSN	Total soluble nitrogen
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acid
VS	Total volatile solids (mg/L)
WAS	Waste activated sludge
WRAP	Waste and Actions Resources Programme
WW	Wastewater

CHAPTER 1

INTRODUCTION

Anaerobic digestion (AD) is a commonly applied process for the treatment and stabilization of high-strength wastes. The process has two outputs, the biogas and the digestate. Generated from wastes, biogas is a renewable energy source and mainly composed of a mixture of methane and carbon dioxide gasses. The industry related to the anaerobic digestion of wastes, known as biogas sector, has a growing trend and is expected to get larger due to the policies and the incentives provided for the investors to support energy production from wastes. On the other hand, the industry still lacks a feasible and cost effective treatment process for the environmental management of the digestate which remains as a challenge.

Digestate is the slurry effluent leaving the anaerobic digester which hosts high nutrients levels, chemical oxygen demand and total solids concentrations. Advanced treatment processes such as vacuum evaporation, membrane processes, struvite precipitation, and ammonia (NH_3) stripping have been offered so far for the management of digestates (Drosg et al., 2015; Fechter and Kraume, 2016). These processes offered are either costly, low-yielded or require chemical addition which limits the applicability of such processes. Digestate is commonly being applied to land as a fertilizer or soil conditioner as a consequence of the lack of feasible and cost-effective methods for digestate management.

On the other hand, the land application of the digestates has several concerns. Digestates when applied on land creates potential chemical, physical and biological pollution problems (Xia and Murphy, 2016). Even though the pollution aspect of land

application is underestimated as it is a commonly employed practice, the management of the land application process has additional challenges. Digestates are mostly applied to near-by agricultural land. When large volumes of digestate production which range between several hundreds to several thousands of cubic meters in AD plants are considered, the available agricultural land may become limited. The digestate may be transported to the lands where it can be used as a fertilizer or soil conditioner. However, composed of 90-95% of water (Fechter and Kraume, 2016), the transportation may create logistical problems. Even if the digestate can be dried and marketted as a fertilizer, the transportation of dried digestate may shade the value of digestate as a fertilizer replacement. Another logistical problem may be created by the storage of digestates. The digestate is required to be stored due to its seasonal applicability on land which is limited to a few months of the year (Section 2.1.3.3.4). The reserved area for the plant may not be adequate for long storage periods that is ruled by the regulations. Thus, it is required to be transported and stored elsewhere from the reserved area of the plant. Moreover, several analysis should be performed on both digestate, crop and soil to prevent overloading and to maintain the soil balance. The analysis may be problematic and even may not be applicable depending on the variable digestate compositions. Digestate compositions may alter by each change of the operational conditions of the AD plants such as HRT and organic loading rate (OLR). Any change applied on the AD process ends up with a digestate of different quality and requires to be re-analyzed. In addition to the limitations regarding the applicability on land, the regulations rule several obligations (Section 2.1.3.4). These obligations can be addressed as the applicable maximum nitrogen load on land, the stability, solid content and heavy metal concentrations of the digestate. The legal limitations governed by regulations and more specifically strict interpretations of these regulations which can change from country to country can be considered as a barrier in the development of AD processes.

Biological waste management is to be grounded on the reuse and recyle approaches based on the regulatory improvements world wide. Being a biological waste, digestates have high organic contents due to incomplete or partial degradation of organic matters and short-circuiting in the digester (Section 2.1.4). The presence of biodegradable organics in the digestates may result in the production of an unstable high organic loaded waste stream. This waste stream has a potential to be further treated to reduce high residual organic loads it preserves. It is also probable to obtain residual biogas during the treatment in the meantime if anaerobic conditions are maintained.

Anaerobic treatment of the wastes are commonly employed in continuously stirred tank reactors (CSTRs) which are known as first generation digesters (Section 3). The retention of the microorganisms that degrade organics (solids retention time, SRT) within the digester is directly related to the hydraulic retention time of the wastewater in these type of digesters. Thus, hydraulic retention time (HRT) is required to be kept long enough to prevent wash-out of the microorganisms. The long HRTs can be maintained in the digester installations that have large footprints. The recent applications for anaerobic treatment of wastes, second and third generation digesters, generally uncouple SRT and HRT. The extended SRT by immobilization or entrapment of the biomass within the digester provides a means of uncoupling of these two parameters. Holding larger biomass in such digesters, the HRT and the related footprint of the digester can be decreased. The decrease in the areal requirement as well as digester volumes consequently decreases the investment costs associated with the new installations, thereby increasing the applicability of an additional process. Being a second generation high-rate anaerobic reactor, anaerobic fixed-film reactors (AFFRs) can be operated in relatively short HRTs whilst they can retain large amounts of biomass.

Even though digestates are the waste streams produced at the end of AD processes, they contain high concentrations of nutrients. The nutrients in digestate composition are mostly in the form that can be easily consumed by microalgal species. The microalgal uptake of nutrients from the digestate is advantageous in terms of both clarifying of the nutrient content and accumulating them into biomass through metabolization of microalgal species. The microalgal biomass produced can further be employed in downstream processes as energy products.

The treatment requirement of the digestates is grounded on the limitations regarding the digestate application on land and the pollutional concerns associated with land application. On the other hand, further AD of the digestates offers both the reduction of residual organic loads and the residual biogas production associated with the decomposition of this organic content. An additional microalgal nutrient removal process has a potential to further removal of nutrients from the wastewater as well as build up microalgal biomass. Therefore, an integration of an addional anaerobic treatment unit with a microalgal nutrient removal process can be applied for digestate treatment and have a potential to maximize the energy profit from the treatment process. Likewise, a digestate treatment scheme covering the AFFRs and microalgal photobioreactors (PBRs) were integrated in this Thesis study (Figure 1.1). To this purpose, six digestate samples of full-scale anaerobic digesters digesting animal manures, mixtures of organic wastes and manures, and sewage sludge were investigated for their residual biogas production potential and further treatability under anaerobic conditions. The digestate having the highest residual biogas yield (RBY) was used in the high-rate treatment using anaerobic fixed-film reactors. The effluent of the AFFRs was then employed in microalgal nutrient removal process. The scope of the Thesis from sampling to microalgal treatment is given in Figure 1.2.

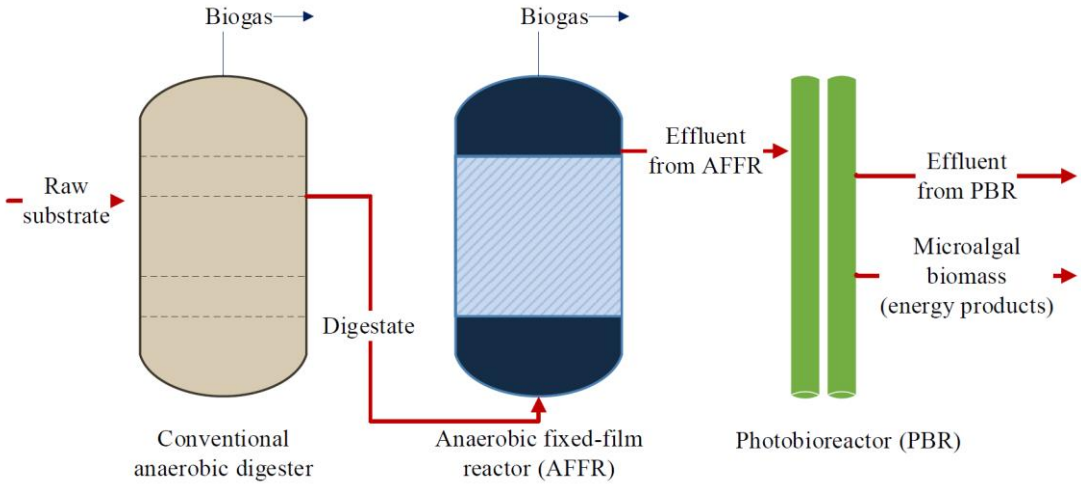


Figure 1.1. Proposed digestate treatment scheme

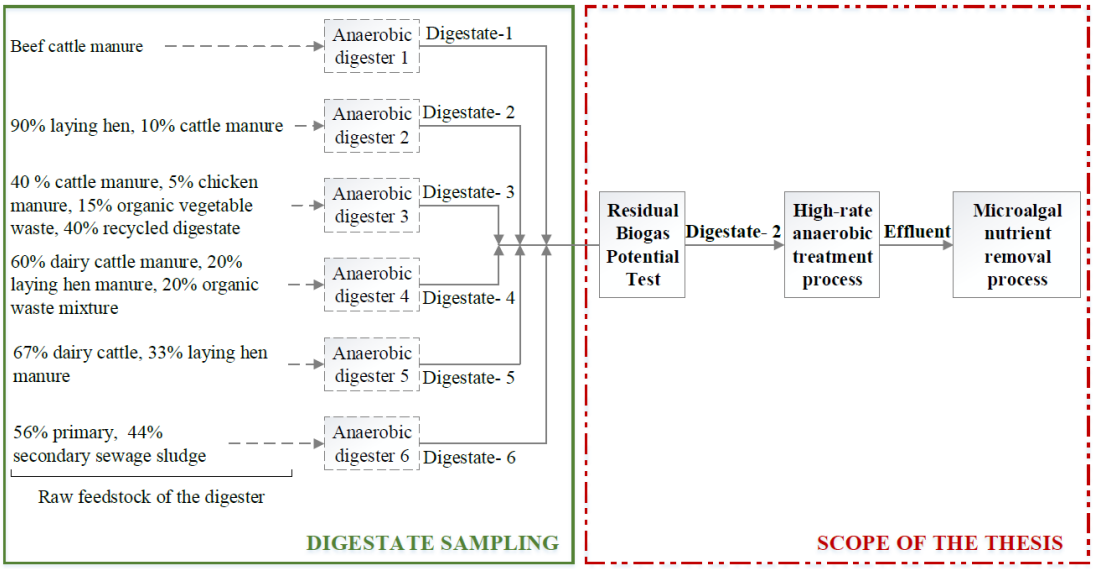


Figure 1.2. The basic flowchart representing the scope of the Thesis.

Similar studies on the residual biogas production potential of the digestates mostly focused on the capture of additional biogas associated with the undigested organic content that could possibly be produced during storage of the digestates. These studies did not broadly cover the further treatment potential of the digestates under anaerobic

conditions. The further anaerobic treatability investigation has a potential to open a gate for new approaches for digestate management. Moreover, high-rate anaerobic treatment of the digestates has firstly been investigated under the scope of this Thesis. Additionally, the integration of high-rate treatment with a microalgal nutrient removal process for the management of digestates has been firstly proposed. The proposed integrated process can create a cost-effective and feasible management solution for the digestates based on the additional energy capture potential.

This Thesis study involves three main chapters about experimentation and results-discussions on treatment and valorization of anaerobic digestate. Further anaerobic treatability and residual biogas production potential of six different digestates are given in Chapter 2. Chapter 3 includes the application of high-rate treatment of a digestate sample using anaerobic fixed-film reactors. Microalgal nutrient removal process applied to the effluent of the AFFRs is described in Chapter 4. The conclusions derived from the overall applications and the related recommendations are covered in Chapter 5.

CHAPTER 2

ANAEROBIC TREATABILITY AND RESIDUAL BIOGAS POTENTIAL OF DIGESTATES

Liquid digestates have a potential to favor the growth of many microalgal species due to their high nutrient concentrations. However, the solid content of the digestate may be considerably high (3-30%) depending on the substrate composition and its degradability and digestion process (Drosg et al., 2015) which may prevent the microalgal growth. A preliminary study on decreasing the solids content of a liquid digestate sample had shown that an effective reduction could not be achieved using a cost-effective method such as gravity sedimentation, filtration, coagulation-flocculation and even centrifugation (Appendix A). Approximately 70% of the particles contained in digestates was previously reported to be less than 1 mm in size and cannot be separated easily due their small sizes, negatively charged surfaces and density (Fechter and Kraume, 2016). Poor settleability of the solids in digestate content represents one of the main obstacles in digestate management (Camilleri-Rumbau et al., 2013) which decreases the efficiency in downstream processing of digestates.

Digestates are also known to hold high chemical oxygen demand (COD) concentrations besides high solid contents. COD concentration of the digestates can range between 50 and 120 kg/t which prevents it to be treated by a classical wastewater treatment method (Fechter and Kraume, 2016). On the other hand, high COD concentrations of the digestates is an indication for the requirement for further treatment as well as the potential for additional biogas production which is mostly lost with the discharge or storage of the digestates.

The requirement to decrease COD and the potential for additional biogas production was investigated under anaerobic batch treatment of digestates called RBP test. To this purpose, the samples taken from six full-scale anaerobic digesters digesting animal manures, mixtures of organic wastes and manures, and sewage sludges were subjected to the RBP test. This chapter covers the literature background for further anaerobic treatment requirement of the digestates and the results of the RBP test applied with the background methodology.

2.1 Literature Background

AD is a valuable well-established technology which combines the treatment of high-strength wastes with the production of renewable energy. AD can be applied in many types of wastes such as animal manures, sewage sludges, municipal solid wastes, agricultural products or residue of these products, food residues, household wastes, industrial wastes and by-products that have high organic content (Lukehurst et al., 2010). AD process is briefly described in Section 2.1.1.

2.1.1 Anaerobic digestion process

Biodegradable organic matters in the waste are converted into methane, carbon dioxide, inorganic nutrients and biomass under anaerobic conditions through hydrolysis, acidogenesis, acetogenesis and methanogenesis processes. Hydrolysis is the breakdown of the biopolymers into soluble compounds. Soluble organic compounds are converted into volatile fatty acids (VFAs) and carbon dioxide in acidogenesis phase. Acetate and hydrogen gas are produced by the decomposition of VFAs in acetogenesis. Methanogenesis is the phase in which acetate, carbon dioxide

and hydrogen gas are converted into methane (de Mes et al., 2003). Simple conversion diagram of substrates during AD process is given in (Figure 2.1).

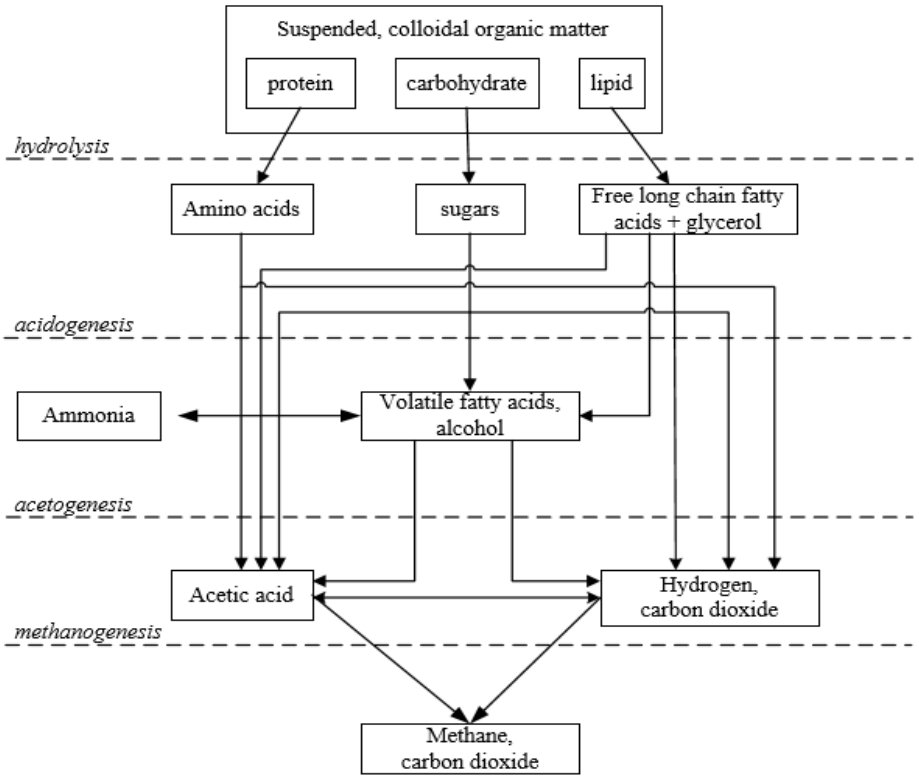


Figure 2.1. Degradation of the substrates during anaerobic digestion process (de Mes et al., 2003).

Anaerobic digestion has two outputs, biogas and digestate. Biogas is mainly the mixture of methane and carbon dioxide gases. Significant biogas yields can be obtained by decomposition of the organics under anaerobic conditions (Table 2.1). Digestate is the slurry effluent leaving the digester which is rich in nutrients, mainly in terms of nitrogen, phosphorus and potassium (McPhail et al., 2012). Digestates can be considered as a waste stream of AD processes. Several processing options have been developed so far for digestate management (Section 2.1.2).

Table 2.1. Biogas yields obtained from various raw feedstocks.

Raw feedstock	Biogas yield, m^3/kg VS	Reference
Unscreened dairy manure	0.076-0.470	Demirer and Chen, 2005a
Dairy cattle manure and agricultural residues	0.087-0.324	Alkaya et al., 2010
Cattle manure	0.15-0.35	Braun, 2007
Chicken manure	0.35-0.6	
Secondary sludge (municipal)	0.2-0.35	
Molasses distillery slops	0.42	
Maize distillery slops	0.4	
Potato distillery slops	0.47	
Municipal biowaste (Source separated)	0.40	
Grey waste	0.08-0.15	
Municipal wastewater sludge	0.3-0.5	Zupančič and Grilc, 2012
Pig stomach content	0.3-0.4	
Vegetable wastes	0.3-0.4	
Straw from cereals	0.2-0.5	
Cattle manure (liquid)	0.1-0.8	
Pig excreta	0.2-0.5	
Sheep excreta	0.3-0.4	

2.1.2 Digestate processing options

Digestates can be processed completely or partially after liquid-solid phase separation (Figure 2.2). Complete treatment technologies require more energy input, more investment and operating expenditures. These technologies are also of variable maturity. The technologies applied for partial treatment, which mostly aim at volume reduction, are relatively simple and economical which commonly ends up with the land application of the digestate (Drosg et al., 2015).

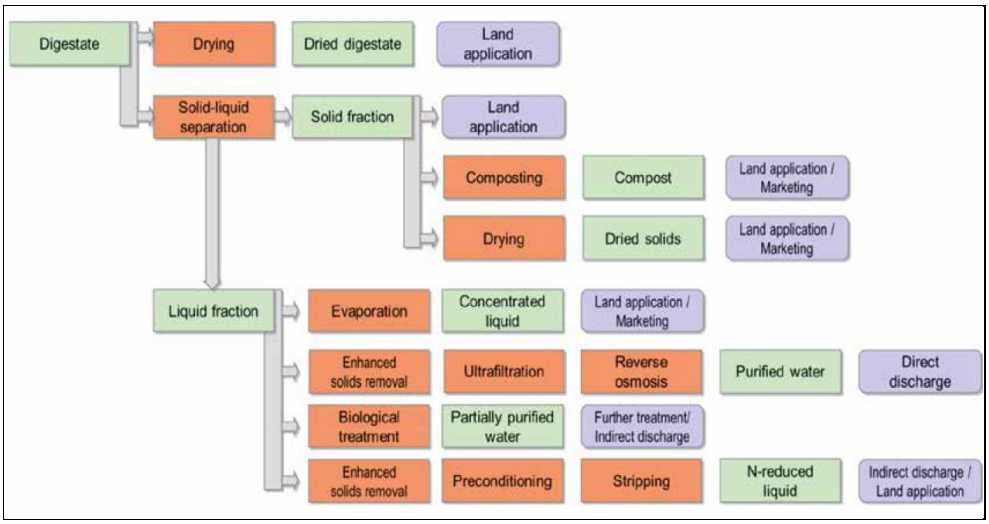


Figure 2.2. Digestate processing options (Drosg et al., 2015).

2.1.2.1 Digestate processing for volume reduction

The separation of solid-liquid phase is generally the first step in digestate processing. Screw presses, vibrating screens, decanters, belt filter presses or flotation can be used for solids removal (Fechter and Kraume, 2016). Some flocculants or precipitants may be added to improve the efficiency of phase separation. Digestates can be composted or dried before land application or marketing. Drying can be applied on the whole digestate or on the phase separated digestate (Drosg et al., 2015). A maximum 15 % dry matter content can be achieved for the liquid digestates of mesophilic digesters (Fechter and Kraume, 2016).

2.1.2.2 Digestate processing for the recovery of nutrients

Struvite precipitation and ammonia stripping are the two methods applied for the recovery of nutrients from the digestates.

2.1.2.2.1 Struvite precipitation

Struvite precipitate (magnesium ammonium phosphate) can be achieved by the addition of magnesium oxide and phosphoric acid into digestates (Drosg et al., 2015). The formation of struvite enables the removal and recovery of nitrogen and phosphorus from the digestates in the form of a valuable slow releasing fertilizer (Uludag-Demirer et al., 2005). The large amount of chemical necessity is the main drawback of this nutrient recovery method which corresponds to high operational costs (Drosg et al., 2015).

2.1.2.2.2 Ammonia stripping

Ammonia stripping is applied on the liquid portion of the digestates. The principle behind is the conversion of ammonium ions (NH_4^+) into ammonia gas by increasing the pH and the heat of the liquid digestate. When ammonia is formed, it is then stripped using a stripping gas (usually steam) and introducing sulfuric acid in a column to enable the formation of ammonium sulfate (Fechter and Kraume, 2016). Clogging of the packed columns by the residual solids in the digestate is a major problem in ammonia stripping. Therefore, an efficient solids removal is required before the process (Drosg et al., 2015). Such a process requires 7 kWh electrical energy for a cubic meter of digestate (Fechter and Kraume, 2016).

2.1.2.3 Digestate processing for complete purification of the liquid phase

Vacuum evaporation and membrane processes have been offered for complete purification of the liquid digestates.

2.1.2.3.1 Vacuum evaporation

Liquid digestate is treated in a vacuum evaporator that reduces the boiling point of water to 40-70^{o}C. Evaporated water contains high ammonia content which is stripped by an acidic scrubber to obtain ammonium sulfate. The water content is then condensed in a condenser. 13 kWh of electrical energy for a meter cube of digestate is required for such a process (Fechter and Kraume, 2016).

2.1.2.3.2 Membrane processes

Membrane processes enables physical separation of the solids from the digestates. The process is called either micro-, ultra- or nano-filtration depending on the pore sizes of the membranes used. Nano-filtration and additionally reverse osmosis membrane processes can even separate dissolved salts (ions) from the water. The digestate is typically solid-liquid phase separated using screw presses or decanter centrifuges. The particles in the liquid fraction of the digestate is further removed by enhanced solids removal processes such as precipitation/flocculation, flotation, screens and filters, etc. The liquid fraction of the digestate is then micro- or ultra-filtrated and followed by reverse osmosis in a typical membrane application for digestate processing (Drosg et al., 2015). Ultra-filtration can handle waste streams of up to 2.5 % dry matter content which requires an elaborate solids removal system before the application of ultra-filtration. Such a process should be capable of removing all particles from the waste stream because reverse osmosis taking place right after ultra-filtration can be clogged by the particles reaching to the unit (Fechter and Kraume, 2016).

Membrane processes offer purified water as well as a nutrient concentrate which can further be used as a liquid fertilizer. However, the purified water is only 50 % of the

treated digestate. The rest 50% is composed of the separated solids before membrane application and the concentrates in membrane filtration processes (Drosg et al., 2015). Approximately 21 kWh electricity is required for one cubic meter of digestate for membrane processing including the solids removal prior to membrane application (Fechter and Kraume, 2016). Ion-exchange can also further be applied for the removal of ions from the membrane-reverse osmosis treated liquid digestate (Drosg et al., 2015).

2.1.2.4 Digestate processing to reduce chemical oxygen demand

An integrated flocculation-aeration-chemical oxidation process has also been proposed for further treatment of digestates. The process reduces the solids and COD contents (Camarero et al., 1996). The biological oxidation processes have high-operational costs (Peng and Pivato, 2017) which is a major drawback in their applicability.

2.1.3 Drivers for digestate treatment

Anaerobic digestion is a well-established treatment process for the high-strength wastes. On contrary, the treatment and disposal of its effluent, digestate, are still challenges for the industry which might represent a barrier against the improvement of wet fermentation processes (Li et al., 2015). The problems in the treatment and the disposal of the digestates can be addressed as the lack of viable digestate treatment processes, pollution concerns regarding the storage, land application and disposal of the digestates, limited applicability of digestates to land and regulatory restrictions covering the management of the digestates.

2.1.3.1 Lack of a viable treatment method for the management of digestates

The advanced digestate processing options such as vacuum evaporation, membrane processes, struvite precipitation, and ammonia stripping either require chemical addition or high energy supply. Thus, such treatment options end up with a considerable investment on installation for the supply of proper equipment and for the treatment of digestates (Drosg et al., 2015). As a consequence, the most widely used option for digestate management remains as either direct disposal to the environment or land application as a fertilizer or soil conditioner (Monnet, 2003; McPhail et al., 2012; Cheng et al., 2015; Romero-Güiza et al., 2016a; Xia and Murphy, 2016).

2.1.3.2 Pollution concerns

Digestates have high nutrient concentrations (nitrogen and phosphorus), light metals such as magnesium, aluminum and heavy metals like cadmium, copper, manganese, zinc, chromium in their composition (Table 2.2). The application of digestates on land has a potential to cause chemical (i.e. heavy metals), biological (i.e. pathogens) and physical (i.e. plastics) pollution (Xia and Murphy, 2016).

The high nutrient concentrations in the digestate composition may lead to eutrophication when transported into receiving water bodies (García-Albacete et al., 2014). Heavy metal concentration of the digestates may also reach to high levels. The application of the digestates of food wastes as a fertilizer were reported to be problematic and likely to affect the economy of biogas producers due to the high cadmium to phosphorus ratio (mean value of 37 mg/kg P) of the food wastes (Karlsson et al., 2014). Some trace elements such as copper and zinc are intentionally added as a metabolic nutrient to the feed of the livestock resulting in the high concentrations in

digestates. The application of such digestates as a fertilizer may result in the accumulation of the related elements and pose a risk of entering the food chain of humans (Sigurnjak et al., 2015). Even though digestion process has a sanitation effect on many pathogens in the waste, the pathogens may even survive after digestion which requires additional measures such as pasteurization or pressure sterilization (Al Seadi and Lukehurst, 2012).

Greenhouse gasses such as carbon dioxide, methane and nitrous oxide and general atmospheric pollutants such as ammonia gas can also be emitted to the atmosphere during storage or land application of the digestates (Menardo et al., 2011).

Table 2.2. General characterization of the liquid digestates (Xia and Murphy, 2016).

Parameter	Range	Parameter	Range
pH	6.7-9.2	Cobalt (Co), mg/L	0.02-0.04
Chemical oxygen demand (COD), mg/L	210-900	Copper (Cu), mg/L	0.09-21.4
Total organic carbon (TOC), mg/L	939-353	Iron (Fe), mg/L	0.9-65
Total nitrogen (TN), mg/L	139-456	Lead (Pb), mg/L	0.03-2.8
Percentage of ammonia nitrogen (TAN/TN)	65-98%	Magnesium (Mg), mg/L	3-659
Total phosphorus (TP), mg/L	7-381	Manganese (Mn), mg/L	0.1-17
Percentage of phosphate (PO_4-P/TP)	82-90%	Molybdenum (Mo), mg/L	<1.8
Aluminum (Al), mg/L	0.1-34	Nickel (Ni), mg/L	<1.4
Boron (B), mg/L	0.9-4	Potassium (K), mg/L	102-2707
Cadmium (Cd), mg/L	<1	Silicon (Si), mg/L	26-72
Calcium (Ca), mg/L	65-1044	Sodium (Na), mg/L	126-709
Chlorine (Cl), mg/L	160-438	Sulfur (S), mg/L	111-115
Chromium (Cr), mg/L	<1.2	Zinc (Zn), mg/L	0.9-13

2.1.3.3 Limitations regarding the land application

The volume of anaerobic digesters ranges between a hundred to several thousand cubic meters (de Mes et al., 2003) which already results in the large volumes of digestate production. A typical anaerobic digester in Germany with 500 kW installed capacity was previously reported to have 7,600 m^3 digestate production in a year

(Dahlin et al., 2017). The large volumes of digestate production present a challenge for the industry in terms of management, disposal or even the storage of the digestates.

The number of AD plants is increasing worldwide which is mainly related to the biogas policies of the countries. Germany, providing incentives for the farmers, increased the total capacity of the AD plants by more than 150% between the years of 2006 and 2011 which corresponded to the doubling of the number of plants (Appel et al., 2016). The total number of biogas installations had reached to 48,269,864 by the end of 2014 in five countries: China, India, Nepal, Vietnam and Bangladesh (REN21, 2016). The growth of the biogas sector is also expected to expand on occasion that many countries have declared a target of installed capacity and/or generation for biogas power (REN21, 2018). Nevertheless, the increasing number of AD plants results in the higher volumes of digestate production. The increasing volumes of digestate production also increases the requirement for the development of a feasible and cost-effective management process for the digestates. The following sections point out the limitations regarding the land application of the digestates as a widely applied way for handling of the digestates.

2.1.3.3.1 Limited available agricultural land for digestate application

Digestate is commonly used as a fertilizer on nearby agricultural land since it has high nutrient levels that can be easily metabolized by crops. Large agricultural fields should be reserved for digestate to be used as a fertilizer (García-Albacete et al., 2014). The applicability of digestates as fertilizers, thus, becomes limited with the availability of nearby agricultural lands. The presence of limited agricultural land for the produced digestate together with the large and increasing volumes of the digestates have a potential to result in the oversupply of the digestate for local scale.

2.1.3.3.2 Limitations on digestate transportation

Digestate can be transported to the agricultural fields of nutrient deficit or to the plants where it can be further processed when it is in excess of local demand. However, the water content of the digestate is approximately 90-95% (Fechter and Kraume, 2016) which may create logistic problems in the transportation of the digestate without processing. Digestate can be packed and carried over long-distances to be used as a fertilizer after getting concentrated or dried as a marketing option. Long-distance transportation may not be a cost-effective solution which may shade the value of the digestate (Xia and Murphy, 2016). Thus, the cost of transportation to be used elsewhere is probable to offset the economic value of the digestate. Marketing of the digestates may even be limited (WRAP, 2012).

2.1.3.3.3 Limitations on the management of land application procedure

Land application should be carried out depending on the type of soil, nutrient status and the need of crops (DEFRA, 2016). Each crop requires different nutrient concentrations and each digestate differ in the composition depending on the feedstock used and the operating parameters/conditions of the plants (Nkoa, 2014). The quality and the quantity of the organic matter to be applied are also to be known to maintain the soil humus balance (Gong et al., 2010). Thus, the land application of the digestates should be carried out depending on the results of various analysis and additional knowledge on both soils, crops and digestates.

Additionally, the digestates should be stabilized before applying on land to prevent further methane, carbon dioxide and ammonium emissions (Wojnowska-Baryla et al., 2018). The stability of the digestate is too much interrelated with the operational

conditions of the plants. High organic loading rate and short HRT lead to large amounts of organic matter to be left undigested (Menardo et al., 2011) which in turn ends up with an unstable digestate. If the digestate is unstable, digestate produced will not also be a good soil amendment material (Makádi et al., 2012). Therefore, the land application of the digestates also requires good management practices even during the operation of the digesters. Therefore, the land application becomes a multi-parameter task covering the information, analysis and experimental evaluations as well as the management of the digester operation.

2.1.3.3.4 Seasonal applicability on land

The digestates can be applied on land seasonally which is limited to a few months in a year. The land application during the periods of excess precipitation especially in winter months results in the pollution of water bodies. Therefore, the digestates are required to be held in a storage tank at the seasons of high precipitation. The storage tank should be sized according to the volume of the digestate produced during the entire storage period and should be capable of retaining the whole volume of the produced digestate (URL 1). The minimum storage period of the digestates is determined by regulations and may result in the requirements of large volumes for storage. The reserved area of the individual plants may not be adequate for storing the digestate for the entire storage period. The digestate produced is to be stored in other areas rather than the reserved area of the plant in such cases (Fechter and Kraume, 2016). Transportation and the associated costs of transportation can be considered as additional challenge for these plants. The storage of the digestates also bring about some necessities such as hygienization. Digestate is required to be pasteurized which means heating up the digestate to a certain temperature for a time period (Liu et al., 2017). Such an application is probable to create a financial burden for individual anaerobic digesters.

2.1.3.4 Regulatory concerns

National and international regulations that are related to the digestate management are briefly described in the following sections.

2.1.3.4.1 National regulations

The land application of the digestates has been regulated in Turkey depending on the conditions ruled by the Notification on Mechanical Separation, Bio-Drying and Bio-Methanation Plants and Fermented Product Management (Official Gazette No: 29498, date 10.10.2015). This notification restricts the application of fermentates to land in terms of the total annual nitrogen (max. 170 kg N/ha) and the dry weight (minimum 30%). Fermentates having less than 30% dry weight should be phase-separated and dried before disposal. The liquid phase of the digestate is not directly allowed to be applied on land.

The stability of the digestate is evaluated in three terms in the Notification as respirometric index, organic acids content and residual biogas potential. The respirometric index is to be less than 50 mmol O_2 /kg organic matter/hour. The organic acids content is to be lower than 1500 mg/L acetic acid equivalent. Residual biogas potential is to be less than 0.25 L/ g VS. The digestate is expected to meet at least one criterion among these three. If the residual biogas potential test is employed to decide on the stability of the digestates, the ones that have more than 0.25 L/ g VS residual biogas can be decided as unstable. The unstable digestates cannot be applied on land. The concentrations of heavy metals; cadmium, chromium, copper, mercury, nickel, lead zinc and PAH16, are also regulated under the Notification. The Notification also rules the hygienization requirement for the digestates of animal manures. These

digestates should be hygienized for 15 days at 55^{o}C or for 7 days at 60^{o}C or for 5 days at 65^{o}C or for 1 hour at 70^{o}C.

2.1.3.4.2 International regulations

Anaerobic digestion of animal wastes and digestate application on land has been regulated under Animal By-Products Regulation (EC, 2009) in the member states of European Union (EU). The interpretation of this regulation changes from country to country in the member states. Ireland is one of the countries which interpret the regulation stringently such as avoiding the use of slaughterhouse wastes in AD processes. However, the controls related to the limitations of the feedstocks and processing of the feedstocks create barriers for the development of industry. Additionally, the land applications of the digestates have a potential to be severely limited by Animal By-Products Regulation which may bring about new challenges to the industry (Smyth, 2013). Total annual nitrogen load applied to land has already been limited by Nitrates Directive (91/676/EEC) covering the member states.

European Union has also adopted a legislative proposal involving the phasing out of landfilling of bio-wastes in non-hazardous waste landfills by 2025 (EC, 2014). Since aerobically and anaerobically degradable wastes are also defined as bio-waste (EC, 2014), landfilling of digestates will no longer be a disposal method for digestate management. Thus, digestates will not be landfilled leaving the attention on the choice of the recovery and reuse of nutrients from the digestates (de Mes et al., 2003).

2.1.4 Anaerobic treatment potential of the digestates

Digestates leaving anaerobic digesters still contain considerable amounts of undigested organic matter (Gioelli et al., 2011; Menardo et al., 2011; Rico et al., 2011). The organic matter content of digestates in terms of volatile solids can be up to 70% of its total solids content (Drosg et al., 2015). Operating conditions of anaerobic digesters such as high organic loading rate and low HRT (Menardo et al., 2011; Rico et al., 2011) as well as short circuiting within digester (Angelidaki et al., 2005) and partial degradation of recalcitrant organic matters in anaerobic digesters (Thygesen et al., 2014) were pointed out as the reasons for the presence of undigested organics in digestates. The presence of undigested organics in digestates provides additional potential for further biogas production (Angelidaki et al., 2005; Gioelli et al., 2011; Thygesen et al., 2014). The studies related to RBYs of digestates conducted in batch reactors are compiled in Table 2.3. These studies involved the digestates obtained from the plants operated with different feedstocks. The total test period was very variable (21-136 days). The testing methodology was also different from each other. Some tests were conducted with the use of inoculum (Schievano et al., 2008; Thygesen et al., 2014) and some were without (Menardo et al., 2011; Rico et al., 2011). Therefore, an available standard test methodology, RBP test was preferred as a guideline to quantify the additional biogas production and the further treatment potentials of the digestates. The RBP test was developed by United Kingdom Waste and Resources Action Programme (WRAP) (WRAP, 2010) and is used to provide an evidence for an effective AD process. It was stated that it could also provide an indication of the environmental impacts arising from the use of digestates and could potentially be used to control these environmental impacts (WRAP, 2013). The RBP test is a compulsory component of British Standards Institution's Publicly-Available Specification (BSI PAS 110). The related test methodology is described under the Materials and Methods section.

Table 2.3. The residual biogas yields (RBYs) of various digestates previously studied in anaerobic batch reactors.

Feedstock of the AD process	Duration, d	Biogas Yield	Reference
Animal effluents and energy crops	70	0.021-0.082 NL/g VS	Menardo et al., 2011
Animal manure and mixture of animal manure and industrial waste and sewage sludge	60	0.156-0.240 NL CH_4 /g VS	Thygesen et al., 2014
Agricultural wastes (cattle, horse wastes silages etc.)	50	0.134 ± 0.039 NL CH_4/g VS	Ruile et al., 2015
Waste from commercial and municipal sources	28	0.032-0.381 L/ g VS	WRAP, 2013
Liquid cow manure and its mixture with maize	125–136	0.075-0.140 NL CH_4 /g VS	Seppälä et al., 2013
Liquid fraction of dairy manure	60	0.013-0.102 L CH_4 /g VS	Rico et al., 2011
Municipal organic waste	125	0.225 NL CH_4 /g VS (55ºC)	Hansen et al., 2006
Liquid and solid manures, maize and grass silage, and grain	35	0.023 ± 0.003 LCH_4/g VS (untreated) approx. 0.084 LCH_4/g VS (mechanically treated)	Lindner et al., 2015
Hay/straw mixture	35	0.229 ± 0.019 L CH_4/g VS (untreated) 0.267 ± 0.008 L CH_4/g VS (mechanically treated)	
Maize silage	35	0.291 ± 0.028 L CH_4/g VS (untreated) 0.318 ± 0.019 L CH_4/g VS (mechanically treated)	
Animal manures, maize and sorghum silages and olive waste	65	0.070-0.090 NL CH_4 /g VS (untreated) 0.057-0.079 NL CH_4 /g VS (thermal treatment) 0.102-0.106 NL CH_4 /g VS (enzyme treatment) 0.042-0.081 NL CH_4 /g VS (alkali treatment)	Sambusiti et al., 2015
Solid fractions of digestate, composted and dried solid fraction of digestate from 14 plants	40	<0.0005-0.094 NL CH_4 /g VS	Maynaud et al., 2017
A mixture of energetic crops, pig manure slurry, agro-industrial waste and organic fraction of municipal solid waste	60	0.066-0.437 NL/g TS	Schievano et al., 2008
Multi-component agri-food	120	0.051-0.115 L/g TS (20ºC)	Wojnowska-Baryla et al., 2018
Domestic wastewater treatment plant sludge	24	0.048 L CH_4 /g VS (unprocessed) 0.088 L CH_4 /g VS (ultrasonicated)	Garoma and Pappaterra, 2018

2.2 Materials and Methods

2.2.1 Digestate sampling and characterization

The digestate samples were collected from six anaerobic digesters operated under the conditions given in Table 2.4. The samples were preserved at 4^{o}C before use and characterized for pH, total solids (TS), volatile solids (VS), COD_t, soluble chemical oxygen demand (COD_s), total kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4^+-N), total phosphorus (TP), dissolved reactive phosphorus (DRP), intermediate alkalinity (IA), partial alkalinity (PA) and total alkalinity (TA) concentrations (Table 2.5).

Table 2.4. The capacities and operating conditions of the anaerobic digesters.

Anaerobic Digester	Raw Feedstock	HRT, d	Digestate production, tons/d	Installed capacity
1	Beef cattle manure	30	278-288	1.4 MW
2	90% laying hen and 10% cattle manure	48-50	83	1.8 MW
3	40 % dairy cattle manure, 5% chicken manure, 15% organic vegetable waste, 40% recycled digestate	28	550	6.4 MWh
4	60% dairy cattle manure, 20% laying hen manure and 20% organic waste mixture (orange pulp, grain silage etc.)	38-40	450	4x1067 kWh (4268 kWh)
5	67% dairy cattle manure and 33% laying hen manure	44	83	330 kWh
6	56% primary and 44% secondary sewage sludge	14-21	3197-3996	8x1.5 MW (12 MW)

2.2.2 Anaerobic Inoculum

Anaerobic inoculum was collected from the inside of an anaerobic digester located at the same plant of the anaerobic digester 6. The digester of anaerobic inoculum sampling was operated under the same conditions of anaerobic digester 6 (Table 2.4). Anaerobic inoculum was sieved through 1mm pore-sized screen before use and characterized for the constituents given in Table 2.5.

Table 2.5. The initial characterization of the digestates and inoculum.

Constituent	The digestate of anaerobic digester						Inoculum
	1	2	3	4	5	6	I
pH	8.78	8.50	8.72	8.87	8.60	8.36	8.71
TS, mg/L	105,250±1152	53,263±593	59,053±321	74,830±255	49,920±524	18,773±176	14,107±255
VS, mg/L	72,630±1139	25,970±260	38,013±213	50,953±82	28,180±425	9,837±160	7,330±264
Solid content, %	10.2	5.1	6.0	7.4	4.9	1.9	1.4
Density, kg/m^3	1,028±4.1	1,035±9.3	989±8.0	1,014±1.5	1,010±4.8	999±4.0	1,005±5.0
COD_t, mg/L	111,056±6992	39,010±913	57,334±2635	76,675±4025	43,751±488	21,079±798	12,893±540
COD_s, mg O_2/L	14,007±941	14,809±761	3,642±647	10,969±805	11,310±5417	656±51	548±16
TKN, mg/L	3,694±35.3	8,394±283.6	2,285±37.9	5,147±100.5	4,815±469.7	1,051±21.7	1,274±197.3
NH_4^+ - N, mg/L	3,288±24.9	7,703±41.8	1,782±11.7	4,569±66.8	4,071±17.8	826±14.9	892±6.0
TP, mg/L	2,314±5.8	2,786±0.0	1,340±0.0	1,555±0.0	1,725±0.0	352±5.1	409±0.0
DRP, mg/L	1,156.1±3.74	1,097.9±3.74	289.7±0.00	465.6±3.74	548.9±1.87	33.7±0.04	27.2±0.04
TA, mg/L as $CaCO_3$	15,555	30,529	9,740	18,012	16,573	3,620	3,227
IA/PA	0.12	0.11	0.13	0.12	0.09	0.06	0.07
COD:TKN	30:1	5:1	25:1	15:1	9:1	20:1	10:1
COD:TP	48:1	14:1	43:1	49:1	25:1	60:1	32:1

2.2.3 Experimental setup

RBP test was conducted in glass reactors with 400 mL effective and 200 mL empty volume. All reactors were set up in triplicates. Each reactor was only fed at the beginning of the experiment and operated in batch for 70 days. The reactors containing digestates from all six digesters (Table 2.4) were named R1-R6. The amount of inoculum and digestate added into reactors were based on their VS concentrations. The inoculum to substrate (i/s) ratio (g $VS_{inoculum}$ /g $VS_{digestate}$) of 4 was used in all digestate containing reactors (WRAP, 2010). The quantities of inoculum and digestate used were calculated using equations 1, 2 and 3 for a total test volume of 400 mL (WRAP, 2010).

$$\text{Digestate added, g} = \frac{400}{1+(\frac{R * VS_{digestate}}{VS_{inoculum}})} \quad [2.1]$$

$$R = \frac{g\ VS\ of\ inoculum}{g\ VS\ of\ digestate} \quad \text{where R=4} \quad [2.2]$$

$$\text{Inoculum added, g} = 400 - \text{digestate added} \quad [2.3]$$

Cellulose (Merck microcrystalline cellulose for thin-layer chromatography) containing reactors (C) were also set up as positive controls with an i/s ratio of 6 to test the activity of the inoculum (WRAP, 2010). The amount of cellulose and inoculum used as positive controls were calculated using the following formula (WRAP, 2010):

$$\text{Cellulose added, g} = \frac{400}{1+(\frac{R * VS_{cellulose}}{VS_{inoculum}})} \quad [2.4]$$

$$R = \frac{g\ VS\ of\ inoculum}{g\ VS\ of\ cellulose} \quad \text{where R=6} \quad [2.5]$$

$$\text{Inoculum added, g} = 400 - \text{cellulose added, g} \quad [2.6]$$

RBP test consisted of two sets of reactors to observe the effect of nutrient supplementation. The first set was supplemented with nutrients while the second set was not. The two sets were identical except nutrient supplementation. The composition of the nutrient solution is given in Table 2.6. Each set included inoculum-only reactors (I) which were set to observe and to exclude the contribution of inoculum in residual biogas production from digestate and cellulose containing reactors (WRAP, 2010).

Table 2.6. Nutrient medium used in RBP test (modified from WRAP, 2010).

Major elements (10 mL added)	Concentration, g/L
KH_2PO_4	13.2
NH_4Cl	13.5
$CaCl_2.2H_2O$	1.88
$MgCl_2.6H_2O$	2.5
Trace elements (1 mL added)	Concentration, mg/L
$FeCl_2.4H_2O$	50
H_2BO_3	1.25
$ZnCl_2$	12
$CuCl_2.2H_2O$	1.7
$MnCl_2.4H_2O$	160
$(NH_4)_6Mo_7O_{24}.4H_2O$	2.5
$AlCl_3.6H_2O$	2.5
$CoCl_2.6H_2O$	5.0

After all the components were added into the reactors, the reactors were capped with rubber stoppers and the headspace of the reactors was flushed with pure nitrogen for 3 minutes to achieve anaerobic conditions. The reactors were then placed on a rotary shaker and kept at 50 rpm in a constant temperature room at 35±1^{o}C. Biogas production was measured by a water displacement device.

2.2.4 The methodology of the treatment measurements and calculations

Anaerobic treatability of digestates was evaluated in terms of TS, VS, COD_t, TKN, NH_4^+-N, TP and DRP. To this purpose, the initial concentrations of the relevant constituents (Table 2.5) of the digestates and inoculum as well as the final concentrations at the end of reactor operation were determined. One reactor from each triplicate was randomly selected for the analysis of the relevant constituent for the final characterization of the reactors.

All triplicates of R2 both with and without nutrient supplementation were also characterized for the relevant constituents. The data obtained for R2 reactors were subjected to statistical analysis using Minitab 17 software by normal and t-test distribution tools. The relevant results obtained are given in Appendix B. The statistical analysis indicated that the results were representative within the 95% confidence interval. Therefore, random selection of one reactor over the triplicates ensured the reliability and representability of the data.

The concentrations of all constituents at the beginning and at the end of the experiment were determined for inoculum-only reactors in order to assess self-removal due to inoculum activity. The initial concentrations in digestate containing reactors were calculated based on the volumes and the constituent concentrations of the digestates, inoculum and nutrient medium (Appendix C). The final concentrations of the constituents were measured at the end of the 70-days of operation. Final characterization of the constituents in the inoculum-only reactors showed that large fractions still remained at the end of 70 days. Additionally, the volumes of inoculum added into the reactors were comparably higher than the digestates in digestate

containing reactors. The final concentrations of the constituents measured at the end of 70 days of operation in the digestate containing reactors were the summation of the retained amounts after the activity of the inoculum itself and the non-degraded portion of the digestate. Therefore, the removal of the constituents was calculated for both the overall reactor content and for only from the content of the digestates. The removal calculations of the constituents from digestates were calculated by excluding the retained amounts corresponding to the inoculum (Appendix C). This estimation was done based on the specific removal of the constituents in inoculum-only reactors which represented the amount of removed constituent per unit volume of the inoculum. The removed amount of the constituents due to inoculum activity was calculated by multiplying the specific removal of the constituents with the volume of inoculum used in the digestate containing reactors. The retained amounts due to inoculum addition were found from the difference between the overall constituent amount in the inoculum added and the removed amount of the constituent due to inoculum activity. When the retained amounts of the constituents were subtracted from the overall reactor content, the final concentration of the digestates could be obtained. Thus, the retained amounts of the constituents corresponding to the inoculum in digestate containing reactors could be excluded (Juncà, 2010) in order to estimate the actual removal amounts of the constituents from digestates. The formula derived for the exclusion of the retained amounts corresponding to the inoculum and the related calculations are presented in Appendix C.

The interference of chloride ions in COD measurement was also tested to eliminate the probability of the effect of chlorides on large COD concentrations of the digestates (Table 2.5). It was found that chloride ions did not interfere with the COD measurements (Appendix D).

2.2.5 Analytical Methods

Standard Methods (APHA, 2005) were used to determine TS, VS, COD_t, TKN, NH_4^+-N, TP and DRP concentrations. COD_s was measured photometrically as described in the manual provided by the manufacturer (Aqualytic, 2014). The samples were first filtered from 0.45 μm pore-sized filters for COD_s and DRP determination. The digestion of the samples before TP analysis was performed based on the procedure described in International Organization for Standardization BS EN ISO 15587-1:2002 (ISO, 2002). PA is representative of the bicarbonate alkalinity and measured to the pH of 5.75. IA is measured to the pH of 4.3 as described by Ripley et al. (1986). pH was measured with Oakton PC 450 portable pH meter.

2.3 Results and Discussion

2.3.1 Digestate characterization

The solids content of the digestates showed high variability in the range of 1.9-10.2 % at the initial characterization (Table 2.5). The digestates of anaerobic digesters 1-5 were in between 4.9 and 10.2 % which were in agreement with the ones previously reported for the digestates of the mixtures of animal manures and energy crops (3.7-9.6%) (Menardo et al., 2011). The digestate of anaerobic digester 6 operated with sewage sludge had the lowest solid content (1.9 %) compared to that of obtained from anaerobic digesters operated with animal manures at high proportions.

The ammonification ratio (NH_4^+-N/TKN) was 0.78-0.92 for digestates of anaerobic digesters 1-6. High ammonification indicated the high ammonium concentrations in the composition of the digestates. The digestate of anaerobic digester 2 had the highest

ammonium and TKN concentrations (7,703±41.8 and 8,394±283.6 mg/L, respectively) which could be attributed to feedstock composition having 90 % of laying hen manure. Poultry manures are characterized as having high NH_4^+-N/TN ratios (Möller and Müller, 2012).

COD_s/COD_t ratio of digestates of anaerobic digesters 1-5 were 12.6, 38.0, 6.4, 14.3 and 25.9, respectively. The soluble COD content of total COD was within the range of previously reported ones for the digestates of commercial and municipal wastes (6.8-53.6%) (WRAP, 2013). The lowest ratio of COD_s/COD_t was observed in the digestate of anaerobic digester 6 (3.1%) was probably due to operation of the digester with sewage sludges.

Total phosphorus contents of the digestates ranged between 0.4-2.7 g/Mg fresh matter which were also found to be comparable to that of previously reported for various digestates as 0.4-2.6 g/Mg fresh matter (Möller and Müller, 2012).

The alkalinity ratio of intermediate to partial alkalinity was in the range of 0.06-0.13 and found to be lower than 0.3 for all digestates. Being lower than 0.3, IA/PA ratios indicated the operational stability of the sampling digesters (Alcaraz-Gonzalez et al., 2015). IA/PA is an indicator of VFA accumulation within the reactor which is encountered before pH drop and the failure of the reactor (Monhonval, 2015).

2.3.2 Suitability of the inoculum

The RBYs of the cellulose positive controls with and without nutrient supplementation were found as 0.496±0.125 L_{biogas}/g VS and 0.631±0.005 L_{biogas}/g VS, respectively, at

the end of 28th day of operation. WRAP (2013) stipulated a minimum biogas yield for cellulose positive control reactors as 0.5 L_{biogas}/ g VS using i/s ratio of 6 on VS basis as applied in this study. Therefore, inoculum used in this study was found to be active to utilize cellulose. The suitability of the inoculum and the efficacy of the test procedures are to be controlled by determination of the RBP of a standard reference material such as cellulose using the same inoculum employed in the test (WRAP, 2010).

2.3.3 Eliminated data in calculation of residual biogas yields

One R1 and R2 reactor with nutrient supplementation had at least 5 days of negative biogas production period (Appendix E). The data obtained from these reactors were excluded from the calculations due to potential inhibition of the inoculum (WRAP, 2010). Additionally, RBP graphs of one R6 with nutrient supplementation and one R5 without nutrient supplementation had spikes (Appendix F) possibly due to a leakage problem. The data obtained from these two reactors were also eliminated from the RBP calculations as suggested by WRAP (2010).

2.3.4 Residual biogas production from digestates

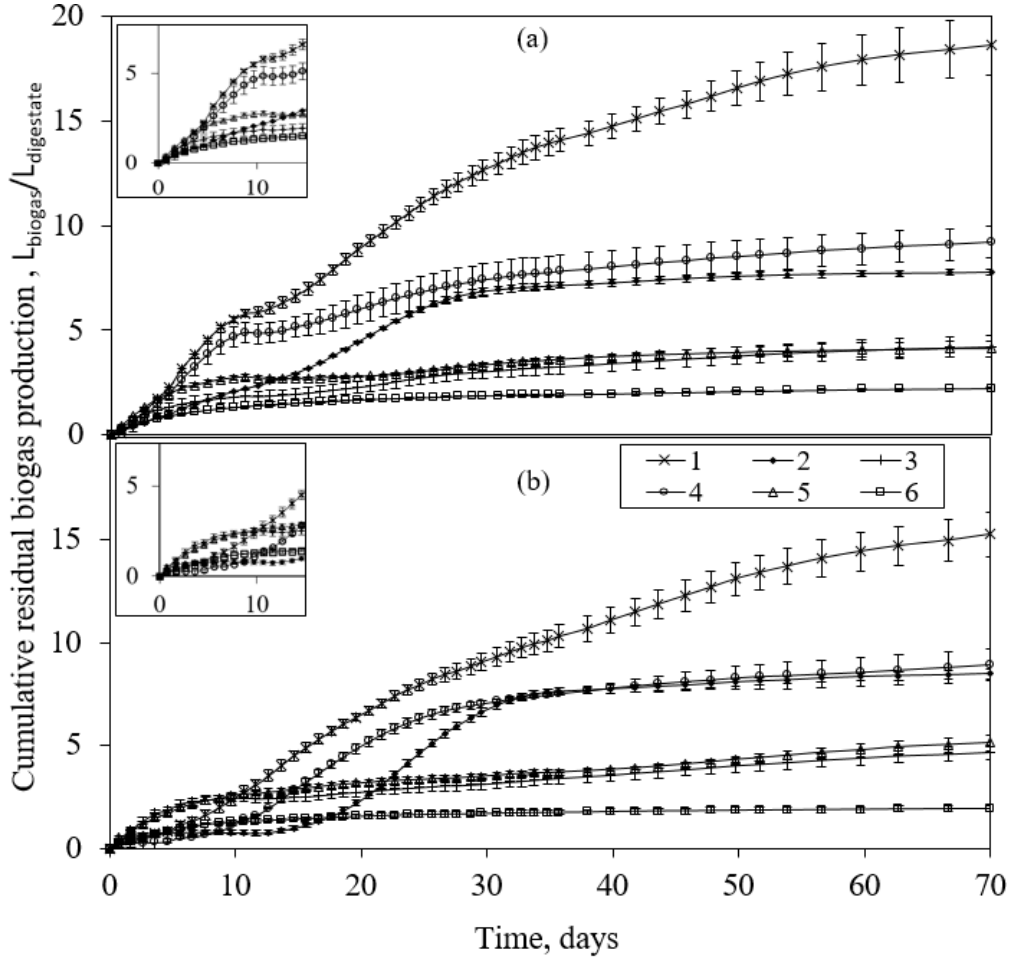
Biogas production was evaluated in terms of total biogas volume produced per unit volume of digestate at the end of the total test period of 70 days (Table 2.7 and Figure 2.3). 2.23-18.62 and 1.91-15.26 $L_{biogas}/L_{digestate}$ were produced with and without nutrient supplementation, respectively, for the digestates of anaerobic digester 1-6. The digestate of anaerobic digester 1 which was operated with beef cattle manure had the highest production with and without nutrient supplementation. This digestate was characterized as having the highest TS, VS, COD_t and DRP concentrations (Table

2.5). The lowest volume of biogas per volume of digestate was recorded for the digestate of anaerobic digester 6 (1.91 ± 0.13 $L_{biogas}/L_{digestate}$ with and 2.23 ± 0.01 $L_{biogas}/L_{digestate}$ without nutrient supplementation). This digestate had the lowest VS and COD_t concentrations. On the other hand, the digestate of anaerobic digester 2 and 4 had approximate residual biogas production (8.48 ± 0.23 and 8.92 ± 0.78 $L_{biogas}/L_{digestate}$, respectively) even if the VS and COD_t concentrations of the former were almost the half of the latter (Table 2.5). Thus, residual biogas production could not be correlated with the VS and COD_t concentrations of the digestates. This fact can be attributed to variable biodegradability of the digestates. The biodegradability of the digestates and the effect of nutrient addition were comprehensively evaluated in the following section considering the biogas production per unit volatile solids added, so-called biogas yield, as applied by WRAP (2013). Such evaluations are mostly based on the biogas yields not the productions since the economic feasibility of AD plants is linked to biogas yields in general (Romero-Güiza et al., 2016b).

Table 2.7. Total biogas production per unit volume of digestate ($L_{biogas}/L_{digestate}$) at the end of 70 days.

Digestate of anaerobic digester	Biogas production, $L_{biogas}/L_{digestate}$	
	with nutrient supplementation	without nutrient supplementation
1	18.62 ± 1.40	15.26 ± 1.08
2	7.77 ± 0.13	8.48 ± 0.23
3	4.23 ± 0.50	4.67 ± 0.34
4	9.21 ± 0.73	8.92 ± 0.78
5	4.12 ± 0.37	5.11 ± 0.36
6	2.23 ± 0.01	1.91 ± 0.13

Note: Refer also Table 2.5 for the relevant characteristics of the digestates.



Note: Refer also Table 2.5 for the relevant characteristics of the digestates.

Figure 2.3. Biogas production from digestates (a) with (b) without nutrient supplementation per unit volume of digestate ($L_{biogas}/L_{digestate}$).

2.3.5 Residual biogas yields of digestates related to volatile solids content of digestates

RBYs with respect to the VS concentration of the digestates (calculated as described by WRAP, 2010) were evaluated for the 28th and 70th day of operation (Table 2.8). 28 day is the indicated test period by WRAP (2013). The biogas yields of digestates were observed to be variable and ranged between 0.078-0.257 and 0.081-0.234 L_{biogas}/g VS

with and without nutrient supplementation, respectively, at the 28th day of operation. The RBYs increased by further incubation for 42 days, as expected, and reached to 0.111-0.299 and 0.123-0.326 L_{biogas}/g VS with and without nutrient supplementation, respectively. The RBYs of the digestates were found to be comparable to the ones obtained in other studies as complied in Table 2.3.

Table 2.8. The residual biogas yields with respect to volatile solids concentration.

Digestate of anaerobic digester	28th day			70th day		
	RBY, L_{biogas}/g VS		% RBY increase[b]	RBY, L_{biogas}/g VS		% RBY increase
	with n.s.[a]	without n.s.		with n.s.	without n.s.	
1	0.168 ± 0.007	0.120 ± 0.005	40	0.256 ± 0.024	0.210 ± 0.015	22
2	0.257 ± 0.007	0.234 ± 0.007	10	0.299 ± 0.005	0.326 ± 0.009	-8
3	0.078 ± 0.008	0.081 ± 0.007	-4[c]	0.111 ± 0.013	0.123 ± 0.009	-10
4	0.142 ± 0.015	0.134 ± 0.004	6	0.181 ± 0.014	0.175 ± 0.015	3
5	0.115 ± 0.003	0.122 ± 0.005	-6	0.146 ± 0.013	0.181 ± 0.013	-19
6	0.188 ± 0.001	0.169 ± 0.012	11	0.227 ± 0.001	0.195 ± 0.014	16

[a] n.s.: nutrient supplementation.
[b] % RBY increase indicates the increase in biogas yields of digestates when nutrients were added with respect to the RBYs of the digestates without nutrient supplementation.
[c] Minus sign indicates the decrease in RBYs of digestates when nutrients were added.

2.3.5.1 Residual biogas yields without nutrient supplementation

Technical digestion time of 90%, that is the time at which 90% of the total biogas yield was recorded, was observed on the 53.8, 35.7, 56.6, 43.6, 56.6 and 33.7th day of operation in the RBP test for the digestates of anaerobic digesters 1-6, respectively. The time required to obtain 90% of the biogas yield from five different animal manures (dairy, horse, goat, chicken and swine manures) was found to range between 17-44 days in a biochemical methane potential (BMP) test (Kafle and Chen, 2016). The time required to further digest the digestates of animal manures (digestates obtained from anaerobic digesters 1-5) were comparably longer than the digestion of raw feedstocks, as expected.

The biogas yield of the digestate of anaerobic digester 1 operated with beef cattle manure was moderate (0.210 ± 0.015 L_{biogas}/g VS) at the end of the 70 days. The digestate did not have the highest biogas yield, in spite of having considerably higher COD_t and VS concentrations in its initial characterization (Table 2.5) and the high biogas production per unit volume of the digestate (Table 2.7). Additionally, 42 days more incubation yielded 75 % more biogas compared to 28 days of incubation. Moreover, the RBP curve of this digestate (Figure 2.4-b) did not plateaued at the end of the 70th day. These facts suggested the presence of slowly degradable materials at high proportion in the digestate of anaerobic digester 1. The slow degradation of this digestate can be attributed to the presence of rigid lignocellulosic fibers materials in cattle manures (Langone et al., 2018) which have low biodegradability (Nasir et al., 2012).

The digestate of anaerobic digester 2 operated with 90 % laying hen manure had the highest COD_s, NH_4^+-N, DRP and considerable total alkalinity and DRP concentrations (Table 2.5). The RBP curve without nutrient supplementation (Figure 2.4-b) indicated a stationary phase between the days of 4-13 for this digestate. Despite the stationary biogas production period of approximately 9 days, the digestate reached to the highest yield both at the 28th day (0.234 ± 0.007 L_{biogas}/g VS) and 70th day (0.326 ± 0.009 L_{biogas}/g VS). The biogas yield increased by 39 % at the end of 70 days compared to the yield of 28th day of operation. The digestate was evaluated as a moderately biodegradable one depending on the time required to obtain 90 % of the biogas yield (35.7 days).

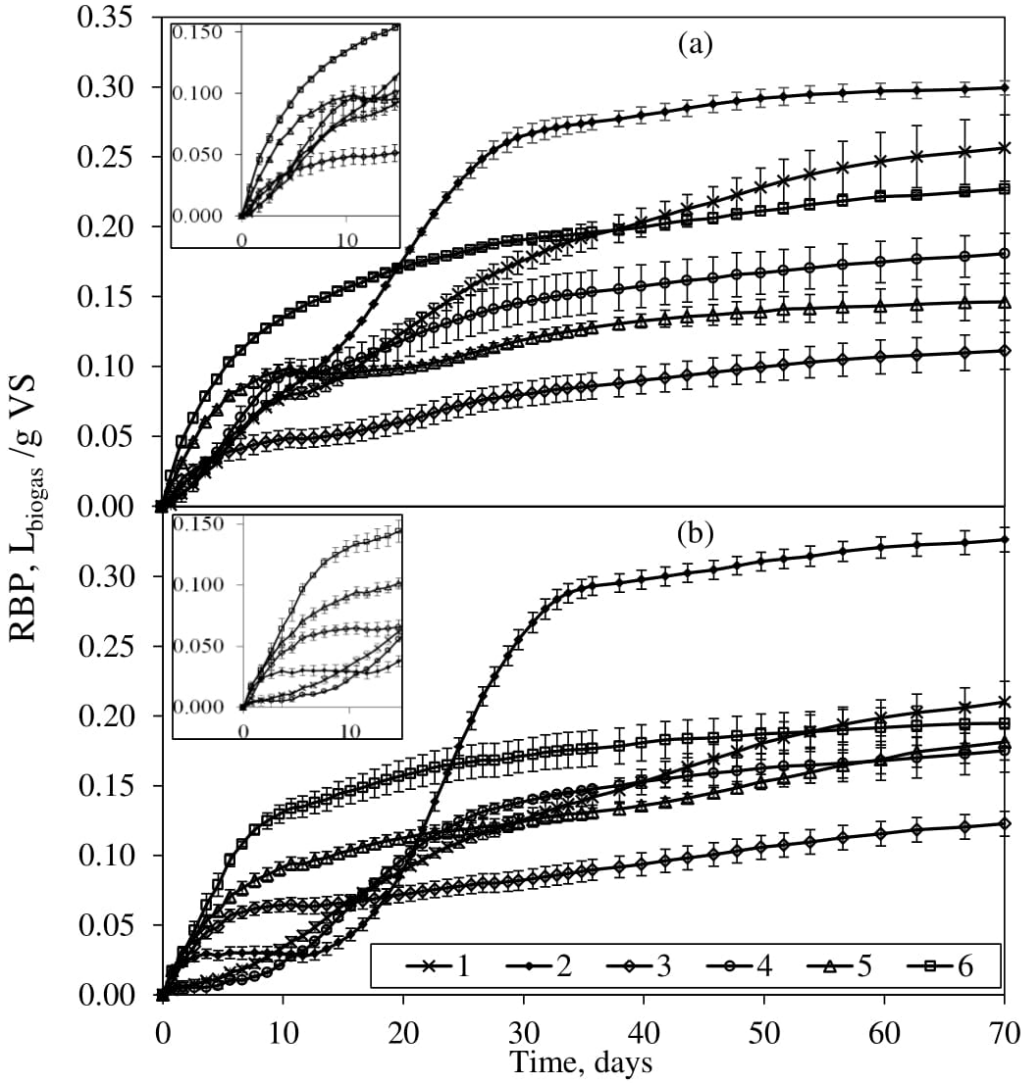


Figure 2.4. RBP curve for the digestates (a) with (b) without nutrient supplementation.

The lowest biogas yields for short-run and long-run operation was obtained from the digestate of anaerobic digester 3 (0.081 ± 0.007 and 0.123 ± 0.009 L_{biogas}/g VS, respectively). The increased concentration of recalcitrant materials in the digestate as a result of recycling of the digestate within the plant (by 40% proportion) (Table 2.4) can be a reason for the low biogas yields obtained from this digestate. Recalcitrant VS may be recycled back into the digester and may lead to the accumulation of the recalcitrant materials in the digester (Estevez et al., 2014). Therefore, it is probable to

observe low residual methane production as a result of digestate recycling as previously noted by Nges et al. (2015). The recalcitrance of the digestate could possibly avoided a better degradation performance even if COD:TKN ratio represented an optimum value (25:1) for anaerobic degradation. Even though a carbon to nitrogen (C:N) ratio between 20:1 and 30:1 was previously reported as an optimum ratio for anaerobic degradation (Zhang et al., 2008), it may not represent the bioavailable or biodegradable fractions (Puyuelo et al., 2011) as in the case of the digestate of anaerobic digester 3.

The digestates of anaerobic digesters 4 and 5 had biogas yields of 0.175 ± 0.015 and 0.181 ± 0.013 L_{biogas}/g VS, respectively, at the end of 70 days. The proximity of the yields may be due to slightly alike compositions of feedstocks used in both digesters and similar HRTs of the plants compared to the other digesters (Table 2.4). The yields obtained at the end of 70 days were comparably lower than the other digestates which is probably due to the composition of the raw feedstock dominated by dairy manure. Longer periods were required to obtain 90 % of the total biogas yield for the digestates of anaerobic digesters 4 and 5 (43.6 and 56.6 days, respectively) when compared to the ones of the digestates of anaerobic digesters 2 and 6 (35.7 and 33.7 days, respectively). This fact indicated the presence of slowly degradable material in the digestates of anaerobic digesters 4 and 5.

The digestate of anaerobic digester 6 had the second highest RBY (0.169 ± 0.012 L_{biogas}/g VS) at the end of 28 days even though it had considerably low concentrations of VS, COD_t and COD_s compared to the other digestates at the initial characterization (Table 2.5). The time required to obtain 90 % of the biogas yield was the least of all digestates (33.7 days) which indicated better biodegradability of this digestate compared to the others. Higher biodegradability of this digestate was probably due to raw feedstock of the digester composed of sewage sludges which is not a feedstock

containing animal manures or mixtures. The biogas yield for sewage sludge (0.310-0.740 L/ g VS) was previously reported to be higher than the ones for animal manures such as pig (0.340- 0.550 L/ g VS), sheep and cow manure (0.090-0.310 L/ kg VS) for comparably shorter operation periods of 10 to 20 days (ISAT and GTZ, 1998).

The biogas yields of the digestates were also comparable with the biogas yields of many raw feedstocks. These raw feedstocks are grey waste (0.08-0.15 L_{biogas}/g VS), dairy manure (0.076-0.470 L_{biogas}/g VS), cattle manure (0.15-0.35 L_{biogas}/g VS), horse manure (0.222 L_{biogas}/g VS), municipal secondary sludge (0.20-0.35 L_{biogas}/g VS), sheep excreta (0.3-0.4 L_{biogas}/g VS) and vegetable wastes (0.3-0.4 L_{biogas}/g VS) (Demirer and Chen, 2005a; Braun, 2007; Zupančič and Grilc, 2012; Kafle and Chen, 2016).

2.3.5.2 Effects of nutrient supplementation

The effects of nutrient supplementation on biogas yields of digestates were evaluated for the operation period of 28 days and 70 days. Nutrient supplementation increased the RBYs of the digestates of anaerobic digesters 1, 2, 4 and 6 by 40, 10, 6, and 11%, respectively, in short-run operation (Table 2.8). The digestates of anaerobic digesters 3 and 5 were negatively affected from the addition of nutrients resulting in a decrease of RBYs but at a lower proportion (4-6 %) compared to that of positively affected digestates (6-40%) at the end of 28 days.

The average daily biogas yields of the digestates were also compared for the period of 0-14 days of operation for the cases of with and without nutrient supplementation. The digestates of anaerobic digesters 1-6 had an average daily biogas yields of 6.3, 7.7, 3.6, 7.0, 6.8 and 10.8 mL_{biogas}/g VS.d with nutrient supplementation and 4.1, 2.5, 4.6,

3.6, 7.1 and 10.1 mL_{biogas}/g VS.d without nutrient supplementation, respectively. The digestates that are positively affected from the addition of nutrients for 28 days of operation (the digestates of anaerobic digesters 1, 2, 4 and 6) were also observed to have higher average daily biogas yields when nutrients were supplemented in the first 14 days of operation. Moreover, the addition of nutrients to the digestate of anaerobic digester 2 eliminated the stationary phase observed in the residual biogas production which lasted for approximately 9 days between the 4^{th} and 13^{th} day of operation (Figure 2.4-a and b).

When the operation period was further extended to 70 days, the nutrient supplementation had comparably different results (Table 2.8). The digestate of anaerobic digester 2, which yielded 10 % more biogas by nutrient supplementation in short-run, had 8% less yield in long-run operation compared to the case of without nutrient supplementation. Nutrient supplementation decreased the RBYs of the digestates of 3 and 5 at a higher proportion (10 and 19 %) at the 70^{th} day of operation than that of 28^{th} day of operation (4 and 6 %, respectively). The digestates of anaerobic digesters 2, 3 and 5 (the ones negatively affected from nutrient addition in long-run) had a COD:TP ratio in the range of 14:1-43:1 (Table 2.5). The high phosphorus concentration relative to the COD concentration (lower COD:TP ratios) and the addition of phosphorus via nutrient medium were probable to have a cumulative inhibitory effect when COD:TP was in the range of 14:1-43:1. High concentrations of phosphorus create phosphorus inhibition on methanogenesis causing a decrease in the production of biogas as previously noted by Mancipe-Jiménez et al. (2017). The digestates of anaerobic digesters 1, 4 and 6 yielded 22, 3, 16% more biogas at the end of 70^{th} day, respectively. The increase in the RBYs due to the addition of nutrients to the digestates of anaerobic digesters 1 and 4 was observed to be lower at the 70^{th} day of operation (22 and 3 %, respectively) compared to the 28^{th} day of operation (40 and 6 %, respectively). The only digestate having higher increase in RBYs at the 70^{th} day of operation as an effect of nutrient supplementation was the digestate of anaerobic

digester 6. This fact was probably due to the distinct raw feedstock composition (sewage sludges) compared to the other digestates containing animal manures.

As a result of the analysis and discussions on the effect of nutrient supplementation, nutrient supplementation was found to be more effective for comparably shorter operation periods. Therefore, nutrient supplementation is recommended for the RBP tests of less than 28 days. Consequently, resulting in the release of more biogas within a relatively shorter time period, the addition of nutrients has a potential to decrease the HRT of a full-scale plant installed with the aim of capturing the residual biogas from digestates.

2.3.5.3 Regulatory evaluation on the RBYs of digestates

British Standard Institute (BSI) had set an RBP limit of 0.45 L_{biogas}/g VS in 2014 by PAS 110 to decide on the stability of the digestate (PAS110:2014). Even though the RBYs of the digestates (Table 2.8) tested did not exceed the limit of PAS110:2014, the digestates were regarded as having a significant potential for biogas capture depending on the comparable biogas yields of digestates to many raw feedstocks used in AD plants.

The digestates having a maximum RBY of 0.25 L/g VS can be considered as stable according to the Notification on Mechanical Separation, Bio-Drying and Bio-Methanation Plants and Fermented Product Management (Official Gazette No: 29498, date 10.10.2015) in Turkey. The only digestate missing the stability criterion is the digestate of anaerobic digester 2, thus the land application would not be allowed before its stabilization.

2.3.6 Residual biogas yields related to COD_t removed from the digestates

RBYs were additionally evaluated with respect to the removed COD_t (COD_r) from the digestates at the end of the total operation period (Table 2.9). The digestate of anaerobic digester 1 and 6 had very close biogas yields in terms of COD_t removed from each digestate (0.203 ± 0.032 and 0.194 ± 0.023 m^3 biogas/kg COD_t removed, respectively). The raw feedstock composition and the operating conditions of the digesters were completely different from each other (Table 2.4). Therefore, no clear reason could be identified for the almost identical biogas yields with respect to the COD_t removal.

The digestate of anaerobic digester 2 had 0.420 ± 0.154 and 0.466 ± 0.171 m^3 biogas/kg COD_t removed from digestate with and without nutrient supplementation. These yields were comparably higher than the yields of other digestates and observed to be close to the theoretical biogas production of 0.5 m^3 per kg COD_r as previously given by Jingura and Kamusoko (2017).

The biogas yields of the digestates of anaerobic digesters 3, 4 and 5 were obtained as 0.308 ± 0.079, 0.262 ± 0.078 and 0.166 ± 0.118 m^3 biogas/kg COD_r. The decreasing biogas yields of these digestates with respect to the COD_r can be attributed to the increasing contents of dairy cattle manures in raw feedstock compositions (by 40, 60, 67 %, respectively). The increase of share of dairy cattle manure is probable to increase the amount of lignocellulosic materials in the digestate. High lignin content of the raw feedstock may result in low biogas yields as well as low biodegradation rate (Wang et al., 2017).

Table 2.9. The residual biogas yields with respect to COD_r concentrations.

Digestate of anaerobic digester	Residual biogas yields, m^3 biogas/kg COD_t removed from digestate	
	with nutrient supplementation	without nutrient supplementation
1	0.237 ± 0.041	0.203 ± 0.032
2	0.420 ± 0.154	0.466 ± 0.171
3	0.279 ± 0.076	0.308 ± 0.079
4	0.270 ± 0.079	0.262 ± 0.078
5	0.201 ± 0.018	0.166 ± 0.118
6	0.216 ± 0.025	0.194 ± 0.023

2.3.7 Anaerobic treatability of digestates

Anaerobic treatability of the digestates was evaluated in terms of COD_t, TS, VS, TKN, NH_4^+-N, TP and DRP at the end of the 70-day of operation. pH and alkalinity measurement were done to control the operational stability of the anaerobic digestion process.

2.3.7.1 pH and alkalinity

The initial pHs of the digestates and inoculum were in the range of 8.36-8.87 (Table 2.5). The final pH values in the reactors after AD were observed as 7.97-8.22 and 8.00-8.24 with and without nutrient supplementation, respectively. Anaerobic processes can tolerate to the pHs of 6.5-8.0 (Cioabla et al., 2012) and the measured pHs were close to the tolerable range.

The overall alkalinity in the reactors were increased by 13-18 % and 15-20 % with and without nutrient supplementation, respectively (Table 2.10). Alkalinity is produced with the consumption of hydrogen ions during methanogenesis (Acharya et al., 2008). When the initial and final alkalinities of the digestates were calculated, it

was found that all digestates except the one including the digestate of anaerobic digester 3 built up additional alkalinity (Table 2.10). Alkalinity was rather removed from the digestate of anaerobic digester 3 which was a sign of its acidification. This was probably due to the recalcitrance of the digestate which was discussed in detail in the following sections.

2.3.7.2 The changes in total chemical oxygen demand concentrations

The reactors containing only inoculum had 12-16 and 15-17% COD_t removal which corresponded to 10775±225 and 10850±150 mg/L of retained COD_t with and without nutrient supplementation, respectively (Table 2.11). The digestate containing reactors (R1-R6) had 16-27 and 17-24 % overall COD_t removal efficiency with and without nutrient supplementation, respectively. Higher removal efficiencies compared to the inoculum-only reactors and the corresponding removal amounts of COD_t (Table 2.11) indicated the degradation of the digestates within the reactors, as expected.

Table 2.10. The change in alkalinity concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	Alkalinity concentration in the reactor, mg/L as $CaCO_3$			Alkalinity accumulation in the reactor, %	The digestate of anaerobic digester	Alkalinity concentration of the digestate, mg/L as $CaCO_3$			Alkalinity accumulation of digestates, %
		Initial	Final	Accumulation			Initial	Final	Accumulation	
		Calculated	Measured	Calculated	Calculated		Measured	Calculated	Calculated	Calculated
with	I	3141	3693	552	18					
	R1	3437	3983	546	16	1	15555	15892	337	2
	R2	4891	5505	614	13	2	30529	32048	1519	5
	R3	3432	3954	522	15	3	9740	9629	-111	-1
	R4	3642	4187	545	15	4	18012	18359	347	2
	R5	3933	4448	515	13	5	16573	16516	-57	0
	R6	3200	3722	522	16	6	3620	3971	351	10
without	I	3227	3867	640	20					
	R1	3532	4187	655	19	1	15555	16812	1257	8
	R2	5027	5805	778	15	2	30529	33260	2731	9
	R3	3526	4100	574	16	3	9740	8934	-806	-8
	R4	3740	4390	650	17	4	18012	18946	934	5
	R5	4042	4681	639	16	5	16573	17200	627	4
	R6	3289	3838	549	17	6	3620	3682	62	2

*The alkalinity measurements were done once, not replicated.

The efficiency of COD_t removal without nutrient supplementation was the highest for the digestate of anaerobic digester 1 and 2 (57-64 and 37-60 %, respectively). The corresponding removed COD_t concentrations were 67144 ± 4005 and 18955 ± 3463 mg/L. These two digestates were initially characterized as having the highest COD_s, TP and DRP concentrations (Table 2.5). Even though the digestate of anaerobic digester 1 had considerably higher removed concentrations of COD_t, the biogas yield in terms of COD_t removed from digestate (0.203 ± 0.032 m^3 biogas/kg COD_t) was less than a half when compared to that of digestate of anaerobic digester 2 (0.466 ± 0.171 m^3 biogas/kg COD_t). Therefore, the digestate of anaerobic digester 2 had a much better biogas conversion from the removed COD_t compared to the digestate of anaerobic digester 1 as its unit indicated. The biogas yield of the digestate of anaerobic digester 1 (digestate of beef cattle manure) can be increased by the application of pretreatment methods to break down the lignocellulosic structures. These applications were previously addressed as aqueous ammonia soaking (Lymperatou et al., 2015), co-digesting with other raw feedstocks or by-products of other processes (Simm et al., 2017) or other thermal, mechanical, chemical or biological pretreatment methods (Borgström, 2011). On the other hand, the addition of nutrients has also potential to increase both the biogas yield and COD_t removal efficiency for such digestates. The biogas yield of the digestate of anaerobic digester 1 could be improved to 0.237± 0.041 m^3 biogas/kg COD_t (approximately 17 %) by nutrient supplementation (Table 2.9) with a corresponding increase in the COD_t removal efficiency from 57-64% to 61-84%. If the biogas yield can be improved, better COD_t removals can be obtained and additional anaerobic treatment of the digestate becomes viable to be implemented by supporting the economics of the plants.

The digestate of anaerobic digester 3 had a moderate COD_t removal efficiency when nutrients were not supplemented (45-45 %). The addition of nutrients considerably decreased the COD_t removal efficiency to 21-35 % which also resulted in a decrease in RBY from 0.308 ± 0.079 to 0.279 ± 0.076 m^3 biogas/kg COD_t removed from

digestate. This fact was probably due to the accumulated recalcitrant matters in the sample as a result of 40% recycling of the digestate within the plant and the probable inhibition due to high phosphorus content as previously mentioned in Section 2.3.5.2. The COD_t removal efficiency from the digestates of anaerobic digesters 4, 5 and 6 without nutrient supplementation were 25-47 %, 25-27 % and 45-46 % respectively. The addition of nutrients promoted the removal of COD_t for these three digestates to 35-62, 47-47 and 44-55%, respectively (Table 2.11) with the corresponding increase in the biogas yields with respect to the removed COD_t from digestates (Table 2.9).

As a general assessment, the initial COD_t concentrations of the digestates were significantly lowered by AD (from the range of 21079-111056 to 11522-43912 mg/L) (Table 2.11). The corresponding removal efficiencies ranged between 25-64% without nutrient supplementation. COD removal efficiency between 32-78% and 37.9-94% can be obtained by AD of raw feedstocks of poultry manures and cattle manures, respectively, as compiled by Sakar et al. (2009). The removal efficiencies obtained in the digestate treatment were lower compared to these ranges probably due to the relative recalcitrance of the digestates compared with raw feedstocks. Nutrient supplementation reduced the COD_t content of the digestates further to the range of 10609-30512 mg/L corresponding to a removal efficiency in the range of 21-84% which was more comparable to the COD removal efficiencies of raw feedstocks given by Sakar et al. (2009). Therefore, nutrient addition was decided to have a potential to enhance the COD_t removal during anaerobic treatment of the digestates.

Table 2.11. The change in COD_t concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	COD_t concentration in the reactor, mg/L			COD_t removal in the reactor, %	The digestate of anaerobic digester	COD_t concentration of the digestate, mg/L			COD_t removal from digestates, %
		Initial	Final	Removal			Initial	Final	Removal	
		Calculated	Measured	Calculated	Calculated		Measured	Calculated	Calculated	Calculated
with	I	12567	10775±225	1792	(12)-(16)					
	R1	14925	11240±302	3685	(23)-(27)	1	111056±6992	30512	80544	(61)-(84)
	R2	14239	11142±326	3098	(18)-(25)	2	39010±913	16853	22157	(35)-(75)
	R3	14556	12122±176	2434	(16)-(18)	3	57334±2635	41202	16132	(21)-(35)
	R4	14729	11744±353	2985	(18)-(23)	4	76675±4025	39707	36968	(35)-(62)
	R5	14398	11495±8	2903	(20)-(20)	5	43751±488	23247	20504	(47)-(47)
	R6	13816	10699±185	3118	(21)-(24)	6	21079±798	10609	10470	(44)-(55)
without	I	12893	10850±150	2043	(15)-(17)					
	R1	15318	11667±99	3651	(23)-(24)	1	111056±6992	43912	67144	(57)-(64)
	R2	14615	11457±228	3158	(20)-(24)	2	39010±913	20055	18955	(37)-(60)
	R3	14933	11795±4	3138	(21)-(21)	3	57334±2635	31438	25896	(45)-(45)
	R4	15107	12174±288	2933	(18)-(21)	4	76675±4025	48997	27678	(25)-(47)
	R5	14777	12172±25	2605	(17)-(18)	5	43751±488	32507	11244	(25)-(27)
	R6	14178	10956±27	3223	(23)-(23)	6	21079±798	11522	9557	(45)-(46)

2.3.7.3 The changes in volatile and total solids concentrations

Volatile solids degradation obtained in inoculum-only reactors were 15-16 and 13-14% with and without nutrient addition, respectively (Table 2.12). The overall VS removal efficiencies in the digestate containing reactors (R1-R6) accounted for more VS removals from these reactors (13-23% and 16-25% with and without nutrient supplementation, respectively) compared to the inoculum-only reactors. VS removal efficiencies without nutrient supplementation for R1-R6 (13-23%) were observed to be improved to 16-25 % with the addition of nutrients.

Volatile solids were destroyed from the digestate content by 19-65% with and 13-64% without nutrient supplementation (Table 2.12). VS removal obtained from the digestate content was found to be comparable with the raw feedstocks of cattle manures and their mixtures with kitchen waste, fish offal, lipids, whey, agricultural residues, beef manure and dairy manure previously reported in the range of 7.3-78% (Nasir et al., 2012). The removal efficiency of VS from the digestate of anaerobic digester 2 was the highest both with (50-65%) and without nutrient supplementation (42-64%). These high ratios of VS removals also corresponded to the highest RBYs with and without nutrient supplementation at the end of the 70 days (Table 2.8). High VS reduction in the digestate of anaerobic digester 2 operated with 90% laying hen manure, can be attributed to better hydrolysis of the digestate (Demirer and Chen, 2005a). The digestate of anaerobic digester 1 was observed to have the second highest VS removal efficiency both for the cases of with and without nutrient supplementation (34-41%) (Table 2.12) which corresponded also the second highest RBY of the digestate at the end of the 70 days (Table 2.8). The digestate of anaerobic digester 3 had the lowest VS removal efficiency from the digestate content with and without nutrient supplementation (19-20 and 16-18%, respectively) which was probably due to recalcitrant nature of the digestate as previously mentioned.

Depending on the fact that high VS degradation yielded more biogas regarding the digestates of anaerobic digesters 1, 2 and 3, a correlation between these two variables was also evaluated including all the digestates (Appendix G). The coefficient of determination (R^2) was found as 0.8956 and 0.9247 with and without nutrient supplementation, respectively, excluding the digestate of anaerobic digester 6. Following the approach of eliminating the digestate of anaerobic digester 6, the higher VS removal amounts corresponded to higher RBYs for the digestates of anaerobic digesters 1-5. This observation was agreed with the one stated as methane production is directly related to the VS degradation (Jingura and Kamusoko, 2017). However, the R^2 values were altered to 0.6214 and 0.8026 with and without nutrient supplementation, respectively, when the digestate of anaerobic digester 6 was included. The created effect as a result of the inclusion the digestate of anaerobic digester 6 in the correlation analysis was probably based on the more biodegradable nature of the digestate of anaerobic digester 6 (sewage sludge) compared to the digestates containing animal manures. Even though the removed VS amounts from the digestates of anaerobic digesters 3 and 6 were approximately same (117 mg for each) (Appendix G), the removed amounts from the digestate of anaerobic digester 6 was obtained by adding less VS at the initial setup. Thus, the RBY obtained was found to be higher for the digestate of anaerobic digester 6 (0.195 ± 0.014 $L_{biogas}/g\ VS_{added}$) compared to that of 3 (0.123 ± 0.009 $L_{biogas}/g\ VS_{added}$). VS removal and the RBYs of the digestates have a potential to be correlated on the condition that the digestates obtained should not originate from anaerobic digesters operated with too distinctive feedstock compositions which has a considerable effect on the biodegradability of digestates. On oppose to the idea that methane production is directly related to the VS degradation (Jingura and Kamusoko, 2017), the correlation analysis demonstrated that the biogas yields was dependent on both degraded VS and biodegradability of the digestates.

Table 2.12. The change in VS concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	VS concentration in the reactor, mg/L			VS removal in the reactor, %	The digestate of anaerobic digester	VS concentration of the digestate, mg/L			VS removal from digestates, %
		Initial Calculated	Final Measured	Removal Calculated	Calculated		Initial Measured	Final Calculated	Removal Calculated	Calculated
with	I	7522	6345±15	1177	(15)-(16)					
	R1	9093	7285±65	1808	(19)-(21)	1	72630±1139	45208	27422	(34)-(41)
	R2	8719	6668±89	2051	(22)-(25)	2	25970±260	11174	14796	(50)-(65)
	R3	8898	7445±5	1453	(16)-(16)	3	38013±213	30693	7320	(19)-(20)
	R4	9003	7470±20	1533	(17)-(17)	4	50953±82	39282	11671	(22)-(24)
	R5	8762	7215±75	1547	(17)-(19)	5	28180±425	20786	7394	(22)-(31)
	R6	7906	6600±30	1306	(16)-(17)	6	9837±160	7817	2020	(19)-(23)
without	I	7330	6350±10	980	(13)-(14)					
	R1	8943	7315±65	1628	(17)-(19)	1	72630±1139	45408	27222	(34)-(41)
	R2	8559	6737±111	1823	(19)-(23)	2	25970±260	12214	13756	(42)-(64)
	R3	8738	7510±20	1228	(14)-(14)	3	38013±213	31620	6393	(16)-(18)
	R4	8844	7485±105	1359	(14)-(17)	4	50953±82	39053	11900	(17)-(29)
	R5	8603	7280±50	1323	(15)-(16)	5	28180±425	21581	6599	(21)-(26)
	R6	7724	6605±85	1119	(13)-(16)	6	9837±160	7974	1863	(13)-(24)

The overall total solids removal in inoculum-only reactors was observed to be 10% and 7% with and without nutrient supplementation, respectively (Table 2.13). AD resulted in the reduction of the overall TS concentration in all reactors including digestate samples. TS removals in the reactors containing the digestates of animal manures at a high proportion (R1-R5) were observed to be at least equal to or higher than the inoculum-only reactors for both the cases of with and without nutrient supplementation. This fact suggested the degradation of TS from the digestate content. The addition of nutrients improved the TS removal efficiency for all the reactors from the range of 4-13% to 8-13%. However, the final TS concentrations in the reactors were not significantly altered in nutrient supplemented reactors compared to the ones without nutrient supplementation (Table 2.13). This fact was probably due to the additional solids introduced by the addition of nutrient medium. The TS concentration of the nutrient medium was measured to be 26590±220 mg/L. When the TS amounts corresponding to the retained amounts of inoculum was excluded, TS removals from the digestates were found to be variable (-2)-(29)% with and (-6)-(37)% without nutrient supplementation) and independent of the initial TS concentration of the digestate itself (Table 2.13).

Table 2.13. The change in TS concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	TS concentration in the reactor, mg/L			TS removal in the reactor, %	The digestate of anaerobic digester	TS concentration of the digestate, mg/L			TS removal from digestates, %
		Initial	Final	Removal			Initial	Final	Removal	
		Calculated	Measured	Calculated	Calculated		Measured	Calculated	Calculated	Calculated
with	I	14441	13005±15	1436	(10)-(10)					
	R1	16634	14645±155	1989	(11)-(13)	1	105250±1152	80810	24440*	(17)-(29)
	R2	16955	14967±213	1988*	(10)-(13)	2	53263±593	43223	10040*	(9)-(27)
	R3	16456	14845±25	1611	(10)-(10)	3	59053±321	53719	5334*	(8)-(10)
	R4	16503	14725±95	1778	(10)-(11)	4	74830±255	63316	11514*	(12)-(19)
	R5	16570	14665±65	1905	(11)-(12)	5	49920±524	40595	9325	(16)-(21)
	R6	15155	13940±70	1215*	(8)-(8)	6	18773±176	18781	-8*	(-2)-(2)
without	I	14107	13125±65	982	(7)-(7)					
	R1	16359	14545±105	1814	(10)-(12)	1	105250±1152	70599	34651	(29)-(37)
	R2	16689	14893±232	1796*	(9)-(13)	2	53263±593	39942	13321*	(19)-(37)
	R3	16170	14960±60	1210*	(7)-(8)	3	59053±321	53100	5953*	(8)-(12)
	R4	16215	14940±250	1275*	(6)-(9)	4	74830±255	65420	9410*	(3)-(22)
	R5	16294	15255±75	1039*	(6)-(7)	5	49920±524	48009	1911	(1)-(6)
	R6	14840	14065±125	775*	(4)-(6)	6	18773±176	19112	-339*	(-6)-(2)

*The values calculated for VS removal were higher than that of TS removals based on both standard deviations of the measured values and the estimation of the initial concentrations within reactors from the average concentrations of the constituents in the related component of the reactor (not measured).

2.3.7.4 The changes in ammonium and total kjeldahl nitrogen concentrations

The initial NH_4^+-N concentration in the reactors was in the range of 946-1393 and 882-1341 mg/L with and without nutrient supplementation, respectively. These NH_4^+-N concentrations in the reactors were expectedly increased due to the addition of nutrient medium (Table 2.14) which involves ammonium in its composition (Table 2.6). The ammonium concentration further increased with the decomposition of organic nitrogen within the reactors (negative signs in Table 2.14 indicates accumulation). The increase in NH_4^+-N concentrations during digestion can be explained by anaerobic degradation of proteins into amino acids and then to ammonia (Demirer and Chen, 2005a). The NH_4^+-N accumulation corresponded to 6-19 and 9-19 % with and without nutrient supplementation, respectively. It was observed that the accumulation of the NH_4^+-N concentration due to degradation was in a good agreement with the ones (12.7-37.9%) obtained from 7 different types of on-site anaerobic digesters operated with variable feedstock composition (Gooch et al., 2006). Final NH_4^+-N concentrations in the reactors were in the range of 1120-1363 and 1037-1470 mg/L with and without nutrient supplementation, respectively, which were below the inhibitory level of 1700-1800 mg/L for anaerobic processes without the acclimation of the inoculum to the substrate (Yenigün and Demirel, 2013).

Table 2.14. The change in NH_4^+-N concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	NH_4^+-N concentration in the reactor, mg/L			NH_4^+-N removal in the reactor, %	The digestate of anaerobic digester	NH_4^+-N concentration of the digestate, mg/L			NH_4^+-N removal from digestates, %
		Initial Calculated	Final Measured	Removal Calculated	Calculated		Initial Measured	Final Calculated	Removal Calculated	Calculated
with	I	956	1120±3	-164	(-17)-(-17)					
	R1	1014	1196±12	-181	(-19)-(-17)	1	3288±24.9	4186	-898	(-41)-(-13)
	R2	1393	1537±61	-144	(-17)-(-6)	2	7703±41.8	7554	149	(-16)-(13)
	R3	996	1139±2	-142	(-14)-(-14)	3	1782±11.7	1456	326	(17)-(20)
	R4	1081	1212±1	-130	(-12)-(-12)	4	4569±66.8	3733	836	(18)-(19)
	R5	1145	1285±2	-140	(-12)-(-12)	5	4071±17.8	3836	235	(5)-(7)
	R6	946	1118±2	-171	(-18)-(-18)	6	826±14.9	1035	-209	(-27)-(-24)
without	I	892	1037±0	-145	(-16)-(-16)					
	R1	951	1108±13	-156	(-18)-(-15)	1	3288±24.9	3878	-590	(-33)-(-2)
	R2	1341	1470±14	-129	(-11)-(-9)	2	7703±41.8	7600	103	(-1)-(4)
	R3	933	1074±7	-141	(-16)-(-14)	3	1782±11.7	1840	-58	(-12)-(5)
	R4	1020	1166±9	-146	(-15)-(-13)	4	4569±66.8	4739	-170	(-9)-(2)
	R5	1086	1280±14	-194	(-19)-(-17)	5	4071±17.8	5007	-936	(-29)-(-17)
	R6	882	1044±2	-162	(-19)-(-18)	6	826±14.9	1080	-254	(-33)-(-29)

NH_4^+-N removal efficiencies varied in the range of (-12)-(5), (-9)-(2) and (-29)-(-17)% for the digestates of anaerobic digesters 3, 4 and 5, respectively. These digestates share a common feature of including dairy cattle manure at a large proportion in their raw feedstock composition (Table 2.4).The variable NH_4^+-N removal efficiencies indicated both the accumulation and removal potential of NH_4^+-N from the content of the digestates. The addition of nutrients provided the removal of NH_4^+-N with an efficiency of 17-20, 18-19 and 5-7%, respectively, from these digestates. However, the overall final NH_4^+-N concentrations in the related reactors were higher in the nutrient supplementated ones (1139-1285 mg/L) compared to the ones without nutrient supplementation (1074-1280 mg/L) (Table 2.14). Therefore, nutrient supplementation to the digestates resulted in higher NH_4^+-N concentrations in the effluent which would develop the pollutional concerns related to increased ammonium concentrations.

The overall TKN removal in reactors was in the range of 2-14 and 7-14% with and without nutrient supplementation, respectively (Table 2.15). TKN is a lumped parameter composed of organic nitrogen and ammonium nitrogen. The degradation of organic nitrogen results in the increase in ammonium content (Ghyselbrecht et al., 2017). The ammonium loss from the reactor potentially reduces the TKN content. The probable pathways of ammonium reduction under anaerobic conditions can be the volatilization of ammonium to ammonia gas above pHs of 7 (Evangelou, 1998), denitrification of oxidized nitrogen (Acharya et al., 2008) and/or anaerobic ammonia oxidation (Anammox) (Li et al., 2017).

Table 2.15. The change in TKN concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	TKN concentration in the reactor, mg/L			TKN removal in the reactor, %	The digestate of anaerobic digester	TKN concentration of the digestate, mg/L			TKN removal from digestates, %
		Initial	Final	Removal			Initial	Final	Removal	
		Calculated	Measured	Calculated	Calculated		Measured	Calculated	Calculated	Calculated
with	I	1328	1189±5	139	(10)-(11)					
	R1	1386	1237±12	149	(10)-(12)	1	3694±35.3	3139*	555	(1)-(29)
	R2	1785	1630±73	155	(2)-(14)	2	8394±283.6	8005	389	(-16)-(21)
	R3	1373	1214±21	160	(10)-(13)	3	2285±37.9	1692	593	(5)-(46)
	R4	1460	1300±1	160	(11)-(11)	4	5147±100.5	4412	735*	(14)-(15)
	R5	1539	1362±3	176	(11)-(12)	5	4815±469.7	4052	763	(15)-(17)
	R6	1294	1185±9	109	(8)-(9)	6	1051±21.7	1107	-56	(-11)-(0)
without	I	1274	1124±29	151	(10)-(14)					
	R1	1334	1194±35	140	(8)-(13)	1	3694±35.3	3979	-285	(-46)-(31)
	R2	1744	1547±21	197	(9)-(13)	2	8394±283.6	7543*	851	(4)-(16)
	R3	1320	1146±6	175	(13)-(14)	3	2285±37.9	1605*	680	(24)-(36)
	R4	1408	1272±17	136	(8)-(11)	4	5147±100.5	5406	-259*	(-14)-(4)
	R5	1490	1383±0	107	(7)-(7)	5	4815±469.7	5375	-560	(-12)-(-12)
	R6	1239	1106±14	133	(10)-(12)	6	1051±21.7	1011*	40	(-4)-(12)

*The values calculated for NH_4^+-N removal were higher than that of TKN removals based on both standard deviations of the measured values and the estimation of the initial concentrations within reactors from the average concentrations of the constituents in the related component of the reactor (not measured).

Free ammonia nitrogen (FAN) concentrations were also calculated to evaluate the probability of volatilization of ammonia nitrogen (Appendix H). The initial FAN concentrations in the reactors with nutrient supplementation were estimated to be in the range of 370-409, 365-509, 364-369, 395-491, 354-418 and 193-346 mg/L for R1-R6, respectively. The ones for the case of without nutrient supplementation were between 347-384, 351-490, 341-346, 373-463, 335-397 and 180-322, respectively. On the other hand, the removal of TKN from the reactor content (Table 2.15) were in the range of 109-176 mg/L with and 107-197 mg/L without nutrient supplementation. Thus, the estimated initial FAN concentrations were much higher than the removed TKN concentrations (Table 2.15). When TKN removal was assumed to follow only the pathway of organic nitrogen (if any) to ammonium, ammonium to ammonia gas conversion, complete removal of FAN via volatilization was not feasible due to lower removed TKN concentrations than FAN. The final FAN concentrations were estimated to be 44, 128, 56, 72, 169 and 130 with and 62, 148, 90, 77, 86 and 93 without nutrient supplementation for R1-R6, respectively. These final FAN concentration estimates were always lower than the TKN removals in the related reactors (Table 2.15) except R6 with nutrient supplementation. Even if all final FAN concentrations volatilized, TKN removal could not be equilibrated. This fact can be speculated to be dependent on two main reasons: (1) the decrease in pH and increase in ammonium concentration during operation may have led to FAN concentrations different from the estimated ones and (2) the other removal mechanisms than the volatilization of ammonium. In fact, the volatilization of ammonia under the pH values less than the pK_a of the volatilization column has been evaluated as a minor mechanism in the literature (Al Nozaily, 2000). A study on pH-based ammonia control in the biogas composition during the treatment of synthetic medium demonstrated that the increase of pH from 7.43 to 7.74 resulted in an increase in FAN concentration to 600 mg/L in a laboratory-scale thermophilic (60^{o}C) continuous flow stirred tank reactor (Strik et al., 2006). However, the corresponding NH_3 in biogas composition was reported as having a maximum of 95 ppm. The authors also addressed a huge liquid/gas transfer limitation for NH_3 in the anaerobic digestion process because of the

concentration of ammonia being lower than theoretical liquid/gas equilibrium. Thus, volatilization of NH_3 was expected to have a minor impact on ammonium removal depending on the pH ranges (7.53-8.87 in overall) (Section 2.3.7.1) lower than the pK_a at 35^oC (8.95) and liquid/gas transfer limitations of NH_3 in anaerobic digestion. Anammox process can represent another potential removal mechanism for NH_4^+-N from the digestate content. It is an autotrophic conversion of ammonium to dinitrogen gas using nitrite as an electron acceptor under anaerobic environment (Henze et al., 2008). The process reported to be succeptible to inhibition in terms of organic content when COD concentration was above than 290 mg/L (Molinuevo et al., 2009). The COD concentrations of above 10000 mg/L in the reactor content (Table 2.11) can thus be expected to inhibit anammox process in the RBP test of the digestates. Sabumon (2007) investigated an additional route for the anaerobic ammonia removal in the presence of organic matter. The author reported that the oxygen required for nitrite and nitrate compounds under anaerobic conditions could be formed during the catalase enzyme activity under oxidative stress and anoxic conditions. Further denitrification step could either be autotrophic or heterotrophic; the latter using organic matter in the conversion of nitrate to dinitrogen gas (Sabumon, 2007). Therefore, ammonium removal in the anaerobic treatment of the digestates can be speculated to be achieved via the pathway of the formation of oxidized nitrogen compounds by enzymatic catalase activity followed by autotrophic and/or heterotrophic denitrification. This conversion pathway for ammonium still remains as another research field.

2.3.7.5 The changes in dissolved reactive and total phosphorus concentrations

The treatment potential of the phosphorus was evaluated for dissolved reactive and total phosphorus parameters. Phosphates that can be detected in colorimetric tests without the application of hydrolysis or oxidative digestion as a pretreatment are defined as the reactive phosphorus which includes various forms of orthophosphates (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4) (Gu et al., 2011). The overall DRP concentrations in

reactors decreased by 67-94% and 68-83% with and without nutrient supplementation, respectively (Table 2.16). The removed DRP concentrations in the reactors were 1.75-5.01 folds higher with nutrient supplementation than without nutrient supplementation. AD processes are known to increase the availability of the phosphorus (Moody et al., 2009), thus the DRP content. Reactive phosphorus represents the readily available form of phosphorus to chemical reactions via coulombic attraction (Venkiteshwaran et al., 2018). On the other hand, Güngör and Karthikeyan (2008) could not observe a consistent increase in DRP concentrations in the investigation of the influent and effluent phosphorus concentrations of 6 full-scale on-farm anaerobic digesters processing dairy manures, even though the breakdown of dissolved unreactive phosphorus in AD suggested mineralization. The authors rather observed a decrease in the influent DRP concentrations after AD by a majority of measurements which approximately ranged between 10-64%. They concluded that DRP might have been precipitated or incorporated in particulate matter and subsequently removed from the dissolved phase (Güngör and Karthikeyan, 2008). Phosphate removal in AD processes were also reported as precipitation and co-precipitation as well as biological phosphorus uptake (van Langerak et al., 1998). The high removal amounts of DRP and the expectation of phosphorus release rather than removal by microorganisms under anaerobic conditions also suggested similar removal mechanisms for DRP in the anaerobic reactors. Thus, the removal amounts of DRP can be linked to the prevalence of readily available precipitating or incorporable matters in the anaerobic environment. This may also be reason why the DRP removal from the digestates of anaerobic digesters 1, 3, 4 and 6 with nutrient supplementation reflected itself as being over 100% in calculation (Table 2.16). Over 100% removal indicated the constituent removed from the other contents in the reactor, which was probably from the nutrient solution added. The addition of the nutrients may have enhanced the formation of readily precipitating matters, thus the precipitation of DRP in the overall reactor content may have promoted.

Table 2.16. The change in DRP concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	DRP concentration in the reactor, mg/L			DRP removal in the reactor, %	The digestate of anaerobic digester	DRP concentration of the digestate, mg/L			DRP removal from digestates, %
		Initial	Final	Removal			Initial	Final	Removal	
		Calculated	Measured	Calculated	Calculated		Measured	Calculated	Calculated	Calculated
with	I	115.0	24.4±0.06	90.6	(79)-(79)					
	R1	142.7	14.1±0	128.5	(90)-(90)	1	1156.1±3.74	-519.9	1676.0	(145)-(145)
	R2	184.3	58.8±2.94	125.5	(67)-(71)	2	1097.9±3.74	459.8	638.0	(55)-(64)
	R3	127.2	7.6±0.04	119.5	(94)-(94)	3	289.7±0.00	-450.4	740.1	(255)-(256)
	R4	130.4	22.3±0.04	108.1	(83)-(83)	4	465.6±3.74	-145.6	611.2	(131)-(132)
	R5	146.5	32.5±0	113.9	(78)-(78)	5	548.9±1.87	62.4	486.5	(89)-(89)
	R6	116.4	20.6±0	95.8	(82)-(82)	6	33.7±0.04	-92	125.7	(373)-(373)
without	I	27.2	6.5±0.07	20.7	(76)-(76)					
	R1	55.1	11.3±0	43.7	(79)-(79)	1	1156.1±3.74	203.5	952.6	(82)-(82)
	R2	97.8	26.3±0.79	71.5	(72)-(74)	2	1097.9±3.74	306.7	791.2	(71)-(74)
	R3	39.2	7.6±0.04	31.7	(81)-(81)	3	289.7±0.00	30.7	259	(89)-(90)
	R4	42.4	8.7±0.04	33.7	(79)-(80)	4	465.6±3.74	70.5	395.1	(85)-(85)
	R5	59.1	10±0.04	49.0	(83)-(83)	5	548.9±1.87	64.5	484.4	(88)-(88)
	R6	28.2	9.1±0.04	19.1	(68)-(68)	6	33.7±0.04	23.0	10.7	(31)-(32)

Initial total phosphorus content of the digestate including reactors was between 480-641 mg/L with and 400-566 mg/L without nutrient supplementation. The high phosphorus concentration of the nutrient medium (Table 2.6) resulted in higher initial TP content in the reactors with nutrient supplementation (Table 2.18). TP was removed by 14-24 % and 19-29 % with and without nutrient supplementation, respectively, in digestate containing reactors. TP removal from unscreened dairy manure was previously reported as 27-61 % in a conventional one phase digester (Demirer and Chen, 2005a). TP content in the reactor had a potential to be removed from the reactor by volatilization since the only mechanism allowed as an outlet from the reactor was volatilization during the ejection of the biogas produced. Volatile phosphine compounds were previously noted to be present in anaerobic digesters but at very low concentrations in the orders of nanograms per cubic meter gas (Roels and Verstraete, 2001). Such a removal mechanism is not explanatory for the overall TP removals from the reactors. It can thus be speculated that the compounds that are resistant to acid digestion in TP determination may be formed at the end of the reactor operation. Organophosphorus compounds such as AMP (adenosine monophosphate) may require more time to digest to be degraded (APHA, 2005). The reactors with nutrient medium had lower or at most equal TP removal efficiency compared to the ones without nutrient medium (Table 2.18). This fact can be attributed to the increased initial phosphorus concentration via addition of nutrients into the reactors which eventually resulted in higher final TP concentrations.

The TP content of the digestates were either removed or accumulated by -50 to 34% with and -17 to 26% without nutrient supplementation (negative values indicates the accumulation) (Table 2.18). The highest TP accumulation observed was in the digestate of anaerobic digester 6 (50-46%) which was probably due to the comparably much lower TP removal within the reactor (69.2 mg/L) than the other reactors (104.9-124.9 mg/L). Accumulation of TP was previously observed in two-phase anaerobic

treatment of unscreened dairy manure with a TP removal range of (-14)-42% (Demirer and Chen, 2005a).

The solubilization of TP into reactive form (DRP/TP) was found to be 0.500, 0.394, 0.216, 0.299, 0.318 and 0.096 for the digestates of anaerobic digesters 1-6, respectively, at the initial stage of the experiment. The DRP fractionation of the total phosphorus were significantly reduced to the range of 0.031-0.113 after AD process (Table 2.17). Thus, dissolved reactive phosphorus released in the anaerobic environment did not have a potential to remain in a soluble state and rather removed as particulates or incorporated forms in particulates.

Table 2.17. DRP/TP ratio for the digestate before and after RBP test.

Digestate of anaerobic digester	Initial DRP of digestate, mg/L	Initial TP of digestate, mg/L	Initial P solubilization of digestate	Final DRP of digestate, mg/L	Final TP of digestate, mg/L	Final P solubilization of digestate
1	1156	2314	0.500	203	1934	0.105
2	1098	2786	0.394	307	2711	0.113
3	290	1340	0.216	31	985	0.031
4	466	1555	0.299	71	1191	0.059
5	549	1725	0.318	65	2024	0.032
6	34	352	0.096	23	360	0.064

Table 2.18. The change in TP concentrations after 70 days of anaerobic batch treatment.

Nutrient supplementation	Reactor	TP concentration in the reactor, mg/L			TP removal in the reactor, %	The digestate of anaerobic digester	TP concentration of the digestate, mg/L			TP removal from digestates, %
		Initial Calculated	Final Measured	Removal Calculated	Calculated		Initial Measured	Final Calculated	Removal Calculated	Calculated
with	I	488	376.7±0.94	111.7	(23)-(23)					
	R1	535	423.3±0.94	111.2	(21)-(21)	1	2314±5.8	2220.6	93.4	(3)-(6)
	R2	641	516.7±1.63	124.6	(19)-(20)	2	2786±0.0	2472.0	314	(9)-(13)
	R3	530	408.7±0.94	121.7	(23)-(23)	3	1340±0.0	1004.6	335.4	(23)-(26)
	R4	528	402.7±0.94	124.9	(23)-(24)	4	1555±0.0	1052.0	503.0	(30)-(34)
	R5	567	462.0±0.00	104.9	(19)-(19)	5	1725±0.0	1727.5	-2.5	(0)-(0)
	R6	480	410.7±0.94	69.2	(14)-(15)	6	352±5.1	517.9	-165.9	(-50)-(-46)
without	I	409	288.0±0.00	121.0	(30)-(30)					
	R1	456	328.7±0.94	127.4	(28)-(28)	1	2314±5.8	1934.0	380.0	(14)-(18)
	R2	566	447.8±6.83	118.0	(19)-(22)	2	2786±0.0	2710.9	75.1	(-3)-(6)
	R3	452	320.0±0.00	131.7	(29)-(29)	3	1340±0.0	985.1	354.9	(26)-(26)
	R4	449	319.3±0.94	129.4	(29)-(29)	4	1555±0.0	1190.8	364.2	(22)-(26)
	R5	489	394.0±0.00	95.4	(19)-(19)	5	1725±0.0	2024.0	-299.0	(-17)-(-17)
	R6	400	299.3±0.94	100.7	(25)-(26)	6	352±5.1	360.2	-8.2	(-4)-(0)

2.4 The decision on the digestate selection on further anaerobic treatment

The digestate of anaerobic digester 2 operated with 90 % laying hen manure was selected as the digestate to be used in high-rate anaerobic treatment process. This digestate had the highest residual biogas yield with respect to both the added amounts of volatile solids in digestate (0.326 ± 0.009 L_{biogas}/g VS) and the removed amount of COD_t from the digestate (0.466 ± 0.171 m^3/kg COD_r) without nutrient supplementation. Higher biogas yields may increase the economic feasibility of AD plants. The biogas yield of the digestate was very close to the theoretical one and did not require any pretreatment step to increase the yield. Additionally, the biogas yields obtained from this digestate was higher than the limit value (0.25 L/g VS) set by the Notification on Mechanical Separation, Bio-Drying and Bio-Methanation Plants and Fermented Product Management to be applicable on land. Thus, the digestate required further stabilization before land application. Moreover, significant COD_t removals (37-60%) from the digestate could be obtained by further anaerobic treatment which has a potential to decrease the environmental aspects associated with its land application or storage.

2.5 Conclusions

The biogas yields obtained in the range of 0.111-0.326 L_{biogas}/g VS were found to be comparable to the raw feedstocks such as cattle, dairy cattle, horse manure. The highest biogas yield was obtained from the digestate of the raw feedstock containing 90% laying hen manure with the highest VS degradation. The biogas yields were considerably different from the biogas production per unit volume of digestates probably due to their variable biodegradability. The digestate of sewage sludge was more easily biodegradable relative to the digestates of animal manures. The digestate obtained from the plant in which digestate was recycled by 40% had the lowest biogas

yield due to accumulation of the recalcitrant matters even if the digestate had an optimum COD:TKN ratio for further anaerobic degradation. The addition of nutrients resulted in phosphorus inhibition when nutrients were added for the digestates having a COD:TP ratio in the range of 14:1-43:1.

Further AD of the digestates resulted in a COD_t removal efficiency in the range of 25-64% which had a potential to be increased to 35-84% by nutrient supplementation. Even if nutrient supplementation enhanced the COD_t removal, it resulted in higher nutrient concentrations in the effluent. Thus, nutrient addition may not be considered as a viable option for decreasing the environmental impacts associated with the land application, storage or disposal of digestates.

Volatile solids content of the digestates could be degraded by 19-65% and 13-64% with and without nutrient supplementation, respectively. VS degradation was found to have a potential to be correlated with the residual biogas yields only if the digestates were not obtained from the AD plants that were operated with too distinct feedstocks such as the ones of sewage sludges and animal manures. The highest COD_t and VS removals were obtained for the digestates of raw feedstocks of 100% beef cattle manure and 90% laying hen manure which had the highest COD_s, TP and DRP concentrations and the highest RBYs at the end of 70 days.

CHAPTER 3

HIGH-RATE ANAEROBIC TREATMENT OF DIGESTATE USING FIXED-FILM REACTORS

The results of the RBP test (Chapter 2) indicated that digestates contained high COD concentrations and had significant residual biogas production potential. The digestate taken from an anaerobic digester operated with a manure mixture of 90% laying hen and 10% cattle manure (digestate of the anaerobic digester 2 in Chapter 2) was found to have the highest RBY (0.299 ± 0.005 with and 0.326 ± 0.009 L_{biogas}/g VS_{added} without nutrient supplementation) among 6 digestate samples. This yield was found to be comparable to that of many raw feedstocks such as cattle manure (0.15-0.35 L/g VS), municipal secondary sludge (0.2-0.35 L/g VS), cereal straw (0.2-0.5 L/g VS), liquid cattle manure (0.3-0.8 L/g VS) and pig excreta (0.2-0.5 L/g VS) (Braun, 2007; Zupančič and Grilc, 2012). Furthermore, considerable COD_t removal (35-75% with and 37-60 % without nutrient supplementation) was also achieved in the 70-day batch anaerobic treatment of this digestate sample. It was therefore concluded that additional COD removal and biogas production from digestates could be practiced to decrease the pollution load as well as to capture the residual biogas associated with the degradation of residual organics. Having the highest RBP and considerable COD removal, digestate of the anaerobic digester 2 was selected for further treatment and residual biogas capture by using high rate fixed-film anaerobic reactors.

The reason behind the selection of a high-rate anaerobic reactor was mainly related to recent studies demonstrating the possibility of reducing the HRT and thus increasing the applicable organic loading rates. Operating an AD process using shorter HRTs creates an opportunity for further COD removal and biogas capture from digestates with minimum footprint and installation costs.

An anaerobic fixed-film reactor which is known with its small area requirement for installation, simplicity in construction and operation as well as durability against process instabilities was selected as a well-suited configuration for high-rate treatment of the digestate. The commonly used high-rate anaerobic digesters are reviewed in this chapter with a special emphasis on the digestate treatment using AFFRs.

3.1 Literature Background

3.1.1 High-rate anaerobic reactors

Low-rate anaerobic digesters can be referred as slow reactors which require 4-6 weeks of HRT to maintain the digestion at a significant extent. Unstirred, semi-continuous digesters in rural areas, septic tanks and Imhoff tanks can be considered as low-rate digesters (Tauseef et al., 2013).

The rate of digestion processes was increased by the invention of the high-rate anaerobic reactors which dated back to 1960s with the introduction of anaerobic filters for the treatment of wastes. High-rate anaerobic configurations can be operated with shorter HRTs while maintaining high solids retention time within the reactors (Abbasi et al., 2012) which eventually results in the reduction of reactor footprint and volumes (Barber and Stuckey, 1999). High solids retention time can be obtained by bacterial sludge entrapment between the spaces and the attachment of the bacteria to the walls of the supporting media and/or bacterial immobilization on fixed or mobile particulate surfaces, and/or sludge blankets (Lettinga et al., 1984). The prolonged solids retention time in high-rate anaerobic digesters enables the reactors to be operated at high organic loadings. The applicability of high organic loadings and less sludge production are the two drivers for the growing interest on high-rate anaerobic treatment of wastes/wastewaters (Rajagopal et al., 2013). Many different configurations of high-

rate reactors have been introduced and used for the treatment of wastewaters from the time of invention. The studies on high-rate AD of various wastes are compiled in Table 3.1. High-rate anaerobic reactors can be classified as first, second and third generation based on their evolution. A brief description on the configurations of high-rate anaerobic reactors is given in the following sections.

3.1.1.1 First generation high-rate anaerobic reactors

Anaerobic CSTRs and anaerobic contact reactors (ACTs) can be considered as the first generation high-rate anaerobic digesters (Abbasi and Abbasi, 2012; Tauseef et al., 2013). CSTRs have been widely applied in the treatment of high strength wastewaters such as liquid animal manure and organic wastewaters (Mao et al., 2015). However, the performance of these reactors is affected by the microbial wash-out along with the effluent. ACT has been developed for the purpose of increasing the solids retention time by recycling the microbial biomass back to the reactor in order to prevent the wash-out of the microorganisms (Tauseef et al., 2013). ACT reactor design was inspired from aerobic activated sludge processes and therefore can be referred as anaerobic activated sludge processes. Both the CSTRs and ACTs have been widely used for the treatment of wastewaters of high suspended solid concentrations (Mao et al., 2015).

3.1.1.2 Second generation high-rate anaerobic reactors

Anaerobic filters, downflow stationary fixed film reactors, upflow anaerobic sludge blankets, fluidized bed and expanded bed reactors, sequencing batch reactors and baffled reactors can be considered as second generation high-rate reactors (Tauseef et al., 2013).

Table 3.1. The studies on high-rate anaerobic treatment of various wastewaters.

Reactor type	Wastewater	Support material	T,°C	HRT	OLR	Biogas	COD removal	Reference
Hybrid SB/FFR	Synthetic sewage WW	Packed pumice stone	12	8-24 h	0.4-1.5 kg COD/m^3.d		COD_t 84% COD_s 87%	Keating et al., 2016
AAF	Dairy cattle WW	*limestone gravel *non-woven polyester matting *mixture of the two WWs	10-37	33 d	0.12 kg VS/m^3.d	*0.012-0.054 m^3 / m^3.d *0.009-0.037 m^3 CH_4/ m^3.d *0.05-0.21 m^3 CH_4/kg COD_r	76-94%	Vartak et al., 1997
UASB	Molasses WW	-	35	7 d	2.3-9.4 kg COD/m^3.d	0.33-0.91 m^3 CH_4/ m^3.d	(-20)-60%	Lo et al., 1991
Hybrid UASB (fixed-film)		Rope			2.3-9.1 kg COD/m^3.d	0.44-1.12 m^3 CH_4/ m^3.d		
UASB	*Molasses waste *Synthetic volatile acids wastes	-	35	0.34-30 d	0.8-20.4 kg COD/m^3.d	0.21-0.51 m^3/kg COD_r	52-98%	Jhung and Choi, 1995
AFBR	Cotton textile WW (supplemented)	Pumice	35	24-50 h	0.38-5 kg COD/m^3.d		27-89%	Şen and Demirer, 2003
AFBR + Ultrasonication	Thickened waste activated sludge	High density polyethylene	37	4, 8 d	4.5- 11.5 kg COD/m^3.d	0.28-0.35 m^3 CH_4/kg COD_r	38-43%	Chowdhury et al., 2017
AFBR						0.33-0.36 m^3 CH_4/kg COD_r	43-65%	

Table 3.1. The studies on high-rate anaerobic treatment of various wastewaters.

Reactor type	Wastewater	Support material	T,°C	HRT	OLR	Biogas	COD removal	Reference
AFBR	Synthetic carbohydrate rich WW	Anox Kaldness-K1	37	15-20 d		0.08-0.41 m^3 CH_4/kg COD_a.d	95%	Yeshanew et al., 2016
Anammox UASB	Chicken manure digested in CSAR	-	35	13 d	-	-	63% COD_t 26 % COD_s	Yangin-Gomec et al., 2017
UASB	Raw and filtered pig slurry	-	36	1.5, 3 d	7.2-16.4 kg COD/m^3.d	0.236-0.248 m^3 CH_4/kg COD		Rico et al., 2017
UASB	Cattle manure	-	37	5.3-16 d	2.35-8.63 kg COD/m^3.d	64.7-73.7 % CH_4	36.2-75.5%	Marañón et al., 2001
AF	Poultry slaughterhouse	PVC ring	29-35	8-36 h (start-up)	0.77-3.43 kg COD/m^3.d	0.024 m^3 biogas/d 46-56% methane	70 % COD_t 79 % COD_s	Rajakumar et al., 2011
				12 h	2.27-15.4 kg COD/m^3.d	0.24 m^3 CH_4/kg COD_r	59-78 % COD_t 64-83.5%COD_s	
MBBR	Winery wastewater	PE carriers	31-39	1.55-4.66 d	1.3-29.59 kg/m^3.d	0.45-14.06 m^3 / m^3.d 45.11-82.61% CH_4	30.6-91%COD_s	Chai et al., 2014

AAF: Anaerobic attached film, AF: anaerobic filter, AFBR: fluidized bed reactor, MBBR: moving bed biofilm reactor, SB: sludge bed, UASB: upflow anaerobic sludge blanket, WW: wastewater. COD_r: COD removed, COD_a: COD added, CSAR: continuously stirred anaerobic reactor.

3.1.1.2.1 Anaerobic filters (fixed-bed) and anaerobic fixed-film reactors

A stationary packing material is present in both anaerobic filters (AFs) and anaerobic fixed-film reactors. The main distinction between each type of anaerobic reactors is that a large fraction of sludge is entrapped between the spaces of packing material in AFs which makes the shape of the packing material less important. On the other hand, AFFRs are designed to avoid the entrapment of suspended solids among the packing material to enable the treatment of wastes with considerable amounts of suspended solids such as screened manure (Lettinga et al., 1984). The shape of the packing material thus becomes the most important component of the reactor configuration in AFFRs. Ideally the packing material is required to have high porosity and large surface area, to be light in weight and to enable biomass attachment to the surface as well as to be economical (Acharya et al., 2008). AFs were previously tested for the treatment of landfill leachate, food processing wastes, pharmaceutical wastes, high strength acid wastewater, wheat starch gluten plant waste, organic particulates, pig slurry and waste, poultry slaughterhouse wastewater and cattle slurry which was observed to yield considerable COD removals (Tauseef et al., 2013). Clogging is one of the main problems in the operation of the AFs, but, can be overcome by a primary settler or pre-acidification step (van Lier et al., 2008).

AFFRs have several advantages compared to other high-rate anaerobic reactors. Hydraulic retention time of fixed film anaerobic reactors can be lower than 5 days which may decrease the area requirement of installations (Hamilton, 2012). AFFRs also show better stability at high organic loading rates and are durable against large toxic shock loads (Acharya et al., 2008). Large amounts of biomass can be retained within the reactor (Rao et al, 2005) which is due to the both attached and granular growth of microorganisms (Salkinoja-Salonen et al., 1983). Therefore, the sludge production is minimized (Nandy and Kaul, 2001; Kocadagistan et al., 2005). AFFR was also found to be more adaptable to different substrates than upflow anaerobic

sludge blanket reactors (UASB) (Jhung and Choi, 1995). These reactors were also reported as stable against intermittent operation with weekend breaks and also observed to recover quickly and satisfactorily after stopping the operation for four months (del Pozo et al., 2000). Moreover, AFFRs does not require any mechanical mixing. These reactors are also simple to construct (Acharya et al., 2008). Apart from these advantages, clogging may be considered as a disadvantage of AFFRs. Recirculation of the effluent and gas, supplying a relatively thin layer of immobilization media near the inlet and improving the distribution of flow in order to avoid too low velocities are the measures that can be taken against the problem of clogging (Kishore, 2010). On the other hand, a recent tracer study applied in the treatment of dairy effluent revealed that AFFRs were not clogged during operation and even the reactors were close to CSTR in flow pattern inside the reactor (Koshta et al., 2017).

3.1.1.2.2 Upflow anaerobic sludge blanket reactors

The main principle of an UASB is the treatment of wastewaters by the anaerobic granules formed during the start-up period of the reactors. The sludge with good settling properties settles at the bottom of the reactors and forms anaerobic sludge granules. These granules compose a sludge blanket (or bed) in the lower part of the reactors (Lettinga et al., 1984). The retention of the biomass within the reactor is, thus, dependent on the formation of well settleable, granular microbial biomass (O'Flaherty et al., 2006). Various physical, chemical and biological parameters such as the type of the wastewater, the operating conditions, active microbial population in the seed sludge can be effective in the formation of the granules (Singh and Singh, 2015). The selection of the pressure, low surface tension, the presence of the inorganic nuclei and readily acidifiable chemical oxygen demand can also be considered as factors effecting the granulation of the biomass (O'Flaherty et al., 2006). Some wastes may not develop granules or result in poor granular development (Tauseef et al., 2013). Even the low

concentration of substrates may result in the disintegration of the granules (Grotenhuis, 1992). UASB and AF filter designs are based on the suspended growth of the microorganisms, thus, same types of wastes are applicable for both designs (Tauseef et al., 2013).

3.1.1.2.3 Anaerobic fixed-film expanded bed (anaerobic expanded bed) and fluidized bed reactors

Extended biomass retention in the reactor is supplied by mobile biomass carriers such as sand and clay particles in both anaerobic fixed-film expanded bed (AFFEBR) and fluidized bed reactors (AFBR). The biomass carriers are lifted up in the reactor by the inlet flow. The effluent is recycled back to the inlet to provide sufficient flow rate for both lifting the carriers and providing the feed to attached biomass. The sludge is present at the lower part of the reactor in expanded bed reactors whereas it is distributed almost over the entire reactor volume in fluidized bed reactors (Lettinga et al., 1984). The wastewaters of highly soluble organic content or of suspended material which is easily biodegradable such that whey, permeate of whey, condensate of black liquor can be successfully treated by AFFEBRs and AFBRs (Tauseef et al., 2013). Operational complexity and high costs are the disadvantages of the reactors due to the expansion of the bed by incoming flow (Barber and Stuckey, 1999)

3.1.1.2.4 Anaerobic sequencing batch reactors

Anaerobic sequencing batch reactors (ASBRs) are operated discontinuously in five discrete steps: fill, react, settle, draw and idle, in a single batch type of reactor. The requirement of mixing for increasing the transfer of the substrate to microorganisms is handled by mechanical stirring or recirculation of the liquid or gas. ASBRs are simple to operate and flexible for use, and offer efficient quality control of the effluent while

high biogas yields are obtained (Tauseef et al., 2013). These reactors have channeling and clogging problems and may have poorly settleable effluents due to inadequate self-immobilization and biogas entrapped in the sludge (Mao et al., 2015). Dairy, textile, brewery, pulp mill, tannery and petrochemical industry and other effluents have been tested for the treatment in ASBRs (Tauseef et al., 2013).

3.1.1.2.5 Anaerobic baffled reactors

The design of anaerobic baffled reactors (ABRs) includes hanging and standing baffles in a pattern. The baffles make the wastewater flow upward and downward from one compartment to another while flowing through the reactor (Tauseef et al., 2013). ABRs act as a two-phase system in which acidogenesis and methanogenesis occurs at different stages, due to baffled compartmentalization of the reactor (Barber and Stuckey, 1999). (A detailed description of the two-phase systems is given in Section 3.1.1.3.3). ABRs are simple and inexpensive to construct and operate; durable to hydraulic shock loads and shows high stability to organic shocks. Uneven distribution of the inflow wastewater and the shallow reactor design to maintain convenient upward flow velocities for the liquid and the gas are the main drawbacks of the ABRs (Barber and Stuckey, 1999).

3.1.1.3 Third generation high-rate anaerobic reactors

Third generation high-rate anaerobic reactors have evolved to provide solutions for the problems associated with the operation of the second generation high-rate reactors. Clogging of the reactors, wash-out of the biomass from the reactor, insufficient mixing, poor settleability of the granules and inapplicability to certain types of wastewaters were the main driver problems in the development of third generation

high-rate anaerobic reactors. These reactors are generally modified versions or hybrids of second generation anaerobic reactors (Tauseef et al., 2013).

3.1.1.3.1 Modifications on UASB reactors

The modifications were mostly made on the UASB reactors. Expanded bed granular sludge blanket (EGSB), internal circulation reactor (IC), anaerobic mitigating blanket reactor (AMBR) and electrolysis enhanced anaerobic digestion (EEAD) are among these modifications. The granules are partially or fully expanded in EGSB reactors, thus, the mass transfer between granular sludge and wastewater is increased (Zheng et al., 2014). IC reactor was developed as a solution for the wash-out problems in conventional UASB reactors. A single IC reactor is composed of two UASB compartments on top of each other working in series (Tay et al., 2010). AMBRs require at least three compartments and mechanical mixing at multiple points to avoid clogging problems and to improve the distribution of the substrate (Tay et al., 2010). Water electrolysis inside UASB reactors creates micro-aerobic conditions in EEAD reactors which improve the hydrolysis of the organic matters, COD removal and methane production (Tartakovsky et al., 2011).

3.1.1.3.2 Hybrid anaerobic reactors

The configurations of hybrid anaerobic reactors have been developed to integrate the unique features of two or more anaerobic processes (Bajpai and Kondo, 2012). Hybrid reactors can be composed of an anaerobic filter or an anaerobic fixed film located at the upper part of an UASB (Tauseef et al., 2013). Such a configuration can minimize the limitations of both reactors in their individual use (Demirer and Chen, 2005b). The sludge bed remaining at the lower part takes the advantage of suspended biomass and act as a buffer zone against toxicity and inhibitory effects of influents. The upper part

can thus deal with relatively harmless feed with the biomass attaching on the surface of bio-filter media (Tauseef et al., 2013).

3.1.1.3.3 Multi-phase anaerobic reactors

The reaction steps of anaerobic digestion pathway are hydrolysis, acidification, acetogenesis and methanogenesis. All these reaction steps occur simultaneously in one phase reactors (Jo et al., 2018). Two phase anaerobic reactors have been developed with the aim of partitioning the hydrolysis/acidification step in the first phase and acetogenesis/methanogenesis step in the second phase regarding the different growth requirements of the microorganisms specific to each phase (Demirer and Othman, 2008; Nasir et al., 2012). Acidifying (acidogenic) bacteria has an optimum pH environment of 5.2-6.5 and the growth rate of these microorganisms is approximately 2 days. On the other hand, methanogens are slow growing microorganisms which require an environmental pH of 7.5-8.5 as an optimum growth condition (Solera et al., 2002). These two consortia differ also in terms of their nutritional requirements. Organic matters are the metabolized by acidogenics forming carbon dioxide, hydrogen and fatty acids. The simplest form of fatty acids (acetic acid) and hydrogen are required for the growth of methanogens. Additionally, acidogens and methanogens differ in their resistance to environmental stresses as well as their physiology, growth and nutrient uptake kinetics (Demirer and Chen, 2004). The operation conditions in one-phase anaerobic digesters such as pH adjustment to near neutral and the extended retention times (usually more than 20-30 days) (Jo et al., 2018) are adjusted depending on the requirements of slow growing consortium, methanogens. The acidic conditions developing in the reactor as a result of acidogenic activity may have an inhibitory effect on the growth of methanogenic organisms (Demirer and Chen, 2005a). As a consequence of the differences in their growth requirements and in reactor operation, two-phase AD processes have been developed. Two-phase reactors usually consist of two reactors operated in series. The first reactor is operated at pHs around 5-6 and at

short HRTs (less than 5 days) to favor the growth of acidogenic organisms and the second reactor is operated to dominate the methanogens (Jo et al., 2018).

Separating the reactors for each phase provides the selection and enrichment of the related bacteria within the specific phase (Demirer and Othman, 2008). The enhanced stability as a result of controlling the acidification phase and the ability to be operated at low HRTs and high OLRs compared to conventional reactors can be accounted as the advantages of the two-phase AD processes (Demirer and Chen, 2005a). Additionally, the installations of two-phase digesters can be cost-effective and smaller in size (Demirer and Chen, 2005a). Acidification in the first phase also acts as a pretreatment step for the waste before methanogenesis (Demirer and Othman, 2008). This fact gains importance especially for the AD of high solids containing wastes. Acidogenesis also results in the liquefaction of the wastes reducing the liquid to be added and the related energy requirements for heating, storing and spreading (Demirer and Chen, 2005a). Studies on the treatment of two-phase reactors are illustrated in Table 3.2.

In addition to two-phase reactors, three-phase digesters have been developed for the purpose of separating three reaction steps in the AD pathway as hydrolysis, acidogenesis and methanogenesis (Abbasi and Abbasi, 2012). Zhang et al. (2017) has recently developed a vertical three-phase digester separated into 3 chambers for high-solids containing wastes. The authors reported 24-54% more methane yield compared to one- and two-phased reactors at an organic loading rate of 10 g VS/L of food waste.

Table 3.2. The studies on two-phase anaerobic treatment of wastewaters.

Wastewater	First phase					Second phase					COD_t removal enhancement, %	Overall removal, %	Reference
	Type	T,°C	HRT	OLR, g/L.d	COD removal	Type	T,°C	HRT	OLR, g/L.d	COD removal, %			
WAS	CM	60	2,4 d		18.2-33.3%	Batch	35			34.8-55.4	26.2-49.4		Demirer and Othman, 2008
Cheese whey	UASB		2.06-4.95 d	11.7-24.6 COD	91.9-97 %	UASB		1.76-3.50 d	0.82-2.52 COD	27.2-59.6	27.2-59.6		Ergüder et al., 2001
Unscreened dairy manure	CM	35	1.25-4 d	4-30 VS		Batch	35					30.3-62.4 VS	Demirer and Chen, 2004
Unscreened dairy manure	Daily fed	35		0.96-6.02 COD		Daily fed	36		1.19-15.06 COD			30-71 COD	Demirer and Chen, 2005a
Vinasses	CSTR	55	1.7-4 d	3.79-9.17 COD	30.1-31.9%	CSTR	55	4 d	2.43-2.65 COD	61.5-71.7			Solera et al., 2002
Food waste	Daily or semi-daily fed	35	4 d	0.5 -5 COD		Daily or semi-daily fed	35	16-96 d	0.5 -5 COD			70.6-86.3 COD 78-84.4 VS	Jo et al., 2018
Sugarcane vinasse	APBR	55	7.5 h	84.2 COD	~20%	UASB ASTBR	Th	18-37 h	15-30 COD	54.8-62.9 55.-73.9		58-75.5 COD 63.2-82.6 COD	Fuess et al., 2017
Chicken manure	CSTR	37	2 d	1.9-4.67 VS		CSTR	53	10 d	1.9-4.67 VS			60-67 VS 49-52 TS	Dalkılıç and Uğurlu, 2015

WAS: waste activated sludge, CM: completely mixed, CSTR: continuously stirred tank reactor, APBR: anaerobic packed bed reactor, ASTBR: anaerobic structured bed reactor, Th: thermophilic.

3.1.2 Anaerobic fixed-film treatment of the waste streams

AFFR reactors are known to hold large biomass in the reactor (Rao et al., 2005) which considerably reduces the HRT (Nikolaeva et al., 2013). Upflow configurations may be preferred to retain more biomass in the reactor since upflow configurations are faster in biofilm development due to lower wash-out effect of the suspended biomass compared to the downflow configurations (Tritt and Kang, 2017). In spite of many different types of packing media used in AFFRs (Table 3.3), the media is required to have a large surface to volume ratio for the immobilization of the microorganisms. High porosity and higher surface area help efficient biomethanation by predomination of methanogenic organisms as well as acidogenics in bacterial biofilm (Acharya et al., 2008).

AFFRs have been previously used for the treatment of wastewaters such as distillery spent wash (Acharya et al., 2008), pharmaceutical (Nandy and Kaul, 2001; Rao et al., 2005), molasses (Jhung and Choi, 1995), cheese whey (Patel et al., 1995), dairy (Qazi et al., 2011; Nikolaeva et al., 2013), food processing (Murray and van den Berg, 1981), petrochemical based (Nel et al., 1985; Noyola et al, 1990; Patel and Madammar, 2002), slaughterhouse (del Pozo et al., 2000) wastewaters as well as various phenolic compounds (Latkar et al., 2003) (Table 3.3). 28.2-82.1% COD removal efficiency was previously obtained in the treatment of dairy wastewater using batch anaerobic fixed bed reactors. The removal efficiency was reported as increasing by decreasing the OLR from 24 to 4.4 kg COD/m^3.d with a corresponding increase in HRT from 1 to 5.5 days (Nikolaeva et al., 2013). Even if the configuration used by the authors was named as anaerobic fixed bed reactor, the media employed in the reactor aimed at immobilization of the microorganisms on the surface and thus could be considered as an AFFR. AFFRs were also applied to high solids containing wastewaters such as distillery spent wash having a TS concentration in the range of 110000-190000 mg/L without any dilution. 80% COD removal was obtained at an OLR of 6.2 kg COD/m^3.d

at a HRT of 30 days (Acharya et al., 2008). COD treatment efficiency was in the range of 60-70% in the treatment of the wastewater from a bulk drug industry at a HRT and an OLR of 1.7 days and 10 kg COD/m^3.d, respectively. HRT was not further reduced below 1.7 days which was probably due to comparably longer time requirement for the hydrolysis of the particulate matters (Rao et al., 2005). Anaerobic reactors can be operated with wastewaters containing high solids content but only at reduced organic loading rates (Lettinga et al., 1984). AFFR treatment was applied as a pre-treatment step for high-strength wastes in various studies (del Pozo et al., 2000; Nandy and Kaul, 2001). Besides the treatment of the wastes, AFFRs has been observed to yield a methane production close to the theoretical one (Hamoda and Kennedy, 1987; Noyola et al., 1990; Rao et al., 2005). 0.36 L CH_4/g COD_r yield could be obtained in the treatment of synthetic wastewater (Michaud et al., 2002). The treatment of wastewater from a bulk drug industry also yielded 0.3-0.5 L_{biogas}/ g COD_r (Rao et al., 2005)

The acclimation of the anaerobic seed sludge to the wastewater is the first step in the start-up of the AFFRs. To this purpose, anaerobic seed sludge is fed with an easily utilizable carbon source such as glucose. This carbon source is gradually replaced by the wastewater to be applied to acclimate the microorganisms to wastewater (Nel et al., 1985; Rao et al., 2005). Another approach to acclimate the microorganisms is to feed the microorganisms with diluted wastewater and gradually increase the organic loading rate by decreasing the dilution (Rao et al., 2005; Nikolaeva et al., 2013). Using diluted wastewater in acclimation previously reported to provide 30 days of start-up period for an AFFR to treat wastewater from a bulk drug industry (Rao et al., 2005). Incremental increase in the organic load is a common practice during the start-up of the high-rate anaerobic reactors (de Lemos Chernicharo, 2007) to prevent overloading (Escudié et al., 2011). The time required for the start-up of an anaerobic reactor can be reduced by using anaerobic seed sludge that is previously acclimated to the wastewater (de Lemos Chernicharo, 2007).

The organic loading rate can also be progressively increased during the operation of the AFFRs to test the durability to high OLRs. The progressive increase of OLR from 8 to 30 kg COD/m^3.day in the treatment of poultry slaughterhouse wastewater using upflow AFFR resulted in a decrease of COD removal efficiency from 85-95% to 55-75% but without any sign of destabilization of the reactors (del Pozo et al., 2000). Additionally, being common in most of industries, a shock load, which carried 50 kg COD/m^3.day, was applied for 12 hours with an HRT of 3 h in the same study. This application ended up with a decrease in the COD removal efficiency from 72 to 58% and the reactor could be recovered after 8 days into its original working performance. On the other hand, destabilization of the reactor was not experienced as tracked by alkalinity ratios and pH levels (del Pozo et al., 2000). Nandy and Kaul (2001) pointed out that AFFRs were durable against a hydraulic overloading that is the twice of the influent flow and could be stabilized back in 5 days during the treatment of herbal-based pharmaceutical wastewater. Not the OLR but the HRT had a great influence in the process instability in AFFRs (del Pozo et al., 2000). Hydraulic overloading created by the application of excessively reduced HRTs may result in the sloughing of the biofilm attached on the immobilization medium and washout of the biomass from the reactor (Chua et al., 1997). On the other hand, AFFRs observed to be slightly affected from 2-5 times hydraulic overloading in terms of COD removal efficiency (Chua et al., 1997). The authors reported that the reactors could recover after the reactor failure by the application of 10 times hydraulic shock load. The process stability under the operation with lower HRTs could also be maintained during the intermittent operation of the AFFRs with weekend breaks (del Pozo et al., 2000). The preservation of the stability was attributed to the decreased volatile fatty acids concentration during the breaks (del Pozo et al., 2000). A maximum 5 days with no-feed period was reported to be applicable in AFFR treatment of herbal-based pharmaceutical wastewater as a demonstration of intermittent breaks. The increase in the no-feed period beyond 10 days required more than 7 days to regain the stability of the reactor (Nandy and Kaul, 2001). AFFR reactors were additionally reported as being more adaptable to the changes in feedstock characteristics than the UASB reactors (Jhung and Choi, 1995).

Table 3.3. The studies on the anaerobic treatment of various waste streams using anaerobic fixed film reactors.

Reactor type	Wastewater	Support material	Temp, °C	HRT	OLR	Biogas	COD removal	Reference
AFFR (upflow)	Distillery spent wash WW	*Charcoal *Coconut coir *Nylon fibers	37	6-30 d	6.2-31 kg COD/m^3.d	7.25 m^3/m^3.d	80 %	Acharya et al., 2008
AFFR/ AFBR	Synthetic WW containing glucose as carbon source	Mineral granules mainly composed of silica and alumina	35	1.5-6.4d	1.1-6 kg COD/m^3.d	0.05-0.36 m^3 CH_4/kg COD_r		Michaud et al., 2002
AFFR (upflow)	a bulk drug industry WW	PVC pall rings	35	Min 1.7 d	0.5-11 kg COD/m^3.d	0.25 m^3/kg COD_r (58-62% CH_4 content) 0.3-0.5 m^3/kg COD_r (65-70% CH_4 content)	35-60% (start-up) 47-66% (operation)	Rao et al., 2005
AFFBR (upflow)	Dairy WW	Hybrid material of tire rubber and zeolite	22-26	1-5.5 d	4.4-24 kg COD/m^3.d	0.07-0.18 m^3 CH_4/kg COD_a	28.2-82.1%	Nikolaeva et al., 2013
AFFR (upflow, downflow)	Poultry slaughterhouse WW	Vertical corrugated PVC tubes	35	6 h	2-12 kg COD/m^3.d	-	approx. 30-90%	del Pozo et al., 2000
AFFR (upflow)	Herbal-based pharmaceutical WW	Nylon scrubber	35	20 h- 6 d	1 - 48 kg COD/m^3.d	*0.42-15.10 m^3 biogas/ m^3.d *0.200-0.350 m^3 CH_4/kg COD_r *51.5-66.5% CH_4 content	36.5-98%	Nandy and Kaul, 2001

Table 3.3. The studies on the anaerobic treatment of various waste streams using anaerobic fixed film reactors.

Reactor type	Wastewater	Support material	Temp, °C	HRT	OLR	Biogas	COD removal	Reference
AFFR (downflow)	Acetic acid based synthetic WW	Oriented needle-punched polyester	35	0.4-5.7 d	1.3-17 kg COD/m^3.d	50-86% methane content	*62.9-98.1% (soluble) *56.8-97.0% (total)	Hamoda and Kennedy, 1987
Baffled AFFR AFFFBR (upflow)	Synthetic WW	High density polyethylene	25	12-36 h	1.387-4.162 g COD/m^2.d	0.21-0.44 m^3 CH_4/kg COD_r 0.22-0.42 m^3 CH_4/kg COD_r	67-90% 58-87%	Yousefzadeh et al., 2017
AFFR (downflow)	*Molasses waste *Synthetic volatile acids wastes	Kock plastic media	35	0.3-30 d	0.75-21.4 kg COD/m^3.d	0.30-0.74 m^3/kg COD_r	49-95%	Jhung and Choi, 1995
AFFR (downflow)	Petrochemical effluent	Inert cylindrical polyethylene carrier	35	3d	4.26 kg COD/m^3.d	0.296-0.355 m^3/kg COD_r	69-93.5% with trace element supplementation	Nel et al., 1985

Table 3.3. The studies on the anaerobic treatment of various waste streams using anaerobic fixed film reactors.

Reactor type	Wastewater	Support material	Temp, °C	HRT	OLR	Biogas	COD removal	Reference
AFFR (upflow)	Cheese whey	Charcoal	37	1-5 d	-	6.0-6.7 m^3/d. m^3 digester (64-72% CH_4 content)	76.6-81.5%	Patel et al., 1995
		Brick pieces, pumice stone, gravel, PVC pieces		1 d	-	3.8-5.4 m^3/d. m^3 digester (64-68% CH_4 content)	67.5-73.7%	
AFFR (batch, repeated batch)	Dairy WW	Foam cubes, bamboo rings, fire bricks, PVC rings, gravels	35	1-8 d	2-21 kg COD/m^3.d		96% maximum	Qazi et al., 2011
AFFR (upflow)	Simulated dairy WW	Fire expanded clay spheres	28-30	5d	1800 mg COD/d	0.25 m^3/kg COD_r	98.1%	Chua et al., 1997
AFFR (upflow)	Acidic petrochemical WW	Bone charcoal	25	6-15 d	3.6-9.0 kg COD/m^3.d	0.20-0.33 m^3 CH_4/kg COD_a.d	95-98%	Patel and Madamwar, 2002
			37	1.5-15 d	3.6-27.2 kg COD/m^3.d	0.37-0.45 m^3 CH_4/kg COD_a.d	45-98%	
			45	3-15 d	3.6-18.1 kg COD/m^3.d	0.33-0.41 m^3 CH_4/kg COD_a.d	89-96%	
			55	2.5-15 d	3.6-21.7 kg COD/m^3.d	0.11-0.66 m^3 CH_4/kg COD_a.d	77-98%	

Table 3.3. The studies on the anaerobic treatment of various waste streams using anaerobic fixed film reactors.

Reactor type	Wastewater	Support material	Temp, °C	HRT	OLR	Biogas	COD removal	Reference
AFFR (downflow)	Terephthalic acid plant WW	PVC tubes	33	3.4, 5.8 d	1.08, 1.89 kg COD/m^3.d	0.36 Nm^3 CH_4/kg COD_r	74.5-77.4%	Noyola et al., 1990
AFFR (downflow)	Food processing waste	Inert needle-punched polyester	-	0.38-1.0	-	0.0137-0.280 m^3/d	-	Murray and van den Berg, 1981
AFFFBR (upflow)	resorcinol	Basalt chips	33-37	3-6 h	1.91-12.34 kg COD/m^3.d	110-515 mL/d	77.59-95.34%	Latkar et al., 2003
	catechol				0.88-13.88 kg COD/m^3.d	46-305 mL/d	56.40-90.81%	
	hydroquinone				1.88-12.05 kg COD/m^3.d	65-290 mL/d	29.15-82.75%	
FBR	Slaughterhouse wastewater	Bamboo ring		2-8 d	1-4 kg COD/m^3.d	0.35-1.5 m^3 CH_4/ m^3.d 72-75 % CH_4	74-95 %	Tritt and Kang, 2017
AFFR (upflow)	Lactate containing synthetic substrate	polypropylene pall rings	20	20 h	1-12 kg ThOD/m^3.d	-	-	El Bayoumy et al., 1999

*FBR: Anaerobic fixed bed reactor, AFFBR: Anaerobic fixed film bed, AFFFBR: anaerobic fixed film fixed bed reactor, COD_a: COD added, ThOD: theoretical oxygen demand.

3.2 Materials and Methods

3.2.1 Liquid digestate

Digestate used in high-rate AD experiments was selected based on the RBP test applied on six different digestates (Chapter 2). The digestate of a manure mixture of 90% laying hen and 10% cattle manure (digestate 2 in Chapter 2) which had the highest RBY was selected for further processing. It was settled for one day before used. Liquid digestate (supernatant) was collected after settling and was then used as the feed for high-rate anaerobic fixed-film reactors. Digestate (before settling) and liquid digestate were characterized for the parameters given in Table 3.4.

3.2.2 Anaerobic seed sludge

Anaerobic seed sludge and digestate were obtained from the same anaerobic digester. Anaerobic seed sludge was taken from the inside of the digester and concentrated by settling for 1 day to obtain a dense culture of anaerobic microorganisms. Settled portion was sieved from a 1 mm screen to remove large particles. Each AFFR was inoculated with 500 mL (517.5 g) of settled and sieved anaerobic seed sludge (will be referred as seed sludge) which corresponded to 63% and 57.5% of effective volume of R1 and R2, respectively.

Table 3.4. Seed sludge and digestate characterization.

Parameter	Seed sludge	Digestate	
		Before settling	After settling (liquid digestate)
pH	8.4	7.85	8.01
Density, kg/m^3	1,035 ± 5.37	1,025 ± 1.13	997 ± 5.27
TS, mg/L	88,455 ± 425	57,670 ± 240	40,138 ± 400
VS, mg/L	38,065 ± 125	34,625 ± 135	22,640 ± 294
Solid content, %	8.54 ± 0.003	5.62 ± 0.017	4.03 ± 0.022
COD_t, mg/L	74,991 ± 1,458	76,795 ± 548	65,066 ± 1,527
COD_s, mg/L	19,460 ± 449	33,006 ± 196	40,304 ± 1051
TKN, mg/L	12,236 ± 0	7,092 ± 85	6,977 ± 189
NH_4^+- N, mg/L	7,434 ± 42	6,339 ± 53	6,637 ± 140
TP, mg/L	18,992 ± 292	8,242 ± 42	7,180 ± 100
DRP, mg/L	253 ± 0.8	157 ± 2.1	151 ± 0.2
Alkalinity, mg/L as $CaCO_3$	23,275 ± 247	21,406 ± 472	19,248 ± 116
NH_4^+- N/TKN	61 %	89 %	95 %

3.2.3 Analytical methods

TS, VS, COD_t, COD_s, TKN, NH_4^+-N, TP and DRP were analyzed as described in the Standard Methods (APHA, 2005). Samples were first filtered from 0.45 μm pore-sized filters for COD_s and DRP measurement. Alkalinity was measured by following the procedure described by Ripley et al. (1986). pH was monitored by Oakton pH/CON 450 pH meter.

Methane, carbon dioxide and nitrogen contents in biogas were determined using Agilent Technologies 6890N Gas Chromatograph (Agilent Technologies, California, USA) with thermal conductivity detector (TCD). The device was equipped with a HP-Plot Q capillary column with dimensions of 30.0 m x 530 μm x 40.0 μm. Gas contents were measured three times at 2-3-day intervals in the last cycle when steady-state COD_t removal was achieved. Each measurement was done in two replicates (except pH) and the related measurements are given in average with standard deviations.

3.2.4 Experimental setup

Anaerobic fixed-film reactors were made of glass in cylindrical shape (Figure 3.1). The first reactor (R1) had 793 mL of effective volume and 56 cm of height. The second reactor (R2) had 870 mL of effective volume and 55.5 cm of height. 150 pieces of polypropylene biomass immobilization (bio-filter) media (500 m^2/m^3 efficient surface and 0.96-0.98 g/cm^3 density) (Figure 3.2) were fixed to a spiral cord which was extended through the reactor in order to prevent moving of the media inside the reactors. These types of immobilization media have been commonly applied in moving bed biofilm reactors (Yeshanew et al., 2016). The media was filled up to approximately 2/3 of the effective height of the reactors. Total volume occupied by biomass immobilization media was 61.5 ± 1.5 mL within the reactors which corresponded to 0.41 ± 0.01 mL of volume per piece of medium.

Reactors were equipped with a gas-liquid-solid (GLS) separator, which was located 1-2 mm close to the deflected surface of the reactors (Figure 3.1). Deflection of reactor surface enabled the direction of biogas produced into GLS separator. Gas flow was then transmitted into a liquid displacement setting (gasmeter) where it was quantified in terms of volume. Biogas volume was recorded daily. The solution used in gasmeter contained 270 g/L of salt and was acidified to pH 2 using concentrated H_2SO_4 (WRAP, 2010). Reactors were purged with nitrogen for half an hour before start-up to ensure the strict anaerobic conditions developed within the reactor. Two AFFRs were operated in parallel.

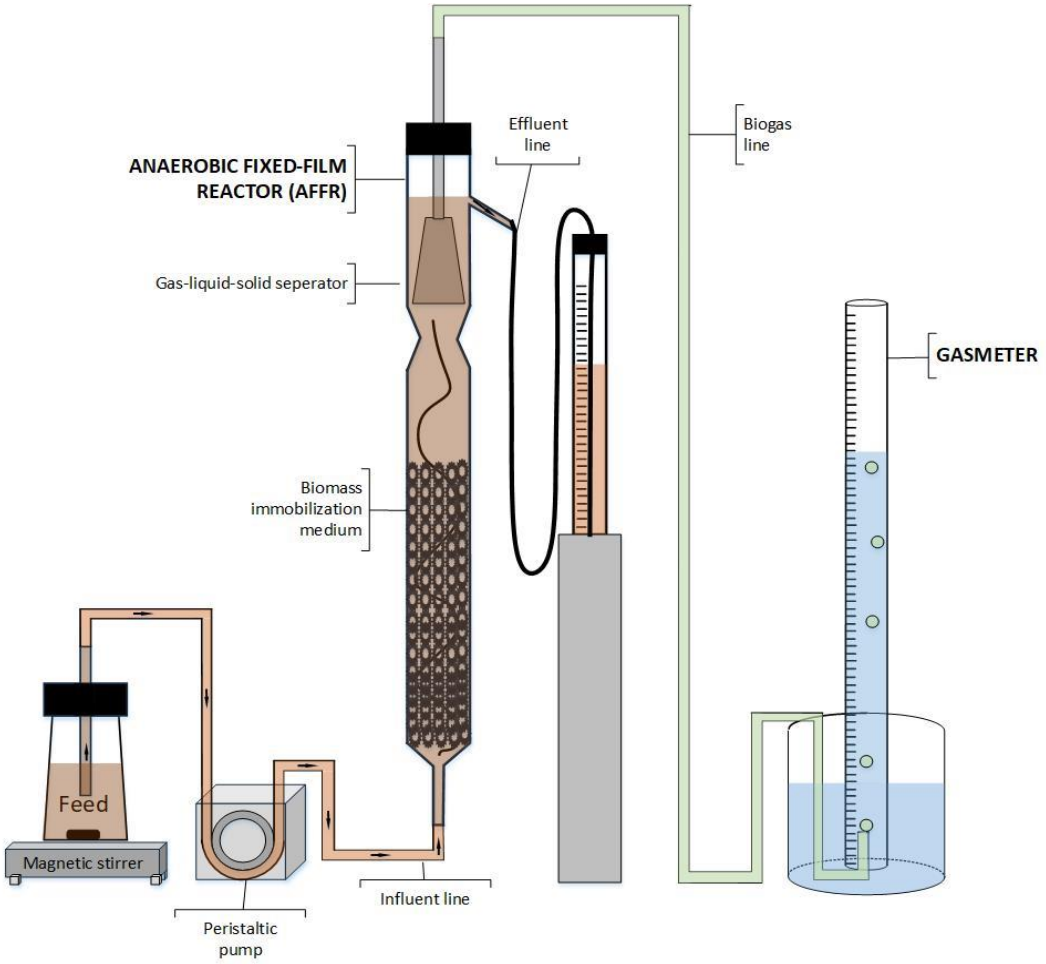


Figure 3.1. Experimental setup of AFFRs.

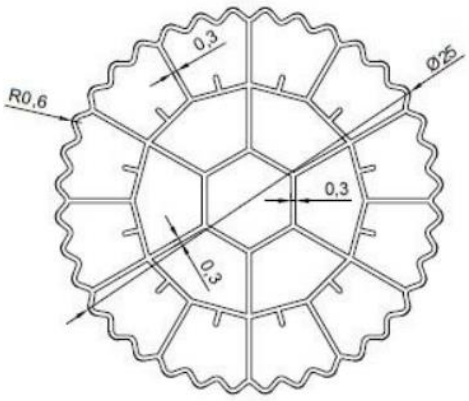


Figure 3.2. Biomass immobilization medium.

3.2.5 Operation of anaerobic fixed-film reactors

The reactors were continuously operated in a constant temperature room ($35 \pm 2^{o}C$) by feeding the liquid digestate using a peristaltic pump (Masterflex) in upflow mode. The feed was refreshed daily. AFFRs were operated for 81 days in six cycles which corresponded to different operational parameters (Table 3.5). Total duration of the cycles 1-6 was 7, 12, 15, 15, 15 and 17 days, respectively. The organic loading rate was incrementally increased at the end of each cycle either by decreasing the dilution of liquid digestate or by increasing the inflow rate. The organic loading rate was doubled by decreasing the dilution of liquid digestate between the cycles 1 and 3. The flow rate of the feed and hydraulic retention time within the reactors were not altered during the first three cycles. Organic loading rate was then increased by increasing the flow rate of the feed at 0.1 mL/min intervals in the last three cycles (4-6), and the dilution ratio for liquid digestate was kept constant at 1/3. The difference between the organic loading rates of R1 and R2 (Table 3.5) was due to the different effective volumes of the reactors used. All dilutions were made with tap water. The decision on increasing the organic loading rate was made based on the criteria set about the COD_t removal efficiency. The COD_t content of the digestate could be reduced by 37-60% in the RBP test (Chapter 2). The organic loading rate was increased when more than 50% COD_t removal was achieved in two consecutive measurements. At least 60% COD or BOD reduction was advised to increase the organic loading rate at the start-up of anaerobic reactors (de Lemos Chernicharo, 2007). However, 60% represented the uppermost COD_t removal efficiency from the digestate in the RBP test applied at the end of 70 days of incubation for the digestate and might have not be achieved. Thus, the 50% COD_t removal criteria was selected as an approximate mid-range COD_t removal efficiency. The removal efficiencies were calculated based on the removed amounts of constituents with respect to the influent concentrations.

Table 3.5. Operational Parameters (as calculated) of AFFRs.

Reactor	Cycle	Time, days	Flow rate, mL/min	HRT, days	Organic loading rate, g/L.d	Dilution ratio applied
R1	1	0-7	0.1	5.09	1.07	1/12
	2	7-19	0.1	5.09	2.13	1/6
	3	19-34	0.1	5.09	4.28	1/3
	4	34-49	0.2	2.53	8.56	1/3
	5	49-64	0.3	1.69	12.83	1/3
	6	64-81	0.4	1.27	17.11	1/3
R2	1	0-7	0.1	5.64	0.96	1/12
	2	7-19	0.1	5.64	1.92	1/6
	3	19-34	0.1	5.64	3.86	1/3
	4	34-49	0.2	2.81	7.71	1/3
	5	49-64	0.3	1.88	11.57	1/3
	6	64-81	0.4	1.41	15.42	1/3

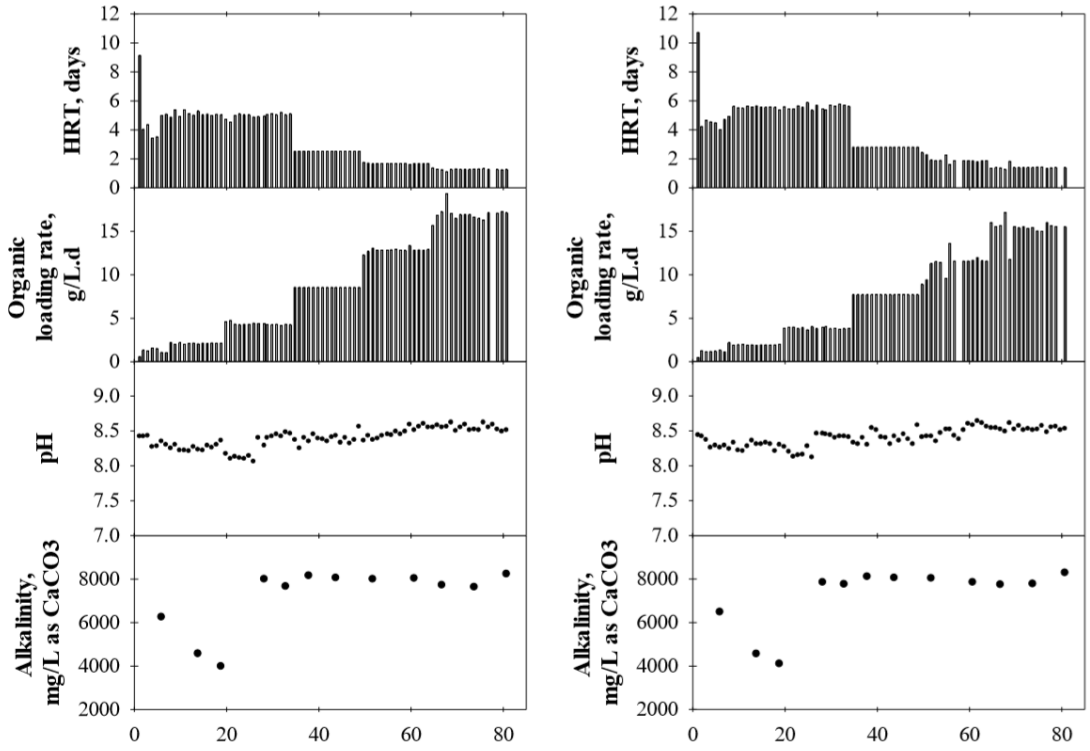
3.3 Results and Discussion

3.3.1 pH and alkalinity

Liquid digestate sample had a pH of 8.01. AD process resulted in an increase in the effluent pH to 8.39 ± 0.14 and 8.41 ± 0.13 for R1 and R2, respectively (Figure 3.3). These values stayed almost stable during the course of the operation. The increase in the pH during anaerobic digestion can be attributed to methanogenic activity since hydrogen and H_3O^+ ions are consumed during methanogenesis resulting in the increase in pH and alkalinity (Acharya et al., 2008). The pH of the AFFR effluents was higher than the optimum pH range for AD. The optimum pH for AD processes was previously reported to be in the range of 6.8-7.2, and the process can tolerate a pH range of 6.5-8.0 (Cioabla et al., 2012). Highly alkaline pHs may result in the disintegration of microbial granules and consequently failure of the process (Franke-Whittle et al., 2014). However, the effluent pH was stable during the operation of the reactors at around 8.40 and the methane composition of the biogas was at appreciable levels (70.7-80.3 % of biogas composition). In addition, the ratio of intermediate to partial alkalinity (IA/PA) ranged between 0.05-0.16 for R1 and 0.06-0.16 for R2 during the

operation. The IA/PA values lower than 0.3 indicated the operational stability of the AFFRs (Alcaraz-Gonzalez et al., 2015). IA/PA is an indicator of VFA accumulation within the reactor which is encountered before pH drop and the failure of the reactor (Monhonval, 2015). The increase in pH during digestion was concluded not to affect the process stability based on the almost stable pH values of the effluent and IA/PA values less than 0.3.

The liquid digestate was initially characterized as having high alkalinity (19,248 ± 116 mg/L as $CaCO_3$) (Table 3.4). The inflow alkalinities in cycles 1-6 (Table 3.5) corresponded to 1604, 3208 and 6416 mg/L as $CaCO_3$ for the dilution ratios of 1/12, 1/6 and 1/3, respectively. The alkalinity of R1 and R2 effluent remained on the range of 7943 ± 206 and 7940 ± 177 mg/L as $CaCO_3$ (Figure 3.3) for the cycles 3-6 when the inflow alkalinity was 6416 mg/L as $CaCO_3$ (Table 3.5). The increase in alkalinity in cycles 3-6 during digestion of the liquid digestate can be attributed to methanogenic activity as previously mentioned. The alkalinity level of 2000-4000 mg/L as $CaCO_3$ is typically required in AD processes to be able to keep the pH at neutral levels (Tchobanoglous et al., 2003) to ensure the stability of the process (Córdoba et al., 2017). The stability of the process was therefore preserved for approximately 53 days for 1/3 diluted liquid digestate indicating sufficient buffering capacity of the liquid digestate against acidic releases.



(a) (b)

(Data points for HRT, OLR and pH are given for a single measurement, data points for alkalinity are given as the average of two measurements)

Figure 3.3. The change of HRT, OLR and the effluent pH and alkalinity (a) R1 and (b) R2.

3.3.2 Hydraulic retention time and organic loading rate

The reactors were operated in six cycles. Each AFFR was initiated with 0.1 mL/min inflow rate which corresponded to an HRT of 5.09 and 5.64 days for R1 and R2, respectively (Table 3.5). R1 had 1.07 g/L.d and R2 had 0.96 g/L.d OLR at the initial cycle. The initial organic loading rate applied was consistent with the ones used in the studies using AFFR (Table 3.3). OLR applied ultimately reached to 17.11 g/L.d and 15.42 g/L.d at 6^{th} cycle for R1 and R2, respectively, which was slightly above to the typical OLR range of 10-15 g/L.d reported for anaerobic fixed-film reactors (Hall, 1992).

HRT was reduced from 5.09 to 1.27 days for R1 and from 5.64 to 1.41 days for R2 at the end of 81 days. The minimum HRTs applied at the 6th cycle of operation (1.27-1.41 days) resulted in slightly lower COD_t removal efficiencies at 6th cycle (57-63% for R1 and 56-62% for R2) compared to the previous cycle (52-63% for R1 and 64-66% for R2). COD_t removal efficiencies indicated that further HRT reduction may end up with lower COD_t removal efficiencies. Therefore, the HRTs applied at the 6th cycle was considered as the least applicable HRT in the operation of AFFRs.

1.27-1.41 days of HRTs applied in high-rate treatment of the digestate corresponded a moderate minimum HRT application in the treatment using AFFR. Variable HRTs were previously applied for the treatment of different wastewaters in AFFRs with a minimum ranging between 6 hours to 6 days (Table 3.3). Hydraulic retention time is a biodegradability or recalcitrance dependent parameter. Longer retention times are required to decompose comparably difficult to degrade feedstocks (Kim et al., 2018). Thus, wastewaters having comparably better biodegradability can be expected to be treated in a shorter HRT in similar reactor configurations. The digestate can therefore be evaluated as having a moderate biodegradability compared to that of the feedstocks such as wastewaters of slaughterhouse, distillery spent wash, bulk drug industry, herbal-based pharmaceutical and molasses used in previous studies (Table 3.3). This digestate also found to be moderately biodegradable compared to the other digestates in the RBP test (Chapter 2).

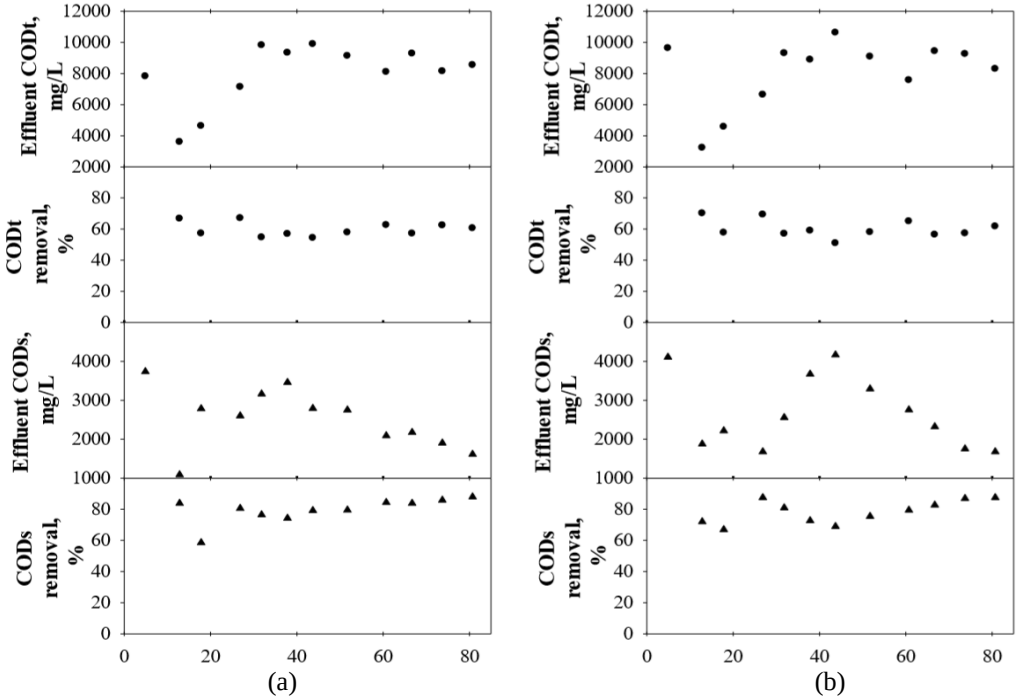
3.3.3 The changes in total and soluble chemical oxygen demand concentrations

COD content of the digestate in the influent and effluent was evaluated both in terms of total and soluble COD. COD_t reduction was approximately preserved during the cycles 2-4 for both reactors (53-57% for R1 and 51-59% for R2) considering the end-cycle measurements even though the OLR was increased from 2.13 to 8.56 g/L.d for

R1 and from 1.92 to 7.71 g/L.d for R2 (Table 3.5). COD_t reduction was increased with the increase in flow rate from 0.2 to 0.3 mL/min in cycle 5 (62-63 and 64-66 % for R1 and R2, respectively). Additional increase in the flow rate to 0.4 mL/min resulted in a slight reduction in COD_t removal efficiencies in cycle 6 in the first measurement (57-57% for R1 and 55-56% for R2). However, these removal efficiencies were observed in the application of 1.33 fold higher organic loading rate in cycle 6 (17.11 g/L.d for R1 and 15.42 g/L.d for R2) compared to those of cycle 5 (12.83 g/L.d for R1 and 11.57 g/L.d for R2) (Table 3.5). Thus, the optimum loading rate was achieved at the 6^{th} cycle. Since the change in COD_t removal between 5^{th} and 6^{th} cycle was not significant, the higher OLRs applied can be preferred to further decrease the footprint of installations. OLR was not further increased to avoid the wash-out of the microorganisms by the increase in the flow rate.

The steady-state COD_t removal at the sixth cycle was achieved between 74 and 81 days of operation (Table 3.6 and Figure 3.4) which corresponded a COD_t removal efficiency of 61.5±1.12 and 59.5±2.5%. The OLR was 17.11 and 15.42 g COD/L.d and the HRT was 1.27 and 1.41 days for R1 and R2, respectively, under steady-state. These COD_t removal efficiencies corresponded to the reduction of 13137 – 13538 and 12429 – 13389 mg/L COD_t from the 1/3 diluted liquid digestate for R1 and R2, respectively. Same digestate (before settling, not the liquid portion) could be treated by 35-75% and 37-60% (COD_t removals) with and without nutrient supplementation in 70 days using anaerobic batch reactors (Chapter 2). The COD_t removal efficiency of the liquid digestate using AFFRs (56-63%) was representative of the batch test without nutrient supplementation (37-60 %) (Chapter 2, Section 2.3.7.2). The batch RBP test applied can be resembled by a hydraulic retention time of 70-days. Therefore, similar COD_t removals were attained using AFFRs, but, in a comparably much shorter retention time (1.27 and 1.41 days for R1 and R2, respectively). It is therefore possible to treat more digestate in a relatively short time using AFFRs which in turn decreases the required footprint of the plant aiming anaerobic treatment of the digestates.

COD_t reduction efficiencies obtained in this study were found to be comparable and higher than several studies employing anaerobic high-rate treatment. Nikolaeva et al. (2013) previously used semi-continuous anaerobic fixed film bed reactor for the treatment of dairy wastewater and had a COD removal efficiency of 46.2% at 12 g/L.d OLR for an HRT of 2 days. When the authors increased the OLR to 24 g/L.d (HRT of 1 d), they observed a lower COD removal (28.2%). Even though COD_t and COD_s concentrations of the pre-digested chicken waste (lab-scale) were lowered by 1/25 dilution ratio to be convenient for anammox process, Yangin-Gomec et al. (2017) found 63 % COD_t reduction (initial COD_t of 807 mg/L) using anammox UASB which was comparable to the one obtained in this study (57-63%). However, COD_s reduction obtained by Yangin-Gomec et al. (2017) was very low (26% at an initial concentration of 295 mg/L) which may be due to high level pre-degradation of chicken manure under laboratory conditions. Digestate used in this experiment was taken from a full-scale plant which retained a high portion of organics that could further be decomposed (Chapter 2).



(Data points for alkalinity are given as the average of two measurements)

Figure 3.4. The change of COD_t and COD_s concentrations (a) R1 and (b) R2.

Table 3.6. The influent and effluent COD_t concentrations and the corresponding removal efficiencies.

Cycle	Time, days	R1				R2			
		Influent COD_t, mg/L	Effluent COD_t, mg/L	COD_t Removed, mg/L	COD_t Removal, %	Influent COD_t, mg/L	Effluent COD_t, mg/L	COD_t Removed, mg/L	COD_t Removal, %
1	**5**	**5422**	**7827±117**	**-2405±117**	**(-47)-(-42)**	**5422**	**9638±234**	**-4216±234**	**(-82)-(-73)**
2	13	10844	3607±50	7238±50	(66)-(67)	10844	3236±124	7609±124	(69)-(71)
2	**18**	**10844**	**4640±0**	**6204±0**	**(57)-(57)**	**10844**	**4582±116**	**6262±116**	**(57)-(59)**
3	27	21689	7139±74	14550±74	(67)-(67)	21689	6645±25	15044±25	(69)-(69)
3	**32**	**21689**	**9828±0**	**11861±0**	**(55)-(55)**	**21689**	**9310±230**	**12378±230**	**(56)-(58)**
4	38	21689	9338±49	12351±49	(57)-(57)	21689	8893±99	12796±99	(59)-(59)
4	**44**	**21689**	**9898±283**	**11791±283**	**(53)-(56)**	**21689**	**10633±0**	**11055±0**	**(51)-(51)**
5	52	21689	9141±50	12549±50	(58)-(58)	21689	9091±198	12598±198	(57)-(59)
5	**61**	**21689**	**8104±119**	**13586±119**	**(62)-(63)**	**21689**	**7581±119**	**14108±119**	**(64)-(66)**
6	67	21689	9289±50	12401±50	(57)-(57)	21689	9437±0	12252±0	(56)-(56)
6	74	21689	8152±149	13538±149	(62)-(63)	21689	9259±0	12429±0	(57)-(57)
6	81	21689	8552±118	13138±118	(60)-(61)	21689	8300±0	13389±0	(62)-(62)

Note: The bold numbers indicate the measurements at end of the cycle.

In spite of the preserved COD_t reduction during the cycles 2-4, COD_s reduction had a general increasing trend at each increase in the organic loading rate (Table 3.7 and Figure 3.4). COD_s removal efficiency increased from 58-59 % (cycle 2) to 84-88 % (cycle 6) for R1 and from 67-67 % (cycle 2) to 79-88 % (cycle 6) for R2. COD_s removal at the 6^{th} cycle was observed to reached to steady-state between 74 and 81^{th} day of operation corresponding to a removal efficiency of 86-88% for both of the reactors (Table 3.7). Approximated to 87%, the COD_s removal obtained in this study was found to be comparable to the one obtained in the treatment of acetic acid based synthetic wastewater (83% COD_s removal) using a downflow AFFR at a similar HRT and OLR (1.3 days and 17.10 g COD/L.d, respectively) (Hamoda and Kennedy, 1987).

The inflow COD_t and COD_s concentrations of the liquid digestate applied to AFFRs at 1/3 dilution were 21689 and 13435 mg/L, respectively, representing a COD_s/COD_t ratio of 62%. The steady-state COD_s reduction thus corresponded to COD_s removals of 11534-11822 and 11682-11755 mg/L for R1 and R2, respectively (Table 3.7). COD_s concentration can also be expected to increase during the decomposition of organic matters as the removal of TS and VS suggested (Section 3.3.5). The complex organic compounds are solubilized by fermentative bacteria (de Lemos Chernicharo, 2007) which would result in the increased COD_s concentration. Therefore, COD_s degradation could be speculated to be higher when the solubilized COD from the organic content of the digestate is considered. The ratio of COD_s/COD_t of the removed COD_t ranged between 85-90% for R1 and 88-94% for R2, respectively. Thus, COD_t removal was mainly due to its soluble content. This result indicated the fact that high-rate treatment of the digestate can be used to further degrade the COD_s content to readily extract the associated biogas amounts. It is therefore advisable to test the COD_s content of the digestate as well as COD_t for the application of high- rate processes as a further treatment option.

Table 3.7. The influent and effluent COD_s concentrations and the corresponding removal efficiencies.

Cycle	Time, days	R1				R2			
		Influent COD_s, mg/L	Effluent COD_s, mg/L	COD_s Removed, mg/L	COD_s Removal, %	Influent COD_s, mg/L	Effluent COD_s, mg/L	COD_s Removed, mg/L	COD_s Removal, %
1	**5**	**3359**	**3738±350**	**-380±351**	**(-22)-(-1)**	**3359**	**4108±58**	**-750±59**	**(-24)-(-21)**
2	13	6717	1087±99	5631±99	(82)-(85)	6717	1877±33	4840±33	(72)-(73)
2	**18**	**6717**	**2784±29**	**3933±29**	**(58)-(59)**	**6717**	**2219±15**	**4499±15**	**(67)-(67)**
3	27	13435	2602±33	10833±33	(80)-(81)	13435	1680±33	11755±33	(87)-(88)
3	**32**	**13435**	**3161±0**	**10274±0**	**(76)-(76)**	**13435**	**2557±86**	**10877±86**	**(80)-(82)**
4	38	13435	3458±33	9976±33	(74)-(75)	13435	3673±82	9762±82	(72)-(73)
4	**44**	**13435**	**2790±75**	**10645±76**	**(79)-(80)**	**13435**	**4167±94**	**9268±94**	**(68)-(70)**
5	52	13435	2750±16	10685±17	(79)-(80)	13435	3294±198	10141±198	(74)-(77)
5	**61**	**13435**	**2091±48**	**11344±48**	**(84)-(85)**	**13435**	**2757±24**	**10678±24**	**(79)-(80)**
6	67	13435	2174±33	11261±33	(84)-(84)	13435	2322±16	11113±17	(83)-(83)
6	74	13435	1901±17	11534±17	(86)-(86)	13435	1753±99	11682±99	(86)-(88)
6	81	13435	1613±67	11822±67	(87)-(88)	13435	1680±101	11755±101	(87)-(88)

Note: The bold numbers indicate the measurements at end of the cycle.

3.3.4 The changes in nutrient concentrations

NH_4^+-N, TKN, TP and DRP concentrations (Figure 3.5) were analyzed to evaluate the removal of nutrients from liquid digestate using AFFRs.

3.3.4.1 Ammonium and total kjeldahl nitrogen concentrations

Influent NH_4^+-N concentration of the AFFRs varied between 553-2212 mg/L through the cycles 1-6 (Table 3.8). Total ammonia nitrogen (TAN) levels of 1700-1800 mg/L can have inhibitory effect with unacclimated inoculum but if the inoculum is acclimated, inhibitory TAN concentrations can be up to 5000 mg/L (Yenigün and Demirel, 2013). The inflow carried a NH_4^+-N concentration of 1106 mg/L (1422 mg/L NH_4^+) for 12 days at the second cycle which was below the inhibitory concentration of 1700-1800 mg/L. The organic load of the inflow was doubled by decreasing the dilution ratio of the liquid digestate which also resulted in the doubling of NH_4^+-N concentration after 12 days. The stability of the reactor thereafter indicated no inhibition arising from the increase of the inflow ammonium content relying on the pH, alkalinity (Section 3.3.1) and biogas measurements (Section 3.3.7). Lower NH_4^+-N concentrations applied during the first two cycles may have provided the acclimation of the microorganisms to high ammonium concentrations.

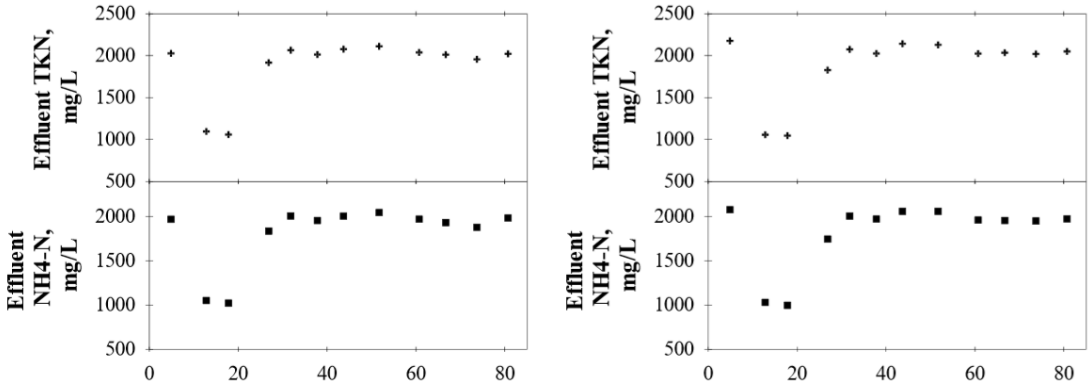
The ammonification (NH_4^+-N/TKN) of the digestate before settling was found to be 89 % (Table 3.4). The ammonification ratio of 46.2 – 57.6 % for the digestates of the mixtures of animal manure, energy crops and by-products of food industries (if included) were previously reported (Menardo et al., 2011). The authors also observed the highest ammonification (77.9 %) in the digestate of pig slurry and energy crops which was probably due to the feedstock composition. Likewise, the feedstock composition having 90% laying hen manure and 10 % cattle manure probably resulted in high ammonification of the

digestate used in this setup. It is expected to have high ammonium nitrogen to total nitrogen ratio in poultry manures (Möller and Müller, 2012). The settling of the digestate resulted in the increase in the NH_4^+-N/TKN ratio (95 %) which indicated the partial removal of organic nitrogen via settling. The particulate fraction of organic nitrogen can be removed in gravitational sedimentation (U.S. EPA, 2007) leading to an increase in the fraction of the ammonium content in TKN.

The ammonification ratios of the AFFR effluents (anaerobically treated liquid digestate) were in the range of 96-98% for R1 and 95-97% for R2 for cycles 1-6 considering the end-cycle measurements. The treatment of poultry slaughterhouse wastewater using anaerobic fixed-film reactors previously resulted in the ammonification rates of 85-96% (del Pozo et al., 2000) which were found to be comparable with this study. The lowest ratios of NH_4^+-N/TKN were observed in the first two cycles when the biogas production was also the lowest (Figure 3.8). As the organic loading increased at each cycle change, ammonification was found to be in the ranges of 96-98% which was slightly higher than the one of the influent (liquid digestate, 95%). This fact may be considered to be due to the development of acclimated culture of microorganisms after second cycle.

The influent concentrations of NH_4^+-N and TKN in cycle 6 were 2212 and 2326 mg/L, respectively. The ammonium nitrogen was removed by 9-15% for R1 and 10-12% for R2 at the 6^{th} cycle of operation (Table 3.8). The corresponding TKN removals were 12-16 % and 11-14 % of TKN in R1 and R2, respectively (Table 3.9). The removed concentrations of TKN were always higher than that of NH_4^+-N (Figure 3.5). Since TKN is the sum of ammonium nitrogen and organic nitrogen, the removed concentrations of TKN being more than ammonium nitrogen indicated simultaneous removal of organic and ammonium nitrogen. When the organic nitrogen is biologically degraded, ammonium is produced (Ghyselbrecht et al., 2017). As the pH of the effluent of AFFRs ranged between 8.06-8.64

(Section 3.31) which was lower than the pK_a of ammonia (8.95 at 35°C), volatilization was expected to have a minor impact on ammonium removal as previously discussed in Section 2.3.7.4. Nitrogen gas content (N_2) of the biogas produced in AFFRs varied between 2.7-12.2 % for R1 and 3.0-16.0 % for R2 at steady-state COD removal period at the 6th cycle of operation (Table 3.14 in Section 3.3.7.1). Variable nitrogen contents detected in biogas can be an indication for Anammox and denitrification processes within reactors. Even though the Anammox process could be inhibited by the high organic matter concentration of the digestate, the potential pathway of the formation of oxidized nitrogen compounds by enzymatic catalase activity followed by autotrophic and/or heterotrophic denitrification can be explanatory for the ammonium removal from the AFFRs (Sectin 2.3.7.4).



(Data points for alkalinity are given as the average of two measurements)

Figure 3.5. Effluent NH_4^+-N and TKN concentrations of (a) R1 and (b) R2.

Table 3.8. The influent and effluent NH_4^+-N concentrations and the corresponding removal efficiencies.

Cycle	Time, days	R1				R2			
		Influent NH_4^+-N, mg/L	Effluent NH_4^+-N, mg/L	NH_4^+-N Removed, mg/L	NH_4^+-N Removal, %	Influent NH_4^+-N, mg/L	Effluent NH_4^+-N, mg/L	NH_4^+-N Removed, mg/L	NH_4^+-N Removal, %
1	**5**	**553**	**1970±1**	**-1417±1**	**(-256)-(-256)**	**553**	**2079±13**	**-1526±13**	**(-278)-(-274)**
2	13	1106	1051±7	55±7	(4)-(6)	1106	1030±6	76±6	(6)-(7)
2	**18**	**1106**	**1022±6**	**85±6**	**(7)-(8)**	**1106**	**997±15**	**110±16**	**(8)-(11)**
3	27	2212	1838±7	374±7	(17)-(17)	2212	1746±13	467±13	(21)-(22)
3	**32**	**2212**	**2009±1**	**204±2**	**(9)-(9)**	**2212**	**2008±3**	**205±3**	**(9)-(9)**
4	38	2212	1957±20	256±20	(11)-(12)	2212	1973±15	240±16	(10)-(12)
4	**44**	**2212**	**2006±1**	**207±2**	**(9)-(9)**	**2212**	**2061±6**	**152±6**	**(7)-(7)**
5	52	2212	2047±17	166±17	(7)-(8)	2212	2061±17	152±17	(6)-(8)
5	**61**	**2212**	**1971±8**	**242±9**	**(11)-(11)**	**2212**	**1961±7**	**251±7**	**(11)-(12)**
6	67	2212	1935±22	278±23	(12)-(14)	2212	1957±8	256±9	(11)-(12)
6	74	2212	1880±4	332±4	(15)-(15)	2212	1952±8	261±9	(11)-(12)
6	81	2212	1985±20	228±20	(9)-(11)	2212	1975±10	237±10	(10)-(11)

Note: The bold numbers indicate the measurements at end of the cycle.

Table 3.9. The influent and effluent TKN concentrations and the corresponding removal efficiencies.

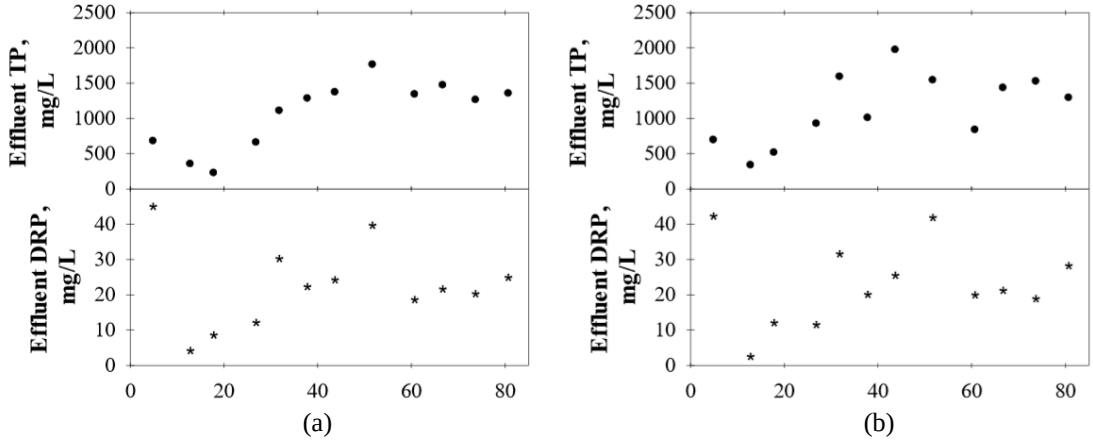
Cycle	Time, days	R1				R2			
		Influent TKN, mg/L	Effluent TKN, mg/L	TKN Removed, mg/L	TKN Removal, %	Influent TKN, mg/L	Effluent TKN, mg/L	TKN Removed, mg/L	TKN Removal, %
1	**5**	**581**	**2028±1**	**-1447±1**	**(-249)-(-249)**	**581**	**2177±43**	**-1596±44**	**(-282)-(-267)**
2	13	1163	1096±10	67±10	(5)-(7)	1163	1057±1	106±2	(9)-(9)
2	**18**	**1163**	**1059±12**	**104±12**	**(8)-(10)**	**1163**	**1047±6**	**117±7**	**(9)-(11)**
3	27	2326	1918±8	408±9	(17)-(18)	2326	1828±28	497±28	(20)-(23)
3	**32**	**2326**	**2066±11**	**259±11**	**(11)-(12)**	**2326**	**2075±3**	**251±3**	**(11)-(11)**
4	38	2326	2015±18	311±18	(13)-(14)	2326	2027±8	299±9	(12)-(13)
4	**44**	**2326**	**2079±1**	**247±2**	**(11)-(11)**	**2326**	**2142±3**	**184±3**	**(8)-(8)**
5	52	2326	2113±24	213±24	(8)-(10)	2326	2129±15	197±16	(8)-(9)
5	**61**	**2326**	**2040±1**	**286±2**	**(12)-(12)**	**2326**	**2026±1**	**300±2**	**(13)-(13)**
6	67	2326	2013±31	313±31	(12)-(15)	2326	2036±3	290±3	(12)-(13)
6	74	2326	1957±6	369±6	(16)-(16)	2326	2020±13	306±13	(13)-(14)
6	81	2326	2022±17	304±17	(12)-(14)	2326	2051±21	275±21	(11)-(13)

Note: The bold numbers indicate the measurements at end of the cycle.

3.3.4.2 Dissolved reactive and total phosphorus concentrations

Phosphorus removal was investigated in terms of DRP and TP concentrations. There was not an exact removal profile in the DRP and TP concentrations as the organic loads altered. DRP (Table 3.10) and TP (Table 3.11) concentrations in the influent at the sixth cycle were 50 mg/L and 2393 mg/L, respectively. Significant DRP removal was obtained in R1 and R2 (54-64 and 48-66 %, respectively) in high-rate anaerobic treatment of the digestate (Table 3.10). The corresponding retained concentrations of DRP in the effluent were in the range of 17-23 and 17-27 mg/L (Figure 3.6). Total phosphorus concentration was also reduced by 33-48% in R1 and by 34-47% in R2 at the 6^{th} cycle (Table 3.11) leaving 1262-1470 and 1291-1524 mg/L of TP in the effluent (Figure 3.6), respectively. The initial concentration of TP was approximately 48-fold higher than the DRP concentration. Thus, the TP removal could not only be attributed to the removed amount of DRP. Different forms of phosphorus other than DRP can also be removed from the liquid digestate. TP measurement includes the forms of phosphorus as DRP, dissolved unreactive phosphorus (DUP) or dissolved organic phosphorus (DOP) and particulate phosphorus (PP) (Alaica, 2012). Therefore, an AFFR application in the treatment of liquid digestates has also a potential to decrease the concentrations of DUP or DOP and PP as well as DRP. A hybrid sludge bed/fixed film reactor was previously reported to reduce the dissolved phosphate concentration by 28-78% in the treatment of synthetic sewage wastewater at 12^{o}C (Keating et al., 2016). The study indicated that the dissolved phosphate removal was in excess of the requirement for microbial growth. Phosphate was claimed to be removed biologically by the formation of intracellular inorganic polyphosphate (polyP) granules which was mediated by biofilm and fixed-film unit. Even though strictly anaerobic archaeal species had a potential to be applied in phosphorus removal under anaerobic conditions due to their capability of luxury polyP uptake (Keating et al., 2016), it could not clearly be concluded whether these species were responsible for phosphate removal from the wastewater in the study of concern. On the other hand, the formation of calcium

phosphate precipitates was also previously reported as a removal and recovery mechanism of phosphate from black waters using UASB reactors (Tervahauta et al., 2014). The compounds identified were addressed as hydroxyapatite, calcium phosphate hydrate and carbonated hydroxyapatite. These compounds were observed in the granular formations in UASB reactors mostly concentrated at the inner part of the granules. The outer part of the granules was found to be mostly organic in nature. (Tervahauta et al., 2014). Observing the effects of calcium and bicarbonate concentrations in the treatment of black water in UASB reactors, Cunha et al. (2018a) revealed that $Ca_x(PO_4)_y$ precipitation was favored by low bicarbonate concentrations. These precipitates then reported to act as a nucleus for granular formations by which a surface was provided for the attachment of microorganisms. When additional calcium at a Ca^{2+}/PO_4^{3-} molar ratio of 3 was added to the effluent of UASB reactors, phosphate concentration could be reduced by 63% (Cunha et al., 2018a). The addition of calcium during the treatment of black water in UASB was also observed to improve the calcium phosphate precipitation without inhibiting the COD removal and the associated biogas capture capability from the black water (Cunha et al., 2018b). Hence, biologically mediated removal and the formation of calcium phosphate granules under anaerobic treatment could explain the phosphorus removal observed in this experiment.



(Data points for alkalinity are given as the average of two measurements)

Figure 3.6. Effluent TP and DRP concentrations of (a) R1 and (b) R2.

Table 3.10. The influent and effluent DRP concentrations and the corresponding removal efficiencies.

Cycle	Time, days	R1				R2			
		Influent DRP, mg/L	Effluent DRP, mg/L	DRP Removed, mg/L	DRP Removal, %	Influent DRP, mg/L	Effluent DRP, mg/L	DRP Removed, mg/L	DRP Removal, %
1	**5**	**13**	**43±0**	**-31±0**	**(-246)-(-246)**	**13**	**41±0**	**-28±0**	**(-223)-(-223)**
2	13	25	2±0	23±0	(91)-(91)	25	1±0	24±0	(95)-(95)
2	**18**	**25**	**7±0**	**18±0**	**(72)-(72)**	**25**	**10±1**	**15±1**	**(56)-(60)**
3	27	50	10±1	40±1	(77)-(81)	50	10±0	40±0	(79)-(79)
3	**32**	**50**	**29±1**	**22±1**	**(42)-(46)**	**50**	**30±0**	**21±1**	**(40)-(42)**
4	38	50	21±1	30±1	(58)-(62)	50	18±0	32±0	(64)-(64)
4	**44**	**50**	**23±0**	**28±0**	**(56)-(56)**	**50**	**24±0**	**27±1**	**(52)-(54)**
5	52	50	38±0	12±0	(24)-(24)	50	40±0	11±1	(20)-(22)
5	**61**	**50**	**17±1**	**34±1**	**(66)-(68)**	**50**	**18±0**	**32±0**	**(64)-(64)**
6	67	50	20±0	31±1	(60)-(62)	50	20±0	31±1	(60)-(62)
6	74	50	19±0	32±0	(64)-(64)	50	17±0	33±0	(66)-(66)
6	81	50	23±0	27±0	(54)-(54)	50	27±0	24±0	(48)-(48)

Note: The bold numbers indicate the measurements at end of the cycle

Table 3.11. The influent and effluent TP concentrations and the corresponding removal efficiencies.

Cycle	Time, days	R1				R2			
		Influent TP, mg/L	Effluent TP, mg/L	TP Removed, mg/L	TP Removal, %	Influent TP, mg/L	Effluent TP, mg/L	TP Removed, mg/L	TP Removal, %
1	**5**	**598**	**678±25**	**-80±25**	**(-18)-(-9)**	**598**	**695±8**	**-97±9**	**(-18)-(-15)**
2	13	1197	353±0	843±0	(70)-(70)	1197	337±8	860±8	(71)-(73)
2	**18**	**1197**	**227±19**	**971±19**	**(80)-(83)**	**1197**	**515±8**	**682±9**	**(56)-(58)**
3	27	2393	658±13	1736±13	(72)-(73)	2393	924±21	1469±21	(61)-(62)
3	**32**	**2393**	**1107±0**	**1287±0**	**(54)-(54)**	**2393**	**1590±17**	**804±17**	**(33)-(34)**
4	38	2393	1283±4	1111±4	(46)-(47)	2393	1008±21	1386±21	(57)-(59)
4	**44**	**2393**	**1370±8**	**1024±9**	**(42)-(43)**	**2393**	**1970±133**	**424±134**	**(12)-(23)**
5	52	2393	1762±92	632±92	(23)-(30)	2393	1541±54	853±55	(33)-(38)
5	**61**	**2393**	**1341±62**	**1053±63**	**(41)-(47)**	**2393**	**837±0**	**1557±0**	**(65)-(65)**
6	67	2393	1470±125	923±125	(33)-(44)	2393	1433±21	961±21	(39)-(41)
6	74	2393	1262±8	1132±9	(47)-(48)	2393	1524±62	870±63	(34)-(39)
6	81	2393	1353±25	1040±25	(42)-(44)	2393	1291±29	1103±30	(45)-(47)

Note: The bold numbers indicate the measurements at end of the cycle.

3.3.5 The changes in total and volatile solids concentrations

Liquid digestate was characterized by its high TS (40.1±0.40 g/L) and VS concentrations (22.6±0.29 g/L) (Table 3.4) representing a VS/TS ratio of 56%. The solid content of the liquid digestate was initially 4%. AFFRs can handle a solid content less than 2 % at the inflow (Wilkie, 2005). Thus, the liquid digestate was used at least by 1/3 dilution which corresponded to a solids content of 1.34% to comply with the operational condition of AFFR related to the solids content.

Inflow total solids concentration in the last cycle was 13.4 g/L for both of the reactors. TS was reduced by 10-24 and 11-20 % for R1 and R2, respectively, during the treatment using AFFRs (Table 3.13). The reduction efficiencies of VS concentrations (Table 3.12) were observed to be slightly higher (24-33% for R1 and 19-30 % for R2) than that of the TS removal. Total solid is a solids parameter that is composed of volatile and fixed solids. When the removal amounts for both TS and VS were compared for the last two measurements, it could be concluded that total solids removal was mainly due to the destruction of volatile solids (by 78-96%). The large fraction of volatile solids removal addresses to the degradation of organic matter since volatile solids determination is an approximation of organic matter present in the samples (Mata-Alvarez, 2002). The solid content of the effluent in the last measurement was calculated as 1.13 and 1.08 % for R1 and R2, respectively. Thus, solid content could be reduced by 15.7 % for R1 and 19.4 % for R2 by the treatment of 1/3 diluted liquid digestate in AFFRs.

Table 3.12. The influent and effluent VS concentrations and the corresponding removal efficiencies.

Cycle	Time, days	R1				R2			
		Influent VS, mg/L	Effluent VS, mg/L	VS Removed, mg/L	VS Removal, %	Influent VS, mg/L	Effluent VS, mg/L	VS Removed, mg/L	VS Removal, %
1	**5**	**1887**	**3555±45**	**-1668±45**	**(-91)-(-86)**	**1887**	**3855±15**	**-1968±15**	**(-105)-(-104)**
2	13	3773	1885±85	1888±85	(48)-(52)	3773	1640±20	2133±20	(56)-(57)
2	**18**	**3773**	**1945±35**	**1828±35**	**(48)-(49)**	**3773**	**2040±10**	**1733±10**	**(46)-(46)**
3	27	7547	3645±115	3902±115	(50)-(53)	7547	4045±55	3502±55	(46)-(47)
3	**32**	**7547**	**4430±20**	**3117±20**	**(41)-(42)**	**7547**	**4705±35**	**2842±35**	**(37)-(38)**
4	38	7547	5060±150	2487±150	(31)-(35)	7547	4480±0	3067±0	(41)-(41)
4	**44**	**7547**	**4985±5**	**2562±5**	**(34)-(34)**	**7547**	**5390±60**	**2157±60**	**(28)-(29)**
5	52	7547	5275±15	2272±15	(30)-(30)	7547	5245±25	2302±25	(30)-(31)
5	**61**	**7547**	**4920±30**	**2627±30**	**(34)-(35)**	**7547**	**4395±45**	**3152±45**	**(41)-(42)**
6	67	7547	5755±5	1792±5	(24)-(24)	7547	5335±85	2212±85	(28)-(30)
6	74	7547	5130±60	2417±60	(31)-(33)	7547	5855±235	1692±235	(19)-(26)
6	81	7547	5530±20	2017±20	(26)-(27)	7547	5360±0	2187±0	(29)-(29)

Note: The bold numbers indicate the measurements at end of the cycle.

Table 3.13. The influent and effluent TS concentrations and the corresponding removal efficiencies.

Cycle	Time, days	R1				R2			
		Influent TS, mg/L	Effluent TS, mg/L	TS Removed, mg/L	TS Removal, %	Influent TS, mg/L	Effluent TS, mg/L	TS Removed, mg/L	TS Removal, %
1	**5**	**3345**	**7970±10**	**-4625±10**	**(-139)-(-138)**	**3345**	**8350±20**	**-5005±20**	**(-150)-(-149)**
2	13	6690	4285±115	2405±115	(34)-(38)	6690	3880±20	2810±20	(42)-(42)
2	**18**	**6690**	**4070±30**	**2620±30**	**(39)-(40)**	**6690**	**4225±25**	**2465±25**	**(36)-(37)**
3	27	13379	8070±80	5309±80	(39)-(40)	13379	8115±5	5264±5	(39)-(39)
3	**32**	**13379**	**8995±25**	**4384±25**	**(33)-(33)**	**13379**	**9480±70**	**3899±70**	**(29)-(30)**
4	38	13379	10250±70	3129±70	(23)-(24)	13379	9455±5	3924±5	(29)-(29)
4	**44**	**13379**	**10185±15**	**3194±15**	**(24)-(24)**	**13379**	**11320±90**	**2059±90**	**(15)-(16)**
5	52	13379	11345±15	2034±15	(15)-(15)	13379	11125±5	2254±5	(17)-(17)
5	**61**	**13379**	**10185±75**	**3194±75**	**(23)-(24)**	**13379**	**8825±65**	**4554±65**	**(34)-(35)**
6	67	13379	12015±65	1364±65	(10)-(11)	13379	10905±185	2474±185	(17)-(20)
6	74	13379	10280±70	3099±70	(23)-(24)	13379	11610±270	1769±270	(11)-(15)
6	81	13379	11055±5	2324±5	(17)-(17)	13379	10730±10	2649±10	(20)-(20)

Note: The bold numbers indicate the measurements at end of the cycle.

3.3.6 Biofilm

Biofilm formation was evaluated by gently washing the biomass immobilization media using deionized water and measuring the related attached total solids (ATS) and volatile solids (AVS) concentration of the biomass at the end of the reactor operation. ATS and AVS were 0.298 ± 0.082 and 0.118 ± 0.031 g per immobilization media, respectively. Biomass immobilization media at the end of the reactor operation is depicted in Figure 3.7.

Figure 3.7. Immobilized biomass after the reactor operation.

3.3.7 Biogas

The biogas formed during the operation of AFFRs was evaluated in terms of biogas production (Section 3.3.7.1) and biogas yields (Section 3.3.7.2).

3.3.7.1 Biogas production

Biogas production was evaluated in terms of daily production (Figure 3.8) and production per unit volume of the liquid digestate applied (Figure 3.9). Negligible biogas production was observed during the first two cycles for both of the reactors (Figure 3.8). Acclimation and adaptation of the bacteria can result in lower biogas production at the initial stages of the experiment (Acharya et al., 2008). No or negligible biogas production at the initial stages of the start-up of bio-film reactors was also previously noted (Michaud et al., 2002).

Biogas production progressively increased from the third to the sixth cycle. The production was 138 ± 89, 470 ± 98, 1117 ± 187, 1864 ± 216 mL for R1 and 37 ± 31, 320 ± 76, 955 ± 317 and 1713 ± 267 mL for R2 in the cycles 3-6, respectively. The progressive increase in the biogas production together with the COD_t removals attained in the range of 51-69% during cycles 2-6 (Table 3.6) suggested the catabolism of organic compounds by the anaerobic bacteria for respiration rather than anabolic activity for biopolymer synthesis (Michaud et al., 2002).

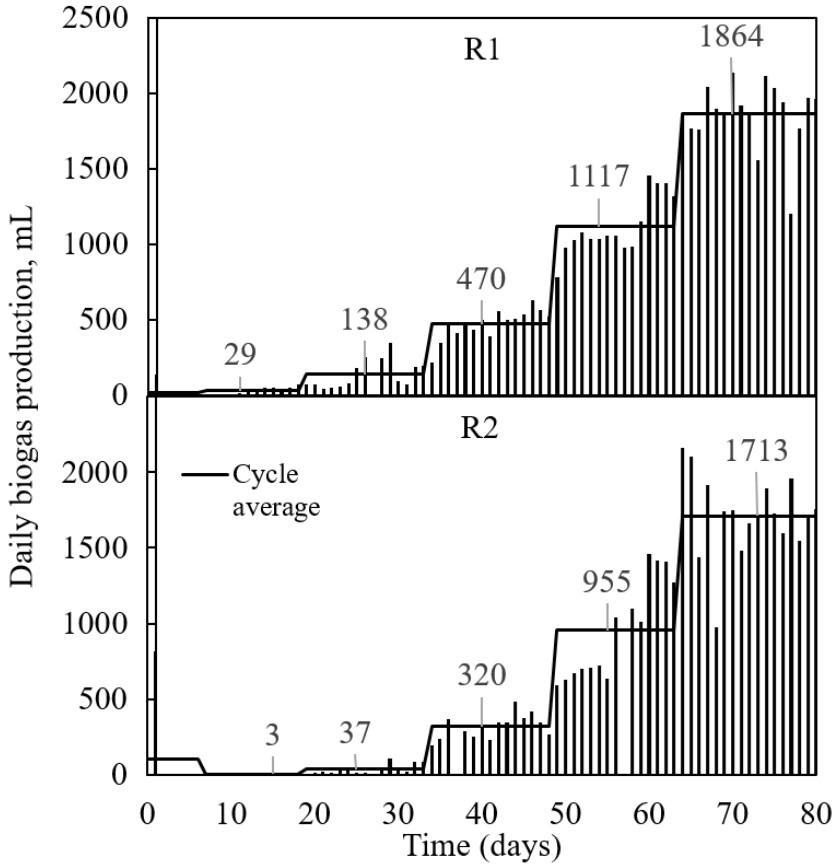


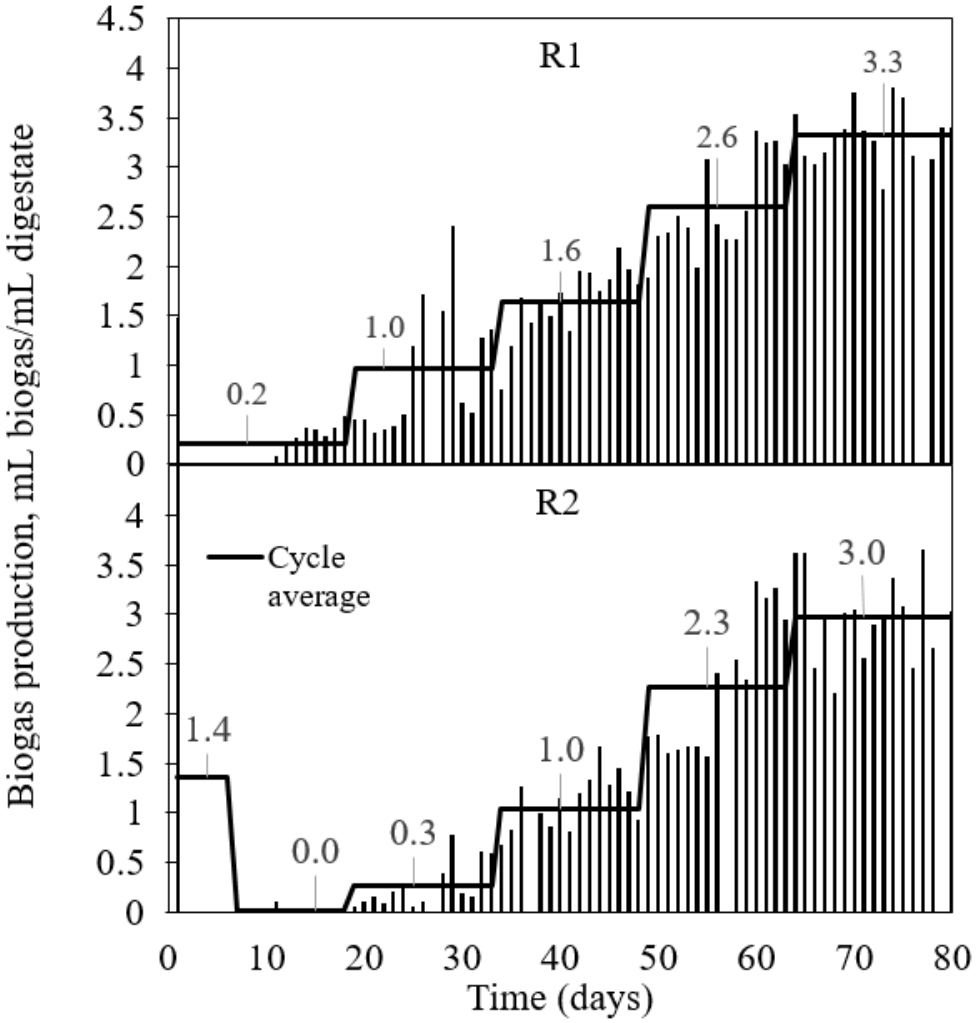
Figure 3.8. Daily biogas production in R1 and R2.

The biogas composition was measured during the steady-state COD_t removal period at the sixth cycle. Methane composition of the biogas was detected to be in the range of 77.5-80.3 % (an average of 78.9 %) and 70.7-78.0% (an average of 74.4 %) in R1 and R2, respectively (Table 3.14). The methane contents were found to be comparable with the ones obtained in the treatment of slaughterhouse wastewater using fluidized bed reactors (72-75 % CH_4) (Tritt and Kang, 2017) and of winery wastewater using moving bed biofilm reactors (45.1-82.6 % CH_4) (Chai et al., 2014). The corresponding carbon dioxide content of the biogas was 9.0-17.0% for R1 and 13.0-19.0% for R2.

Table 3.14. Nitrogen, methane and carbon dioxide contents of the biogas.

Reactor	Measurement	Nitrogen (N_2) Content, %	Methane (CH_4) Content, %	Carbon dioxide (CO_2) Content, %
R1	1	5.5 ± 1.1	77.5 ± 0.7	16.9 ± 0.3
	2	2.7 ± 0.0	80.3 ± 0.0	17.0 ± 0.1
	3	12.2 ± 4.1	78.8 ± 2.5	9.0 ± 1.6
R2	1	3.0 ± 2.0	78.0 ± 1.6	19.0 ± 0.3
	2	7.3 ± 2.1	77.4 ± 1.2	15.3 ± 0.9
	3	16.0 ± 0.4	70.7 ± 0.3	13.0 ± 1.0

An average of 3.3 and 3.0 mL of daily biogas per mL digestate volume was produced during the cycle 6 in R1 and R2, respectively (Figure 3.9). When the total daily volume of digestate produced in the plant is considered (approximately 80 m^3) (Chapter 2- digestate of anaerobic digester 2), daily biogas production would correspond to 240-264 m^3. The volume of biogas produced was converted into energy terms to be illustrative of the power generation capability (Table 3.15). Lower heating value of CH_4 was taken as 36 MJ/ m^3 CH_4 and assumed conversion efficiency of CH_4 to electricity was 35% (Manyuchi et al., 2018). Reporting approximately 80 m^3 of digestate production daily (Chapter 2- digestate of anaerobic digester 2), the plant was found to be capable of generating an additional 21.9-27.4 kW power output via anaerobic treatment of the digestate. The power output of the digestate corresponded to 1/53-1/66 that of obtained from the digestion of raw feedstock which was declared as 12691000 kWh/year (1449 kW). The plant considers increasing the installed capacity from 1.8 MW to 3.11 MW to meet the electricity requirement for 40 000 residences. If the targeted installed capacity and the projection on the energy supply for 40 000 residences were considered, the power output of the digestate treatment process obtained from the plant without capacity increase was predicted to be capable of meeting the energy requirement of 282 to 352 residences (for 80 m^3 of digestate). The capacity increase within the plant in turn results in the increase in the volume of digestate. As the volume of the digestate production increases, the power that can be generated increases via the treatment of more digestate in AFFRs (Table 3.15).



(The influent COD_t, COD_s and VS were 21.7, 13.4 and 7.5 mg/mL digestate, respectively, for both reactors)

Figure 3.9. Biogas production per volume of digestate in R1 and R2.

Table 3.15. Power that can be generated by the treatment of digestate in AFFRs.

Item	Formula	R1		R2	
Biogas production, m^3/m^3 digestate.d	A	3.3	3.3	3.0	3.0
		Min	Max	Min	Max
CH_4 content, %	B	77.5	80.3	70.7	78.0
CH_4 production at 35ºC, m^3 CH_4/m^3 digestate.d	$C=\frac{AxB}{100}$	2.558	2.650	2.121	2.340
CH_4 production at STP[(1)], m^3 CH_4/m^3 digestate.d	$D= C_x \frac{273.15}{308.15}$	2.267	2.349	1.880	2.074
Lower heating value of CH_4[(2)] MJ/m^3 CH_4	E	36	36	36	36
Energy yield, MJ/m^3 digestate.d	F=DxE	81.61	84.56	67.68	74.66
Energy equivalents, kWh/m^3 digestate.d (1kWh=3.6 MJ)	G=F/3.6	22.67	23.49	18.80	20.74
Electrical efficiency, kWh/m^3 digestate.d (conversion efficiency=35%[(2)])	H=Gx0.35	7.93	8.22	6.58	7.26
Power generation, kW/m^3 digestate	I= H/24	0.33	0.34	0.27	0.30
Power generation, kW					
for 80 m^3 digestate	J=Ix80	26.4	27.4	21.9	24.2
for 100 m^3 digestate	J=Ix100	33.1	34.3	27.4	30.2
for 200 m^3 digestate	J=Ix200	66.1	68.5	54.8	60.5
for 500 m^3 digestate	J=Ix500	165.3	171.3	137.1	151.2

(1) : Standard temperature and pressure
(2) : Manyuchi et al., 2018.

3.3.7.2 Biogas yields

Biogas yields were calculated relative to the COD_t removal (Table 3.16 and Table 3.17) and the added amount of volatile solids through the inflow (Table 3.18). The yields in cycle 1 were excluded from the calculations due to wash-out of the seed sludge. The average biogas yields in the cycles increased from 0.030 to 0.249 m^3_{biogas}/kg COD_r for R1 and from 0.003 to 0.235 m^3_{biogas}/kg COD_r for R2 (Table 3.16 and Table 3.17, respectively). The biogas yields obtained in the sixth cycle when the reactors were operated under steady-state COD_t removal were found to be comparable with the ones obtained in the treatment of cattle manure using UASB reactors (Marañón et al., 2001). The authors reported a biogas yield of 0.20-0.29 m^3/kg COD_r when HRT was between 8.9-22.5 days and OLR was in the range of 2.35-4.91 g COD/L.d.

When the biogas yields were converted into methane yields using the average of methane contents in the biogas (78.9 and 74.4 % for R1 and R2, respectively), average methane yields were calculated as 0.196 and 0.175 m^3 CH_4/ kg COD_r for R1 and R2, respectively. The yields were found to be comparable to the ones obtained in the anaerobic treatment of dairy cattle wastewater using attached film media (0.05-0.21 m^3 CH_4/kg COD_r) (Vartak et al., 1997). Even though 0.35 Nm^3/kg COD is the theoretical maximum methane production, the actual methane productions are often much lower than the theoretical level (van Haandel and van der Lubbe, 2012; Nielfa et al., 2015).

Biogas yields were additionally calculated relative to the added VS to the reactor through the influent (Table 3.18). R1 had an average of 0.430 m^3_{biogas}/kg VS_{added} with a maximum 0.494 and a minimum 0.277 m^3_{biogas}/kg VS_{added} at the sixth cycle whereas R2 had 0.395 m^3_{biogas}/kg VS_{added} biogas yield (max. 0.499, min 0.225 m^3_{biogas}/kg

VS_{added}). The yields of the digestate was found to be higher than the ones obtained in the 70-day RBP test for the same digestate (0.299±0.005 m^3/kg VS_{added} and 0.326±0.009 m^3/kg VS_{added} with and without nutrient supplementation, respectively) (Chapter 2). This fact can be due to employment of different portions of the digestate used in each experiment. RBP test was conducted on the digestate itself and AFFR treatment was applied on the liquid portion of the digestate after settling. The biogas yields of the liquid digestate at the end of the fixed film treatment resembled more raw feedstocks compared the ones in RBP test due to higher yields obtained. These raw feedstocks can be accounted as municipal wastewater sludge (0.3-0.5), pig stomach content, sheep excreta and vegetable wastes (0.3-0.4), straw from cereals and pig excreta (0.2-0.5), liquid cattle manure (0.1-0.8) given by Zupančič and Grilc (2012) and molasses, maize and potato distillery slops (approximately 0.40) given by Braun (2007) (the biogas yields in the parenthesis are given in m^3/kg VS_{added}). Thus, an AFFR establishment is capable of extracting more biogas with higher yields especially from the COD_s content of the liquid portion of the digestates.

Table 3.16. Biogas yields relative to the COD_t removal for R1.

Cycle	Time, d	COD_{inf}, mg/L	COD_{eff}, mg/L	COD_r, mg/L	Average COD_r in the cycle, mg/L	Average daily volume of digestate applied in the cycle, mL	Average daily biogas production in the cycle, mL	Biogas yield in the cycle, m^3 biogas/kg COD_r	Max. biogas yield in the cycle, m^3 /kg COD_r	Min biogas yield in the cycle, m^3 /kg COD_r
		A	B	C=A-B	D=average C	E	F	G=(F*1000)/(C*E)		
2	13	10844	3607	7237	6721	142	29	0.030	0.073	0.000
	18	10844	4640	6204						
3	27	21689	7139	14550	13206	147	138	0.071	0.178	0.023
	32	21689	9828	11861						
4	38	21689	9338	12351	12071	286	470	0.136	0.183	0.063
	44	21689	9898	11791						
5	52	21689	9140	12549	13049	433	1117	0.198	0.258	0.139
	61	21689	8140	13549						
6	67	21689	9289	12400	13025	574	1864	0.249	0.286	0.161
	74	21689	8151	13538						
	81	21689	8552	13137						

Table 3.17. Biogas yields relative to the COD_t removal for R2.

Cycle	Time, d	COD_{inf}, mg/L	COD_{eff}, mg/L	COD_r, mg/L	Average COD_r in the cycle, mg/L	Average daily volume of digestate applied in the cycle, mL	Average daily biogas production in the cycle, mL	Biogas yield in the cycle, m^3 biogas/kg COD_r	Max. biogas yield in the cycle, m^3 /kg COD_r	Min biogas yield in the cycle, m^3 /kg COD_r
		A	B	C=A-B	D=average C	E	F	G=(F*1000)/(C*E)		
2	13	10844	3236	7608	6935	146	3	0.003	0.015	0.000
	18	10844	4582	6262						
3	27	21689	6645	15044	13712	145	37	0.019	0.055	0.004
	32	21689	9310	12379						
4	38	21689	8893	12796	11926	286	320	0.094	0.141	0.057
	44	21689	10633	11056						
5	52	21689	4447	17242	15675	417	955	0.146	0.223	0.091
	61	21689	7581	14108						
6	67	21689	9437	12252	12690	575	1713	0.235	0.297	0.134
	74	21689	9259	12430						
	81	21689	8300	13389						

Table 3.18. Biogas yields relative to the added amount of VS (VS_{added}) for R1 and R2.

Reactor	Cycle	Average VS_{added} in the cycle, mg/L	Average daily volume of digestate applied, mL	Average daily biogas production in the cycle, mL	Biogas yield in the cycle, m^3 biogas/kg VS_{added}	Max. biogas yield in the cycle, m^3 biogas/kg VS_{added}	Min. biogas yield in the cycle, m^3 biogas/kg VS_{added}
		A	B	C	D= (C*1000)/(A*B)		
R1	2	3773	142	29	0.053	0.130	0.000
	3	7547	147	138	0.125	0.312	0.041
	4	7547	286	470	0.218	0.293	0.100
	5	7547	433	1117	0.342	0.447	0.240
	6	7547	574	1864	0.430	0.494	0.277
R2	2	3773	146	3	0.005	0.028	0.000
	3	7547	145	37	0.034	0.101	0.007
	4	7547	286	320	0.148	0.223	0.090
	5	7547	417	955	0.303	0.463	0.188
	6	7547	575	1713	0.395	0.499	0.225

3.3.8 Global warming potential of the captured biogas from digestate

Global warming potentials (GWP) were calculated based on the measured average daily biogas production per unit volume of digestate and the minimum and maximum CO_2 and CH_4 contents at the steady-state COD removal period at the 6th cycle for R1 and R2 (Table 3.19). 100-year GWP was taken as 1 for CO_2 and 28 for CH_4 (Myhre et al. 2013). The CO_2 and CH_4 contents of biogas were measured at room temperature (around 25^oC). The densities of CO_2 and CH_4 used for the calculations are taken as 1.7989 and 0.6556 kg/m^3 for 25^oC (Lide, 2006).

Biogas generated was found to have a daily GWP of 47.4-49.7 kg CO_2e/m^3 digestate for R1 and 39.6-44.0 kg CO_2e/m^3 digestate for R2 in terms of total CO_2 and CH_4 captured. CH_4 contributed to the highest portion of GWP (daily 46.9-48.6 and 38.9-43.0 kg CO_2e/m^3 digestate for R1 and R2, respectively) which was obviously due to 28-fold higher GWP of CH_4 compared to CO_2. GWP of the CH_4 captured in this study was much higher than the one calculated for the CH_4 emissions from storage of untreated and anaerobically digested cattle slurry with and without starch addition (Clemens et al., 2006). The authors reported cumulative CH_4 emissions in the range of 0-36 kg CO_2e/m^3 (0-30^oC) during the storage period (55-140 days). The study demonstrated the GWP of the digestate during the storage of cattle slurry, thus, showed the possibility of the further decomposition if anaerobic conditions favored. The high-rate anaerobic treatment of the digestate, on the other hand, revealed the significance of the extent of further anaerobic decomposition in terms of the resultant GWP of the captured gas. Furthermore, the complete sequestration or recovery of CO_2 and CH_4 content of the biogas may result in 1157-1450 tCO_2e/yr for 80 m^3/d digestate production in the plant (Table 3.19). The total emissions of CH_4 and N_2O from anaerobic lagoons were previously reported as 703±195 kg CO_2e/m^2.yr (Owen and Silver, 2015). The potential to capture CO_2 and CH_4 from the digestate used in the high-rate treatment is thus equivalent to the overall emissions of CH_4 and N_2O from

anaerobic lagoons having a total surface area of 1646-2062 m^2. It should also be noted that this estimation was based on the total digestate volume produced (80 m^3/d). Thus, more volumes of digestate produced would eventually result in the folded increase in the GWP of the digestates (Table 3.19).

Another fact to mention here is that, nitrous oxide (N_2O) and NH_3 were not quantified in the biogas composition. Even though NH_3 emission was discussed as having a minor impact in ammonium removal during the treatment, even these emissions may possibly add in the GWP of the captured gas. NH_3 is not a greenhouse gas, however, its deposition results in the conversion of NH_3 into a greenhouse gas, N_2O. Moreover, N_2O has a GWP of 265 (9.5 fold higher than CH_4) (Myhre et al. 2013). The quantification of N_2O and NH_3, thus, becomes important for estimates of the GWP of the captured gas. The capture of the associated gasses would also enhance the reduction of GWP of the digestate if the contents of the biogas produced is properly managed, sequestered or recovered.

Table 3.19. tCO_2e of methane and carbon dioxide fractions of the biogas captured from the digestate.

			R1		R2	
	Unit	Formula	Min	Max	Min	Max
Biogas production	m^3/m^3 digestate.d	A	3.3	3.3	3.0	3.0
		Methane				
Methane content	%	B	77.5	80.3	70.7	78
Methane production	m^3 CH_4/m^3 digestate.d	C=A*B/100	2.558	2.650	2.121	2.340
Density of methane at 25°C	kg/m^3	D	0.6556	0.6556	0.6556	0.6556
Mass of methane	kg CH_4/m^3 digestate.d	E=C*D	1.677	1.737	1.391	1.534
GWP of CH_4	GWP	F	28	28	28	28
CO_2 equivalent of methane	kg CO_2e/m^3 digestate.d	G=E*F	46.9	48.6	38.9	43.0
		Carbon dioxide				
Carbon dioxide content	%	G	7.5	17.2	12.6	19.3
Carbon dioxide production	m^3 CO_2/m^3 digestate.d	H=A*G/100	0.248	0.568	0.378	0.579
Density of carbon dioxide at 25°C	kg/m^3	I	1.7989	1.7989	1.7989	1.7990
Mass of carbon dioxide	kg CO_2/m^3 digestate.d	J=H*I	0.4	1.0	0.7	1.0
GWP of CO_2	GWP	K	1	1	1	1
CO_2 equivalent of CO_2	kg CO_2e/m^3 digestate.d	K=J*K	0.4	1.0	0.7	1.0
Total CO_2 equivalent	kg CO_2e/m^3 digestate.d	L=K+F	47.4	49.7	39.6	44.0
	t CO_2e/m^3 digestate.yr	M=L*0.365	17.3	18.1	14.5	16.1
tCO_2e for 80 m^3/d digestate	t CO_2e/yr	N=M*80	1384	1450	1157	1285
tCO_2e for 100 m^3/d digestate	t CO_2e/yr	O=M*100	1730	1813	1446	1606
tCO_2e for 200 m^3/d digestate	t CO_2e/yr	P=M*200	3460	3626	2892	3212
tCO_2e for 500 m^3/d digestate	t CO_2e/yr	R=M*500	8649	9064	7230	8029

3.4 Conclusions

The application of an AFFR in the treatment of liquid digestates had a potential to decompose COD_t by 56-63% which was mainly composed of the COD_s (85-94 %). Yet, it is still possible to decompose VS portion and remove it in AFFR via COD_s removal. This addresses the fact that it becomes possible to extract the related biogas from the soluble COD in a relatively such time period (HRTs of 1.3-1.4 days). The biogas yields obtained (0.395-0.430 L_{biogas}/g VS_{added}) was relatively high which corresponded to the yields of many raw feedstocks such as municipal wastewater sludge, pig stomach content, sheep excreta, vegetable wastes, straw from cereals and pig excreta, liquid cattle manure molasses, maize and potato distillery slops. Significant phosphorus removal was also attained (47-66% DRP and 36-47% TP) which resembled the formation of calcium phosphate precipitates in anaerobic reactors. Such a removal mechanism is the subject in the most recent studies for phosphate recovery in the form of calcium phosphate precipitates from high-rate anaerobic reactors (Tervahauta et al., 2014; Cunha et al., 2018a and 2018b), even though final characterization of the granular phosphate formations is yet to be further investigated to be employed in agriculture and/or industry in terms of feasibility, safeness and bioavailability (Cunha et al., 2018b).

The treatment of the liquid digestate using AFFRs requires approximately 7-38 times less volume compared to the conventional digesters depending on the hydraulic retention times required for both reactors (1.3-1.4 days for AFFR, 10-50 days for conventional digesters). Therefore, AFFR treatment of digestate would yield less footprint and decrease the associated financial costs. The simplicity of construction and operation and the flexibility against operational instabilities can be considered as additional factors that can promote the application of AFFRs as a treatment unit for digestates. Moreover, alkalinity dosing was not required during the digestate treatment

which can be accounted as a factor to reduce the operational costs. The required alkalinity levels are seldom satisfied in the influent wastewater of AD processes. Alkalinity addition is required to maintain the pH to assure the stability of digestion process which may affect the economics of the plant considerably (Tchobanoglous et al., 2003).

Even though the power generation capacity from the further anaerobic treatment of the digestate was not even close to megawatt levels for the plant, it should be noted that the major consideration in such a process is to reduce the pollution load of the digestate. Energy extraction can be considered as a by-product of the process aiming at digestate treatment. In addition to the reduction of organic load and providing an alternative way to phosphate recovery, yearly 14.5-18.1 tCO_2e/m^3 digestate (CH_4+CO_2) or more (inclusion of NH_3 and N_2O) can be prevented before emitting to the atmosphere if biogas produced form digestate is properly managed.

CHAPTER 4

NUTRIENT REMOVAL FROM THE EFFLUENT OF ANAEROBIC FIXED-FILM REACTOR USING MICROALGAL CULTURES

Liquid digestate, itself, is a waste stream that can be favorably employed in microalgal processes for nutrient removal and valorization of the microalgal biomass grown. High nutrient concentrations that can be easily metabolized by microalgal species and the favorable pH range for fresh water microalgae are the main drivers for liquid digestates to be used in microalgal processes. Additionally, large volumes of digestates produced in AD plants have a potential to provide continous feed supply for microalgal species.

Even if further anaerobic treatment of the digestate would reduce the COD content, the concentration of the nutrients in AFFR effluent would still be high (Chapter 4). The pH range of the AFFR effluent was suitable for freshwater microalgae to survive and grow (approximately 8.40). Moreover, AFFR effluent is produced in large quantities. Therefore, the digestate after being additionally treated using AFFRs have still potential to be employed in nutrient removal processes using microalgal cultures. This chapter covers the literature background, materials, methods, results and discussions on the treatment of AFFR effluent by using microalgal cultures.

4.1 Literature Background

Microalgae are unicellular species having sizes in the range of a few micrometers to a few hundred micrometers. The existing number of microalgae species is estimated to be the range of 200 000-800 000 (Venkatesan et al., 2015). The photosynthesis by

microalgal species contributes to the 50% of the world's oxygen production (Cadoret et al., 2012). Diatoms (Bacillariophyceae), green algae (Chlorophyceae) and golden algae (Chrysophyceae) are the three major classes of microalgae in terms of abundance. Cyanobacteria (blue-green algae) are also regarded as prokaryotic microalgae under the class name of Cyanophyceae (Venkatesan et al., 2015).

Microalgae require water, sunlight, nutrients and some specific environmental conditions for growth such as pH, temperature, salinity and dissolved gases. Microalgae convert light energy into potential chemical energy in the presence of carbon dioxide and water via photosynthesis in the presence of light during a day period (Yildiz et al., 2013). On the other hand, microalgae may exhibit different types of metabolisms (autotrophic, heterotrophic, mixotrophic and photo-heterotrophic growth) as a response to changes in environmental conditions. Light is the sole energy source which is converted to energy by photosynthesis in photoautrophic growth. Organic compounds are carbon and energy sources in heterotrophic cultivation. Microalgae can live autorotrophically or heterotrophically in mixotrophic type of growth. Light is the energy source and organics are the carbon sources in photo-heterotrophic growth of microalgae (Gouveia, 2011).

Microalgal biomass can be used to produce co-products and by-products (Gouveia, 2011) as feed additives for animals and fishes, supplements for health food and dietary, pharmaceutical/medicinal compounds (Berg-Nilsen, 2006). The biomass can also be employed in the production of various kinds of fuel synthesis by taking the advantage of different cellular components such as biohydrogen production by proton and electrons, bioethanol from sugars and starch, biodiesel from oils, biomethane and biomass to liquid fuel (BTL) from biomass (Gouveia, 2011).

Microalgal biomass has been considered as the most important reneweable fuel feedstock for future applications (Gouveia, 2011). It is believed to be capable of replacing fossil fuels (Chisti, 2007; Wijffels and Barbosa, 2010; Liu et al., 2013; Calicioglu and Demirer, 2015, 2016). This fact is due to the many advantages offered by microalgal biomass. These advantages can be regarded as (Gouveia, 2011):

- higher photon conversion efficiency (3-8%) compared to that of terrestrial plants (0.5%).
- high biomass yields and growth rates.
- high carbon dioxide capturing capacity.
- ability to be grown in the fields apart from agricultural lands which eliminates the probability of competing with the crops for arable land.
- ability to be harvested more than once a year which is not applicable for seasonal crops.
- ability to be stimulated to produce a feedstock with high concentration (biomass, oil, starch).
- ability to be grown in a liquid medium or salt and wastewater streams which results in less fresh water consumption.
- ability to remove nitrogen and phosphorus from wastewaters such as industrial and municipal wastewaters, agricultural run-off, concentrated animal feed operations.
- ability to be grown without fertilizers and pesticides reducing the environmental impacts arising from their use.

Wastewaters contain organic and inorganic nutrients which require to be treated before the discharge to decrease the environmental impacts. Microalgae has been proven to have a potential to be grown in wastewater by metabolizing inorganic nutrients such as nitrogen and phosphorus (Kesaano and Sims, 2014; Olguin, 2012). Being a cost-effective process, major nutrients and also micronutrients required for the growth of

microalgae can be supplied from wastewater (Yu et., 2017). Thus, the microalgal wastewater treatment provides both the removal of the associated components from the wastewater via accumulating them into biomass and the growth of the microalgal biomass that can be used in a further downstream process. Microalgal wastewater treatment can be effectively applied for nutrient removal from wastewaters with a lower cost than conventional methods which in turn offers an environmentally friendly nutrient recovery process. On the other hand, algal biofuel production can not be an economically viable option unless algae species are grown in wastewater (Christenson and Sims, 2011).

The integration of microalgae and digestate was first conducted at 1950s (Golueke and Oswald, 1959). However, the interest on the subject has recently developed because of the increasing demand of biogas industry to treat digestates as well as to benefit from the high nutrient content of the digestates (Xia and Murphy, 2016). The constituents of the liquid digestates (liquid portion of digestates after solid-liquid phase separation) make processing of this waste stream easy for microalgal cultures. Slightly alkaline or near neutral pHs of the liquid digestates enables freshwater and alkaliphilic microalgae to survive and grow. The primary nutrients for microalgal growth are present in digestates at high concentrations (total nitrogen: 139-3456 mg/L, total phosphorus: 7-381 mg/L). Even the fractions of easily utilizable ammonium and phosphate has a large proportion in total nitrogen and total phosphorus content (65-98% and 82– 90%, respectively). Additionally, the presence of organic carbon sources such as volatile fatty acids (e.g. acetates) enables microalgae to be grown mixotrophically. This type of algal growth results in higher biomass productivity and concentration and it is less affected from photoinhibition/limitation than phototrophic algal growth. High inorganic carbon concentrations of digestates (939-1353 mg/L) have also potential to be employed in photosynthesis (Xia and Murphy, 2016). Therefore, liquid digestates have a great potential to be used in microalgal nutrient removal processes.

Many studies have been conducted integrating the microalgae with digestate treatment up to date (Table 4.1). *Chlorella* and *Scenedesmus* sp. were the most commonly used species in the digestate treatment. These species were previously reported as being among the top eight pollution-tolerant genera (Palmer, 1969). Digestates required dilution, filtration and/or dilution and autoclaving as a pretreatment before microalgal treatment. The pretreatment methods are applied to reduce the solids content and to eliminate interferences of other microorganisms (like bacteria, protozoa) in microalgal cultivation (Abu Hajar et al., 2016). Digestates are also diluted before microalgal applications in order for decreasing the probability of inhibition due to high ammonium concentrations (Yan et al.,2012).

The researches indicated that the digestate treatment using microalgal cultures can remove 100% TN, NH_4^+-N, TP and PO_4^{3-} (Table 4.1) after the pretreatment methods applied. *Desmodesmus* sp. was reported to remove 4.542-9.494 mg/L total nitrogen and 0.244-0.390 mg/L phosphate phosphorus daily from the filtered and diluted digestate of pig manure in batch and fed-batch cultivation (Ji et al., 2014 and 2015). The removal rate of ammonium nitrogen achieved in these related studies was 5.284-8.920 mg/L.d and the corresponding biomass concentration ranged between 0.324-1.039 g/L. The nitrogen and phosphorus removal rates noted by Åkerström et al. (2014) and Cai et al. (2013a) were significantly larger for *Chlorella* sp. and *Nannochloropsis salina* obtained in the treatment of digestates of sludges and municipal wastewater. 42.6 mg/L total nitrogen and 4.1 mg/L phosphorus was removed daily by *Chlorella* sp. treating the liquor of anaerobically digested sludge in batch treatment of 7-8 days (Åkerström et al., 2014). The semi-continuous operation of the reactors using *Nannochloropsis salina* as inoculum yielded 13.4-56.5 mg/L.d total nitrogen and 2.3-4.3 mg/L.d total phosphorus removal (Cai et al., 2013a).

The digestate treatment using microalgal cultures additionally resulted in microalgal biomass production at variable quantities (Table 4.1). The treatment of the digestate of pig manures using *Desmodesmus* and *Chlorococcum* sp. resulted in the lowest biomass productivity (between 0.024 and 0.029 g/L.d) (Ji et al., 2014; Montero et al., 2018). The highest biomass productivities were observed using *Scenedesmus* sp., *Chlorella pyrenoidosa* and *Chlorella* PY-ZU1 as 0.67, 0.63 and 0.60 g/L.d, respectively (Tan et al., 2014; Cheng et al., 2015; Dickinson et al., 2015). The biomass concentrations covered in the studies given in Table 4.1 was in the range of 0.324-4.81 g/L whereas the studies conducted using *Chlorella* sp. resulted in 0.494-4.81 mg/L of biomass.

Apart from the ability to assimilate the nutrients from the liquid digestate and store the nutrients within their biomass, microalgal cultures can be further employed in downstream processes to make use of their cellular compounds such as carbonhydrates, proteins, fats, lipids, pigments. Even if microalgal biomass production in wastewater treatment has been proved to be viable, there remains a bottleneck for commercialization of microalgal applications. For many years up to now, this bottleneck has been addressed as the cost of harvesting and dewatering of suspended microalgal culture which accounts for approximately 20-30% of the total operating cost (Gudin and Thepenier, 1986; Larronde-Larretche and Jin, 2016; Quijano et al., 2017). The poor settleability of the microalgal biomass is the reason behind the 20-30% share of the harvesting and dewatering costs (Quijano et al., 2017). The algal biomass is in the form of dilute suspensions (Irving and Allen, 2011). The harvesting processes such as centrifugation, filtration and flocculation are not cost-effective solutions for microalgal biomass to obtain a dense culture. On the other hand, the formation of the microalgal bacterial aggregrates during wastewater treatment was noted as having excellent settling characteristics. Such formations of biomass can be easily separated by simple gravitational settling and provides efficient and cost-effective harvesting of the microalgal biomass (Quijano et al., 2017).

Table 4.1. Studies on digestate treatment using microalgae species.

Inoculum	Wastewater Origin	Pretreatment	Type	Specific growth rate	Biomass Productivity/ Concentration	Nutrient removal		Reference
						Nitrogen	Phosphorus	
Chlorella sp.	Liquor of anaerobically digested sludge	Dilution with the effluent water of wastewater treatment plant (12, 25, 40, 50, 70, 100%)	Batch (7-8 d)		*0.45 g/L.d *2.11 g/L	N: 42.6 mg /L.d	P: 4.1 mg /L.d	Åkerström et al., 2014
Nannochloropsis salina	Digestate of municipal WW	*Dilution with deionized water (3, 6, 12, 18, 24%) *Salinity adjustment (artificial seawater)	Batch (10 d)	0.334-0.645 1/d	*0.092 g/L.d *0.92 g/L	TN: 100%	TP: 100%	Cai et al., 2013a
			Semi-cont. (18 d)		0.155 g/L.d	TN: 13.4-56.5 mg/L.d	TP: 2.3-4.3 mg/L.d	
Nannochloropsis salina *Synechocystis* sp.	Digestate of municipal WW	*Dilution with deionized water (3, 6, 12, 18, 24%) *Salinity adjustment (artificial seawater)	Batch (10 d)		0.151 g/L.d	TN: 100 % TAN: 100 %	TP: 100%	Cai et al., 2013b
			Semi-cont. (18 d)		0.212 g/L.d	TN: 100%	TP: 100%	

Table 4.1. Studies on digestate treatment using microalgae species.

Inoculum	Wastewater Origin	Pretreatment	Type	Specific growth rate	Biomass Productivity/ Concentration	Nutrient removal		Reference
						Nitrogen	Phosphorus	
Chlorella PY-ZU1 (mutant)	Digestate of swine manure and sewage	*Centrifugation *Sterilization	Batch (8-13 d)		*0.601 g/L.d *4.81 g/L	NH_3-N: 73%	TP: 99.6%	Cheng et al., 2015
Scenedesmus sp.	*Secondary municipal WW effluent *Municipal WW supplemented with digestate of swine manure and microalgae	* Sterilization	Batch then cont. (45 d)	0.82-1.94 1/d	0.67 g/L.d	NH_3-N: 16.9-41.2 mg/L.d	PO_4-P: 1.68-6.69 mg/L.d	Dickinson et al., 2015
Chlorella sp.	Undigested and digested dairy manure	*Dilution (10, 15, 20, 25x) *Filtration	Batch (21 d)	0.282-0.409 1/d	1.71 g/L	TN: 82.5 % NH_4^+: 81-178 mg/L (100%)	TP: 74.7%	Wang et al., 2010a
Chlorella vulgaris	Undigested and digested dairy manure	*Dilution (20x)	Semi-cont. (30 d)			TN: 93.6 % NH4+-N: 100 %	TP: 92%	Wang et al., 2010b

Table 4.1. Studies on digestate treatment using microalgae species.

Inoculum	Wastewater Origin	Pretreatment	Type	Specific growth rate	Biomass Productivity/ Concentration	Nutrient removal		Reference
						Nitrogen	Phosphorus	
Scenedesmus obliquus	Digestate of piggery waste	*Sedimentation *Filtration *Sterilization *Dilution	Batch (7 d)	0.334-0.486 1/d	0.311 g/L.d	TN: 74.6 %	TP: 88.8%	Xu et al., 2015
Chlorella sp.	Digestate	*Ultraviolet *Filtration	Batch (6 d)		0.494 g/L	TN: 73.1%	TP: 67.6%	Yan and Zheng, 2014
Chlorella pyrenoidosa	Digestate of starch WW mixed with alcohol WW	*Settling *Filtration *Sterilization	Batch (9 d)		*0.58 g/L.d *3.01 g/L	TN: 91.6 %	TP: 90.7%	Yang et al., 2015
Chlorella vulgaris *Scenedesmus obliquus* *Neochloris oleoabundans*	Digestate	*Ultraviolet *Filtration	Batch (7 d)	0.134-0.451 1/d	0.217 g/L.d	TN: 76.0 %	TP: 0.81-1.26 mg/L.d 63.2%	Zhao et al., 2015
Chroococcus sp.	*Chroococcus* sp.	*Settling *Filtration *Dilution (10-100%)	Batch (12 d)		1.42 g/L	TAN: 85.2%	TDP: 89.3%	Prajapati et al., 2014

Table 4.1. Studies on digestate treatment using microalgae species.

Inoculum	Wastewater Origin	Pretreatment	Type	Specific growth rate	Biomass Productivity/ Concentration	Nutrient removal		Reference
						Nitrogen	Phosphorus	
Chlorella minutissima *Chlorella sorokiniana* *Scenedesmus bijuga*	Digestate of poultry litter effluent	*Centrifugation *Dilution (4, 6, 8%)	Batch (12 d)		*0.076 g/L.d *0.612 g/L	TN: 79 mg/L	TP: 5.2 mg/L	Singh et al., 2011
Chlorella pyrenoidosa	Digestate of wheat starch processing WW	*Settling *Filtration	Outdoor batch	0.19-1.02 1/d	*0.63 g/L.d *1.90 g/L	TN: 83.1%	TP: 97.0%	Tan et al., 2014
Mixed microalgae dominated by *Scenedesmus* sp.	Digestate of WW treatment plant	*Dilution	Batch (7 d)	0.04-0.9 1/d	2.6 g/L			Uggetti et al., 2014
Desmodesmus sp.	Digestate of pig manure	*Filtration *Dilution (2.5, 5, 10%)	Batch (14 d)		*0.029 g/L.d *0.412 g/L	TN: 4.542 mg/L.d NH_4^+-N: 5.284 mg/L.d (100 %)	TP: 0.326 mg/L.d PO_4-P: 0.290 mg/L.d	Ji et al., 2014

Table 4.1. Studies on digestate treatment using microalgae species.

Inoculum	Wastewater Origin	Pretreatment	Type	Specific growth rate	Biomass Productivity/ Concentration	Nutrient removal		Reference
						Nitrogen	Phosphorus	
Desmodesmus sp.	Digestate of pig manure	*Filtration *Dilution (2.5, 5,10% for batch)	Batch (10 d)		0.324 g/L	TN: 75.6% 6.227 mg/L.d NH_4^+-N: 92.7% 7.701 mg/L.d	PO_4-P: 100% 0.244 mg/L.d	Ji et al., 2015
			Fed-batch (40 d)		1.039 g/L	TN: 94.2% 9.464 mg/L.d NH_4^+-N: 91.1% 8.920 mg/L.d	PO_4-P: 88.7% 0.390 mg/L.d	
Chlorella vulgaris *Neochloris oleoabundans* *Scenedesmus. obliquus*	Digestate of cattle slurry and raw cheese whey	*Dilution (1:10, 1:15, 1:20, 1:25)	Batch (21 d)	0.23-0.64 1/d	0.26 g/L.d	NH_4^+-N: 99.9% 7.8 mg/L.d	PO_4-P: 97.3% 0.28 mg/L.d	Franchino et al., 2013
Scenedesmus accuminatus	Digestate of the wastes of piggery farm	*Filtration *Sterilization *Dilution	Batch	0.038-0.091 1/d	0.118 g/L.d	NH_4^+-N: 6.46 mg/L.d		Park et al., 2010
			Semi-cont.		0.213 g/L.d	NH_4^+-N: 89% 19.2 mg/L.d		

Table 4.1. Studies on digestate treatment using microalgae species.

Inoculum	Wastewater Origin	Pretreatment	Type	Specific growth rate	Biomass Productivity/ Concentration	Nutrient removal		Reference
						Nitrogen	Phosphorus	
Cholorococcum sp.	Digestate of pig manure	*Filtration *Centrifugation *Dilution (2, 5, 6, 8%)	c-batch (27 d)		0.85 g/L 0.029 g/L.d	NH_4^+-N: 5.3 mg/L.d		Montero et al., 2018
		*Filtration *Centrifugation *Dilution (5.6%)	nc-batch (24 d)		0.64 g/L 0.024 g/L.d		PO_4^{3-}: 1.6 mg/L.d	
Chlorella vulgaris	Digestate of liquid swine manure	*Ammonia stripping *Settling *Centrifugation *Dilution (2, 3, 4x)	au-batch (7 d)		0.10-0.14 g/L.d	TN: 55.4% NH_4^+-N: 99.8 %	TP: 29.7%	Deng et al., 2017
			Nau- batch (7 d)		1.1 g/L	TN: 70.8% NH_4^+-N: 99.9 %	TP: 54.4%	

WW: wastewater; cont: continuous; au-batch: autoclaved and batch; nau-batch: not autoclaved and batch; c-batch: controlled batch; nc-batch: non- controlled batch.

4.2 Materials and Methods

4.2.1 Mixed microalgal culture

Mixed microalgal culture was taken from Lake Eymir in Ankara, Turkey. The culture was initially grown and kept as a stock culture by weekly feeding with liquid digestate taken from an anaerobic digester of Tatlar Wastewater Treatment Plant (Ankara, Turkey) that processed sewage sludges as raw feedstock (56% primary and 44% secondary sludge). The plant had 14-21 days of hydraulic retention time and 8 digesters with 1.5 MW total installed capacity. Microalgal inoculum used was obtained from the mentioned stock microalgal culture and characterized for TS, VS, DRP, total dissolved phosphorus (TDP), NH_4^+-N and chlorophyll-a concentrations (Table 4.2).

4.2.2 Wastewater origin

An anaerobic fixed-film reactor was previously set as an additional treatment step for the digestate taken from a full-scale AD plant (Chapter 3). The plant had a conventional completely mixed anaerobic digester and used a manure mixture of 90% laying hen and 10% cattle manure as a raw feedstock. Processing of the digestate in an AFFR was aimed to treat the residual organic content and to capture the associated biogas during the treatment. The effluent of AFFR was collected during steady-state operation of the last cycle and was settled for 1 day. The liquid phase above the settled portion of AFFR effluent (AFFR liquor) was further used in nutrient removal processes by mixed microalgal culture. The characterization of the AFFR effluent before settling and AFFR liquor is given in Table 4.2.

Table 4.2. The characterization of the AFFR effluent, AFFR liquor and microalgal inoculum.

Parameter	AFFR effluent	AFFR liquor	Microalgal inoculum
pH	8.84	8.84	7.58
TS, mg/L	11580 ± 60	8700 ± 0	2820 ± 40
VS, mg/L	5610 ± 50	4130 ± 150	1320 ± 20
Chlorophyll-a, mg/L	-*	-*	3.6 ± 0.409
NH_4^+-N, mg/L	1950.0 ± 30.00	1616.3 ± 23.75	1.6 ± 0.12
NO_3^--N, mg/L	-*	11.4 ±0.18	103.0 ± 0.50
NO_2^--N, mg/L	-*	0.0 ± 0.00	165.3 ± 1.85
DRP, mg/L	45.40 ± 0.20	41.00 ± 0.00	2.72 ± 0.00
TDP, mg/L	51.25 ± 1.75	42.75 ± 0.25	9.25 ± 0.25
VS/TS, %	48.4	47.5	46.8
DRP/TDP, %	88.6	95.9	29.4

* Not measured.

4.2.3 Analytical methods

TS, VS, chlorophyll-a and DRP concentrations were analyzed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). TDP, NH_4^+-N and NO_3^--N (nitrate nitrogen) were measured photometrically as described in the manual provided by the manufacturer (Aqualytic, 2014). NO_2^--N was measured by Dionex ICS-1000 ion chromatography. The samples were first filtered from 0.45 μm pore-sized cellulose acetate filters for NH_4^+-N, NO_3^--N, NO_2^--N (nitrite nitrogen), DRP and TDP determination. All experiments were performed in replicates. The results were given with the averages and standard deviations.

4.2.4 Particle size distribution analysis

Particle size distribution analysis was performed to determine the agglomerated formations of microalgae species in Central Laboratory of Middle East Technical University using Malvern Mastersizer 2000. Samples for particle size distribution

analysis were collected from randomly selected reactors after DRP exhaustion. They were collected in eppendorf tubes, wrapped with aluminum foil, and kept at 4^{o}C in a refrigerator before analyses. Analyses were completed within 24 hours of sample collection.

4.2.5 Microalgal species characterization

The samples for microscopic species characterization were immediately fixed after collection using Lugol's iodine solution and left at room temperature in the dark. The mixed microalgal culture taken from Lake Eymir and the grown microalgal species in the reactors were identified and counted using Leica DMI 4000 B inverted microscope by Department of Biological Sciences, Biology /Molecular Biology and Genetics.

4.2.6 Settling test

The samples for the settling test were taken from the same reactors which were used in particle size distribution analysis after DRP exhaustion. 100 mL of graduated cylinder was filled with sample and subjected to gravitational sedimentation for 2 days. Liquid-solid phase separation was done by pipetting the liquid phase (supernatant). The chlorophyll-a content and the volume of the collected supernatant was determined.

The chlorophyll-a concentration of the settled biomass (C_b) was calculated using the chlorophyll-a concentrations and the volumes of the overall content and the supernatant obtained after solid-liquid phase separation. To calculate the C_b following formula was used:

$$C_b = \frac{[V_t x C_t] - [V_s x C_s]}{V_b} \qquad [4.1]$$

C_t: Chlorophyll-a concentration at the end of the reactor operation, mg/L

C_s: Chlorophyll-a concentration of the supernatant at the end of the settling test, mg/L

C_b: Chlorophyll-a concentration of the settled biomass, mg/L

V_t: Total volume of the sample after settling, mg/L

V_s: Volume of the pipetted supernatant, mg/L

V_b: Volume of the settled biomass, mg/L

4.2.7 Scanning Electron Microscopy (SEM) Imaging

SEM imaging was performed to visualize the biological formations after the nutrient removal process. The imaging was done in Central Laboratory of Middle East Technical University using QUANTA 400F Field Emission SEM. 1 mL sample was taken from randomly selected reactors and diluted by 1/6 using ethanol within eppendorf tubes. Eppendorf tubes were then wrapped with aluminum foil, and kept at 4^{o}C in a refrigerator before imaging. SEM imaging was performed within 3-4 days of sample collection.

4.2.8 Experimental Setup

Photobioreactors used in the microalgal nutrient removal experiments were made of glass with an outside diameter of 8.6 cm and a height of 25.7 cm. Eight reactors were used in the setup (Table 4.3). Seven reactors were prepared by using 4 different dilutions of the AFFR liquor (Table 4.3). The AFFR liquor was diluted with deionized water by 1/6 for T1a and T1b, by 1/8 for T2a and T2b, by 1/10 for T3a and T3b and by 1/12 for T4. T1a and T1b, T2a and T2b, T3a and T3b were replicates of each other and were operated to investigate the repeatability of the experiments. These seven reactors were inoculated with 40 mL of microalgal inoculum (Table 4.2) and

completed to 1000 mL using the diluted AFFR liquor. One reactor was set as a control reactor (C) with 40 mL of microalgal inoculum and 800 mL of deionized water to observe the pH change during operation. PBRs were operated in batch at room temperature (26 ± 1ºC). Total operation time of the reactors varied for each reactor (Table 4.3) depending on the time required for a DRP removal of 99-100%. DRP is the highly available form of phosphorus to algae (Ekholm and Krogerus, 2003).

Table 4.3. Dilution ratios and total operation periods for reactors.

Reactor	Dilution	Total operation period, days
T1a	1/6	25.2
T1b	1/6	25.2
T2a	1/8	17.6
T2b	1/8	18.9
T3a	1/10	12.6
T3b	1/10	14.2
T4	1/12	11.6

pH was monitored by Oakton pH/CON 450 pH meter and adjusted for 3-4 times a day using concentrated hydrochloric acid (HCl) or sodium hydroxide (NaOH) (6N, 3N or 1N). PBRs were continuously aerated at a flow rate of 1 L/min with ambient air and continuously illuminated at 4.05 ± 0.460 Klux. Light intensity was measured by Extech EasyView^{TM} 31 Light Meter at 13 different locations in the setup where the reactors run. The illumination of the experimental setup is depicted in Figure 4.1.

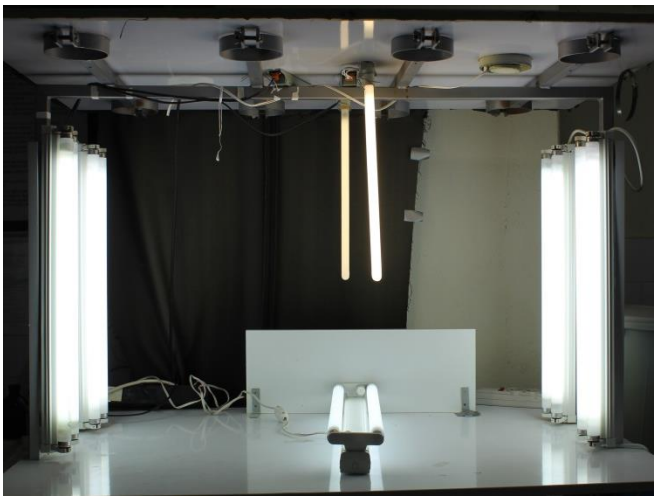


Figure 4.1. Illumination of the experimental setup.

4.3 Results and Discussion

4.3.1 Determination of the microalgae species

Microalgae species were determined in the samples obtained both from the Lake Eymir (Section 4.3.1.1) and from the randomly selected reactors (Section 4.3.1.2).

4.3.1.1 Microalgae species in mixed microalgal culture

The sample obtained from Lake Eymir could be characterized by the presence of 17 different microalgae species (Table 4.4). *Bacillariophyta* was the main group of microalgae (56.76% by biovolume) dominated in the Lake sample. The biovolume of *Cyanophyta* species was 20.67%. *Chlorophyta* species including *Chlorella vulgaris* and *Scenedesmus* sp. was represented by 1.32% by biovolume (Table 4.1). These species were extensively used in the previous studies on microalgal wastewater treatment. The presence of *Chlorella* and *Scenedesmus* species in a wastewater

treatment plant pre-dominantly among 71 species (Renuka et al., 2015) is an indication of their high adaptability to wastewaters.

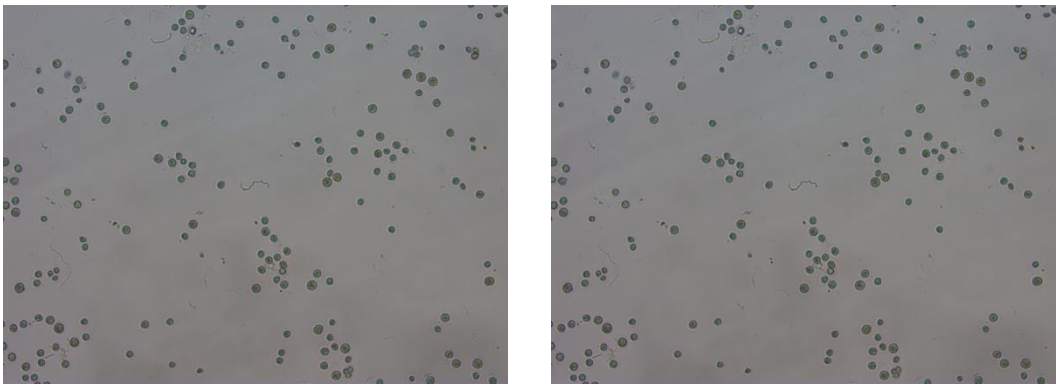
Table 4.4. Microalgae counts and biovolumes in the sample of Lake Eymir.

Group	Species	Count, %	Biovolume, %	Group biovolume, %
Bacillariophyta	*Synedra ulna*	0.16	56.67	56.76
Bacillariophyta	*Nitzschia acicularis*	0.00	0.09	
Cyanophyta	*Anabaena* sp1	4.29	11.75	20.67
Cyanophyta	*Pseudanabaena limneticum*	2.31	4.76	
Cyanophyta	*Anabaena* sp2	0.16	3.41	
Cyanophyta	*Microcystis* sp.	0.05	0.74	
Cyanophyta	*Merismopedia tenuissima*	0.01	0.01	
Picoplankton	Picoplankton - unidentified single cells <2 µm diam.	92.71	13.33	13.33
Dinophyta	*Plagioselmis lacustris*	0.11	2.65	4.10
Dinophyta	*Peridinium* sp.	0.00	1.45	
Cryptophyta	*Cryptomonas* sp.	0.02	3.82	3.82
Chlorophyta	*Chlorella vulgaris*	0.07	0.33	1.32
Chlorophyta	*Closteriopsis longissima*	0.04	0.29	
Chlorophyta	*Scenedesmus* sp.	0.04	0.32	
Chlorophyta	*Ankistrodesmus* sp.	0.01	0.29	
Chlorophyta	*Monoraphidium arcuatum*	0.00	0.01	
Chlorophyta	*Oocystis* sp.	0.00	0.09	

4.3.1.2 Microalgae species grown after nutrient removal processes

T1b and T2b were sampled for microscopic investigation after the nutrient removal process. The microscopic images obtained during the count of the species are given in Figure 4.2. *Chlorella vulgaris* was dominated among the other species by over 99% in both count and biovolume at the end of the operation of the reactors (Figure 4.3). The domination of *Chlorella* sp. by 99.30-99.60 % was previously reported in the treatment of secondary sewage effluent at the end of 30 days of operation (Marchello et al., 2015). *Chlorella vulgaris* is predominantly present in most wastewaters

including temperate climates and it has been widely employed in wastewater treatment studies due to its efficient assimilation of organics and nutrients as well as its and rapid growth (Ge et al., 2018). The survival of *Chlorella vulgaris* as a dominating species among 17 different algal species also indicated the high tolerance and adaptability of *Chlorella vulgaris* to the digestates which contained high ammonium and phosphorus concentrations. *Chlorella* sp. were previously noted as having excellent adaptation to livestock wastewaters (Tripathi and Kumar, 2017).



(a) (b)

Figure 4.2. Microscopic images taken from the samples of (a) T1b and (b) T2b.

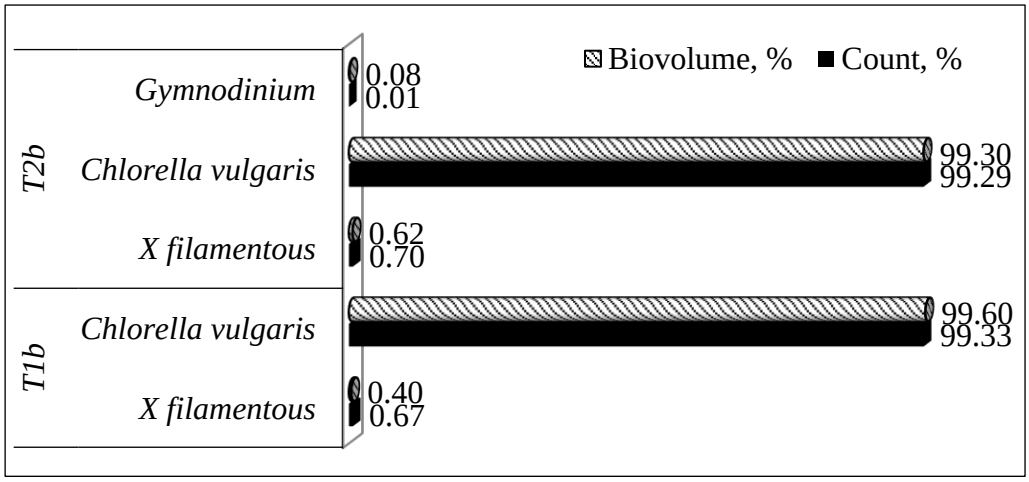


Figure 4.3. Microalgal species dominated at the end of the reactor operations.

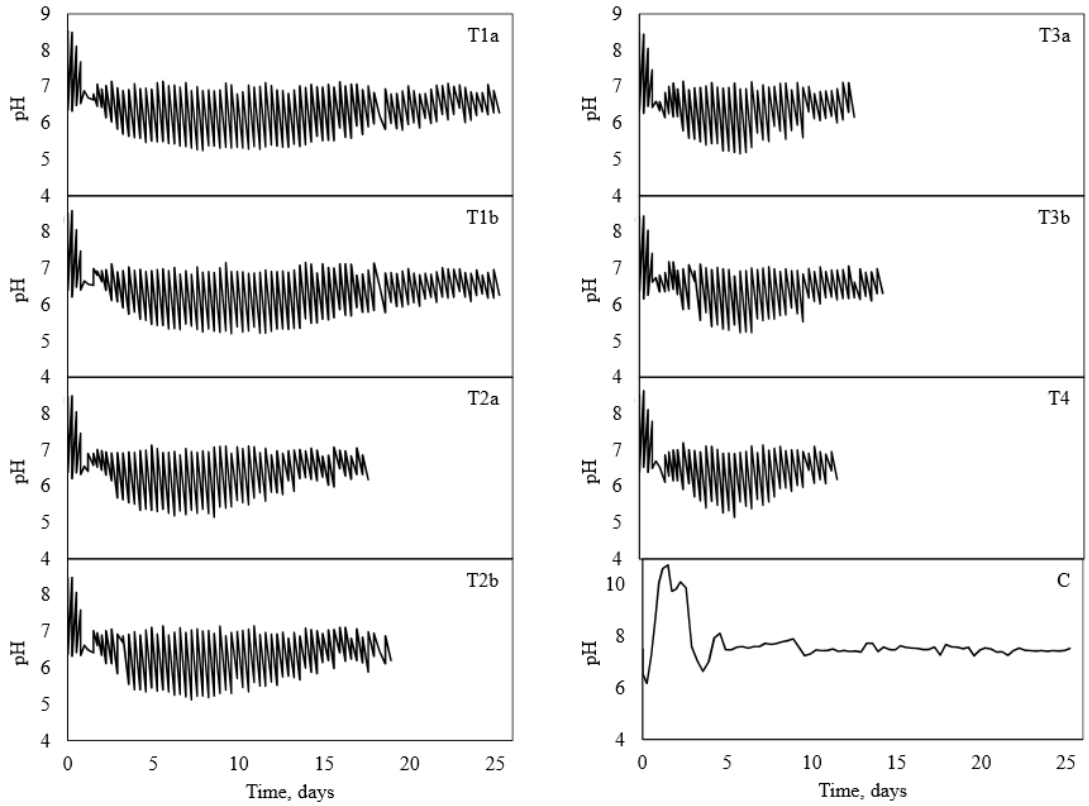
4.3.2 pH control and adjustment

pH was controlled and adjusted manually 3-4 times a day. Initial pHs of the reactors were set to 6.35-6.39 just before the start-up of the reactors. pH levels increased to 8.43-8.62 after 6 hours of operation. The increasing profile of pH after each pH adjustment continued in the first day of experiment, but at comparably lower levels (Figure 4.4). pH was adjusted to below 7 (6.38 ± 0.107) in the first day of operation aiming at decreasing the ammonium loss via conversion to ammonia as a consequence of increasing pHs. Nearly all ammonium is in the ionized form at approximately pH 7 and the ionized form decreases by increasing pHs (Evangelou, 1998). Cheng et al. (2015) also observed a pH increase from 6.0-6.5 to 7.7 at the initial stage of the *Chlorella* PY-ZU1 growth using digestate of swine manure. pH increase was probably due to the degradation of organic matters and urea hydrolysis with aeration of the manure-based product (Park et al., 2005), that is the AFFR liquor. Additionally, microalgal uptake of CO_2 can also give rise to pH of the environment (Delgadillo-Mirquez et al., 2016). Control reactor was operated without pH adjustment except the initial one from 7.50 to 6.57 (Figure 4.4). pH reached to 10.76 at the 1.5^{th} day of operation. Thus, microalgal activity may be an inducing factor for pH increase at the early stages of the experiment. The microalgal species might have survived via uptake of the residual nutrients in the microalgal inoculum content (Table 4.2).

pH tended to decrease after the initial operation day (Figure 4.4) and the adjustment was made by increasing the pH to 6.97 ± 0.092. Increasing the pH to neutral levels aimed to avoid the inhibition of microalgal species due to excessive acidification of the growth environment (Eustance et al., 2013). pH decrease can be attributed to ammonium-based nutrition of microalgal species. For a molecular ammonium uptake of the organism, a proton is released to the environment to maintain the neutrality of the cell which results in the acidification of the environment. This fact significantly

lowers the pH of the environment during the exponential growth of organisms which can further inhibit the growth (Eustance et al., 2013). A rapid decrease in pH from 9.87 to 6.64 was observed between the days 2.6 and 3.6 in the control reactor. pH oscillated between 7.24 and 7.90 from the day 4.9 on in control reactor although pH was adjusted and increased in the reactors including AFFR liquor. Almost stable pH (7.24-7.90) in the control reactor may be an indication of ceasing of the nutrition of the microalgal species.

A similar pattern in pH variation, an increase in pH within the initial days of cultivation followed by a rapid decrease in pH to values near 7, was also previously observed during the cultivation of microalgae using the liquid digestate of a wastewater treatment plant (Uggetti et al., 2014). The authors reported the main reason behind the pH decrease to near neutral levels as nitrification of ammonium. Therefore, both microalgal uptake of ammonium and ammonium conversion into nitrite and nitrate by nitrifiers are expected to have an effect on the decrease of pH during the growth of microalgal species.



(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)

Figure 4.4. pH change and adjustment during the operation of microalgal batch reactors.

4.3.3 The removal of the nutrients by microalgal-bacterial consortium

The removal of the nutrients by microalgal-bacterial consortium was evaluated with respect to the overall change in nutrient concentrations at the end of the operation period of the reactors and then the stepwise change of the nutrient concentrations throughout the process. As will be discussed in detail in the following sections, the profile of the change in nutrient concentrations during the stepwise measurements indicated an increase in the nutrient concentrations (ammonium, dissolved reactive and total dissolved phosphorus) within earlier days after the start-up of the reactors. On the other hand, the microalgal species was in growth state depending on the chlorophyll-

a measurements when the nutrients were increasing (Section 4.3.4.2). The growth of microalgal species together with the increasing amounts of nutrients suggested simultaneous dissolution and removal of nutrients at the initial stages of the operation. Therefore, a reaction kinetics study was performed on the removal of nutrients to estimate the total concentrations and the additional dissolved amounts of the nutrients.

The data points obtained from the measurements during the operation of the reactors were observed to follow two distinct patterns. The first pattern included the increase in the concentration of the constituents followed by a subsequent decrease where dissolution and uptake were simultaneously experienced. The second pattern had a steady reduction in the related concentrations and was considered as being representative of the removal of the constituents. The data points involved in second pattern were subjected to reaction kinetics analysis to estimate the order of removal for each constituent. A minimum of four data points was included in the reaction kinetics analysis.

The reaction kinetics analysis was based on linear line fitting to the data points. Linear line fitting was applied by drawing '√(nutrient concentration) vs time' (Denton and Rostron, 2013), 'nutrient concentration vs time', 'ln (nutrient concentration) vs time' and '1/(nutrient concentration) vs time' (Heldman, 2003) graphs to calculate the R^2 value for the one-half-order, zero-order, first-order and second-order reaction kinetics, respectively. The decision on the representative reaction kinetics was made based on the R^2 values of the fitted linear lines.

The linear fitting line in 'y=ax+b' form enables the rate constant of the reaction (k) to be estimated from the slope (a) of the line. If the line is extended back to the y-axis keeping the rate constant the same (where x=0), the interception is the point where

total concentration of the nutrients (initial concentrations before consumption) can be read. This total concentration is representative of the sum of the measured concentration at the start-up and the amount of the nutrients released due to dissolution. Therefore, the nutrient release and the total nutrient removal rates of microalgal-bacterial consortium could be estimated from the linear fitting line of the related order of kinetics. The overall change and the stepwise change in nutrient concentrations as well as the analysis on reaction kinetics are given in detail in the following sections.

4.3.3.1 The changes in NH_4^+- N, NO_3^--N and NO_2^--N concentrations

NH_4^+- N, NO_3^--N and NO_2^--N concentrations within the reactors were evaluated according to the initial and final concentrations within reactors in Section 4.3.3.1.1 (overall change) and according to the stepwise measurements made during the operation of the reactors in Section 4.3.3.1.2. The estimation of the NH_4^+- N removal kinetics and actual removal rates are given in Section 4.3.3.1.3. The corrected nitrification and microalgal uptake of NH_4^+- N depending on the consideration of additional dissolved amounts are presented in Section 4.3.3.1.4.

4.3.3.1.1 The overall change in NH_4^+-N, NO_3^--N and NO_2^--N concentrations

The initial NH_4^+-N concentration applied in the reactors ranged between 127.5-316.3 mg/L (Table 4.5). The dilutions in the treatment of AFFR liquor using microalgal species were made according to NH_4^+-N concentrations which corresponded to NH_4^+-N concentrations below 350 mg/L. 350 mg/L NH_4^+-N was reported to be toxic for phytoplankton (Barsanti and Gualteri, 2014). The survival of *Chlorella vulgaris* among 17 algal species counted in the mixed microalgal culture as a dominating species indicated the tolerance of *Chlorella vulgaris* to high NH_4^+- N concentrations.

Total NH_4^+-N removal was the highest in T1a and T1b (90.8 and 90.3%, respectively) which also had the highest amount of AFFR liquor at the initial setting (Table 4.5). Consequently, the retained NH_4^+-N concentrations after microalgal treatment of the AFFR liquor was the lowest in T1a and T1b (29.0±1.00 and 26.0±0.50 mg/L, respectively). On the other hand, the lowest total NH_4^+-N removal was observed in T3a (45.6%) and T4 (45.7%) which were operated with the 1/10 and 1/12 dilution ratios of the AFFR liquor, respectively. The retained NH_4^+-N concentrations were 93.0±5.50 and 69.3±1.00 mg/L in these respective reactors. T3a and T4 were operated for a shorter time period (12.6 and 11.6 days, respectively) compared to the others (14.2-25.2 days) due to earlier DRP consumption (Section 4.3.3.2.1). The earlier DRP consumption these reactors may have resulted in lower removal efficiencies for NH_4^+-N. The NH_4^+-N removal was dependent on the availability of phosphorus in the growth environment of microalgal species. T3a and T4 reactors included the most diluted AFFR liquor and this fact may have leaded to phosphorus limited conditions for further removal of NH_4^+-N.

The removal of NH_4^+-N in microalgal nutrient removal processes can be due to nitrification, denitrification and stripping during the growth of microalgal-bacterial consortium (Delgadillo-Mirquez et al., 2016). The reduction of NO_2^--N and NO_3^--N to molecular nitrogen (denitrification) is a biologically mediated process under anoxic conditions (Savaglio and Puopolo, 2012). Denitrification was not considered as a removal mechanism for NH_4^+-N in this study due to continuous aeration and illumination of the reactors which is inhibitory for denitrification. Microalgae produces oxygen continuously under the condition of continuous illumination which potentially inhibit the denitrification process (Jia and Yuan, 2016). Delgadillo-Mirquez et al. (2016) also pointed out that denitrification was not likely to occur mainly due to aerobic conditions and increasing dissolved oxygen concentrations in a

mixed microalgae and bacteria culture for nitrogen and phosphate removal from wastewater. Furthermore, volatilization of ammonia (ammonia stripping) was assumed not to be a removal mechanism for ammonium due to the maintenance of pH around neutral. A similar assumption was previously made by Rada-Ariza et al. (2017) for the operational pH interval between 7.5 and 8.0. Ammonia stripping was previously noted as a removal mechanism for nitrogen in the treatment of the digestate of starch wastewater and alcohol wastewater using *Chlorella pyrenoidosa* at pHs 8.5-9.5 (Yang et al., 2015). Anammox process was also previously discussed as an ammonium removal mechanism under anaerobic conditions (Section 2.3.7.4). However, ammonium removal in microalgal nutrient removal process by Anammox was not expected based on the fact that Anammox can be severely inhibited by oxygen. A dissolved oxygen concentration of 0.25 mM was previously reported to inhibit the Anammox activity by 90% (Carvajal-Arroyo et al., 2013). Additionally, the formation of nitrite and nitrate can possibly be inhibitory for Anammox (Jin et al., 2012; Carvajal-Arroyo et al., 2013) during the operation of the microalgal reactors. Therefore, nitrification and microalgal uptake were accepted as the major removal mechanisms for ammonium in microalgal process and the anammox, denitrification and stripping pathways were excluded for the reasons described above.

NO_2^--N and NO_3^--N concentrations in the reactors (0.9-6.3 and 2.7-3.7 mg/L, respectively) were much lower at the initial stage of the experiment for all reactors compared to NH_4^+-N concentrations (127.5-316.3 mg/L) (Table 4.5). NO_3^--N concentrations increased from the initial concentrations of 2.7-3.7 mg/L to 34.5-146 mg/L at the end of operation considering all reactors. The increasing concentrations of the NO_3^--N within the reactors at the end of the experiment suggested nitrification of ammonium. Nitrification is a process by which reduced nitrogen compounds (mainly ammonia) are sequentially converted into NO_2^- and NO_3^- by nitrifying microorganisms (U.S. EPA, 2002). The mineral forms of nitrogen that can be assimilated by microalgae also include NO_3^- and NO_2^- as well as NH_4^+ (Cadoret et al., 2014; Delgadillo-Mirquez

et al., 2016). However, microalgae prefer to metabolize NH_4^+ rather than other forms of nitrogen (Delgadillo-Mirquez et al., 2016) due to lower energy requirement (Cadoret et al., 2014). Therefore, any NO_2^- or NO_3^- removal by microalgal assimilation was not expected due to the NH_4^+ content remaining at the end of the experiment in each reactor which was in the range of 26-93 mg/L (Table 4.5).

Even if the microalgal nutrient removal process was evaluated in terms of the overall decrease in NH_4^+-N concentration, the stepwise measurements during the operation of the reactors were also examined in Section 4.3.3.1.2. Further discussion on the NH_4^+-N removal was carried out in Sections 4.3.3.1.3 and 4.3.3.1.4 after inclusion of the additional dissolved amounts.

Table 4.5. NH_4^+- N, NO_3^--N and NO_2^--N concentrations at the initial and final stages of the experiment.

Constituent		Reactor						
		T1a (25.2 days)	T1b (25.2 days)	T2a (17.6 days)	T2b (18.9 days)	T3a (12.6 days)	T3b (14.2 days)	T4 (11.6 days)
NH_4^+- N, mg/L	Initial	316.3 ± 89.41	268.8 ± 12.75	222.5 ± 2.00	230.9 ± 2.63	170.9 ± 2.13	166.1 ± 4.63	127.5 ± 6.25
	Final	29.0 ± 1.00	26.0 ± 0.50	84.8 ± 0.25	66.5 ± 3.50	93.0 ± 5.50	71.8 ± 0.25	69.3 ± 1.00
	Removed	287.3	242.8	137.8	164.4	77.9	94.4	58.3
NO_3^--N, mg/L	Initial	3.0 ± 0.10	3.6 ± 0.10	3.1 ± 0.10	3.7 ± 0.25	3.2 ± 0.10	2.7 ± 0.13	2.7 ± 0.40
	Final	146.0 ± 1.00	141.5 ± 0.50	46.6 ± 0.38	54.0 ± 0.00	50.1 ± 1.25	40.6 ± 0.85	34.5 ± 0.45
	Formed	143.0	137.9	43.5	50.4	46.9	37.8	31.8
NO_2^--N, mg/L	Initial	0.9 ± 0.09	2.2 ± 0.18	2.5 ± 0.14	2.2 ± 0.15	4.4 ± 0.26	6.3 ± 0.34	5.4 ± 1.34
	Final	3.1 ± 0.16	0.1 ± 0.01	42.4 ± 1.79	48.7 ± 1.67	0.0 ± 0.00	4.3 ± 0.11	0.0 ± 0.00
	Formed	2.3	-2.1	40.0	46.5	-4.4	-2.0	-5.4
(NO_3^--N)+(NO_2^--N), mg/L		145.3	135.8	83.5	96.8	42.4	35.8	26.4
Total NH_4^+- N removal, %		90.8	90.3	61.9	71.2	45.6	56.8	45.7
NH_4^+- N conversion by nitrification, %		50.6	55.9	60.6	58.9	54.5	38.0	45.3
NH_4^+- N removal by assimilation into biomass, mg/L		142.0	107.0	54.3	67.5	35.4	58.6	31.9
NH_4^+- N removal by assimilation into biomass, %		49.4	44.1	39.4	41.1	45.5	62.0	54.7

Note 1: A sample calculation on NH_4^+-N removal is provided in Appendix I.
Note 2: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

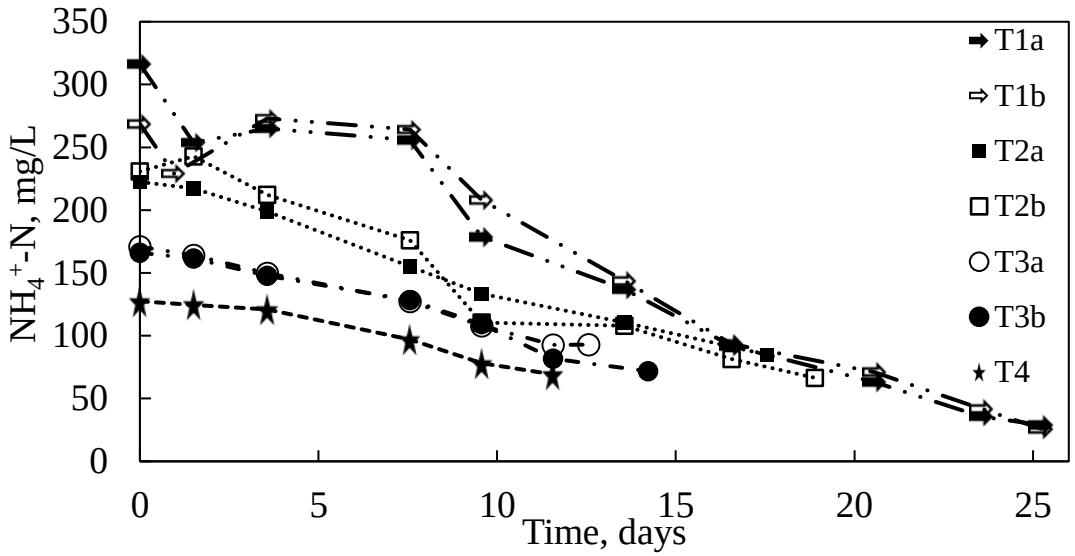
4.3.3.1.2 The changes in NH_4^+-N and NO_3^--N concentrations during the operation of the reactors

NH_4^+- N reduction and NO_3^--N accumulation profiles were investigated by stepwise measurements made during the operational period of the reactors (Figure 4.5 and 4.6, respectively). The reactors containing the most concentrated AFFR liquor (T1a, T1b) experienced an increase in the NH_4^+- N concentration in the first 3.5 days of operation which was followed by a reduction. The concentration of NH_4^+- N decreased to its initial start-up value after 7 to 9 days of operation. Similarly, NH_4^+- N increase was observed for 1.5 days in T2b (1/8 diluted AFFR liquor) which was reduced to its start-up value after one day. NH_4^+- N concentration decreased at the second measurement (on the 1.5th day of operation) for the rest of the reactors (T2a, T3a, T3b and T4). Dissolution of particulate fraction of the digestate may contribute to an increase in the concentration of ammonium (Botheju et al., 2010). The degradation of organic matter or urea hydrolysis can also be accounted for the reason of NH_4^+- N increase as previously discussed in Section 4.3.2. The increase in the concentration of the nutrients (ammonium and phosphate) was previously observed in the treatment of centrate wastewater of anaerobically digested sludge using *Chlorella vulgaris* culture (Ge et al., 2018). The authors linked the reason behind the increase in the concentration of the nutrients to the release of intracellular materials from the dead cells (Ge et al., 2018). The study of concern included a sterilization process as a pre-treatment step and did not involve any solids removal process. On the other hand, the studies on nutrient removal from digestates using microalgal cultures included filtration and/or centrifugation as a general approach (Table 4.1). The removal of solids before microalgal nutrient removal processes may constitute the reason behind the dissolution of nutrients not previously reported and evaluated elsewhere in the studies aiming at nutrient removal from the digestates using microalgal cultures. In these studies, the particulates which might be responsible for the additional dissolution of the nutrients have probably been removed from the wastewater by the application of solids removal

processes as a pretreatment step, thus, the associated dissolution may not have been occur. Likewise, the increase in ammonium concentration suggested the dissolution of nutrients into water as the AFFR liquor was not filtered or centrifuged to remove the particulate matters before the application of microalgal nutrient removal process.

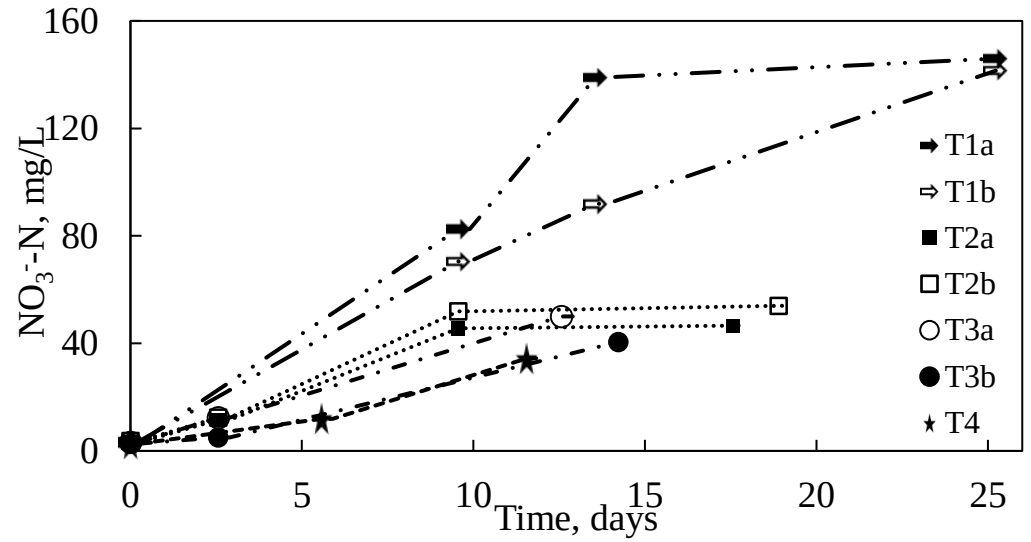
The concentration of NO_3^--N was observed to increase in all reactors from the range of 2.7-3.7 to 34.5-146 mg/L which indicated nitrification (Figure 4.6). The increase in NO_3^--N in T2a and T2b continued until 9.6 days and remained approximately constant to the end of operation (17.6 and 18.9th day, respectively). The stabilized concentration of nitrate justified that the denitrification process was not experienced during the operation.

Microalgae species was in growth phase within the earlier days of experiment (Section 4.3.4.2) when dissolution of nutrients was experienced. Thus, the dissolution and the removal of nutrients were experienced simultaneously within the early days of the experiment. Section 4.3.3.1.3 describes the application of the reaction kinetics on the removal of the nutrients in order to estimate the additional dissolved amounts and actual removal rates of the NH_4^+- N. NH_4^+- N removal via nitrification and microalgal NH_4^+- N removal was re-calculated based on the kinetics fitted in Section 4.3.3.1.4.



(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)

Figure 4.5. The change in NH_4^+- N concentration during the operation.



(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)

Figure 4.6. The change in NO_3^-- N concentration during the operation.

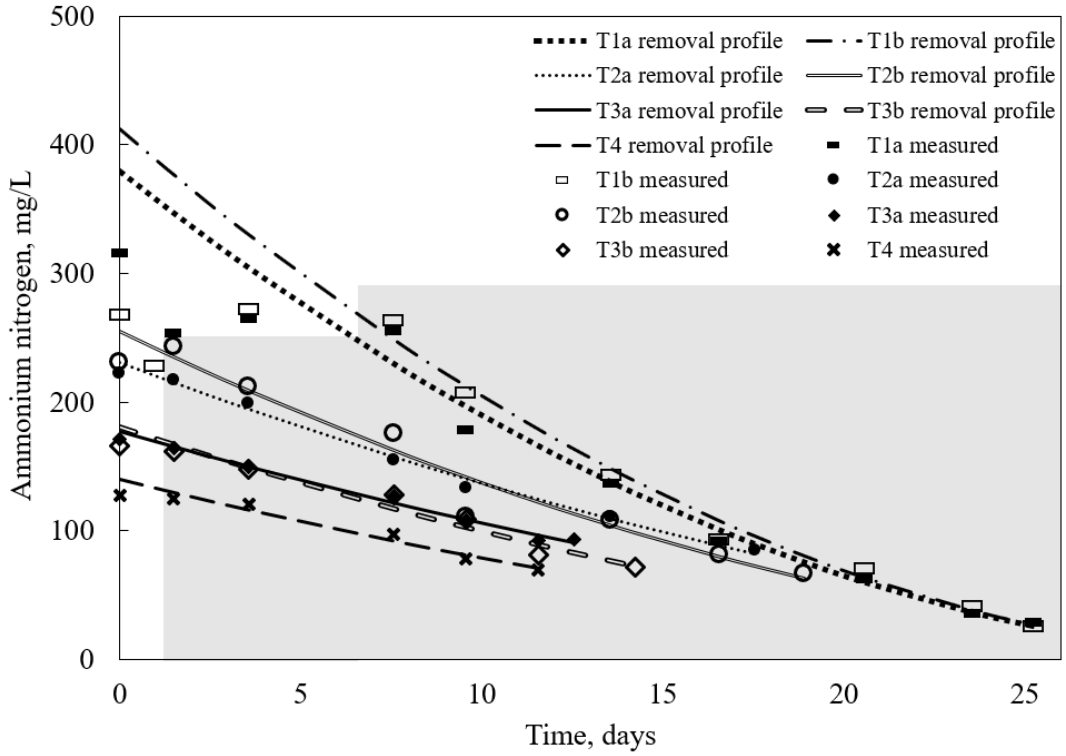
4.3.3.1.3 Estimation of the NH_4^+-N removal kinetics and actual removal rates

The reaction kinetics study on NH_4^+- N removal was performed as described in Section 4.3.3. The details of NH_4^+- N removal kinetics are provided in Appendix J.

One-half-order kinetics was found to represent the removal of NH_4^+- N in all reactors with a coefficient of determination of 0.9516-0.9930. The NH_4^+- N removal profiles obtained by the application of one-half-order kinetics are presented in

Figure 4.7. Total NH_4^+- N concentrations in the reactors were found to range between 140.4-412.1 mg/L which were expectedly higher than the ones measured at the initial start-up of the reactors (127.5-316.3 mg/L) (Table 4.6) due to the dissolution of additional NH_4^+- N from the digestate content. The additional dissolved NH_4^+- N was in the range of 6.7 and 143.4 mg/L (Table 4.6) which increased the NH_4^+- N concentration by 4-53% within the reactors. Even though the dissolution of NH_4^+- N from the digestate content increased the abundance of this constituent in the growth environment of microalgal-bacterial consortium, the total NH_4^+-N concentrations reaching up to 412 mg/L did not have any inhibitory effect. 350 mg/L NH_4^+-N is toxic for phytoplankton (Barsanti and Gualteri, 2014) as previously mentioned. However, the extension of the dissolution process over a time period as well as the simultaneous NH_4^+-N uptake by microalgal-bacterial consortium probably avoided the inhibitory effects of high NH_4^+-N concentrations.

The additional dissolved NH_4^+- N could not be approximated between the replicates of the reactors and the initial concentrations of the AFFR liquor added to the reactors (Table 4.6) which was probably due to uneven distribution of the particulates and/or organics in AFFR liquor.



Note: The shaded area shows the data points included in the estimation of the removal kinetics. (Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)

Figure 4.7. NH_4^+- N removal profiles estimated by one-half-order reaction kinetics analysis.

Table 4.6. The calculations on the removal of NH_4^+- N including the dissolution process.

Reactor	Half-order linear line equation	y values at x=0	Total concentration of NH_4^+- N, mg/L	Measured NH_4^+- N at the initial start-up, mg/L	Additional dissolved NH_4^+- N, mg/L	Final NH_4^+- N (measured) in the reactor, mg/L	Actual NH_4^+- N removed, mg/L	Total operation period, d	Rate of NH_4^+- N removal, mg/L.d	Removal Efficiency, %
	A	B	$C = (B)^2$	D	E = C-D	F	G = C-F	H	I= G /H	J=(C-F)*100/C
T1a	y=-0.5704x+19.493	19.493	379.9770	316.3	63.7	29.0	350.977	25.2	13.9	92.4
T1b	y=-0.5984x+20.300	20.300	412.0900	268.8	143.3	26.0	386.09	25.2	15.3	93.7
T2a	y=-0.3488x+15.192	15.192	230.7969	222.5	8.3	84.8	145.9969	17.6	8.3	63.3
T2b	y=-0.4261x+15.978	15.978	255.2965	230.9	24.4	66.5	188.7965	18.9	10.0	74.0
T3a	y=-0.3014x+13.325	13.325	177.5556	170.9	6.7	93.0	84.5556	12.6	6.7	47.6
T3b	y=-0.3444x+13.444	13.444	180.7411	166.1	14.6	71.8	108.9411	14.2	7.7	60.3
T4	y=-0.2987x+11.849	11.849	140.3988	127.5	12.9	69.3	71.0988	11.6	6.1	50.6

Note 1: x values represented time and y (B) values represented $\sqrt{(NH_4^+- N)}$. Total concentration of NH_4^+- N (C) was calculated from the equation of $y=B=\sqrt{(NH_4^+- N)}$.

Note 2: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

The actual NH_4^+- N removal was found as 351.0, 386.1, 146.0, 188.8, 84.6, 108.9 and 71.1 mg/L for T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively, under the consideration of dissolution (Table 4.6). These actual removals were expectedly higher than that of obtained without considering the dissolution (58.3-242.8 mg/L) (Table 4.5). The highest NH_4^+- N removal efficiency was recorded as 92.4 for T1a and 93.7% for T1b which contained the most concentrated AFFR liquor. Even though complete ammonium removal was previously reported in the treatment of digestates using microalgal cultures (Wang et al., 2010a and 2010b; Ji et al., 2014 and 2015), these high removal efficiencies can be attributed to the uncontrolled increase in pH during operation. pH increase is an inducing factor for ammonia volatilization and decreasing the pH to near neutral levels has a potential to limit the ammonia volatilization. Cheng et al. (2015) previously reported 73% ammonium removal with an operational pH between 7.0 and 7.5 whereas 100% ammonium removal was obtained at higher pHs of 9-10 (Ji et al., 2014).

Actual NH_4^+- N removal could be related to the total concentrations within the reactors with an R^2 of 0.9917 (Figure 4.8). The reactors having higher total NH_4^+- N concentration, thus, presented better NH_4^+- N removal (Table 4.6). The better NH_4^+- N removal with the use of more concentrated AFFR liquor was previously attributed to the elimination of phosphorus deficit environment for microalgal-bacterial consortium (Section 4.3.3.1.1). The NH_4^+- N removal rates calculated in the range of 6.1-15.3 mg/L.d were consistent with the studies integrating digestates with microalgal treatment. Franchino et al. (2013) reported NH_4^+- N elimination capacity of *Chlorella vulgaris* as 3.4-7.8 mg/L.d for different dilutions of digestate of cattle slurry and raw cheese whey. Higher removal rates were also obtained as 19.2 mg/L.d NH_4^+- N using *Scenedesmus accuminatus* in the treatment of digestates of the piggery wastes in semi-continuous reactors (Park et al., 2010). NH_4^+- N removal rates were also found to be increasing with the increasing amount of AFFR liquor used at the start-up of the reactor (13.9-15.3, 8.3-10.0, 6.7-7.7 and 6.1 mg/L.d for T1a-T1b, T2a-T2b, T3a-T3b and T4,

respectively). The dependence of removal to initial concentration of NH_4^+- N was found to be agreed well with that of Wang et al. (2014). The authors observed different nutrient removal rates in wastewater containing different concentrations of nitrogen using the same species of algae.

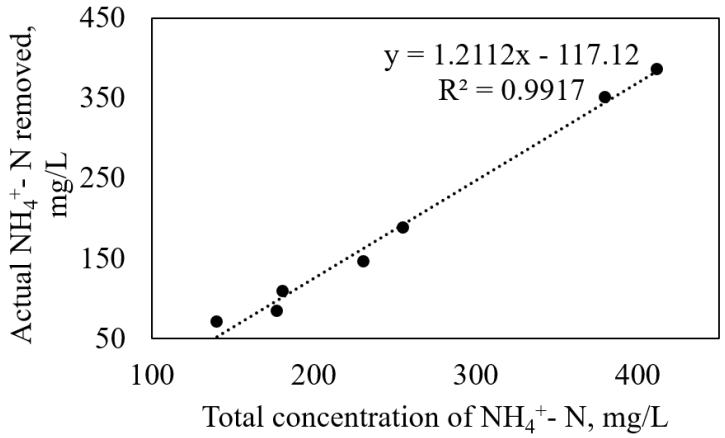


Figure 4.8. Correlation between total and actual removed NH_4^+- N concentrations.

4.3.3.1.4 Nitrification of NH_4^+- N and microalgal NH_4^+-N removal

The nitrified concentrations and biological uptake of ammonium were re-calculated (Table 4.7) based on the total and additional dissolved concentrations estimated using reaction kinetics (Section 4.3.3.1.3). The calculations showed that the nitrified NH_4^+-N concentration was in the range of 32.9-57.2% of the removed NH_4^+- N. The increase in cumulative nitrite and nitrate concentrations corresponded to 26.4-145.3 mg/L which resulted in the final concentrations ranging between 34.5-149.1 mg/L. Uggetti et al. (2014) also reported the nitrification of ammonium in the treatment of the digestate of a wastewater treatment plant using a mixed microalgae culture dominated by *Scenedesmus* sp. The authors observed an increase in the NO_x-N concentration from approximately 30 to 140 mg/L. The abundance of oxygen in microalgal treatment processes has a potential to stimulate ammonium nitrification (Uggetti et al., 2014).

Table 4.7. The corrected nitrified and biomass uptake of NH_4^+- N via dissolution consideration.

Constituent		Reactor						
		T1a (25.2 days)	T1b (25.2 days)	T2a (17.6 days)	T2b (18.9 days)	T3a (12.6 days)	T3b (14.2 days)	T4 (11.6 days)
NH_4^+- N, mg/L	Initial	380.0	412.1	230.8	255.3	177.6	180.7	140.4
	Final	29.0	26.0	84.8	66.5	93.0	71.8	69.3
	Removed	351.0	386.1	146.1	188.8	84.6	109.0	71.2
NO_3^--N, mg/L	Initial	3.0	3.6	3.1	3.7	3.2	2.7	2.7
	Final	146.0	141.5	46.6	54.0	50.1	40.6	34.5
	Formed	143.0	137.9	43.5	50.4	46.9	37.8	31.8
NO_2^--N, mg/L	Initial	0.9	2.2	2.5	2.2	4.4	6.3	5.4
	Final	3.1	0.1	42.4	48.7	0.0	4.3	0.0
	Formed	2.3	-2.1	40.0	46.5	-4.4	-2.0	-5.4
$(NO_3^-$-N)+$(NO_2^-$-N), mg/L		145.3	135.8	83.5	96.8	42.4	35.8	26.4
Total NH_4^+- N removal, %		92.4	93.7	63.3	74.0	47.6	60.3	50.7
NH_4^+- N conversion by nitrification, %		41.4	35.2	57.2	51.3	50.2	32.9	37.1
NH_4^+- N removal by assimilation into biomass, mg/L		205.7	250.3	62.6	92.0	42.1	73.2	44.8
NH_4^+- N removal by assimilation into biomass, %		58.6	64.8	42.8	48.7	49.8	67.1	62.9

Note: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

Despite playing an important role in the ammonium removal process by converting it into oxidized forms, nitrification was not considered in the studies (Wang et al., 2010a; Cai et al., 2013b) achieving 100% ammonium removal. On the other hand, nitrification may possibly represent the main removal mechanism for ammonium removal in microalgal photobioreactors (Rada-Ariza et al., 2017). Investigating the growth of the nitrifying bacteria in the presence of cyanobacteria and algae in a lab-scale continuous flow nitrifying bioreactor seeded with activated sludge, Choi et al. (2010) reported the unchanged community structure of nitrifying bacteria while microalgae and cyanobacteria were grown. The authors addressed *Nitrosospira*, *Nitrospira*, and *Nitrobacter* species as the dominant species. Gammaproteobacteria, which have an ability to oxidize ammonia, were also identified in the algal–bacterial photobioreactors treating piggery wastewaters (Ferrero et al., 2012). *Nitrosococcus oceani* and *Nitrosococcus halophilus* are the two species that represent the gammaproteobacterial ammonia-oxidizing bacteria (Koops et al., 2006). The probable source of the nitrifiers in microalgal nutrient removal process from AFFR liquor can be the mixed microalgal culture obtained from a Lake Eymir which was initially grown in the digestate of Tatlar Wastewater Treatment Plant (Section 4.2.1). Nevertheless, nitrification of ammonium has a potential to open a pathway for further removal of nitrogen by converting nitrite and nitrate into gaseous nitrogen via denitrification. This can be achieved via introducing dark cycles in microalgal reactors in the presence of sufficient organic carbon (Rada-Ariza et al., 2017).

The uptake of NH_4^+- N by microalgal bacterial biomass was in the range of 42.1-250.3 mg/L which constituted 42.8-67.1% of the removed portion of the NH_4^+- N. This finding was in agreement with the study reporting 45-84% of ammonium removal by the assimilation into algal bacterial biomass during the treatment of pretreated swine slurry using *Chlorella sorokiniana* and a mixed bacterial culture (de Godos et al., 2009). The correlation between chlorophyll-a and algal NH_4^+-N consumption for different batches was also evaluated in the treatment of AFFR liquor (Figure 4.9)

which yielded a R^2 value of 0.8499 and a correlation coefficient (R) of 0.9219. The R^2 of 0.8499 obtained for different batches indicated that the ammonium uptake increased with the abundance of microalgal species. However, the uptake could not be directly related to the growth of the microalgal culture as R^2 represented a value not very close to perfect fit. The reason behind not being able to directly correlate the ammonium uptake with microalgal growth may be probably due to uptake of ammonium by other microorganisms in the growth environment. On the other hand, the R^2 value obtained for chlorophyll-a and microalgal NH_4^+-N consumption was in agreement with the ones obtained between 0.85-0.97 for the removal of nutrients ($NH_4^+$and PO_4^{3-}) from wastewater by mixed microalgae-bacteria culture (Delgadillo-Mirquez et al., 2016).

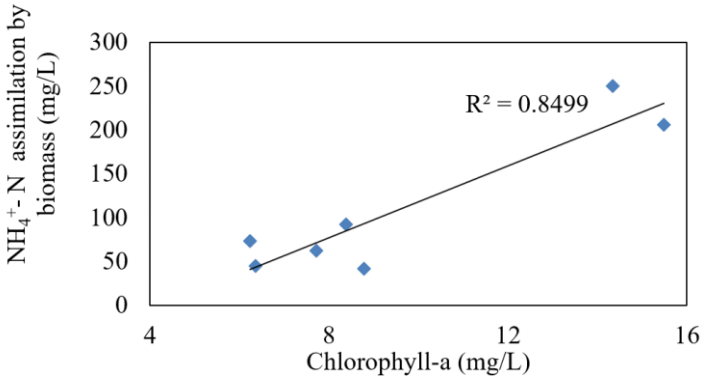


Figure 4.9. Correlation of chlorophyll-a concentration with ammonium assimilation.

4.3.3.2 The changes in DRP and TDP concentrations

DRP and TDP removals were evaluated considering the overall change of concentrations (Section 4.3.3.2.1) and the data obtained during the operation of the reactors (Section 4.3.3.2.2).

4.3.3.2.1 The overall change in DRP and TDP concentrations

AFFR liquor was initially characterized as comprising of 95.9% of the dissolved phosphorus in reactive form (DRP/TDP) (Table 4.2). Therefore, 95.9% of the dissolved phosphorus was readily available for the microalgal species at the initial stage of the experiment. TDP removal efficiencies were observed as 95.6% for T1a and T1b, 95.2 and 95.3% for T2a and T2b, 94.4 and 94.1% for T3a and T3b, respectively, and 93.3% for T4 (Table 4.8). The longer operation periods and the use of more concentrated AFFR liquor resulted in higher removal efficiencies of TDP. Microalgal-bacterial consortium was observed to be capable of removing 7.60-16.01 mg/L TDP with 93-95% treatment efficiencies. Phosphorus can be removed by bacterial or microalgal uptake as well as precipitation in microalgal nutrient removal processes, the latter induced by high pHs (Lau et al., 1995; Cho et al., 2011; Lee et al., 2015). Inorganic phosphates can be coagulated and adsorbed in algal systems at pHs higher than 8 (Li et al., 2011). The pH of the reactors in microalgal nutrient removal setup was kept around 7, thus, the main mechanism for phosphorus removal was considered as biological uptake of microalgal-bacterial consortium. A similar approach for phosphorus precipitation at high pHs was previously used by Su et al. (2012) and Ji et al. (2014).

Table 4.8. DRP and TDP concentrations at the initial and final stages of the experiment.

Constituent		Reactor						
		T1a (25.2 days)	T1b (25.2 days)	T2a (17.6 days)	T2b (18.9 days)	T3a (12.6 days)	T3b (14.2 days)	T4 (11.6 days)
DRP, mg/L	Initial	13.10 ± 0.14	14.63 ± 0.13	11.47 ± 0.17	11.68 ± 0.20	9.46 ± 0.22	9.20 ± 0.16	7.72 ± 0.16
	Final	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.00	0.06 ± 0.02	0.08 ± 0.00	0.08 ± 0.00	0.00 ± 0.00
	Removed	13.10	14.63	11.39	11.62	9.38	9.12	7.72
TDP, mg/L	Initial	16.75 ± 1.00	15.75 ± 0.50	13.63 ± 0.38	12.60 ± 0.40	9.60 ± 0.20	9.45 ± 0.00	8.15 ± 0.05
	Final	0.74 ± 0.06	0.69 ± 0.09	0.65 ± 0.3	0.59 ± 0.01	0.54 ± 0.04	0.56 ± 0.04	0.55 ± 0.05
	Removed	16.01	15.06	12.98	12.01	9.06	8.89	7.60
Total DRP removal, %		100.0	100.0	99.3	99.5	99.2	99.1	100.0
Total TDP removal, %		95.6	95.6	95.2	95.3	94.4	94.1	93.3

Note 1: A sample calculation on the removal efficiencies of DRP and TDP is presented in Appendix K.
Note 2: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

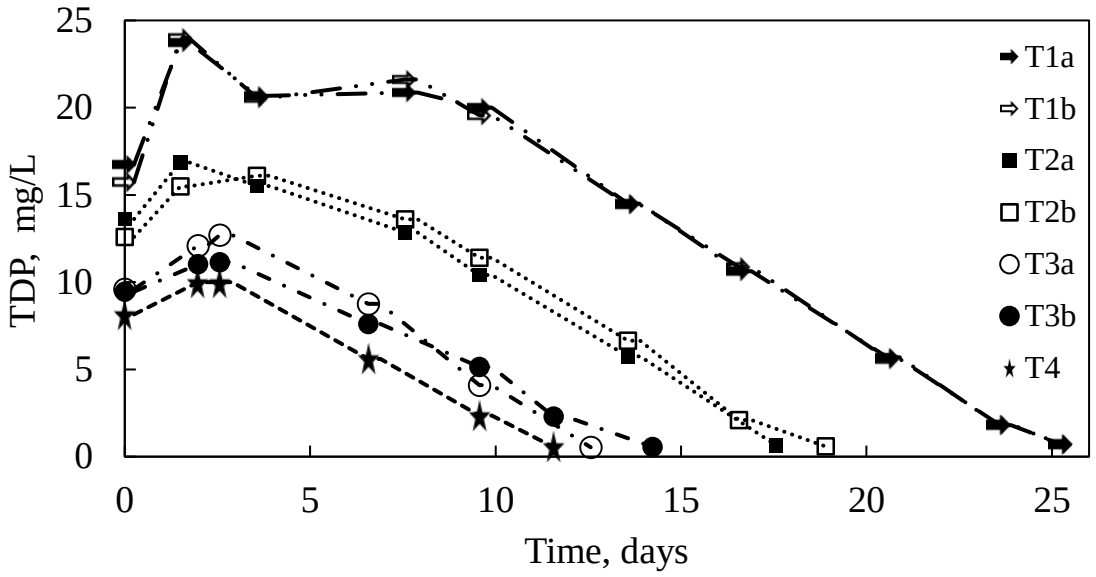
DRP removal efficiency of 99-100% was achieved in all reactors under different operation periods of the reactors (Table 4.8). These removal efficiencies accounted for 7.72-13.10 mg/L of DRP consumption by microalgal-bacterial consortium. DRP and TDP removals were further investigated during the operation of the reactors by stepwise measurements given in Section 4.3.3.2.2.

4.3.3.2.2 The changes in TDP and DRP concentrations during the operation of the reactors

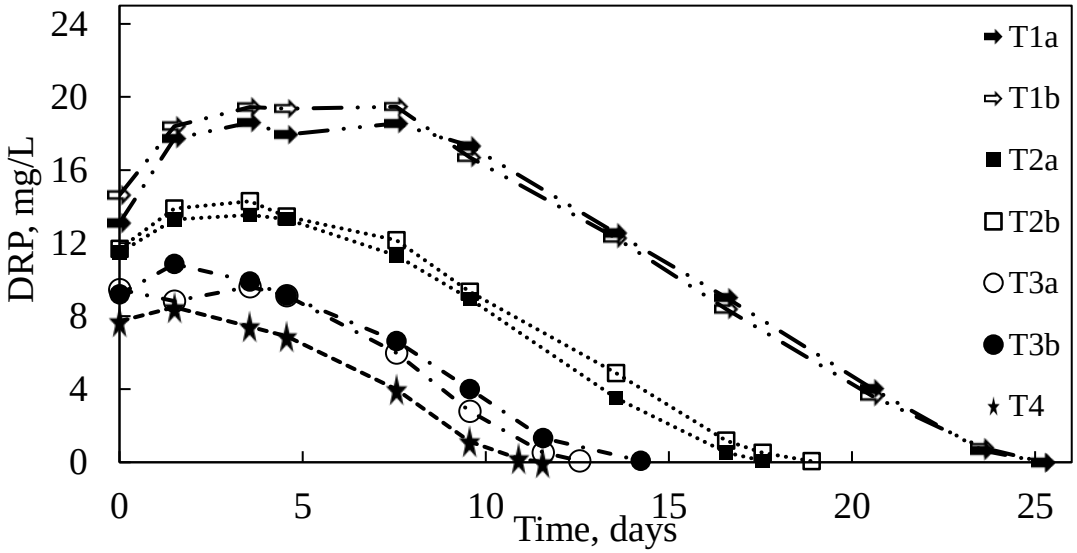
TDP accumulated in all reactors at the early days of the experiment at significant concentrations (Figure 4.10). The maximum concentrations of TDP were observed to be 23.75, 24.00, 16.88, 16.10, 12.70, 11.15, 10.00 mg/L in T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively, after the start-up of the reactors. The peak TDP concentrations were recorded on different days of the experiment (1.5^{th} day for T1a, T1b and T2a, 3.6^{th} day for T2b, 2.6^{th} day for T3a and T3b and 2^{nd} day for T4). These concentrations were accounted for an increase of the TDP concentration in T1a, T1b, T2a, T2b, T3a, T3b and T4 by 42, 52, 24, 28, 32, 18 and 23%, respectively, with respect to the initial TDP concentration within the reactor. The increase in TDP concentrations can be a result of the maintenance of pH at around 7 using HCl. Decreasing the pH using a concentrated acid as used in this study (e.g. HCl) may cause dissolution of the particulate phosphorus into soluble form (Zhang et al., 2010). Total phosphorus in manures is comprised of inorganic phosphorus by a majority and mostly bounded to the particulates as calcium and/or magnesium-phosphorus. The particulates in manures can also contain calcium-phosphorus (Ca-P) compounds due to their low solubility. Acidification would protonate the phosphate ions of Ca-P compounds and would result in the dissolution of bounded phosphorus into solution (Zhang et al., 2010). Therefore, HCl addition to regulate pH and the corresponding TDP increase at the early days of the experiment is an indication of acidification induced dissolution of phosphorus.

The increase in DRP concentrations were also observed after the start-up of reactors (Figure 4.11). The highest DRP concentrations measured were 18.56 mg/L for T1a, 19.46 mg/L for T1b, 13.54 mg/L for T2a, 14.29 mg/L for T2b, 9.61 mg/L for T3a, 10.85 mg/L for T3b and 8.49 mg/L for T4. These concentrations accounted for an increase in the DRP concentrations by 42, 33, 18, 22, 2, 18 and 10% in T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively, with respect to the initial DRP concentrations. The maximum concentrations of DRP in T1a and T1b, which contained the most concentrated AFFR liquor, were observed at a later operation time than that of observed for maximum TDP concentration for the same reactors. This fact suggested the stepwise conversion of particle phosphorus into dissolved form phosphorus and then later conversion into dissolved reactive phosphorus. The dissolved phosphorus from particulates is comprised of dissolved reactive and unreactive phosphorus. Dissolved unreactive phosphorus mainly includes dissolved organic phosphorus and polyphosphates (Güngör and Karthikeyan, 2008). The organic compounds in dissolved phase can be hydrolyzed into inorganic phosphorus with an alkaline enzyme phosphatase by algal species (Åkerström et al., 2014). Polyphosphate compounds may be originally present in the environment or may be formed as a result of the dissociation of the organic compounds. These compounds are unstable and are converted to orthophosphate (DRP) in water eventually (Spellman, 2006). Hence, the stepwise conversion of particulate phosphorus into dissolved reactive phosphorus is probable to be observed in particulate- dissolved unreactive- dissolved organic- dissolved inorganic- dissolved reactive phosphorus pathway.

Sections 4.3.3.2.3 and 4.3.3.2.4 describe the application of the reaction kinetics to estimate the dissolved amounts and actual removal rates of the TDP and DRP, respectively.



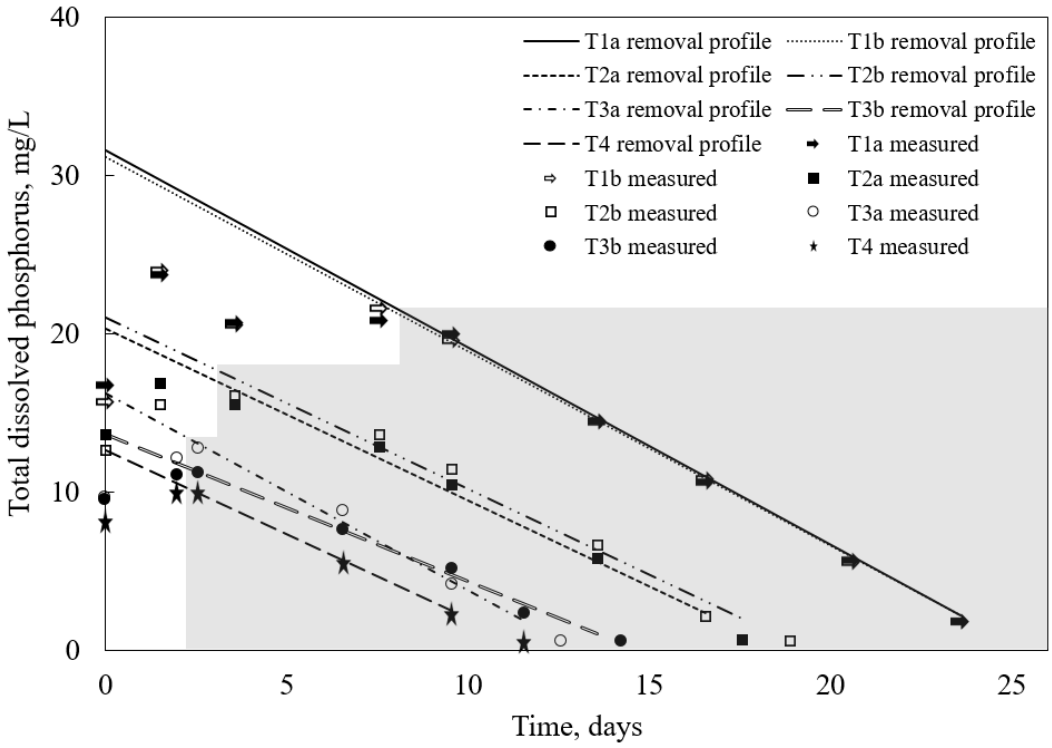
(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)
Figure 4.10. The change in TDP concentration during the operation of the reactors.



(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)
Figure 4.11. The change in DRP concentration during the operation of the reactors.

4.3.3.2.3 Estimation of the TDP removal kinetics and actual removal rates

The TDP removal within the reactors was well represented with zero-order kinetics having a R^2 between 0.9833-0.9988 (Appendix L). TDP removal profiles estimated using zero-order kinetics are given in Figure 4.12. Total TDP concentration was calculated as in the range of 12.631-31.557 mg/L for all the reactors (Table 4.9). TDP removal corresponded to 95.6-97.8% which was in agreement with that of the treatment of the digestate of piggery waste (maximum 93.41-97.16%) using *Scenedesmus obliquus* (Xu et al., 2015).



Note: The shaded area shows the data points included in the estimation of the removal kinetics.
(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)

Figure 4.12. TDP removal profiles estimated by zero-order reaction kinetics analysis.

Additional phosphorus release due to dissolution ranged between 4.169-15.422 mg/L (Table 4.9) which corresponded to an increase by 88, 98, 49, 67, 69, 44 and 55 % for T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively, with respect to the initial measured concentrations within reactors. The dissolution driven increase in phosphorus concentrations (44-98%) were observed to be higher than that of NH_4^+- N (4-53%). This fact may be speculated to be due to different dissolution processes governing for each specific nutrient. Phosphorus dissolution may originate from the particulate fraction containing calcium phosphate precipitates. The calcium phosphate precipitates were probably formed during the high-rate anaerobic treatment of the liquid digestate as previously discussed (Chapter 3, Section 3.3.4.2). These precipitates can be broken down with the acidic treatment enabling the release of bounded phosphorus into solution (Zhang et al., 2010). The addition of HCl to regulate pH may have acted as a chemical treatment for more phosphorus release in the simultaneous dissolution and uptake of the nutrients. On the other hand, ammonium release can be due to degradation of organic matter and urea hydrolysis driven by aeration (Park et al., 2005). Thus, the different dissolution pathways for ammonium and phosphorus may have resulted in the release of associated compounds at different proportions.

Additional dissolved amounts of phosphorus were expectedly higher in the reactors containing the most concentrated AFFR liquor (T1a and T1b) (Table 4.9) which was probably due to inclusion of more solids in the reactor setup. The additional phosphorus dissolved was consumed, not accumulated, depending on the fact that total TDP concentration in the range of 12.631-31.557 mg/L was decreased to the too low levels of 0.54-0.74 mg/L at the end of reactor operation. The concentrations of dissolved phosphorus in T1a and T1b measured initially at the start-up (16.75 and 15.75 mg/L, respectively) almost doubled as a result of additional dissolution from the content of the digestate (31.56 and 31.17 mg/L, respectively) (Table 4.9). Higher TDP concentrations reached via dissolution in T1a and T1b also enabled more NH_4^+- N

removal from AFFR liquor (92.7, 93.7 %, respectively) (Table 4.7). Hence, it can be concluded that more dissolution avoided phosphorus limited conditions within the reactors so that more ammonium could be assimilated. Limited phosphorus concentration below 8 mg/L was previously noted as reducing the biomass productivity of *Chlorella* sp. (Åkerström et al., 2014). On the other hand, excess phosphorus was observed to inhibit the growth of *Chlorella* PY-ZU1 probably because of the high cellular osmotic pressure (Cheng et al., 2015). Hence, simultaneous dissolution and uptake may present an opportunity to avoid phosphorus limited conditions in the growth environment and to treat more phosphorus by its release extended over a time period without inhibition of the species.

Nutrient deficiency in the reactors can also be estimated at the begining of the experiment depending on the molar ratio of DIN/DRP (dissolved inorganic nitrogen to dissolved reactive phosphorus) ratio (Redfield ratio) (Wilcock et al., 2007). DIN:DRP at the initial characterization of the reactor content was 29.3, 33.1, 26.0, 26.5, 21.9, 21.5 and 18.2 for T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively. Phosphorus deficiency was probable to be observed in the reactors based on the fact that average DIN/DRP ratio for phytoplankton was 16:1 derived from the stoichiometric formula of $C_{106}H_{181}O_{45}N_{16}P$ (Choi and Lee, 2015). Microalgal species in the reactors other than T3b and T4 were most probable to be prone to phosphorus limitation since DIN/DRP ratios higher than 22 previously reported as phosphorus limited for microalgae (Hillebrand and Sommer, 1999). Even though the reliability of designing microalgal reactors for nutrient removal purposes depending on a fixed stoichiometry such as Redfield ratio is questionable (Whitton et al., 2016), it can be indicative for nutrient deficiency in the reactors.

Table 4.9. The calculations on the biological TDP uptake including the dissolution process.

Reactor	Zero-order linear line equation	y values at x=0	Total concentration of TDP, mg/L	Measured TDP at the initial start-up, mg/L	Additional dissolved TDP, mg/L	Final TDP (measured) in the reactor, mg/L	Actual TDP removed, mg/L	Total operation period, d	Rate of TDP removal, mg/L.d	Removal Efficiency, %
	A	B	C = B	D	E = C-D	F	G = C-F	H	I= G /H	J=(C-F)*100/C
T1a	y= -1.2469x+31.557	31.557	31.557	16.75	14.807	0.74	30.817	25.2	1.22	97.7
T1b	y= -1.2291x+31.172	31.172	31.172	15.75	15.422	0.69	30.482	25.2	1.21	97.8
T2a	y= -1.0841x+20.264	20.264	20.264	13.63	6.634	0.65	19.614	17.6	1.11	96.8
T2b	y= -1.0836x+20.995	20.995	20.995	12.60	8.395	0.59	20.405	18.9	1.08	97.2
T3a	y= -1.2399x+16.209	16.209	16.209	9.60	6.609	0.54	15.669	12.6	1.24	96.7
T3b	y= -0.9292x+13.619	13.619	13.619	9.45	4.169	0.56	13.059	14.2	0.92	95.9
T4	y= -1.0587x+12.631	12.631	12.631	8.15	4.481	0.55	12.081	11.6	1.04	95.6

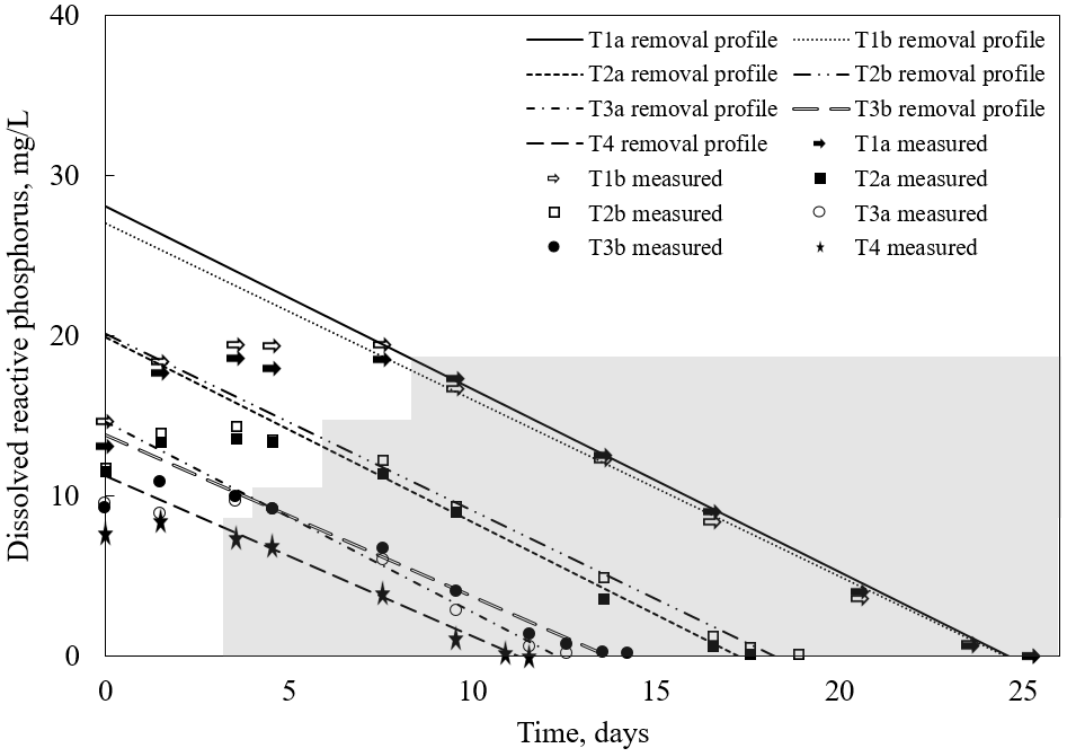
Note 1: x values represented time and y values represented TDP concentration.
Note 2: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

The rate of biological TDP uptake (TDP removal rate) was between 0.92-1.24 mg/L.d (Table 4.9). This rate corresponded to 12.1-30.8 mg/L of TDP removal by microalgal-bacterial consortium in the course of operation period. The TDP removals were observed to be higher than the ones when dissolution was not taken into account (7.60-16.01 mg/L). TDP removal rates agreed with the range of 0.25-4.10 mg/L.d previously reported for the nutrient removal processes from the liquor of anaerobically digested sludge diluted with the effluent water of a wastewater treatment plant using *Chlorella* sp. (Åkerström et al., 2014).

4.3.3.2.4 Estimation of the DRP removal kinetics and actual removal rates

The DRP removal within the reactors was well represented with zero-order kinetics with a R^2 ranging between 0.9802-0.9951. The details of DRP removal kinetics are provided in Appendix M. DRP removal profiles obtained following zero-order kinetics are given in Figure 4.13. The total DRP concentrations was found to range between 11.21-28.01 mg/L which almost completely exhausted at the end of the reactor operation (Table 4.10).

Additional dissolution of DRP resulted in an increase in the DRP concentrations by 114, 84, 73, 72, 54, 50 and 45% for T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively, within the reactors. The regulation of pH by the addition of HCl also led to an increase the readily available form of phosphorus for biological uptake (DRP) within the reactors, even more than two times the measured initial DRP concentrations (Table 4.10). The dissolution of reactive phosphorus varied between 3.494 and 14.910 mg/L and was slightly lower than the total phosphorus dissolution (4.169-15.422 mg/L). This fact suggested the dissolution of unreactive phosphorus as well as reactive phosphorus, but, at a comparably lower amount.



Note: The shaded area shows the data points included in the estimation of the removal kinetics. (Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)

Figure 4.13. DRP removal profiles estimated by zero-order reaction kinetics analysis.

Table 4.10. The calculations on the biological DRP uptake including the dissolution process.

Reactor	Zero-order linear line equation	y values at x=0	Total concentration of DRP, mg/L	Measured DRP at the initial start-up, mg/L	Additional dissolved DRP, mg/L	Final DRP (measured) in the reactor, mg/L	Actual DRP removed, mg/L	Total operation period, d	Rate of DRP removal, mg/L.d	Removal Efficiency, %
	A	B	C = B	D	E = C-D	F	G = C-F	H	I= G /H	J=(C-F)*100/C
T1a	y= -1.1419x+28.010	28.010	28.010	13.10	14.910	0	28.010	25.2	1.11	100.0
T1b	y= -1.101x+26.972	26.972	26.972	14.63	12.342	0	26.972	25.2	1.07	100.0
T2a	y= -1.1582x+19.894	19.894	19.894	11.47	8.424	0.08	19.814	17.6	1.13	99.6
T2b	y= -1.1032x+20.081	20.081	20.081	11.68	8.401	0.06	20.021	18.9	1.06	99.7
T3a	y= -1.1864x+14.576	14.576	14.576	9.46	5.116	0.08	14.496	12.6	1.15	99.5
T3b	y= -1.0088x+13.758	13.758	13.758	9.20	4.558	0.08	13.678	14.2	0.96	99.4
T4	y= -0.9984x+11.214	11.214	11.214	7.72	3.494	0	11.214	11.6	0.97	100.0

Note 1: x values represented time and y values represented DRP concentration.
Note 2: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

4.3.4 Biological Growth

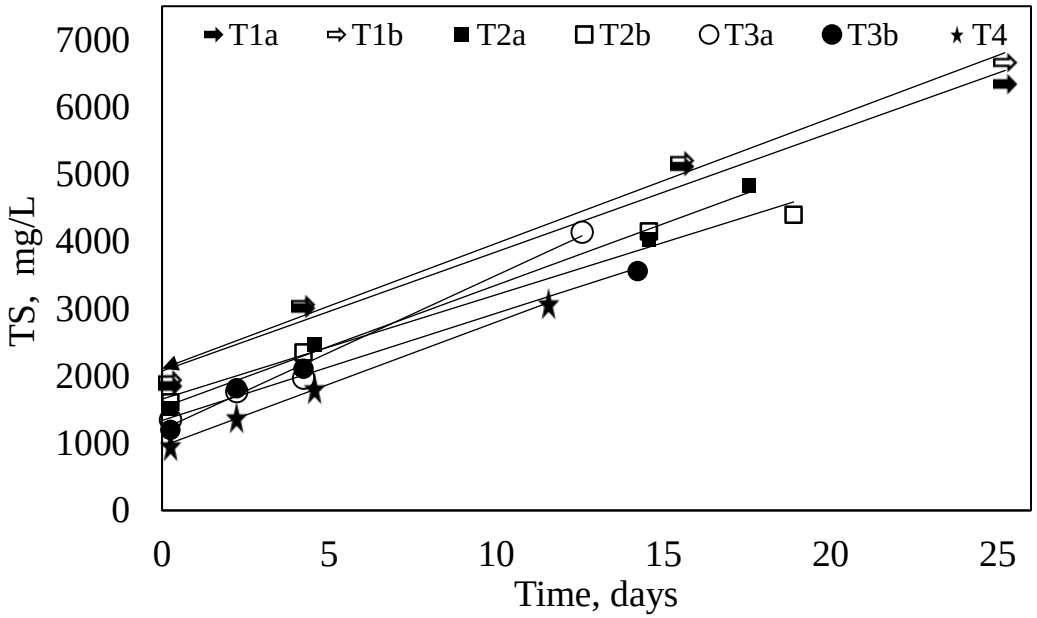
Biological growth was investigated in terms of total solids concentration for microalgal-bacterial consortium in Section 4.3.4.1 and in terms of chlorophyll-a concentration for microalgal species in Section 4.3.4.2.

4.3.4.1 Overall growth of microalgal-bacterial consortium

Biomass production and productivity were evaluated with respect to the concentration of total solids. The concentration of total solids was observed to be increasing during the entire operational period for all the reactors (Figure 4.14). 2.11-4.72 g/L of biomass was produced until the end of the operation of the reactors (Table 4.11). Biomass production was the highest in the reactors containing the most concentrated AFFR liquor (4.49 and 4.72 g/L for T1a and T1b, respectively) and the lowest for the reactor containing the most diluted AFFR liquor (2.11 g/L for T4). Biomass production within reactors were found to be comparable with the ones obtained using *Chlorella* PY-ZU1 (4.81 g/L) in the treatment of the digestate of swine manure and sewage (Cheng et al., 2015), using *Chlorella pyrenoidosa* (3.01 g/L) in the treatment of anaerobically digested starch wastewater mixed with alcohol wastewater (Yang et al., 2015) and using a mixed culture dominated with *Scenedesmus* sp. (2.6 g/L) in the treatment of the digestate of wastewater treatment plant (Uggetti et al., 2014). A linear biomass growth was observed within the reactors with R^2 values of 0.9813, 0.9899, 0.9929, 0.9823, 0.9881, 0.9802 and 0.9992 for T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively. An increase in the concentration of the AFFR liquor ended up with the higher biomass concentrations with extended operation periods as a result of the linear biomass growth. Åkerström et al. (2014) previously observed a linear biomass growth in their study investigating nutrient removal from sludge liquor using *Chlorella* sp. Higher biomass concentrations would probably reduce the harvesting costs of biomass

(Åkerström et al., 2014). The increasing concentration of biomass even in the reactors containing the most concentrated AFFR liquor was also an indication of no interference of the light transmission during the operation.

Biomass productivity could be represented by 0.18 ± 0.021 g/L.d for all the reactors contained in the experiment (Table 4.11). 0.21-0.26 g/L.d of biomass productivity was previously reported for *Chlorella vulgaris* grown in the mixture of the digestate of cattle slurry and raw cheese whey (Franchino et al., 2013). Similar biomass productivities for all the reactors suggested no inhibition due to the high ammonium content (Section 4.3.3.1.3) or the phosphorus concentration (Section 4.3.3.2.3).



(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)

Figure 4.14. The change in the concentration of total solids during operation.

Table 4.11. Biomass production and biomass productivity at the end of the operation.

	T1a	T1b	T2a	T2b	T3a	T3b	T4
Time of operation, d	25.2	25.2	17.6	18.9	12.6	14.2	11.6
Biomass production, mg/L	4490	4720	3310	2790	2790	2360	2110
Biomass productivity, mg/L.d	178	187	188	148	222	166	183

Note: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

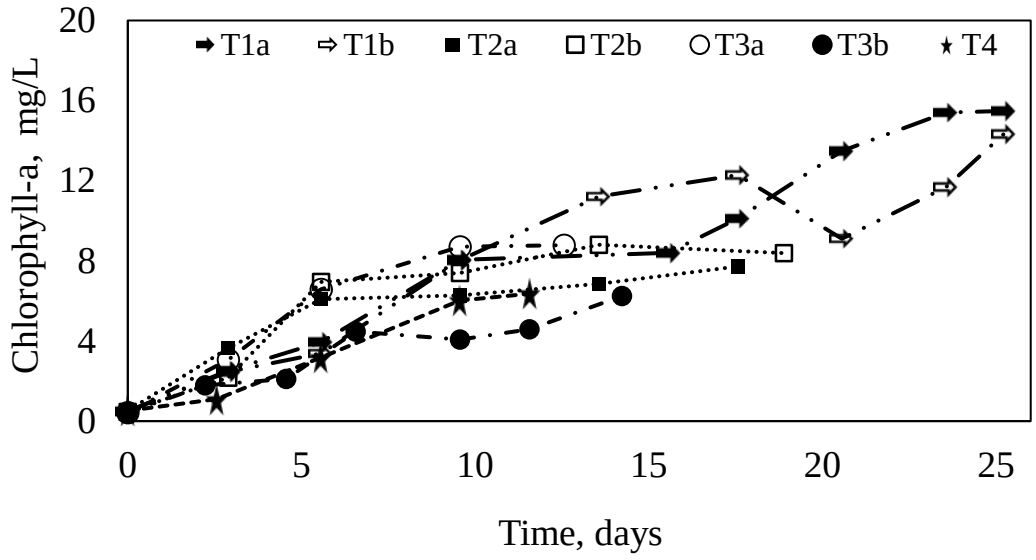
4.3.4.2 Microalgal growth

Microalgal growth was assessed depending on the chlorophyll-a concentrations measured. The chlorophyll-a concentrations increased from 0.42-0.52 to 1.10-3.64 mg/L within 2.23-2.90 days of the operation (Table 4.12). The growth of microalgal species continued till the end of the experiment (Figure 4.15). The increasing chlorophyll-a concentrations in the early days of the experiment indicated that the microalgal species was in growth stage when the additional nutrients dissolved. Therefore, dissolution and uptake of the nutrients were simultaneously experienced as previously discussed in Section 4.3.3.1.2.

Table 4.12. Chlorophyll-a concentrations at the start-up and second measurement.

Reactor	Chlorophyll-a, mg/L		Time of the second measurement, days
	Start-up	Second measurement	
T1a	0.50±0.02	2.52±0.48	2.90
T1b	0.50±0.03	2.46±0.60	2.90
T2a	0.52±0.13	3.64±0.18	2.90
T2b	0.46±0.10	2.17±0.07	2.90
T3a	0.42±0.05	3.06±0.22	2.90
T3b	0.42±0.05	1.79±0.20	2.23
T4	0.52±0.00	1.10±0.06	2.56

Note: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

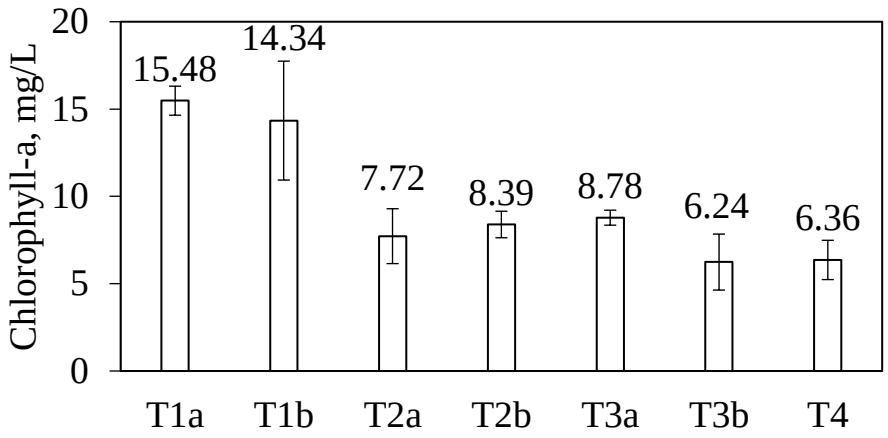


(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)
Figure 4.15. The change in chlorophyll-a concentrations during the operation.

The chlorophyll-a contents at the end of the microalgal treatment of different dilutions of AFFR liquor ranged between 6.36-15.48 mg/L (Figure 4.16). Significant chlorophyll-a buildup (12-31 times increase with respect to the initial chlorophyll-a concentrations) was observed in the reactors. The increase in the chlorophyll-a contents and the maximum chlorophyll-a concentration measured were consistent with the ones reported by Singh et al. (2011). The authors reached to a final chlorophyll-a concentration of 17-42 times higher than the initial measurement with a maximum of 14.05 mg/L in the treatment of anaerobically digested poultry litter using *Chlorella minutissima*, *Chlorella sorokiniana*, *Scenedesmus* sp. A chlorophyll-a concentration of 13.6 mg/L was previously reported during the mixotrophic growth of *Chlorella vulgaris* using a centrate of anaerobically digested sludge (Ge et al., 2018).

The highest chlorophyll-a content was observed in T1a and T1b which had the highest concentration of the AFFR liquor at the start-up. Although dilution of AFFR liquor for T1a-T1b (1/6) and T2a-T2b (1/8) were close to each other, chlorophyll-a content

almost doubled in T1a-T1b compared to T2a-T2b (Figure 4.16). This fact can be due to the increase in the concentration of the limiting substrate, phosphorus, via dissolution (Sections 4.3.3.2.3 and 0). Dissolution mediated total dissolved phosphorus release was 14.807 and 15.422 mg/L in T1a and T1b while that of in T2a and T2b were 6.634 and 8.395 mg/L. TDP concentrations in T1a and T1b approximately doubled compared to T2a and T2b which was in accordance to the chlorophyll-a concentrations. Thus, as more phosphorus dissolved, it was utilized by the species in extended time periods, resulting in more biomass built-up and higher removals in the ammonium content (Section 4.3.3.1.3). The additional phosphorus supply in phosphorus limited wastewaters have a potential to increase the biomass production which is possibly due to contribution to ATP (adenosine triphosphate) synthesis to yield energy to metabolic activities (Cheng et al., 2015).



(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12)
Figure 4.16. The chlorophyll-a contents at the end of the operational periods.

The uptake ratio of NH_4^+-N:TDP by microalgal-bacterial consortium was 6.7, 8.2, 3.2, 4.5, 2.7, 5.6 and 3.7 for T1a, T1b, T2a, T2b, T3a, T3b and T4, respectively. It was observed that significantly higher NH_4^+-N:TDP ratios (6.7 for T1a, 8.2 for T1b) resulted in the highest microalgal biomass concentrations in terms of chlorophyll-a (Figure 4.16).

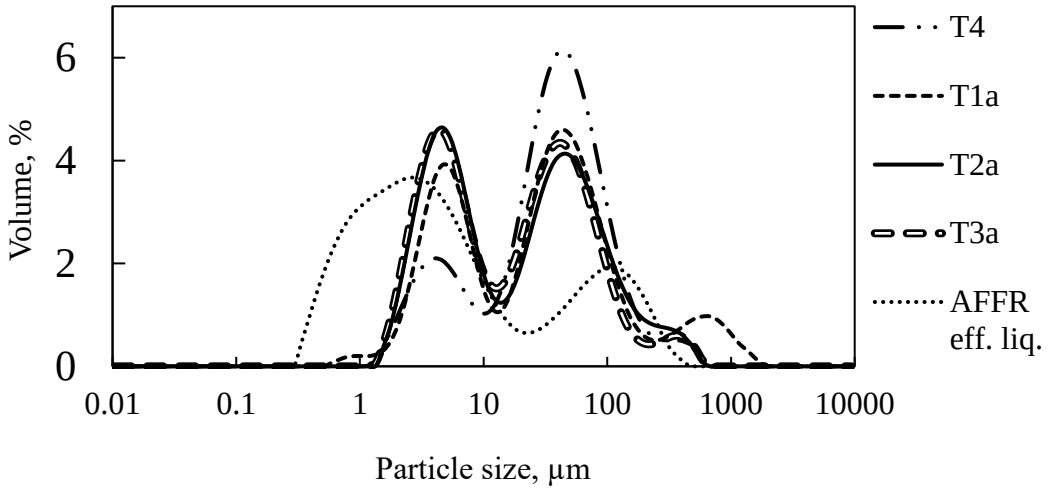
4.3.5 Particle Size Distribution

The samples taken from AFFR liquor (diluted by 1/10 to decrease the viscosity) and from the reactors T1a, T2a, T3a and T4 were characterized by a bimodal particle size distribution (Figure 4.17). 0.01-10 μm sized particles in AFFR liquor was comprised 70.3% of the volume and the larger particles had lower share (Table 4.13). The volume fraction of the particles in 0.01-10 μm particle size range in T1a, T2a, T3a and T4 (35.1, 42.7, 43.4 and 21.7%, respectively) was lower compared to that of AFFR liquor. The major volume was rather occupied by 10-100 μm-sized particles within the reactors (Table 4.13). Examining the rheological properties of *Porphyridium cruentum* and *Chlorella vulgaris*, Bernaerts et al. (2018) also observed biomodal particle size distribution for both species and indicated that the first peak at 1-10 μm was accounted for the individual cells and the second peak at 10-100 μm was for clusters of intact cells.

Larger particle sizes (above 100 μm) were also recorded at signicant volume fractions (14.6, 11.5, 7.9, 11.4 and 11.3 % for T1a, T2a, T3a, T4 and AFFR liquor, respectively). The particles above 100 μm may probably originate from the AFFR liquor itself based on the fact that the share of these particles in AFFR liquor (11.3%) was not too distinct from other samples taken from the reactors.

Additionally, 0.3-0.55 μm-sized particles was 4.7% by volume and no particles were recorded below 0.3 μm in AFFR liquor. On the other hand, there were no 0.55 μm or below-sized particles in the samples of microalgal reactors. The disappearance of the 0.3-0.55 μm-sized particles from the AFFR liquor during the microalgal cultivation may be speculated to be a sign of disintegration of these small-sized particles and to form the background of the dissolution of nutrients. The attachment of the microorganisms and the structuring of biofilms on 0.3-0.55 μm-sized particles can

also be speculated to result in the disappearance of such particles as a consequence of growing sizes. However, these speculations require further investigation due to lack of research on particle size distribution in microalgal wastewater treatment studies.



(Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.)

Figure 4.17. Particle size distribution of the samples.

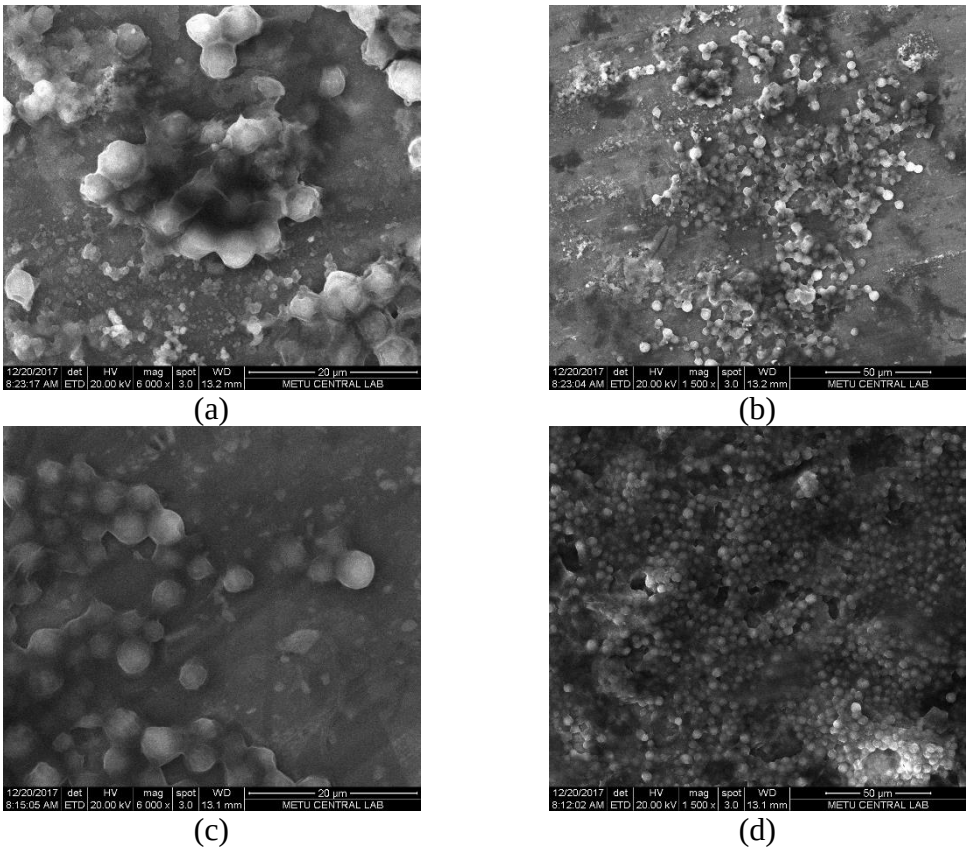
Table 4.13. Volume fraction of the particles within the approximated size ranges.

	Volume Fraction, %				
Particle Size, µm	T1a	T2a	T3a	T4	liquid portion of AFFR effluent
0.01-0.3	0.0	0.0	0.0	0.0	0.0
0.3-0.55	0.0	0.0	0.0	0.0	4.7
0.01-10	35.1	42.7	43.4	21.7	70.3
10-100	49.3	45.8	48.8	66.9	18.4
100-1000	14.6	11.5	7.9	11.4	11.3
1000-10000	1.0	0.0	0.0	0.0	0.0

Note: Dilution ratio of AFFR liquor for T1a-T1b: 1/6, for T2a-T2b: 1/8, for T3a-T3b:1/10, for T4: 1/12.

4.3.6 SEM imaging

SEM imaging was performed to visualize the formations of the microalgal species in clusters (Figure 4.18). SEM visualization also confirmed the results of the particle size analysis by the observation of the individual species and agglomerated forms.



(a) (b)

(c) (d)

Figure 4.18. SEM images for sample from (a) T1b at 6000x, (b) T1b at 1500x, (c) T2b at 6000x, (d) T2b at 1500x.

4.3.7 Settleability of the microalgal biomass

The samples taken from T1a, T2a, T3a and T4 were subjected to settling test to evaluate the settleability of the microalgal biomass after nutrient removal process. Figure 4.19 shows the pictures taken at the initial and final stages of the settling test. The last column included the ones after solid and liquid phase separation.

The chlorophyll-a concentrations of the supernatants were measured as 0.65±0.008, 1.13±0.064, 0.26±0.003 and 0.24±0.026 mg/L for T1a, T2a, T3a and T4, respectively. The chlorophyll-a concentration of the settled biomass was calculated using the formula given in Section 4.2.6 and ranged between 164-503 mg/L which can be sorted in a decreasing order as T4, T1a, T3a and T2a (503, 327, 211 and 164 mg/L, respectively). The initial concentration of AFFR liquor used in the setup could not be directly related to the settleability of the microalgal species. Nevertheless, the sorting of the chlorophyll-a concentrations was in very well agreement with the particle size distribution of these biomasses presented in Section 4.35. The volume fraction of the 10-100 μm-sized particles of the samples (the formations in clusters) had also a decreasing proportion in the order of the samples of T4, T1a, T3a and T2a (Table 4.13). Thus, the higher volumes of 10-100 μm-sized particles, addressing the microalgal formations in clusters (Bernaerts et al., 2018), might have leaded the microalgal biomass to have better settleability.

The overall chlorophyll-a concentration within the reactors before settling (15.48±0.832, 7.72±1.570, 8.78±0.429 and 6.36±1.125 mg/L for T1a, T2a, T3a and T4, respectively) could be concentrated by 21-79 times (2100-7900%) after gravity sedimentation. 21-79 times concentrated biomass and the low residual chlorophyll-a content of the supernatant in the range of 0.24-1.13 mg/L indicated good settleability of the microalgal culture. The good settleability of the algal biomass can be attributed

to the formation of the microalgal bacterial clusters. The cluster formations during wastewater treatment results in an easily settleable biomass which can be separated by simple gravitational settling. These formations provide efficient and cost-effective harvesting of the microalgal biomass (Quijano et al., 2017). Thus, AFFR liquor treatment using *Chlorella* sp. presents an opportunity to produce a well-settled biomass which can potentially harvested by a cost-effective method as simple gravity sedimentation.

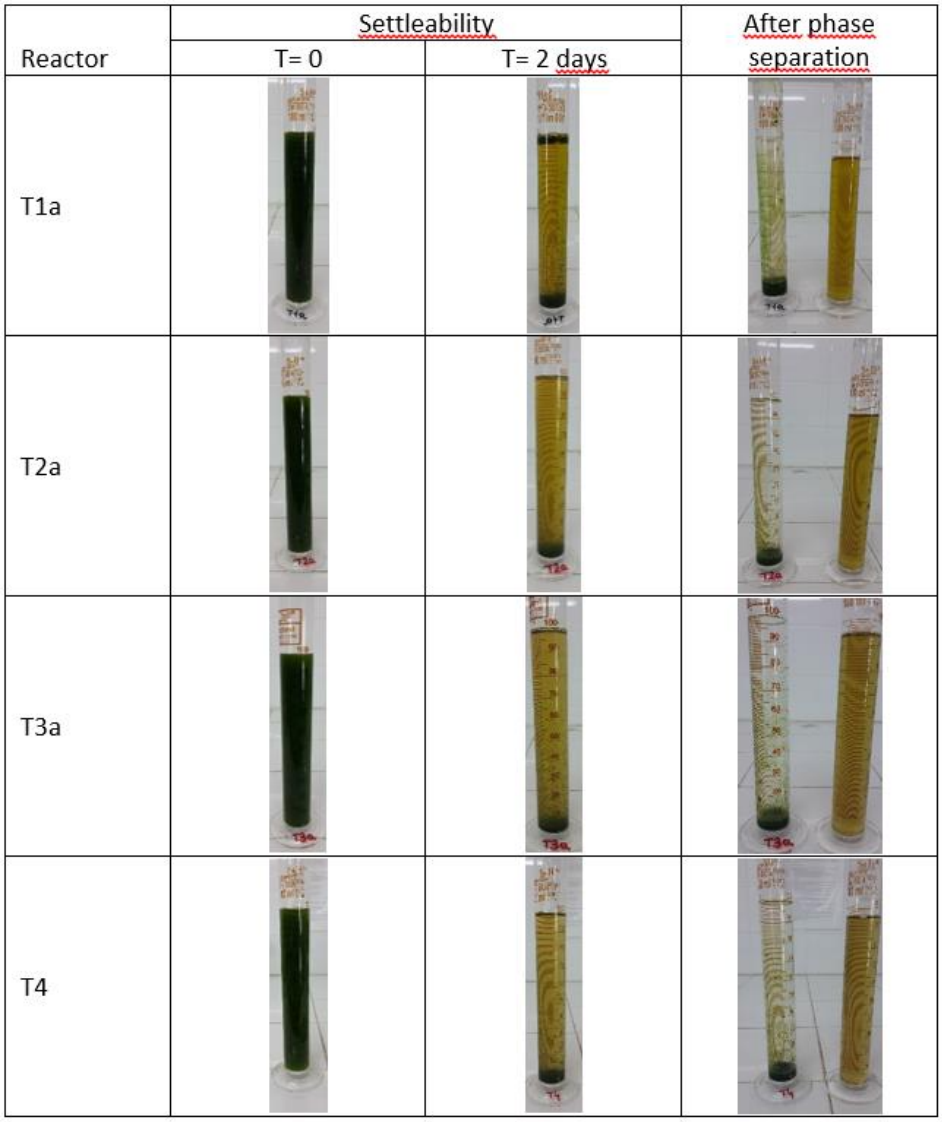


Figure 4.19. Settling test applied to samples from randomly selected reactors.

4.4 Conclusions

AFFR liquor obtained from the high-rate anaerobic treatment of a digestate sample was subjected to a nutrient removal process by using mixed microalgal cultures. The dominating microalgae species was *Chlorella vulgaris* which indicated its well adaptability to such wastewater types (i.e. digestates).

Ammonium nitrogen concentration could be reduced by 92.4-93.7%. The removal of NH_4^+-N was mainly due to microalgal uptake (by 58.6-64.8%) and nitrification (by 35.2-41.4%). 95.6-97.8% TDP removal and the complete removal of the DRP were achieved in the treatment of AFFR liquor using microalgal culture. The additional dissolution of phosphorus from the digestate content in an extended period of time avoided the phosphorus limited conditions as well as the inhibition due to excessive phosphorus loading at the initial stages. Moreover, more ammonium removal could be attained particularly because of the high amounts of additional dissolved phosphorus. Particulates such as calcium phosphate formations contained in the wastewaters were found to act as an additional phosphorus source, thus, the removal of these particulates before application of microalgal treatment have a potential to create phosphorus limited conditions in the growth environment of microalgal species.

The microalgal content of the reactors increased by 12-31 times the initial concentration of the reactors at the end of the growth period. Additionally, microalgal biomass could be concentrated by 2100-7900% via gravity sedimentation which was induced by the formation of microalgal bacterial clusters. Good settleability of microalgal species has a potential to decrease the harvesting costs which is the main bottleneck in large scale application of wastewater treatment using microalgal cultures and the valorization of the biomass.

CHAPTER 5

CONCLUSIONS ON THE ENTIRE PROCESSES APPLIED AND RECOMMENDATIONS

This thesis study covered the residual biogas potential test, high-rate anaerobic treatment and microalgal nutrient removal process applied for the purpose of treatment and valorization of the digestate. The applied processes can potentially reduce the NH_4^+-N concentration from 6637±140 mg/L (Chapter 3 Section 3.2.2) to 26-29 mg/L (Chapter 4 Section 4.3.3.1.1) including the dilutions made. Dissolved reactive phosphorus content of the liquid digestate was decreased from 151±0.2 mg/L (Chapter 3 Section 3.2.2) to the complete exhaustion (Chapter 4 Section 4.3.3.2.1). The plant of sampling for the digestate used in high-rate anaerobic and microalgal treatment processes already produces a commercial fertilizer from the digestate and thus can be regulated under the plants of composite fertilizer production covered in the Regulation on Water Pollution Control (Official Gazette No: 25687, Date: 31.12.2004). The regulation indicates the discharge limits for the nutrients as 50 mg/L ammonium nitrogen, 50 mg/L nitrate nitrogen and 35 mg/L phosphate phosphorus for the composite fertilizer producing plants. The minimum ammonium nitrogen concentration at the end of the microalgal nutrient removal process was 26-29 mg/L and the total dissolved phosphorus was 0.69-0.74 mg/L. Dissolved reactive phosphorus was completely removed at the end. The ammonium nitrogen and phosphate phosphorus concentrations were well below the limits set for the discharge. On the other hand, nitrate concentrations accumulated in the microalgal reactors have a potential to reach 141.5-146.0 mg/L as a consequence of nitrification activity. Nitrifiers potentially originated from the sample obtained from Lake Eymir as mixed microalgal source. Even though molecular identification of the community structure was not included within the scope of the Thesis, it still remains as an attractive research

field. On the other hand, nitrate content should be further reduced to comply with the discharge standards. Further removal can be achieved by providing additional available phosphorus to microalgal culture to assimilate nitrate. Microalgal cultures were previously noted as being capable of consuming nitrate and nitrite in the absence of ammonium (Section 4.3.3.1.1). A denitrification process, which converts nitrate and nitrite to molecular nitrogen, can also be applied to reduce the nitrate concentration.

AFFR treatment of the liquid digestate was found to have a potential to capture more than 38.9-48.6 kg CO_2e/m^3 digestate.d greenhouse gas before being emitted to the atmosphere (Chapter 3, Section 3.3.8). Furthermore, the microalgal process applied after the high-rate treatment of the liquid digestate can capture additional CO_2, in spite of not being quantified. Even the pH regulation below 7 prevented the release of ammonia gas during microalgal nutrient removal process that would have potentially converted into nitrous oxide, a greenhouse gas. Nitrous oxide has 265 times more GWP than CO_2. Thus, the overall process applied for the treatment of liquid digestate would have a positive environmental impact in terms of greenhouse gas capture.

The entire process offered, that is the coupled high-rate anaerobic and microalgal treatment, has also energy production profits as well as the removal of nutrients from the digestate and reduction of greenhouse gas emissions. The average biogas yields obtained (0.395-0.430 $m^3/kg\ VS_{added}$) were found to be comparable to many substrates such as municipal wastewater sludge, sheep excreta, vegetable wastes, straw from cereals, pig excreta, liquid cattle manure, molasses, maize and potato distillery slops. The biogas production corresponded to an additional 24.7-30.9 kW power output for 80 m^3 of daily production of digestate which represented the 1/47-1/59 of the total power output of the plant. The power output from the plant predicted to be capable of meeting the energy requirement of 408 to 512 residences based on the declared

information from the plant. Even though power output obtained by digestate processing was not very high compared to the power output of the plant, meeting the power demand of several units in the installations would favor the applicability of such a process chain when environmental and legal concerns are considered.

On the other hand, the microalgal biomass obtained could be concentrated by simple gravitational sedimentation to 164-503 mg/L which were 2100-7900% higher than the overall microalgal concentration. Good settleability of the microalgal biomass enables the processes to be applied on large scales with reduced harvesting costs. Reducing the harvesting costs, microalgal biomass obtained can further be employed for energy production at large scales such as biodiesel, bioethanol, biomethane and biohydrogen production. Among these energy production options, biomethane production from algal biomass can serve as an on-site management option for the digestates in the plant. Microalgal biomass obtained can be recycled back into the main anaerobic digester after being settled. The liquid portion can be discharged after nitrate concentration is decreased either by microalgal assimilation or denitrification or any other process. The introduction of more biomass into the digester may probably increase the biogas production within the plant after the operational conditions are adjusted, depending on the fact that microalgal biomass promotes biogas production (Perazzoli et al., 2013). Specifically, the methane yield of *Chlorella vulgaris* was previously reported as 0.286 L CH_4/g VS (Lakaniemi et al., 2011). In addition to the recycling of the settled portion of microalgal biomass, the overall content of the microalgal nutrient removal process can be recycled back to the main digester. The recycling of the overall content of the microalgal process have a potential to at least partially meet the water requirement for diluting the raw feedstock. Thus, the water footprint of the overall processes can be reduced. The recycling of the microalgal biomass back into the digester can fulfill the requirement of recovery and reuse of this biological waste which is a core issue in the management of the biological wastes in international agenda. Nevertheless, as previously mentioned, nitrate content is required to be treated by microalgal

assimilation or denitrification or any other process, to prevent nitrate accumulation in the main digester in such a case.

Even if this thesis study has proved the potential applicability of the high-rate anaerobic treatment and the subsequent microalgal nutrient removal from the digestates, there remains additional studies that can be carried out. First of all, the residual biogas potential test revealed that the digestates had a significant biogas potential, but, some may need additional pretreatment for improving the associated biogas yields. The pretreatment methods should be investigated to increase the applicability of such an integrated process chain by improving the economics of the plant. As an additional recommendation, the removal of further nitrate content can be enhanced by a denitrification or microalgal uptake process. These two processes is required to be evaluated considering the economic viability of each to prevent financial burden for the plant investors. The entire digestate management loop may also be investigated with the aim of closing the loop by the recycling of the overall content of the microalgal nutrient removal process back into the main digester. Moreover, the phosphorus removal in the high-rate anaerobic treatment of the liquid digestate was grounded on the formation of the calcium phosphate precipitates. Closer investigation on the phosphate removal in anaerobic digestion is required for understanding the removal mechanisms to enable the recovery of this World's limited reserve in a simultaneous treatment process. Additionally, the enhancement of the simultaneous dissolution and uptake of the nutrients can also be carried out since such a process can have promising results in nutrients removal from the digestate content. The optimization of the process parameters for dissolution-enhanced nutrient removal using microalgal cultures can be investigated within this scope.

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APPENDICES

A. Application and evaluation of solids removal methods for a digestate sample

A liquid digestate sample was taken from a beef cattle manure processing plant with the aim of microalgal nutrient removal. The sample had a high solids concentration (72075 mg/L TS) compared to the applied solids contents in the previous studies conducted using microalgal cultures (Table A.1). The high solids concentration had a potential to prevent the light penetration required for the growth of microalgal cultures. Therefore, the sample was subjected to simple solids removal processes such as gravity sedimentation, filtration, centrifugation, coagulation and flocculation with and without dilution. This appendix includes the preliminary evaluation of the solid removal options for the liquid digestate sample.

Table A.1. Pretreatment methods for solids removal from various digestates and the applied solids concentrations in microalgal processes previously studied.

Pretreatment method	Before pretreatment	After pretreatment	Reference
Filtration	1040 mg/L SS	negligible	Park et al., 2010
Dilution	1.1 g/L TSS	0.4 and 1.8 g TSS/L	Uggetti et al., 2014
Dilution, Filtration	6%	0.24-0.6 %	Wang et al., 2010(a)
Dilution	1590 mg/L TSS	191-1113 mg/L	Åkerström et., 2014
Precipitation, Filtration	high	46-71 mg/L TSS	Tan et al., 2014
Dilution, Centrifugation	4.71 % TS	0.2355-0.471 %	Franchino et al., 2013
Dilution	0.4 g/L TSS	0.04 g /L	Serejo et al., 2015
Sedimentation, Filtration	438 mg/L TSS	307 mg/L TSS	Xu et al., 2015
Dilution	6.80 % TS	0.34 %	Wang et al., 2010 (b)
Dilution		0.0078- 0.12 g/L TSS	Dickinson et al., 2015

Materials and Methods

Liquid digestate

The liquid digestate (LD) sample was obtained from the outlet of the liquid line of a gravitational solid-liquid phase separation unit of an anaerobic digester operated with 100% beef cattle manure. The plant had an approximate hydraulic retention time of 30 days with 278-288 tons of daily digestate production (Chapter 2, digestate of anaerobic digester 1). LD was characterized for TS, volatile solids VS, COD_t, COD_s, TP, total soluble phosphorus (TSP), total nitrogen (TN) and total soluble nitrogen (TSN) concentrations (Table A.2).

Table A.2. The initial characterization of the liquid digestate.

Constituent	Concentration, mg/L
TS	72 075
VS	38 507
COD_t	62 467
COD_s	11 985
TN	3 800
TSN	2 333
TP	1 850
TSP	550

Analytical methods

TS, VS, COD_t, COD_s, TP and TSP were measured according to standard methods (APHA, 2005). TN and TSN were analyzed photometrically according to the instructions given by the manufacturer (Aqualytic, 2014). Samples were first filtered from 0.45 μm pore-sized filter papers for determination of COD_s, TSP and TSN.

Gravity settling analysis

Two cylindrical graduated glass containers of 66 cm height and 4.5 cm radius were used for settling analysis. Three liters of effective volume was filled with LD. LD was allowed to settle for 5 days. Sampling was done at 9 cm and 27.5 cm depths from the initial surface, on the first, second and fifth day of the experiment. The samples were analyzed for their TS concentrations.

Filterability analysis

LD sample was individually filtered from several meshes with pore sizes of 600, 425, 175, 100, 63 and 53 μm. TS concentration of the filtrate (the liquid portion passing through the filter) was measured.

Dilution and sequential filtration analysis

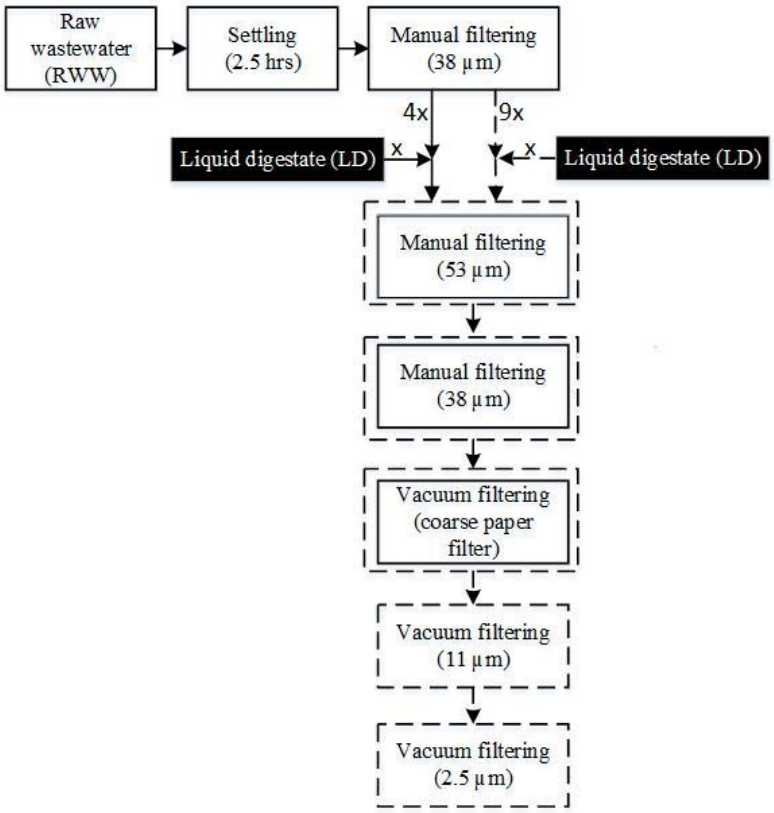
Dilution of LD was done using a raw wastewater (RWW) sample taken from the inlet of a domestic wastewater treatment plant of Middle East Technical University. RWW was settled for 2.5 hours and then filtered from 38 μm mesh filter to remove the large particles in order to obtain dilution wastewater (DWW). TS, VS and solid content of RWW and DWW are given in Table A.3.

Table A.3. Solids characterization of RWW and DWW.

Wastewater	TS, mg/L	VS, mg/L	Solid content, %
RWW	1150	313	0.119
DWW	887	120	0.093

The steps carried out in dilution and sequential filtration analysis is shown in Figure A.1. LD was first diluted with DWW by 1/5 and 1/10 dilution ratios. Diluted LD (DLD) was manually filtered through 53 and 38 μm pore-sized meshes, sequentially,

by revolving a plastic apparatus over the mesh. The mesh-filtered DLD (MFDLD) was additionally filtered from coarse filter paper using a vacuum pump. To examine the further TS removal capability via filtration, MFDLD of 1/10 diluted LD was subjected to further vacuum filtration through 11 and 2.5 µm pore-sized filters, sequentially. The filtrates were analyzed for TS concentrations and solid contents.



Dashed lines show the flow chart of 1/10 diluted LD processing, solid lines flow show the one of 1/5 diluted LD processing

Figure A.1. The flowchart of dilution and sequential filtration analysis.

Centrifugation analysis

Unprocessed LD and 1/10 diluted, sequentially filtered LD from 600, 425, 175, 100, 63 and 53 µm pore-sized filters (DSFLD) were centrifuged at different centrifugation

speeds and times (Table A.4). Total solids concentration was measured for the supernatant of the centrifuged samples.

Table A.4. Centrifugation speed and time applied on LD and DSFLD.

Input	Centrifugation speed, rpm	Centrifugation time, min
LD	10 000	10
DSF LD	1 000	10
	5 000	10
	5 000	20
	5 000	30
	10 000	10
	10 000	20
	10 000	30

Mass centrifugation of LD and subsequent dilution analysis

LD sample of 40 L was first centrifuged at 10000 rpm for 10 min and the supernatant is collected. The supernatant was diluted by 1/10 ratio using raw domestic wastewater obtained from the inlet of a domestic wastewater treatment plant of Middle East Technical University. Domestic wastewater employed in this analysis was not filtered but just settled for 2.5 hours and the liquid phase was used in the dilution of the supernatant of LD.

Coagulation and flocculation analysis

Coagulation and flocculation analysis were performed on both LD and DSFLD. $Al_2(SO_4)_3.18H_2O$ was used as a coagulant. Unprocessed LD was subjected 100, 1000, 5000, 10000, 50000 mg/L of coagulant doses. 100, 1000, 5000, 10000 and 23077 mg/L $Al_2(SO_4)_3.18H_2O$ was used for coagulation of DSFLD. The solid content and the volumetric sludge generation percentage were analyzed after 1 hour settling of the coagulated sample.

Results

Settleability of the LD

The initial TS concentration of the LD was 72 g/L and could only be reduced to approximately 65 g/L by gravitational sedimentation for 5 days. The maximum reduction in TS was in the range of 9.2-9.7 % (Table A.5). The measurements on day 2 at 9 cm depth was found to be higher than the one measured on day 1 which suggested the formation of scum in the settling containers.

Table A.5. TS concentrations given in mg/L in settling analysis.

Day	Container 1		Container 2	
	27.5 cm	9 cm	27.5 cm	9 cm
0	72075	72075	72075	72075
1	69280	65960	69200	67690
2	68500	67020	66490	69500
5	65450	65430	65100	65590
Maximum TS reduction, %	9.2	9.2	9.7	9.0

Filterability of the LD

Filterability of the liquid digestate was tested with the mesh filters of different pore sizes. Gravitational filtration could not be achieved when the LD left idle on the meshes. Hence, a plastic apparatus was revolved over the meshes to enable filtering of LD. Revolving helped the digestate to pass through the filters with pore-sizes of 600, 425, 175, 100 and 63 µm. However, sequestration of the LD while revolving the plastic apparatus was required to enable the filtration of LD through 53 µm pore-sized mesh which resulted in higher concentration of TS compared to that of 63 µm (

Table A.6). Maximum TS removal was achieved using a 63 µm pore-sized mesh (16.4 %) which corresponded to 60 230 mg/L retaining solids concentration.

Table A.6. TS concentrations of the filtered LDs.

Pore size, μm	TS, mg/L	TS reduction, %
600	67555	6.3
425	66585	7.6
175	63900	11.3
100	62090	13.9
63	60230	16.4
53	61290	15.0

Dilution and sequential filtration

The poor settleability and poor filterability of the LD lead to a search for an alternative and economic method for solids reduction. Dilution with raw domestic wastewater by 1/5 and 1/10 was decided to be applied to reduce the solid content of LD. The solid contents were reduced to 1.517 and 0.791 % for 1/5 and 1/10 dilution ratios, respectively (Table A.7). Franchino et al. (2013) reported that microalgae were able to survive at a solid content of 0.942%. However, the experiments they conducted were performed using solids contents in the range of 0.118-0.471% which were obtained by dilution with tap water. A digestate sample having 0.34% solid content was also used by Wang et al. (2010, b). Thus, further reduction in solids content was required for LD. DLD was then sequentially filtered from 53 and 38 μm pore-sized filters manually and then a coarse paper filter under vacuum to provide LD sample with a solid content applicable for microalgal growth. The solid contents of DSFLD after sequential filtration were obtained as 1.114 % for 1/5 diluted and 0.575 % for 1/10 diluted LD which were still higher than the ones applied in the previous studies aiming at nutrient removal from digestates using microalgal cultures.

Table A.7. Solid contents after dilution-sequential filtration processes.

Dilution ratio	Pore size, μm	TS, mg/L	TS reduction, %	Solid content, %
1/5	unfiltered	14725	79.6	1.517
	53	11940	83.4	1.191
	38	11830	83.6	1.181
	paper filter (vacuum)	11140	84.5	1.114
1/10	unfiltered	7860	89.1	0.791
	53	6500	91.0	0.661
	38	6170	91.4	0.632
	paper filter (vacuum)	5710	92.1	0.575

1/10 diluted DSFLD was further subjected to vacuum filtration through 11 and 2.5 μm pore-sized papers sequentially (Table A.8). The solids content could be reduced to 0.45 % at the end of filtration by 2.5 μm pore-sized filter. However, these two filters were immediately clogged and thus decided to be not easily applicable for solids reduction.

Table A.8. Solid contents after 11- and 2.5-micron sequential filtration.

Dilution ratio	Pore size, μm	TS, mg/L	Solid content, %
1/10	11	4830	0.49
	2.5	4460	0.45

Centrifugation

Centrifugation of unprocessed liquid digestate

LD was centrifuged without any processing. The supernatant of the centrifuged samples had 23 975 mg/L TS concentration which corresponded to a solid content of 2.413 %.

Centrifugation of 1/10 diluted and sequentially filtered liquid digestate

Centrifugation was also carried out for 1/10 diluted and sequentially filtered LD with pore sizes 600, 425, 175, 100, 63 and 53 µm. 0.285 % solid content was obtained at 10 000 rpm centrifugation for 10 minutes (

Table A.9). The solid content obtained at 5000 rpm and 10 minutes of centrifugation (0.326 %) was also applicable for microalgal processes when compared to the those of given by Franchino et al. (2013) and Wang et al. (2010, b).

Table A.9. Initial centrifugation analysis

Centrifugation Speed, rpm	Centrifugation Time, min	TS, mg/L	Solid content, %
1000	10	4600	0.462
5000	10	3240	0.326
10000	10	2830	0.285

The time of centrifugation was also extended for 5000 and 10 000 rpm to investigate the effect of centrifugation time on solids removal (Table A.10). The solid contents were in the range of 0.281-0.304% for centrifugation at 5000 rpm and of 0.264-0.277 % for centrifugation at 10 000 rpm. Thus, increasing the time for centrifugation did not result in a considerable reduction in solid contents.

Table A.10. Centrifugation at different time intervals.

Centrifugation Speed, rpm	Centrifugation Time, min	TS, mg/L	Solid content, %
5000	10	2877	0.304
	20	2690	0.290
	30	2703	0.281
10000	10	2470	0.277
	20	2560	0.267
	30	2587	0.264

Mass centrifugation of the liquid digestate and its subsequent dilution with raw domestic wastewater

The centrifugation of large volumes of 1/10 diluted LD was an energy intensive process. The LD was decided to be first centrifuged and then diluted with settled domestic wastewater. The solid content of the resultant mixture was 0.322% in which microalgae was probable to grow (Table A.11). On the other hand, the compositions of the constituents were also determined for raw domestic wastewater, settled domestic wastewater, LD and the centrifuged LD before and after dilution (Table A.11). Even though the solids content for microalgal growth was satisfied by centrifugation, the nutrients especially phosphorus content was scrapped from the liquid phase by centrifugation at significant amounts. The phosphorus is usually a limiting substrate for microalgal species and wasting it via centrifugation was not meeting the scope of microalgal nutrient removal processes.

Table A.11. The constituent compositions of raw domestic wastewater, settled domestic wastewater, LD and the centrifuged LD.

	TS, mg/L	VS, mg/L	Solid content, %	TN, mg/L	TSN, mg/L	TP, mg/L	TSP, mg/L	COD_t, mg/L	COD_s, mg/L
Raw domestic wastewater	806	256	0.083	70	63	8	5	419	196
Settled domestic wastewater	636	133	0.071	60	53	6	5	169	69
LD	72075	38507	N.D.	3800	2333	1850	550	62467	11985
Centrifuged LD	20558	7366	2.503	N.D.	N.D.	80	15	27033	12565
1/10 diluted centrifuged LD (at 10 000 rpm for 10 min)	2406	584	0.322	377	273	13	6	3150	1235

N.D.: not determined

Coagulation

Coagulation of unprocessed liquid digestate

LD was coagulated using coagulant doses between 100-50000 mg/L. Scum formation was measured by the ratio of the volume of scum layer to complete volume of the sample. Scum layer was not observed at the 100 and 1000 mg/L coagulant doses (Table A.12). The scum layer was observed to occupy 24 and 30 % of the total volume at 5000 and 10000 coagulant doses, respectively. Sudden and almost complete scum formation was observed in the application of 50000 mg/L coagulant which resulted in the frothing of the LD. Therefore, solids data could not be obtained for this coagulant dose. Coagulation of the unprocessed LD was found as not being feasible for the high solid content observed even after coagulation (6.998-7.085 %), very high amounts of coagulant doses applied and the large amounts of scum formation.

Table A.12. The solid content after coagulation of the unprocessed LD.

Coagulant dose, mg/L	TS, mg/L	Solid content, %	Scum formation, %
100	70820	6.998	Not observed
1000	72140	7.085	Not observed
5000	66380	6.998	24
10000	63360	6.774	30
50000	Not measurable		

Coagulation of 1/10 diluted and sequentially filtered liquid digestate

Coagulation experiments were also performed for 1/10 diluted and sequentially filtered LD (through 600, 425, 175, 100, 63 and 53 μm pore-sized filters) at different coagulant doses (Table A.13). There observed a clear interface between the coagulated sludge and the liquid portion at the doses of 5000, 10000, 23077 mg/L coagulant (Figure A.2). Volume of sludge generated corresponded to 60, 36 and 23% relative to the total volumes of the samples after 1 hour of settling. The solid content was also found to range between 0.428-7.73 %.

The optimum coagulant dose to reduce the solid content to be applicable in microalgal nutrient removal process was achieved at 5 000 mg/L of coagulant. On the other hand, 5 grams of coagulant application to 1L of LD was decided not to be an economical way for solids reduction. Additionally, the sludge generated (60 % of volume) was comparably higher than the ones for higher doses which may represent a potential problem in disposal.

Table A.13. Coagulation of 1/10 diluted and sequentially filtered digestate.

Dilution	Coagulant dose, mg/L	TS, mg/L	Solid content, %	pH	Sludge generated, %
1/10	100	5825	0.598	7.73	No clear interface
1/10	1000	5055	0.520	7.28	No clear interface
1/10	5000	4175	0.428	6.13	60
1/10	10000	6620	0.695	4.28	36
1/10	23077	17655	1.781	3.89	23

Figure A.2. Samples after 30 minutes settling of coagulated LD.

Conclusions

The direct application of the liquid digestate for microalgal nutrient removal processes was not feasible due to its high solids content that could potentially prevent the growth of microalgal species. Settling and filtration analysis revealed that liquid digestate was poorly settleable and filterable. 1/10 diluted and sequentially filtrated LD could have

been applied before centrifugation process to reduce the solid content. However, the dilution of LD before centrifugation increases the volume to be centrifuged which in turn makes such a process energy intensive. Mass centrifugation of the LD followed by its dilution with domestic wastewater resulted in a solid content of 0.32% which is fairly applicable for microalgal growth. However, it was concluded that the nutrient concentrations were decreased by centrifugation which was not a desired option for microalgal processes. Coagulation of 1/10 diluted and sequentially filtered liquid digestate required a coagulant dose of 5000 mg/L with a 60% of total volume sludge production. Such a process had a potential to increase the chemical costs associated with the large amounts of consumption. Moreover, the large volumes of sludge production were another concern in the management of the LD. Therefore, it was decided that the solids reduction methods implemented did not provide an efficient or cost-effective solution based on the results of the analysis.

APPENDICES

B. The statistical evaluation of the results of the analysis obtained from R2 reactors

The results of the analysis applied for each parameter for R2 reactors were evaluated using normal and t-test methods conducted by Minitab 17 software. The 95% confidence interval for each parameter was given in lower and upper limits in Table B.1. The statistical evaluation showed that the results were representative in 95% confidence interval.

Table B.1. Statistical analysis of the results obtained from R2 reactors at the end of RBP test and the 95% confidence intervals.

Parameter	Nutrient supplementation	Triplicate	Measured	Mean	Standard deviation	Confidence interval within 95% confidence level			
						Normal method		t-test	
						Lower	Upper	Lower	Upper
pH	with	1	7.97	7.91	0.07	7.21	8.60	7.7304	8.08
		2	7.83						
		3	7.92						
	without	1	7.99	8.00	0.04	7.60	8.39	7.90	8.10
		2	8.04						
		3	7.96						
Alkalinity (mg/L as $CaCO_3$)	with	1	5510	5515	8.1	5436	5594	5495	5535
		2	5524						
		3	5510						
	without	1	5786	5771	14.5	5629	5913	5735	5807
		2	5757						
		3	5771						
COD_t, mg/L	with	1	10805	11142	293	8272	14012	10413	11870
		2	11341						
		3	11279						
	without	1	11397	11457	87	10604	12310	11240	11674
		2	11557						
		3	11417						
TS, mg/L	with	1	14810	14967	202	12987	16947	14464	15469
		2	14895						
		3	15195						
	without	1	14985	14893	250	12442	17345	14271	15515
		2	15085						
		3	14610						

Table B.1. Statistical analysis of the results obtained from R2 reactors at the end of RBP test and the 95% confidence intervals.

Parameter	Nutrient supplementation	Triplicate	Measured	Mean	Standard deviation	Confidence interval within 95% confidence level			
						Normal method		t-test	
						Lower	Upper	Lower	Upper
VS, mg/L	with	1	6610	6668	93	5763	7574	6439	6898
		2	6620						
		3	6775						
	without	1	6740	6737	75	6002	7471	6550	6923
		2	6810						
		3	6660						
NH_4^+-N, mg/L	with	1	1491	1537	74	810	2264	1353	1722
		2	1623						
		3	1498						
	without	1	1484	1470	14	1333	1607	1435	1505
		2	1456						
		3	1470						
TKN, mg/L	with	1	1568	1630	84	810	2449	1422	1838
		2	1725						
		3	1596						
	without	1	1561	1547	19	1366	1728	1501	1593
		2	1526						
		3	1554						
TP, mg/L	with	1	513	521	10.8	415	626	494	548
		2	516						
		3	533						
	without	1	441	445	8.4	363	527	425	466
		2	440						
		3	455						

APPENDICES

C. Calculations regarding anaerobic treatment in RBP test

The treatment potential of the digestates were evaluated both considering the difference between the initial and final concentrations in the reactors and the concentrations of the constituents in the digestate content at the beginning and at end of the operation. The second approach, that is the concentrations of the constituents in the digestate content, required the exclusion of the concentrations of the constituents resulting from the inoculum itself at the end of operation. The calculation methodology of the concentrations of the constituents in the digestate content at the end of the operation and the results of these calculations are given in the following tables.

Table C.1. Nomenclatures, units and the calculations for the removal efficiency of the constituents from digestate content.

Quantity	Nomenclature	Unit	Source
Constituent	X	-	COD_t, TS, VS, NH_4^+-N, TKN, DRP, TP
Volume of digestate in reactor	V_d	mL	measured
Volume of inoculum in reactor	V_I	mL	measured
Volume of nutrient and trace metal solution within reactor	V_{NT}	mL	measured
Total effective volume within reactor	V_T	mL	$V_T = V_d + V_i + V_{NT}$
Initial X concentration of digestate	$[X]_{d,i}$	mg/L	measured
Initial X concentration of inoculum	$[X]_{I,i}$	mg/L	measured
Initial X concentration of nutrient and trace metal solution	$[X]_{NT,i}$	mg/L	measured
Initial X amount of digestate	$X_{d,i}$	mg	$X_{d,i} = [X]_{d,i} * V_d / 1000$
Initial X amount of inoculum	$X_{I,i}$	mg	$X_{I,i} = [X]_{I,i} * V_I / 1000$
Initial X amount of nutrient and trace metal solution	$X_{NT,i}$	mg	$X_{NT,i} = [X]_{NT,i} * V_{NT} / 1000$
Initial total X amount within reactor	$X_{T,i}$	mg	$X_{T,i} = X_{d,i} + X_{I,i} + X_{NT,i}$
Initial X concentration within reactor	$[X]_{T,i}$	mg/L	$[X]_{T,i} = (X_{T,i} / V_T)*1000$
Calculations for specific X removal in reactors			
Final concentration of X within inoculum-only reactor	$[X]_{T,f\ (inoculum\text{-}only)}$	mg/L	measured
X amount remained in inoculum-only reactor	$X_{T,f\ (inoculum\text{-}only)}$	mg	$X_{T,f\ (inoculum\text{-}only)}=[X]_{T,f\ (inoculum\text{-}only)} * V_{T\ (inoculum\text{-}only)} /1000$
Initial X amount of nutrient and trace element solution in inoculum-only reactor	$X_{NT,i\ (inoculum\text{-}only)}$	mg	$X_{NT,i\ (inoculum\text{-}only)} = [X]_{NT,i\ (inoculum\text{-}only)}*V_{NT\ (inoculum\text{-}only)} / 1000$
Initial X amount of inoculum in inoculum-only reactor	$X_{I,i\ (inoculum\text{-}only)}$		$X_{I,i\ (inoculum\text{-}only)} = [X]_{I,i\ (inoculum\text{-}only)} * V_{I\ (inoculum\text{-}only)} / 1000$
Initial total X amount within inoculum-only reactor	$X_{T,i\ (inoculum\text{-}only)}$	mg	$X_{T,i\ (inoculum\text{-}only)} = X_{I,i\ (inoculum\text{-}only)} + X_{NT,i\ (inoculum\text{-}only)}$
Initial X concentration within inoculum-only reactor	$[X]_{T,i\ (inoculum\text{-}only)}$	mg/L	$[X]_{T,i\ (inoculum\text{-}only)} = (X_{T,i\ (inoculum\text{-}only)} / V_{T(inoculum\text{-}only)})*1000$
Specific X removal of inoculum	I_s	mg/mL	$I_s = (X_{T,i\ (inoculum\text{-}only)} - X_{T,f\ (inoculum\text{-}only)})/V_{T(inoculum\text{-}only)}$
Calculations for X removal efficiency from digestates by exempting inoculum contribution			
Inoculum X removal amount in each reactor	$X_{I,removed}$	mg	$X_{I,removed} = I_s * (V_I + V_{NT})$
Inoculum X remaining in the reactor	$X_{I,f}$	mg	$X_{I,remained} = (X_{I,i} + X_{NT,i}) - X_{I,removed}$
Final X concentration within reactor	$[X]_{T,f}$	mg/L	measured
Final X amount within reactor	$X_{T,f}$	mg	$X_{T,f} = [X]_{T,f} * V_T/1000$
Digestate X amount remained	$X_{d,f}$	mg	$X_{d,f} = X_{T,f} - X_{I,f}$
Digestate X concentration remained	$[X]_{d,f}$	mg/L	$[X]_{d,f} = X_{d,f} *1000 / V_d$
X removal efficiency	X_{eff}	%	$X_{eff} = ([X]_{d,i} - [X]_{d,f}) *100 / [X]_{d,i}$

d: digestate, NT: nutrient and trace element solution, I: inoculum, i: initial, f:final, T: total

Table C.2. COD_t calculations with and without nutrient supplementation.

Reactor	Triplicate code	Initial COD_t concentrations, mg/L			Volumes used, mL				Initial COD_t amounts, mg				COD_t concentration in reactor, mg/L		Final COD_t amount in reactor, mg	Specific COD_t removal of inoculum, mg/mL	Inoculum COD_t, mg		Digestate COD_t remained, mg	Digestate COD_t concentration remained, mg/L	COD_t removal efficiency from digestates, %
		Digestate	Inoculum	Nutrient medium	Digestate	Inoculum	Nutrient medium	Total volume	Digestate	Inoculum	Nutrient medium	Total in reactor	Initial	Final			Removed	Remained			
		$[X]d,i$	$[X]_{I},i$	$[X]nt,i$	Vd	V_I	Vnt	Vt	Xd,i	X_{I},i	Xnt,i	Xt,i	$[X]t,i$	$[X]t,f$	Xt,f	Is	X_{I},removed	X_{I},f	Xd,f	$[X]d,f$	X eff
I[a]	1	0	12893	700	0	400.00	11	411.00	0	5157	7.7	5165	12567	10775±225	4429±92	1.79±0.225	736.4	4428.5	0.0±0.0	0±0	14±2
R1[a]	1	111056	12893	700	9.83	387.97	11	408.80	1092	5002	7.7	6101	14925	11240±302	4595±124	1.79	714.8	4295.0	299.9±123.6	30512±12579	73±11
R2[a]	1	39010	12893	700	26.17	371.06	11	408.23	1021	4784	7.7	5813	14239	10805±120	4411±49	1.79	684.5	4107.2	303.8±48.9	11610±1868	70±5
R2[a]	2	39010	12893	700	26.18	371.06	11	408.24	1021	4784	7.7	5813	14239	11341±353	4630±144	1.79	684.5	4107.2	522.5±144.0	19958±5502	49±14
R2[a]	3	39010	12893	700	26.19	371.06	11	408.25	1022	4784	7.7	5813	14240	11279±89	4605±36	1.79	684.5	4107.2	497.5±36.4	18992±1391	51±4
R3[a]	1	57334	12893	700	18.34	380.01	11	409.35	1051	4900	7.7	5958	14556	12122±176	4962±72	1.79	700.6	4206.6	755.5±72.2	41202±3938	28±7
R4[a]	1	76675	12893	700	13.88	384.09	11	408.97	1064	4952	7.7	6024	14729	11744±353	4803±144	1.79	707.9	4251.9	551.0±144.3	39707±10399	48±14
R5[a]	1	43751	12893	700	24.28	373.64	11	408.93	1062	4817	7.7	5888	14398	11495±8	4700±3	1.79	689.2	4135.9	564.5±3.3	23247±136	47±0
R6[a]	1	21079	12893	700	62.58	335.93	11	409.51	1319	4331	7.7	5658	13816	10699±185	4381±76	1.79	621.6	3717.3	663.9±75.6	10609±1209	50±6
I[b]	1	0	12893	700	0.00	400.00	0	400.00	0	5157	0	5157	12893	10850±150	4340±60	2.04±0.15	817.2	4340.0	0.0±0.0	0±0	16±1
R1[b]	1	111056	12893	700	9.83	387.97	0	397.80	1092	5002	0	6094	15318	11667±99	4641±39	2.04	792.6	4209.5	431.6±39.4	43912±4005	60±4
R2[b]	1	39010	12893	700	26.21	371.06	0	397.26	1022	4784	0	5806	14616	11397±223	4528±89	2.04	758.1	4026.0	501.6±88.7	19140±3386	51±9
R2[b]	2	39010	12893	700	26.17	371.06	0	397.23	1021	4784	0	5805	14614	11557±194	4591±77	2.04	758.1	4026.0	564.7±77.2	21574±2950	45±8
R2[b]	3	39010	12893	700	26.21	371.06	0	397.27	1022	4784	0	5806	14616	11417±231	4536±92	2.04	758.1	4026.0	509.8±91.9	19451±3507	50±9
R3[b]	1	57334	12893	700	18.28	380.01	0	398.30	1048	4900	0	5948	14933	11795±4	4698±2	2.04	776.4	4123.1	574.8±1.7	31438±91	45±0
R4[b]	1	76675	12893	700	13.81	384.09	0	397.90	1059	4952	0	6011	15107	12174±288	4844±115	2.04	784.7	4167.4	676.6±114.5	48997±8293	36±11
R5[b]	1	43751	12893	700	24.3	373.64	0	397.94	1063	4817	0	5880	14777	12172±25	4844±10	2.04	763.4	4054.0	789.9±10.0	32507±413	26±1
R6[b]	1	21079	12893	700	62.57	335.93	0	398.50	1319	4331	0	5650	14178	10956±27	4366±11	2.04	686.3	3644.9	720.9±10.7	11522±171	45±1

[a] with nutrient supplementation.

[b] without nutrient supplementation.

[c] Removal efficiencies of the constituents in inoculum-only reactors were calculated by ($[X]t,i$ - $[X]t,f$) *100/ $[X]t,i$.

Table C.3. TS calculations with and without nutrient supplementation.

Reactor	Triplicate code	Initial TS concentrations, mg/L			Volumes used, mL				Initial TS amounts, mg				TS concentration in reactor, mg/L		Final TS amount in reactor, mg	Specific TS removal of inoculum, mg/mL	Inoculum TS, mg		Digestate TS remained, mg	Digestate TS concentration remained, mg/L	TS removal efficiency from digestates, %
		Digestate	Inoculum	Nutrient medium	Digestate	Inoculum	Nutrient medium	Total volume	Digestate	Inoculum	Nutrient medium	Total in reactor	Initial	Final			Removed	Remained			
		[X]d,i	[X]ı,i	[X]nt,i	Vd	Vı	Vnt	Vt	Xd,i	Xı,i	Xnt,i	Xt,i	[X]t,i	[X]t,f	Xt,f	Is	Xı,removed	Xı,f	Xd,f	[X]d,f	X eff
I[a]	1	0	14107	26590	0	400.00	11	411.0	0	5643	292.5	5935	14441	13005±15	5345±6	1.44±0.015	590	5345	0±0	0±0	10±0
R1[a]	1	105250	14107	26590	9.83	387.97	11	408.8	1034	5473	292.5	6800	16634	14645±155	5987±63	1.44	573	5193	794±63	80810±6447	23±6
R2[a]	1	53263	14107	26590	26.17	371.06	11	408.2	1394	5235	292.5	6921	16954	14810±30	6046±12	1.44	549	4978	1068±12	40790±468	23±1
R2[a]	2	53263	14107	26590	26.18	371.06	11	408.2	1394	5235	292.5	6921	16954	14895±205	6081±84	1.44	549	4978	1102±84	42109±3197	21±6
R2[a]	3	53263	14107	26590	26.19	371.06	11	408.2	1395	5235	292.5	6922	16956	15195±105	6203±43	1.44	549	4978	1225±43	46771±1637	12±3
R3[a]	1	59053	14107	26590	18.34	380.01	11	409.3	1083	5361	292.5	6736	16456	14845±25	6077±10	1.44	562	5092	985±10	53719±558	9±1
R4[a]	1	74830	14107	26590	13.88	384.09	11	409.0	1038	5418	292.5	6749	16503	14725±95	6022±39	1.44	567	5144	879±39	63316±2800	15±4
R5[a]	1	49920	14107	26590	24.28	373.64	11	408.9	1212	5271	292.5	6776	16570	14665±65	5997±27	1.44	552	5011	986±27	40595±1095	19±2
R6[a]	1	18773	14107	26590	62.58	335.93	11	409.5	1175	4739	292.5	6206	15155	13940±70	5709±29	1.44	498	4533	1175±29	18781±458	0±2
I[b]	1	0	14107	26590	0	400.00	0	400.0	0	5643	0	5643	14107	13125±65	5250±26	0.98±0.065	393	5250	0±0	0±0	7±0
R1[b]	1	105250	14107	26590	9.83	387.97	0	397.8	1034	5473	0	6508	16359	14545±105	5786±42	0.98	381	5092	694±42	70599±4250	33±4
R2[b]	1	53263	14107	26590	26.21	371.06	0	397.3	1396	5235	0	6630	16690	14985±115	5953±46	0.98	364	4870	1083±46	41321±1743	22±3
R2[b]	2	53263	14107	26590	26.17	371.06	0	397.2	1394	5235	0	6629	16687	15085±25	5992±10	0.98	364	4870	1122±10	42870±379	20±1
R2[b]	3	53263	14107	26590	26.21	371.06	0	397.3	1396	5235	0	6631	16690	14610±150	5804±60	0.98	364	4870	934±60	35634±2274	33±4
R3[b]	1	59053	14107	26590	18.28	380.01	0	398.3	1080	5361	0	6441	16170	14960±60	5959±24	0.98	373	4988	971±24	53100±1307	10±2
R4[b]	1	74830	14107	26590	13.81	384.09	0	397.9	1033	5418	0	6452	16215	14940±250	5945±100	0.98	377	5041	903±99	65420±7203	13±10
R5[b]	1	49920	14107	26590	24.30	373.64	0	397.9	1213	5271	0	6484	16294	15255±75	6071±30	0.98	367	4904	1167±30	48009±1228	4±2
R6[b]	1	18773	14107	26590	62.57	335.93	0	398.5	1175	4739	0	5914	14840	14065±125	5605±50	0.98	330	4409	1196±50	19112±796	-2±4

[a] with nutrient supplementation.

[b] without nutrient supplementation.

[c] Removal efficiencies of the constituents in inoculum-only reactors were calculated by ([X]t,i - [X]t,f) *100/ [X]t,i.

Table C.4. VS calculations with and without nutrient supplementation.

Reactor	Triplicate code	Initial VS concentrations, mg/L			Volumes used, mL				Initial VS amounts, mg				VS concentration in reactor, mg/L		Final VS amount in reactor, mg	Specific VS removal of inoculum, mg/mL	Inoculum VS, mg		Digestate VS remained, mg	Digestate VS concentration remained, mg/L	VS removal efficiency from digestates, %
		Digestate	Inoculum	Nutrient medium	Digestate	Inoculum	Nutrient medium	Total volume	Digestate	Inoculum	Nutrient medium	Total in reactor	Initial	Final			Removed	Remained			
		[X]d,i	[X]ı,i	[X]nt,i	Vd	Vı	Vnt	Vt	Xd,i	Xı,i	Xnt,i	Xt,i	[X]t,i	[X]t,f	Xt,f	Is	Xı,removed	Xı,f	Xd,f	[X]d,f	X eff
I[a]	1	0	7330	14520	0	400.00	11	411.0	0	2932	159.7	3092	7522	6345±15	2608±6	1.18±0.01	484	2608	0±0.0	0±0	16±0
R1[a]	1	72630	7330	14520	9.83	387.97	11	408.8	714	2844	159.7	3717	9093	7285±65	2978±27	1.18	470	2534	444±26.6	45208±2704	38±4
R2[a]	1	25970	7330	14520	26.17	371.06	11	408.2	680	2720	159.7	3559	8719	6610±20	2698±8	1.18	450	2430	269±8.2	10266±312	60±1
R2[a]	2	25970	7330	14520	26.18	371.06	11	408.2	680	2720	159.7	3559	8719	6620±80	2703±33	1.18	450	2430	273±32.7	10421±1248	60±5
R2[a]	3	25970	7330	14520	26.19	371.06	11	408.2	680	2720	159.7	3560	8720	6775±5	2766±2	1.18	450	2430	336±2.0	12835±78	51±0
R3[a]	1	38013	7330	14520	18.34	380.01	11	409.3	697	2785	159.7	3642	8898	7445±5	3048±2	1.18	460	2485	563±2.0	30693±112	19±0
R4[a]	1	50953	7330	14520	13.88	384.09	11	409.0	707	2815	159.7	3682	9003	7470±20	3055±8	1.18	465	2510	545±8.2	39282±589	23±1
R5[a]	1	28180	7330	14520	24.28	373.64	11	408.9	684	2739	159.7	3583	8762	7215±75	2950±31	1.18	453	2446	505±30.7	20786±1263	26±4
R6[a]	1	9837	7330	14520	62.58	335.93	11	409.5	616	2462	159.7	3238	7906	6600±30	2703±12	1.18	408	2214	489±12.3	7817±196	21±2
I[b]	1	0	7330	14520	0	400.00	0	400.0	0	2932	0	2932	7330	6350±10	2540±4	0.98±0.01	392	2540	0±0.0	0±0	13±0
R1[b]	1	72630	7330	14520	9.83	387.97	0	397.8	714	2844	0	3558	8943	7315±65	2910±26	0.98	380	2464	446±25.9	45408±2631	37±4
R2[b]	1	25970	7330	14520	26.21	371.06	0	397.3	681	2720	0	3400	8560	6740±40	2678±16	0.98	364	2356	321±15.9	12262±606	53±2
R2[b]	2	25970	7330	14520	26.17	371.06	0	397.2	680	2720	0	3400	8558	6810±110	2705±44	0.98	364	2356	349±43.7	13331±1669	49±6
R2[b]	3	25970	7330	14520	26.21	371.06	0	397.3	681	2720	0	3401	8560	6660±110	2646±44	0.98	364	2356	290±43.7	11049±1667	57±6
R3[b]	1	38013	7330	14520	18.28	380.01	0	398.3	695	2785	0	3480	8738	7510±20	2991±8	0.98	372	2413	578±8.0	31620±436	17±1
R4[b]	1	50953	7330	14520	13.81	384.09	0	397.9	704	2815	0	3519	8844	7485±105	2978±42	0.98	376	2439	539±41.8	39053±3025	23±6
R5[b]	1	28180	7330	14520	24.30	373.64	0	397.9	685	2739	0	3424	8603	7280±50	2897±20	0.98	366	2373	524±19.9	21581±819	23±3
R6[b]	1	9837	7330	14520	62.57	335.93	0	398.5	616	2462	0	3078	7724	6605±85	2632±34	0.98	329	2133	499±33.9	7974±541	19±6

[a] with nutrient supplementation.

[b] without nutrient supplementation.

[c] Removal efficiencies of the constituents in inoculum-only reactors were calculated by ([X]t,i - [X]t,f) *100/ [X]t,i.

Table C.5. NH_4^+-N calculations with and without nutrient supplementation.

Reactor	Triplicate code	Initial NH_4^+-N concentrations, mg/L			Volumes used, mL				Initial NH_4^+-N amounts, mg				NH_4^+-N concentration in reactor, mg/L		Final NH_4^+-N amount in reactor, mg	Specific NH_4^+-N removal of inoculum, mg/mL	Inoculum NH_4^+-N, mg		Digestate NH_4^+-N remained, mg	Digestate NH_4^+-N concentration remained, mg/L	NH_4^+-N removal efficiency from digestates, %
		Digestate	Inoculum	Nutrient medium	Digestate	Inoculum	Nutrient medium	Total volume	Digestate	Inoculum	Nutrient medium	Total in reactor	Initial	Final			Removed	Remained			
		[X]d,i	[X]ı,i	[X]nt,i	Vd	Vı	Vnt	Vt	Xd,i	Xı,i	Xnt,i	Xt,i	[X]t,i	[X]t,f	Xt,f	Is	Xı,removed	Xı,f	Xd,f	[X]d,f	X eff
I[a]	1	0	892	3290	0	400.00	11	411.0	0.0	357	36.2	393	956	1120±3	460±1.2	-0.16±0.003	-67.3	460.3	0±0.0	0±0	0±0
R1[a]	1	3288	892	3290	9.83	387.97	11	408.8	32.3	346	36.2	415	1014	1196±12	489±4.6	-0.16	-65.4	447.6	41±4.6	4186±466	-27±14
R2[a]	1	7703	892	3290	26.17	371.06	11	408.2	201.6	331	36.2	569	1393	1491±7	609±2.9	-0.16	-62.6	429.8	179±2.9	6836±109	11±1
R2[a]	2	7703	892	3290	26.18	371.06	11	408.2	201.7	331	36.2	569	1393	1623±2	662±0.6	-0.16	-62.6	429.8	233±0.6	8887±22	-15±0
R2[a]	3	7703	892	3290	26.19	371.06	11	408.2	201.8	331	36.2	569	1394	1498±14	612±5.7	-0.16	-62.6	429.8	182±5.7	6941±218	10±3
R3[a]	1	1782	892	3290	18.34	380.01	11	409.3	32.7	339	36.2	408	996	1139±2	466±0.6	-0.16	-64.1	439.2	27±0.6	1456±31	18±2
R4[a]	1	4569	892	3290	13.88	384.09	11	409.0	63.4	343	36.2	442	1081	1212±1	495±0.2	-0.16	-64.7	443.5	52±0.2	3733±15	18±0
R5[a]	1	4071	892	3290	24.28	373.64	11	408.9	98.9	333	36.2	468	1145	1285±2	526±0.8	-0.16	-63.0	432.5	93±0.8	3836±34	6±1
R6[a]	1	826	892	3290	62.58	335.93	11	409.5	51.7	300	36.2	388	946	1118±2	457±0.6	-0.16	-56.8	392.7	65±0.6	1035±10	-25±1
I[b]	1	0	892	3290	0	400.00	0	400.0	0.0	357	0	357	892	1037±0	415±0.1	-0.15±0	-58.1	414.9	0±0.0	0±0	0±0
R1[b]	1	3288	892	3290	9.83	387.97	0	397.8	32.3	346	0	378	951	1108±13	441±5.0	-0.15	-56.3	402.4	38±5.0	3878±510	-18±16
R2[b]	1	7703	892	3290	26.21	371.06	0	397.3	201.9	331	0	533	1341	1484±0	590±0.0	-0.15	-53.9	384.9	205±0.0	7810±0	-1±0
R2[b]	2	7703	892	3290	26.17	371.06	0	397.2	201.6	331	0	533	1341	1456±0	578±0.0	-0.15	-53.9	384.9	194±0.0	7393±0	4±0
R2[b]	3	7703	892	3290	26.21	371.06	0	397.3	201.9	331	0	533	1341	1470±14	584±5.6	-0.15	-53.9	384.9	199±5.6	7597±212	1±3
R3[b]	1	1782	892	3290	18.28	380.01	0	398.3	32.6	339	0	372	933	1074±7	428±2.8	-0.15	-55.2	394.2	34±2.8	1840±152	-3±9
R4[b]	1	4569	892	3290	13.81	384.09	0	397.9	63.1	343	0	406	1020	1166±9	464±3.3	-0.15	-55.8	398.4	65±3.3	4739±241	-4±5
R5[b]	1	4071	892	3290	24.30	373.64	0	397.9	98.9	333	0	432	1086	1280±14	509±5.6	-0.15	-54.3	387.5	122±5.6	5007±229	-23±6
R6[b]	1	826	892	3290	62.57	335.93	0	398.5	51.7	300	0	351	882	1044±2	416±0.9	-0.15	-48.8	348.4	68±0.9	1080±15	-31±2

[a] with nutrient supplementation.

[b] without nutrient supplementation.

[c] Removal efficiencies of the constituents in inoculum-only reactors were calculated by ([X]t,i - [X]t,f) *100/ [X]t,i.

Table C.6. TKN calculations with and without nutrient supplementation.

Reactor	Triplicate code	Initial TKN concentrations, mg/L			Volumes used, mL				Initial TKN amounts, mg				TKN concentration in reactor, mg/L		Final TKN amount in reactor, mg	Specific TKN removal of inoculum, mg/mL	Inoculum TKN, mg		Digestate TKN remained, mg	Digestate TKN concentration remained, mg/L	TKN removal efficiency from digestates, %
		Digestate	Inoculum	Nutrient medium	Digestate	Inoculum	Nutrient medium	Total volume	Digestate	Inoculum	Nutrient medium	Total in reactor	Initial	Final			Removed	Remained			
		[X]d,i	[X]ı,i	[X]nt,i	Vd	Vı	Vnt	Vt	Xd,i	Xı,i	Xnt,i	Xt,i	[X]t,i	[X]t,f	Xt,f	Is	Xı,removed	Xı,f	Xd,f	[X]d,f	X eff
I[a]	1	0	1274	3290	0	400.00	11	411.0	0	509.6	36.2	545.8	1328	1189±5	488±1.8	0.14±0.004	57.3	489	0±0.0	0±0	11±0
R1[a]	1	3694	1274	3290	9.83	387.97	11	408.8	36.3	494.3	36.2	566.8	1386	1237±12	506±5.1	0.14	55.6	475	31±5.1	3139±514	15±14
R2[a]	1	8394	1274	3290	26.17	371.06	11	408.2	219.7	472.7	36.2	728.6	1785	1568±28	640±11.4	0.14	53.2	456	184±11.4	7047±437	16±5
R2[a]	2	8394	1274	3290	26.18	371.06	11	408.2	219.7	472.7	36.2	728.7	1785	1725±17	704±6.9	0.14	53.2	456	248±6.9	9491±262	-13±3
R2[a]	3	8394	1274	3290	26.19	371.06	11	408.2	219.9	472.7	36.2	728.8	1785	1596±28	652±11.4	0.14	53.2	456	196±11.4	7479±436	11±5
R3[a]	1	2285	1274	3290	18.34	380.01	11	409.3	41.9	484.1	36.2	562.2	1373	1214±21	497±8.6	0.14	54.5	466	31±8.6	1692±469	26±21
R4[a]	1	5147	1274	3290	13.88	384.09	11	409.0	71.4	489.3	36.2	596.9	1460	1300±1	532±0.4	0.14	55.1	471	61±0.4	4412±28	14±1
R5[a]	1	4815	1274	3290	24.28	373.64	11	408.9	116.9	476.0	36.2	629.1	1539	1362±3	557±1.0	0.14	53.6	459	98±1.0	4052±43	16±1
R6[a]	1	1051	1274	3290	62.58	335.93	11	409.5	65.8	428.0	36.2	529.9	1294	1185±9	485±3.5	0.14	48.3	416	69±3.5	1107±56	-5±5
I[b]	1	0	1274	3290	0	400.00	0	400.0	0	509.6	0	509.6	1274	1124±29	449±11.4	0.15±0.029	60.2	449	0±0.0	0±0	12±2
R1[b]	1	3694	1274	3290	9.83	387.97	0	397.8	36.3	494.3	0	530.6	1334	1194±35	475±13.9	0.15	58.3	436	39±13.9	3979±1417	-8±38
R2[b]	1	8394	1274	3290	26.21	371.06	0	397.3	220.0	472.7	0	692.7	1744	1561±21	620±8.3	0.15	55.8	417	203±8.3	7754±318	8±4
R2[b]	2	8394	1274	3290	26.17	371.06	0	397.2	219.7	472.7	0	692.4	1743	1526±14	606±5.6	0.15	55.8	417	189±5.6	7230±212	14±3
R2[b]	3	8394	1274	3290	26.21	371.06	0	397.3	220.0	472.7	0	692.7	1744	1554±0	617±0.0	0.15	55.8	417	200±0.0	7647±0	9±0
R3[b]	1	2285	1274	3290	18.28	380.01	0	398.3	41.8	484.1	0	525.9	1320	1146±6	456±2.5	0.15	57.1	427	29±2.5	1605±139	30±6
R4[b]	1	5147	1274	3290	13.81	384.09	0	397.9	71.1	489.3	0	560.4	1408	1272±17	506±6.7	0.15	57.8	432	75±6.7	5406±485	-5±9
R5[b]	1	4815	1274	3290	24.30	373.64	0	397.9	117.0	476.0	0	593.0	1490	1383±0	550±0.0	0.15	56.2	420	131±0.0	5375±0	-12±0
R6[b]	1	1051	1274	3290	62.57	335.93	0	398.5	65.8	428.0	0	493.7	1239	1106±14	441±5.4	0.15	50.5	378	63±5.4	1011±86	4±8

[a] with nutrient supplementation.

[b] without nutrient supplementation.

[c] Removal efficiencies of the constituents in inoculum-only reactors were calculated by ([X]t,i - [X]t,f) *100/ [X]t,i.

Table C.7. DRP calculations with and without nutrient supplementation.

Reactor	Triplicate code	Initial DRP concentrations, mg/L			Volumes used, mL				Initial DRP amounts, mg				DRP concentration in reactor, mg/L		Final DRP amount in reactor, mg	Specific DRP removal of inoculum, mg/mL	Inoculum DRP, mg		Digestate DRP remained, mg	Digestate DRP concentration remained, mg/L	DRP removal efficiency from digestates, %
		Digestate	Inoculum	Nutrient medium	Digestate	Inoculum	Nutrient medium	Total volume	Digestate	Inoculum	Nutrient medium	Total in reactor	Initial	Final			Removed	Remained			
		[X]d,i	[X]ı,i	[X]nt,i	Vd	Vı	Vnt	Vt	Xd,i	Xı,i	Xnt,i	Xt,i	[X]t,i	[X]t,f	Xt,f	Is	Xı,removed	Xı,f	Xd,f	[X]d,f	X eff
I[a]	1	0	27	3310	0.00	400.00	11	411.0	0.0	10.9	36	47.3	115	24.4±0.1	10.0±0.0	0.09±0	37.2	10.0	0.0±0.0	0±0	79±0
R1[a]	1	1156	27	3310	9.83	387.97	11	408.8	11.4	10.6	36	58.3	142.7	14.1±0.0	5.8±0.0	0.09	36.1	10.9	-5.1±0.0	-520±0	145±0
R2[a]	1	1098	27	3310	26.17	371.06	11	408.2	28.7	10.1	36	75.2	184.3	60.8±0.4	24.8±0.2	0.09	34.5	12.0	12.9±0.2	492±6	55±1
R2[a]	2	1098	27	3310	26.18	371.06	11	408.2	28.7	10.1	36	75.2	184.3	54.6±0.2	22.3±0.1	0.09	34.5	12.0	10.3±0.1	395±3	64±0
R2[a]	3	1098	27	3310	26.19	371.06	11	408.2	28.8	10.1	36	75.3	184.3	60.8±0.2	24.8±0.1	0.09	34.5	12.0	12.9±0.1	492±3	55±0
R3[a]	1	290	27	3310	18.34	380.01	11	409.3	5.3	10.3	36	52.1	127.2	7.6±0.0	3.1±0.0	0.09	35.4	11.4	-8.3±0.0	-450±1	255±0
R4[a]	1	466	27	3310	13.88	384.09	11	409.0	6.5	10.4	36	53.3	130.4	22.3±0.0	9.1±0.0	0.09	35.7	11.1	-2.0±0.0	-146±1	131±0
R5[a]	1	549	27	3310	24.28	373.64	11	408.9	13.3	10.2	36	59.9	146.5	32.5±0.0	13.3±0.0	0.09	34.8	11.8	1.5±0.0	62±0	89±0
R6[a]	1	34	27	3310	62.58	335.93	11	409.5	2.1	9.1	36	47.6	116.4	20.6±0.0	8.4±0.0	0.09	31.4	14.2	-5.8±0.0	-92±0	373±0
I[b]	1	0	27	3310	0.00	400.00	0	400.0	0.0	10.9	0	10.9	27.2	6.5±0.1	2.6±0.0	0.02±0	8.3	2.6	0.0±0.0	0±0	76±0
R1[b]	1	1156	27	3310	9.83	387.97	0	397.8	11.4	10.6	0	21.9	55.1	11.3±0.0	4.5±0.0	0.02	8.0	2.5	2.0±0.0	203±0	82±0
R2[b]	1	1098	27	3310	26.21	371.06	0	397.3	28.8	10.1	0	38.9	97.8	26.2±0.0	10.4±0.0	0.02	7.7	2.4	8.0±0.0	305±0	72±0
R2[b]	2	1098	27	3310	26.17	371.06	0	397.2	28.7	10.1	0	38.8	97.7	25.4±0.3	10.1±0.1	0.02	7.7	2.4	7.7±0.1	294±5	73±0
R2[b]	3	1098	27	3310	26.21	371.06	0	397.3	28.8	10.1	0	38.9	97.8	27.2±0.2	10.8±0.1	0.02	7.7	2.4	8.4±0.1	321±3	71±0
R3[b]	1	290	27	3310	18.28	380.01	0	398.3	5.3	10.3	0	15.6	39.2	7.6±0.0	3.0±0.0	0.02	7.9	2.5	0.6±0.0	31±1	89±0
R4[b]	1	466	27	3310	13.81	384.09	0	397.9	6.4	10.4	0	16.9	42.4	8.7±0.0	3.5±0.0	0.02	8.0	2.5	1.0±0.0	71±1	85±0
R5[b]	1	549	27	3310	24.30	373.64	0	397.9	13.3	10.2	0	23.5	59.1	10.0±0.0	4.0±0.0	0.02	7.7	2.4	1.6±0.0	65±1	88±0
R6[b]	1	34	27	3310	62.57	335.93	0	398.5	2.1	9.1	0	11.2	28.2	9.1±0.0	3.6±0.0	0.02	7.0	2.2	1.4±0.0	23±0	32±1

[a] with nutrient supplementation.

[b] without nutrient supplementation.

[c] Removal efficiencies of the constituents in inoculum-only reactors were calculated by ([X]t,i - [X]t,f) *100/ [X]t,i.

Table C.8. TP calculations with and without nutrient supplementation.

Reactor	Triplicate code	Initial TP concentrations, mg/L			Volumes used, mL				Initial TP amounts, mg				TP concentration in reactor, mg/L		Final TP amount in reactor, mg	Specific TP removal of inoculum, mg/mL	Inoculum TP, mg		Digestate TP remained, mg	Digestate TP concentration remained, mg/L	TP removal efficiency from digestates, %
		Digestate	Inoculum	Nutrient medium	Digestate	Inoculum	Nutrient medium	Total volume	Digestate	Inoculum	Nutrient medium	Total in reactor	Initial	Final			Removed	Remained			
		[X]d,i	[X]ı,i	[X]nt,i	Vd	Vı	Vnt	Vt	Xd,i	Xı,i	Xnt,i	Xt,i	[X]t,i	[X]t,f	Xt,f	Is	Xı,removed	Xı,f	Xd,f	[X]d,f	X eff
I[a]	1	0	409	3373	0.00	400.00	11	411.0	0.0	163.6	37.1	200.7	488.3	377±0.9	155±0.4	0.11±0.001	45.9	154.8	0.0±0.0	0±0.0	23±0[c]
R1[a]	1	2314	409	3373	9.83	387.97	11	408.8	22.7	158.7	37.1	218.5	534.6	423±0.9	173±0.4	0.11	44.6	151.2	21.8±0.4	2221±39	4±2
R2[a]	1	2786	409	3373	26.17	371.06	11	408.2	72.9	151.8	37.1	261.8	641.3	516±0.0	211±0.0	0.11	42.7	146.2	64.4±0.0	2462±0.0	12±0
R2[a]	2	2786	409	3373	26.18	371.06	11	408.2	72.9	151.8	37.1	261.8	641.3	519±0.9	212±0.4	0.11	42.7	146.2	65.5±0.4	2503±15	10±1
R2[a]	3	2786	409	3373	26.19	371.06	11	408.2	73.0	151.8	37.1	261.8	641.4	515±0.9	210±0.4	0.11	42.7	146.2	64.2±0.4	2450±15	12±1
R3[a]	1	1340	409	3373	18.34	380.01	11	409.3	24.6	155.4	37.1	217.1	530.4	409±0.9	167±0.4	0.11	43.7	148.9	18.4±0.4	1005±21	25±2
R4[a]	1	1555	409	3373	13.88	384.09	11	409.0	21.6	157.1	37.1	215.8	527.6	403±0.9	165±0.4	0.11	44.1	150.1	14.6±0.4	1052±28	32±2
R5[a]	1	1725	409	3373	24.28	373.64	11	408.9	41.9	152.8	37.1	231.8	566.9	462±0.0	189±0.0	0.11	43.0	147.0	42.0±0.0	1728±0	0±0
R6[a]	1	352	409	3373	62.58	335.93	11	409.5	22.0	137.4	37.1	196.5	479.9	411±0.9	168±0.4	0.11	38.7	135.8	32.4±0.4	518±6	-47±2
I[b]	1	0	409	3373	0.00	400.00	0	400.0	0.0	163.6	0	163.6	409.0	288±0.0	115±0.0	0.12±0.000	48.4	115.2	0.0±0.0	0±0.0	30±0
R1[b]	1	2314	409	3373	9.83	387.97	0	397.8	22.7	158.7	0	181.4	456.1	329±0.9	131±0.4	0.12	46.9	111.7	19.0±0.4	1934±38	16±2
R2[b]	1	2786	409	3373	26.21	371.06	0	397.3	73.0	151.8	0	224.8	565.8	443±0.9	176±0.4	0.12	44.9	106.9	69.3±0.4	2643±14	5±1
R2[b]	2	2786	409	3373	26.17	371.06	0	397.2	72.9	151.8	0	224.7	565.6	443±0.9	176±0.4	0.12	44.9	106.9	69.0±0.4	2635±14	5±1
R2[b]	3	2786	409	3373	26.21	371.06	0	397.3	73.0	151.8	0	224.8	565.8	457±0.9	182±0.4	0.12	44.9	106.9	74.8±0.4	2855±14	-2±1
R3[b]	1	1340	409	3373	18.28	380.01	0	398.3	24.5	155.4	0	179.9	451.7	320±0.0	127±0.0	0.12	46.0	109.4	18.0±0.0	985±0.0	26±0
R4[b]	1	1555	409	3373	13.81	384.09	0	397.9	21.5	157.1	0	178.6	448.8	319±0.9	127±0.4	0.12	46.5	110.6	16.4±0.4	1191±27	23±2
R5[b]	1	1725	409	3373	24.30	373.64	0	397.9	41.9	152.8	0	194.7	489.4	394±0.0	157±0.0	0.12	45.2	107.6	49.2±0.0	2024±0.0	-17±0
R6[b]	1	352	409	3373	62.57	335.93	0	398.5	22.0	137.4	0	159.4	400.1	299±0.9	119±0.4	0.12	40.6	96.7	22.5±0.4	360±6.0	-2±2

[a] with nutrient supplementation.

[b] without nutrient supplementation.

[c] Removal efficiencies of the constituents in inoculum-only reactors were calculated by ([X]t,i - [X]t,f) *100/ [X]t,i.

APPENDICES

D. Effect of chloride ion concentration on COD measurements

Chloride and chemical oxygen demand were measured according to Standart Methods (APHA, 2005). $HgSO_4$:Cl^- ratio (by weight) ranged between 175-2285 according to the results of the analysis (Table D.1). A $HgSO_4$:Cl^- (mercury sulfate:chloride) of 10:1 (by weight) is offered in the measurement of COD to eliminate the interference of chloride ions (Gopal, 2007). Chloride ions were found to be not interfering with the results due to high $HgSO_4$:Cl^- ratios during COD measurements.

Table D.1. Chloride concentrations of the digestates and related $HgSO_4$:Cl^- ratios during the COD measurements.

Digestate of anaerobic digester	Chloride, mg/L	Sample volume taken for COD analysis, mL	Chloride amount of sample, mg	$HgSO_4$ added, g	$HgSO_4$:Cl^-
1	3374	0.2141	0.722	1.0771	1491
	3374	0.2627	0.886	2.0254	2285
2	4724	1.2121	5.725	1.0040	175
	4724	1.1415	5.392	2.0017	371
3	2849	0.3458	0.985	1.0085	1024
	2849	0.3791	1.080	2.0145	1865
4	8797	0.3420	3.009	1.0069	335
	8797	0.2967	2.610	2.0064	769
5	4599	0.3256	1.497	1.0150	678
	4599	0.3207	1.475	2.0023	1358
6	1425	1.3840	1.972	1.0091	512
	1425	1.5222	2.168	2.0021	923

APPENDICES

E. Negative biogas production periods in the related reactors

The negative biogas production periods of 5 or more days was observed in one of the nutrient supplemented R1 and one of R2 reactors and given in Figure E.1 and Figure E.2, respectively.

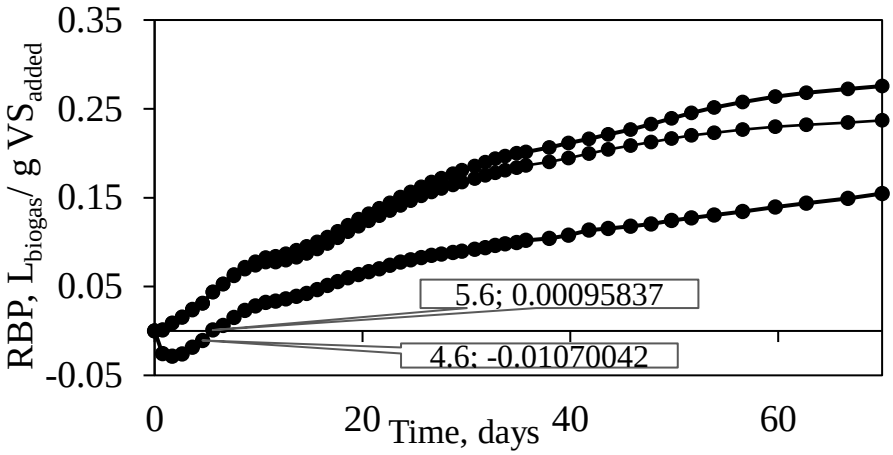


Figure E.1. Negative biogas production period experienced in one of the nutrient supplemented R1 reactor.

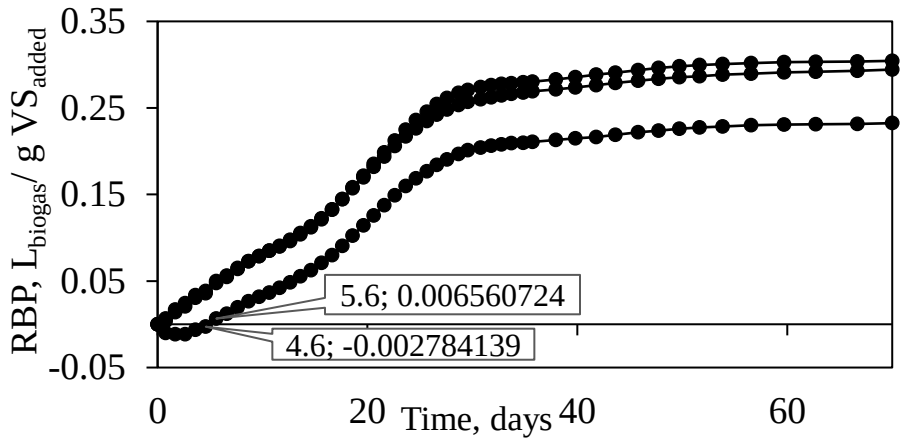


Figure E.2. Negative biogas production period experienced in one of the nutrient supplemented R2 reactor.

APPENDICES

F. Inconsistent data observed in RBP test

Inconsistent data, i.e. spike formation, observed in the residual biogas potential graphs is given in Figure F.1 and Figure F.2.

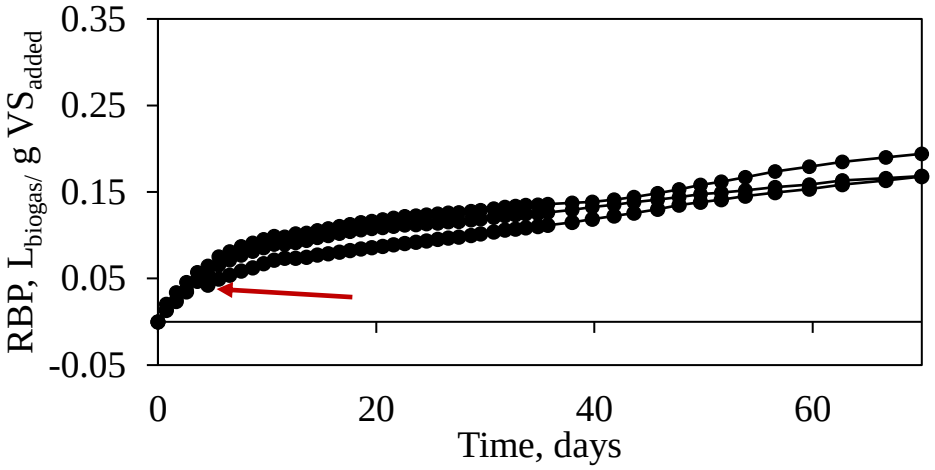


Figure F.1. The formation of a spike in one R5 reactor without nutrient supplementation.

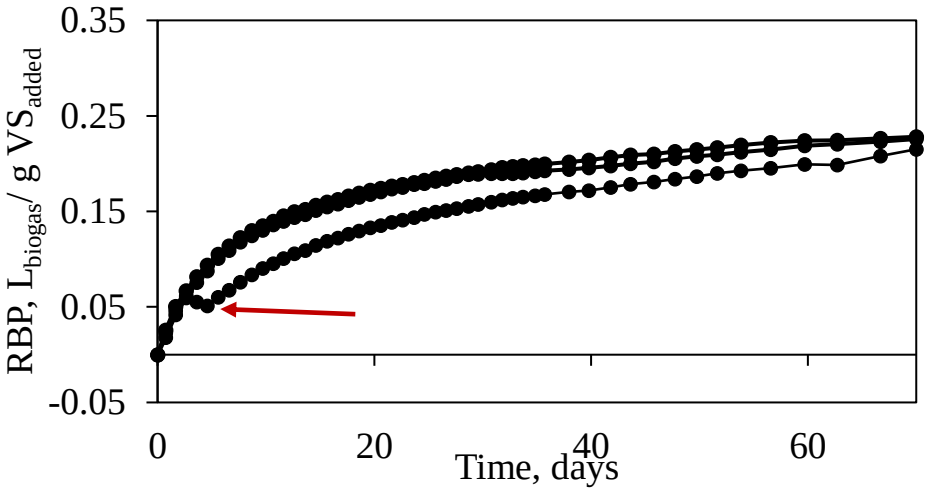


Figure F.2. The formation of a spike in one R6 reactor with nutrient supplementation.

APPENDICES

G. Correlation between biogas yields and removed volatile solids from digestate samples

The residual biogas yields calculated based on the added VS of the digestates and the corresponding removed amounts of VS from the digestates are given in Tables G.1 and G.2 with and without nutrient supplementation, respectively.

Table G.1. Residual biogas yields and VS removed from digestates with nutrient supplementation.

The digestate of anaerobic digester	Residual biogas yield, L_{biogas}/g VS_{added}	VS removal amounts from the digestate, mg
1	0.256 ± 0.024	270±27
2	0.299 ± 0.005	387±37
3	0.111 ± 0.013	134±2
4	0.181 ± 0.014	162±8
5	0.146 ± 0.013	179±31
6	0.227 ± 0.001	127±12

Table G.2. Residual biogas yields and VS removed from digestates without nutrient supplementation.

The digestate of anaerobic digester	Residual biogas yield, L_{biogas}/g VS_{added}	VS removal amounts from the digestate, mg
1	0.210 ± 0.015	268±26
2	0.326 ± 0.009	361±44
3	0.123 ± 0.009	117±8
4	0.175 ± 0.015	165±42
5	0.181 ± 0.013	161±20
6	0.195 ± 0.014	117±34

The correlation between RBYs and removed VS was evaluated using the average values for both variables. The coefficient of determinations (R^2) were found as 0.6214 (Figure G.1) and 0.8026 (Figure G.2) with and without nutrient supplementation, respectively.

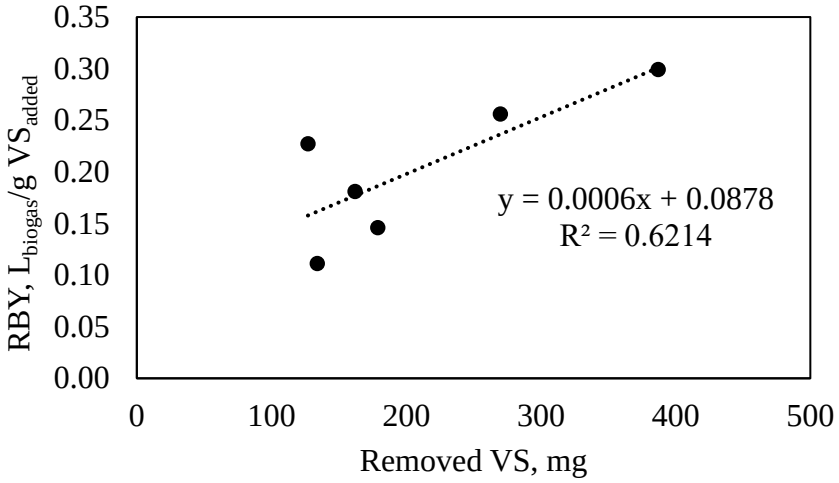


Figure G.1. Correlation between RBY and VS removed from digestate with nutrient supplementation including the digestate of anaerobic digester 6.

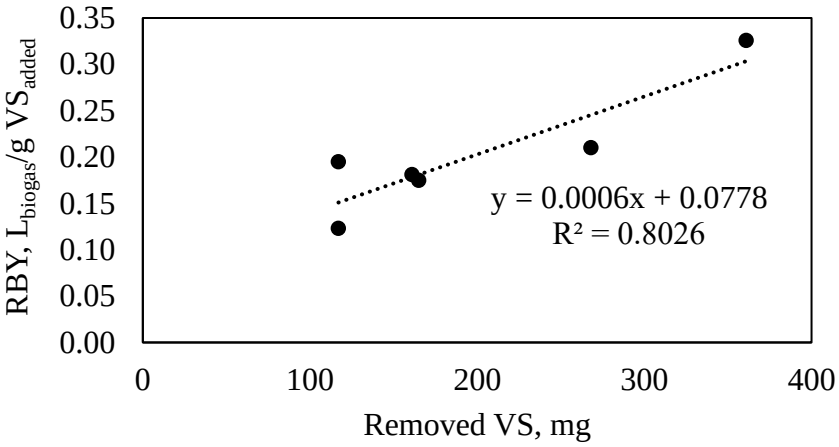


Figure G.2. Correlation between RBY and VS removed from digestate without nutrient supplementation including the digestate of anaerobic digester 6.

When the data related to digestate of anaerobic digester 6 is eliminated, R^2 values increased from 0.6214 to 0.8956 (Figure G.3) and from 0.8026 to 0.9247 (Figure G.4) with and without nutrient supplementation, respectively.

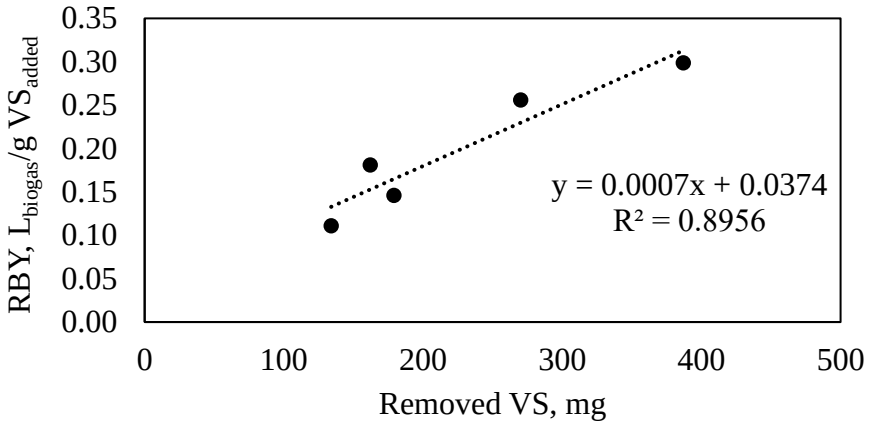


Figure G.3. Correlation between RBY and VS removed from digestate with nutrient supplementation excluding the digestate of anaerobic digester 6.

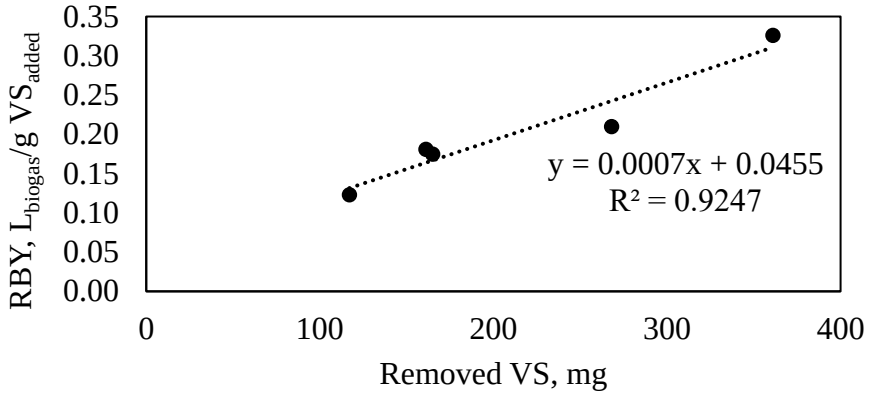


Figure G.4. Correlation between RBY and VS removed from digestate without nutrient supplementation excluding the digestate of anaerobic digester 6.

APPENDICES

H. Calculations on the concentration of free ammonia

Free ammonia nitrogen (FAN) concentrations were theoretically calculated using the following formula (Anthonisen et al., 1976; Calli et al., 2005):

$$FAN = \frac{TAN}{1+10^{(pK_a - pH)}} \quad [H.1]$$

where, TAN is the concentration of total ammonia nitrogen and pK_a is the acid dissociation constant (8.95 at 35^{o}C).

The estimation of FAN concentrations at the beginning of the RBP test

FAN concentrations were calculated depending on the individual pHs of the components and the initial NH_4^+-N concentrations in the reactors (Table H.1). pH in the reactors were initially not measured to preserve the anaerobic conditions after nitrogen purging. Thus, the FAN concentrations were calculated based on the pH of the digestate and the pH of the inoculum independently for each reactor (Table H.1).

Table H.1. FAN estimates at the beginning of the test.

Nutrient supplementation	Reactor	Initial pH of digestate	Initial pH of inoculum	pK_a at 35ºC	Initial TAN concentration in the reactor, mg/L	Potential FAN concentration due to digestate, mg/L	Potential FAN concentration due to inoculum, mg/L
		A	B	C	D	$E=D/(1+10^{C-A})$	$F=D/(1+10^{C-B})$
with	R1	8.78	8.71	8.95	1014	409	370
	R2	8.50	8.71	8.95	1393	365	509
	R3	8.72	8.71	8.95	996	369	364
	R4	8.87	8.71	8.95	1081	491	395
	R5	8.60	8.71	8.95	1145	354	418
	R6	8.36	8.71	8.95	946	193	346
without	R1	8.78	8.71	8.95	951	384	347
	R2	8.50	8.71	8.95	1341	351	490
	R3	8.72	8.71	8.95	933	346	341
	R4	8.87	8.71	8.95	1020	463	373
	R5	8.60	8.71	8.95	1086	335	397
	R6	8.36	8.71	8.95	882	180	322

The estimation of final FAN concentrations at the end of the RBP test

Final FAN concentrations were calculated depending on the measured pH and NH_4^+-N concentrations at the end of operation (Table H.2).

Table H.2. FAN estimates at the end of the test.

Nutrient supplementation	Reactor	Final pH	pK_a at 35ºC	Final TAN concentration in the reactor, mg/L	Potential FAN concentration in the reactor, mg/L
		A	B	C	$D=C/(1+10^{B-A})$
with	R1	7.53	8.95	1196	44
	R2	7.91	8.95	1537	128
	R3	7.66	8.95	1139	56
	R4	7.75	8.95	1212	72
	R5	8.13	8.95	1285	169
	R6	8.07	8.95	1118	130
without	R1	7.72	8.95	1108	62
	R2	8	8.95	1470	148
	R3	7.91	8.95	1074	90
	R4	7.8	8.95	1166	77
	R5	7.81	8.95	1280	86
	R6	7.94	8.95	1044	93

APPENDICES

I. A sample calculation for NH_4^+-N removal in T1a

The major mechanisms for ammonium removal were assumed as nitrification and microalgal-bacterial uptake. The related calculations were given in the following table.

Table I.1. Sample calculation for NH_4^+-N removal

Constituent		T1a	Formula	Calculation
NH_4^+- N, mg/L	Initial	316.3 ± 89.41	A	316.3
	Final	29.0 ± 1.00	B	29.0
	Removed	287.3	C= (A-B)	316.3-29.0= 287.3
NO_3^--N, mg/L	Initial	3.0 ± 0.10	D	3.0
	Final	146.0 ± 1.00	E	146.0
	Formed	143.0	F= (E-D)	146.0-3.0=143.0
NO_2^--N, mg/L	Initial	0.9 ± 0.09	G	0.9
	Final	3.1 ± 0.16	H	3.1
	Formed	2.3*	I= (H-G)	3.1-0.9= 2.2*
(NO_3^--N)+(NO_2^--N), mg/L		145.3	J= F+I	143.0+2.3= 145.3
Total NH_4^+- N removal, %		90.8	K= C*100/A	287.3*100/316.3= 90.3
NH_4^+- N conversion by nitrification, %		50.6	L= J*100/C	145.3*100/287.3= 50.6
Microalgal NH_4^+- N removal, mg/L		142.0	M= (C-J)	287.3-145.3= 142.0
Microalgal NH_4^+- N removal, %		49.4	N= M*100/C	142.0*100/287.3= 49.4

* The difference between values was based on the significant digits used in actual calculations.

APPENDICES

J. NH_4^+-N removal kinetics

Linear fitting lines according to zero-, one-half-, first- and second-order kinetics on NH_4^+- N removal are shown in Figure J.1 and the related R^2 values of these lines are given in Table J.1. The maximum and minimum R^2 values obtained by the application of zero-, one-half- and first-order kinetics were observed to be very close to each other (Table J.1). The variation between R^2 values for each reactor was then calculated by taking the difference between maximum and minimum R^2 values. The related variations were found as 0.0558, 0.0414 and 0.0532 according to zero-, one-half- and first-order kinetics, respectively. The application of one-half-order kinetics resulted in respectively less variation between maximum and minimum R^2 values, thus, found to be the best representative on the removal of NH_4^+- N.

Table J.1. R^2 values of the linear fitting lines for NH_4^+- N removal.

	Zero-order	One-half-order	First-order	Second-order
T1a	0.9382	0.9844	0.9859	0.8747
T1b	0.9546	0.9893	0.9748	0.8
T2a	0.9800	0.9930	0.9972	0.976
T2b	0.9342	0.9516	0.9592	0.936
T3a	0.9900	0.9867	0.9808	0.962
T3b	0.9721	0.9602	0.9440	0.9012
T4	0.9753	0.9693	0.9612	0.9387
max	0.9900	0.9930	0.9972	0.9760
min	0.9342	0.9516	0.9440	0.8000
variation between R^2 values	0.0558	0.0414	0.0532	0.1760

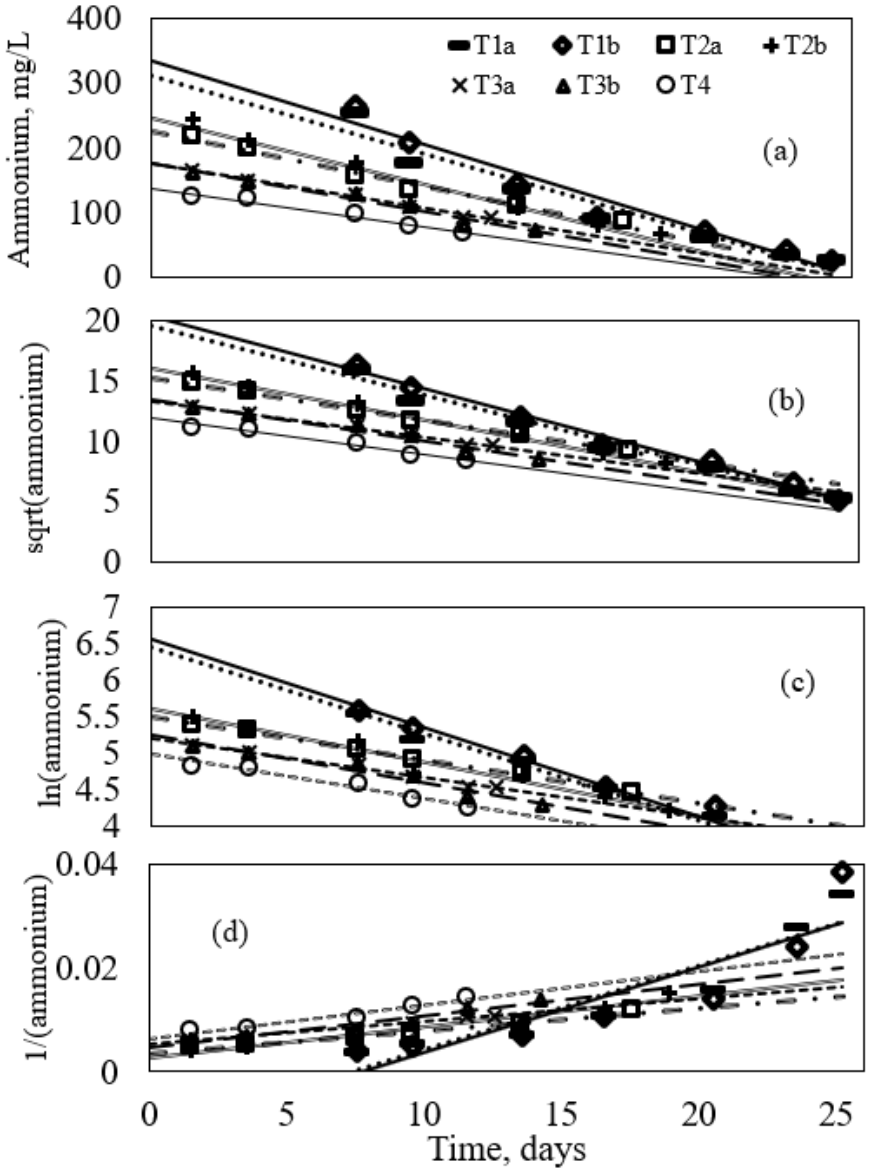


Figure J.1. NH_4^+- N removal linear fitting lines: (a) zero-order, (b) one-half-order, (c) first-order, (d) second-order kinetics.

APPENDICES

K. A sample calculation on DRP and TDP removal

DRP and TDP removal was calculated based on the assumption that the only removal mechanism was the biological uptake (Table K.1).

Table K.1. Sample calculation on DRP and TDP removal.

Constituent		T1a	Formula	Calculation
DRP (mg/L)	Initial	13.10 ± 0.14	A	13.10
	Final	0.00 ± 0.00	B	0.00
	Removed	13.10	C= (A-B)	13.10-0.00= 13.10
TDP (mg/L)	Initial	16.75 ± 1.00	D	16.75
	Final	0.74 ± 0.06	E	0.74
	Removed	16.01	F= (D-E)	16.75-0.74= 16.01
Total DRP removal (%)		100.0	G= (C*100)/A	13.10*100/13.10=100
Total TDP removal (%)		95.6	H= (F*100)/D	16.01*100/16.75= 95.6

APPENDICES

L. TDP removal kinetics

Linear fitting lines according to zero-, first- and second-order kinetics for TDP removal are shown in Figure L.1 and the related R^2 values of these lines are given in Table L.1. The first and second order kinetics had a lower coefficient of determination in the range of 0.8062-0.8894 and 0.5701-0.6237, respectively, compared to the zero-order kinetics (0.9833-0.9988). Hence, zero-order kinetics was found to be representative for the removal of TDP in all reactors and used for further estimation of total concentration of TDP within the reactors.

Table L.1. R^2 values of the linear fitting lines for TDP removal.

	Zero-order	First-order	Second-order
T1a	0.9971	0.8894	0.6076
T1b	0.9981	0.8803	0.5859
T2a	0.9853	0.8062	0.6145
T2b	0.9833	0.8438	0.5701
T3a	0.9924	0.8356	0.6237
T3b	0.9923	0.8533	0.5911
T4	0.9988	0.8822	0.6217
Max	0.9988	0.8894	0.6237
Min	0.9833	0.8062	0.5701

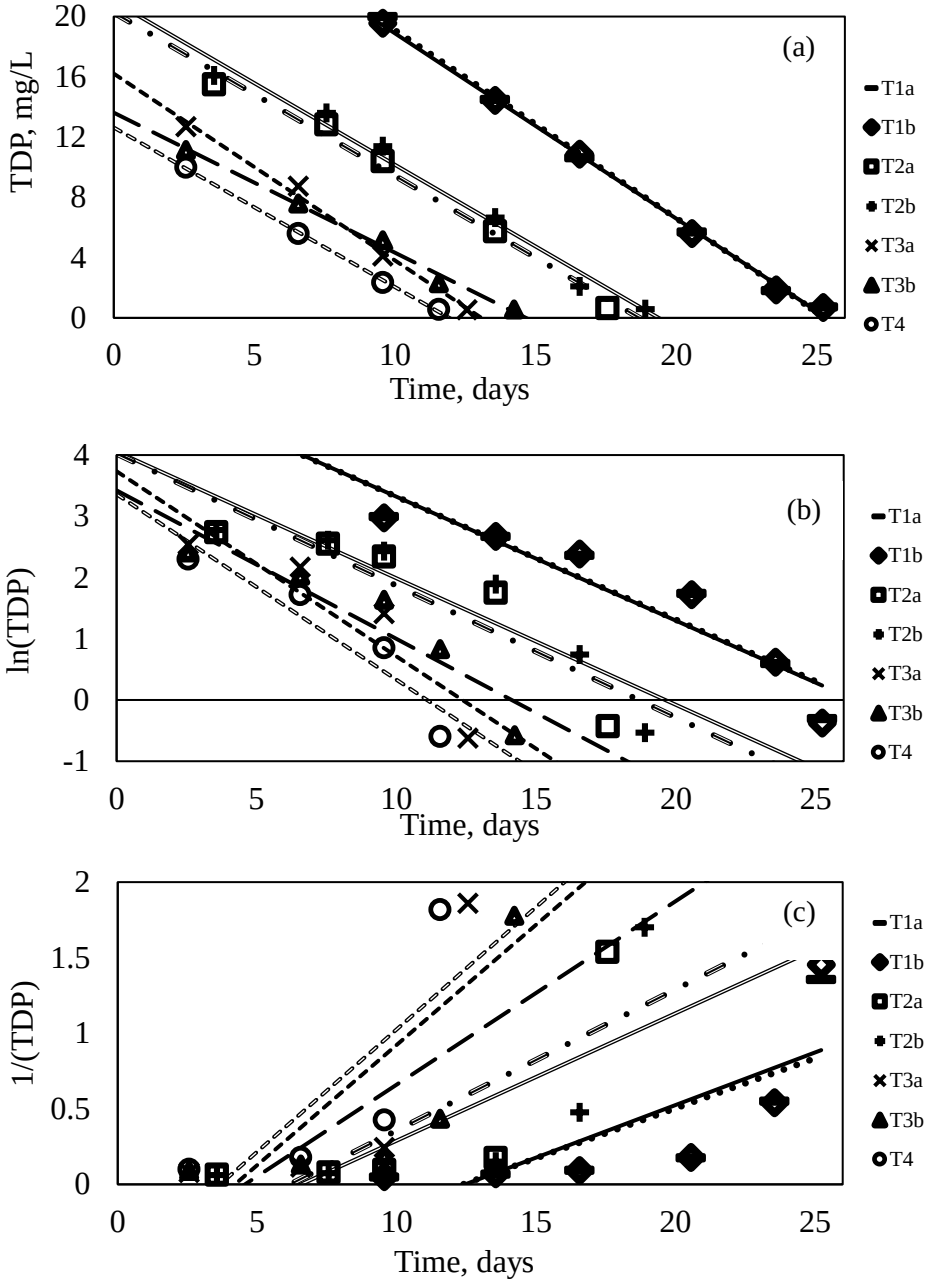


Figure L.1. TDP removal linear fitting lines: (a) zero-order, (b) first-order, (c) second-order kinetics.

APPENDICES

M. DRP removal kinetics

Linear fitting lines according to zero-, first- and second-order kinetics of DRP removal are shown in Figure M.1 and the related R^2 values of these lines are given in Table M.1. The DRP removal within all the reactors was well represented with zero-order kinetics with a R^2 ranging between 0.9802-0.9951. The first and second order kinetics had a lower coefficient of determination in the range of 0.8223-0.8799 and 0.4461-0.6758, respectively. Zero-order kinetics was found to be representative for the removal of DRP in all reactors and used for further estimation of total concentration of DRP within the reactors.

Table M.1. R^2 values of the linear fitting curves for DRP

	Zero-order	First-order	Second-order
T1a	0.9951	0.8406	0.5746
T1b	0.9934	0.8799	0.6202
T2a	0.9921	0.8578	0.4629
T2b	0.9892	0.8279	0.6758
T3a	0.9898	0.8223	0.453
T3b	0.9802	0.8543	0.4461
T4	0.9890	0.8441	0.5491
Max	0.9951	0.8799	0.6758
Min	0.9802	0.8223	0.4461

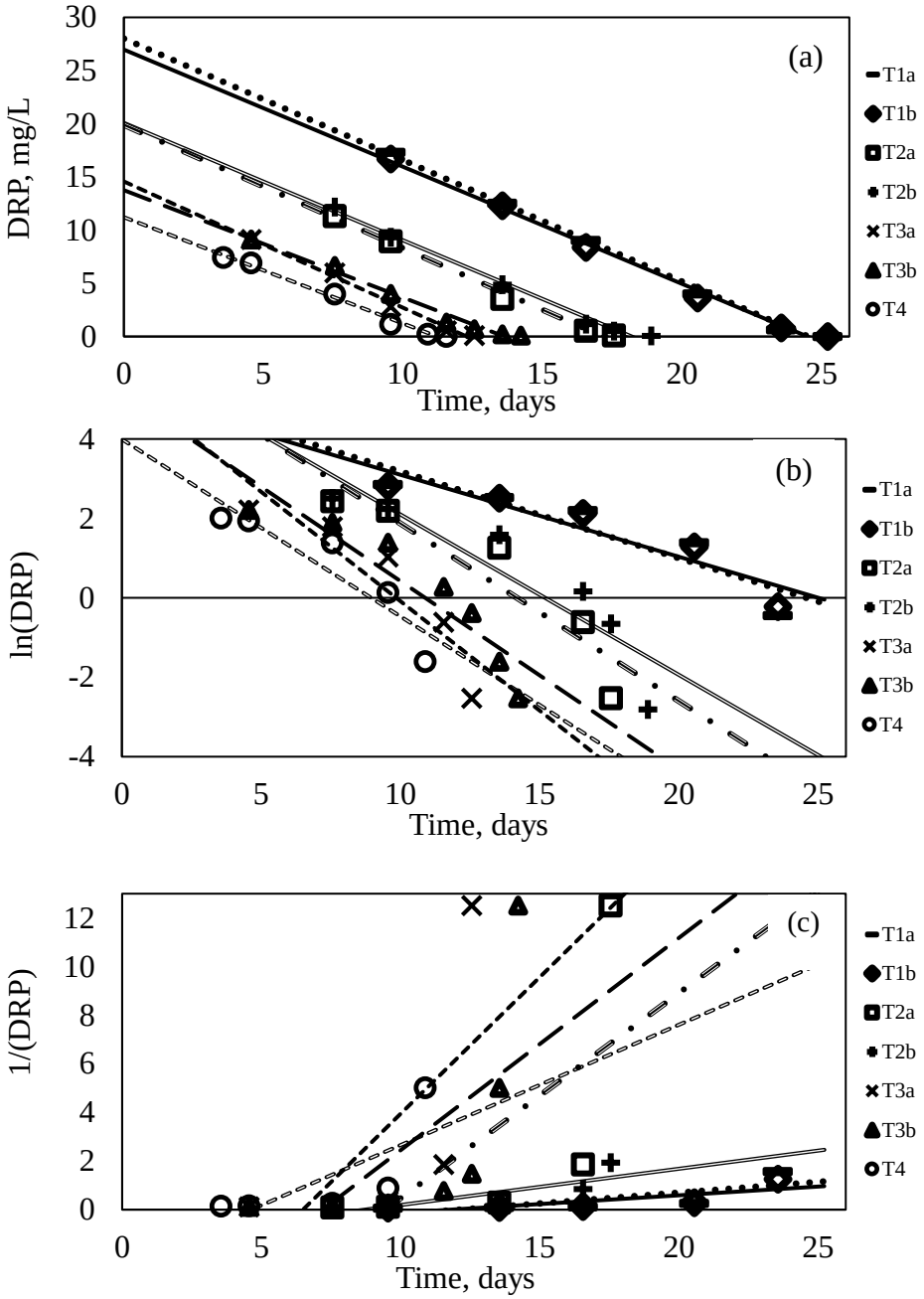


Figure M.1. DRP removal linear fitting lines: (a) zero-order, (b) first-order, (c) second-order reaction.

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2005-2006	MASS Inc. Co.	Management Systems
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PUBLICATIONS

1. Ulgudur, N., Ergüder, T. H., Demirer, G. N. (submitted to a journal). Simultaneous dissolution and uptake of nutrients in the microalgal treatment of a digestate.

2. Ulgudur, N., Ergüder, T. H., Uludağ-Demirer, S., Demirer, G. N. (submitted to a journal). Anaerobic treatment and valorisation of digestate using high-rate fixed film reactors.

3. Ulgudur, N., Demirer, G. N. (2019). Anaerobic treatability and residual biogas potential of the effluent stream of anaerobic digestion processes. Water Environment Research, 91(3), 259-268. DOI: 10.1002/wer.1048.

4. Arslan, B., Ulgudur N., Erdogan M., Imamoglu I., Karakaya I. (2014). Comparison of structual properties of copper deposits from sulfate and pyrophosphate electrolytes. ECS Transactions, 58 (32), 105-113.

INTERNATIONAL CONFERENCE PROCEEDINGS

Ülgüdür N., Karakaya, İ., İmamoğlu, İ. "Environmentally friendly copper electroplating process development", IWA Biennial World Water Congress 2014 Lisbon, Portugal, 21-26 September, 2014.

Ülgüdür N., Erdoğan, M., Karakaya, İ., İmamoğlu, İ. "Environmentally friendly copper strike electroplating process development", ISGMA 2014 International Symposium on Green Manufacturing and Applications, Busan, S.Korea, 24-28 June, 2014.

Ülgüdür N., İ. Karakaya, İ. İmamoğlu "Evaluation of non-cyanide alternative in a lab-scale copper strike electroplating process by monitoring bath parameters and product quality assessment", ISGMA 2014 International Symposium on Green Manufacturing and Applications, Busan, S.Korea, 24-28 June, 2014.

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Ülgüdür N., Karakaya İ., İmamoğlu İ., "Laboratuvar ölçekli bakır elektrokaplama sürecinde temiz üretim uygulamaları", 10. Ulusal Çevre Mühendisliği Kongresi, Ankara, 12-14 Eylül 2013.