Lappeenranta University of Technology
School of Energy Systems
Degree Program in Environmental Technology

Sara Merin

REJECT WATER MANAGEMENT AT BIOGAS PLANTS AND THE UTILIZATION OF REJECT WATER AS A CULTURE MEDIUM FOR MICROALGAE

Examiners: Professor Risto Soukka
Senior Research Scientist Kristian Spilling

ABSTRACT

Lappeenranta University of Technology
School of Energy Systems
Degree Program in Environmental Technology

Sara Merin

Reject water management at biogas plants and the utilization of reject water as a culture medium for microalgae

Master's thesis
2016

105 pages, 63 figures, 18 tables and 6 appendices

Examiners: Professor Risto Soukka
Senior Research Scientist Kristian Spilling

Keywords: reject water, microalgae, anaerobic digestion, ammonium nitrogen, polymers

In this master's thesis, the potentiality of microalgae cultivation in effluent liquid (reject water) from a biogas plant was studied. The thesis examines influencing factors to the quality of reject water and considers reject water management around the world and in Finland. In addition, the capability of microalgae to remove nutrients from reject water was investigated.

Reject water samples from a co-digestion plant owned by Envor Group Oy and Viikinmäki municipal wastewater treatment plant were investigated experimentally as a potential microalgae culture medium. The typically high ammonium nitrogen and total solids content in reject water were observed to limit partially the growth of microalgae. Ammonia stripping and solid particle removal methods by addition of polymers or ferric sulfate were found to constitute functional methods for improving reject water quality. The most adaptable species for reject water were *Scenedesmus obliquus*, *Chlorella sp., Monoraphidium contortum* and *Scenedesmus quadricauda. Scenedesmus obliquus* also showed its capability to remove nitrogen and phosphorus from ammonia stripped and diluted reject water of Viikinmäki wastewater treatment plant.

TIIVISTELMÄ

Lappeenrannan teknillinen yliopisto
School of Energy Systems
Ympäristötekniikan koulutusohjelma

Sara Merin

Rejektiveden käsittely biokaasulaitoksilla ja rejektiveden hyödyntäminen mikrolevän kasvualustana

Diplomityö
2016

105 sivua, 63 kuvaa, 18 taulukkoa ja 6 liitettä

Tarkastajat: Professori Risto Soukka
Erikoistutkija Kristian Spilling

Avainsanat: rejektivesi, mikrolevät, anaerobinen mädätys, ammoniumtyppi, polymeerit

Diplomityössä tarkastellaan mikrolevän viljelyn potentiaalia mädätyslaitoksen rejektivedessä. Työssä perehdytään rejektiveden laatuun vaikuttaviin tekijöihin ja tarkastellaan sen yleisempiä käsittelymenetelmiä maailmalla ja Suomessa. Lisäksi tutkitaan mikrolevien potentiaalia poistaa rehevöittäviä typpi- ja fosforiravinteita rejektivedestä.

Työn kokeellisessa osiossa tutkittiin Envor Group Oy:n yhteismädätyslaitoksen ja Viikinmäen yhdyskuntajätevedenpuhdistamon lietteiden mädätyslaitoksen rejektivettä mikrolevän kasvualustana. Rejektivedelle tyypillisesti korkea ammoniumtyppi ja kiintoainepitoisuus huomattiin rajoittavan osittain mikrolevän kasvua. Ammoniakkistrippaus ja kiintoaineksen poisto polymeereillä tai ferrisulfaatilla todettiin toimiviksi menetelmiksi rejektiveden laadun parantamiseen. Mikrolevälajit *Scenedesmus obliquus*, *Chlorella sp.*, *Monoraphidium contortum* ja *Scenedesmus quadricauda* sopeutuivat parhaiten kasvualustaan rejektivedessä. Lisäksi *Scenedesmus obliquus* poisti tehokkaasti typpi- ja fosforiravinteet ammoniakkistripatusta ja laimennetusta Viikinmäen puhdistamon rejektivedestä.

ACKNOWLEDGEMENTS

This thesis was part of the LEVARBIO project and it was co-operated with Finnish Environment Institute (SYKE). Senior research scientist Kristian Spilling from SYKE supervised me during the project and writing process. I want to give the largest thank you for Kristian who was ready to give professional advice close to around the clock. Your advice and ideas were most valuable and inspiring! I am especially grateful for my professor Risto Soukka from Lappeenranta University of Technology (LUT) for supervising me to transform this project into written form of master's thesis. Your wide experiment as a professor and supervisor of numerous masters' theses was priceless.

A huge thank you is for Jonna Piiparinen from SYKE who introduced me professionally to microalgae cultivation and strict laboratory work. I am extremely grateful for Jukka Seppälä who gave me the opportunity to write this thesis at SYKE. I specially thank you Katariina Natunen for the advice and helping me in this project. I am thankful for Pia Varmanen who did wide nutrient analyses just for this project. In addition, I thank you all SYKE colleagues that were friendly and supporative during my project time at SYKE. Thank you SYKE Marine Research Centre for giving me an opportunity to work at the laboratory and offering me a work desk at Kumpula around the scientific atmosphere.

Thank you biogas plant Envor Group Oy, Viikinmäki wastewater treatment plant, Helsinki Region Environmental Services Authority (HSY), Anna Kuokkanen (HSY), Kemira, Jukka Lehtovuori (Kemira), Satu Nokkonen (Häme University of Applied Sciences (HAMK)), Maritta Kymäläinen (HAMK), Jarkko Nummela (HAMK) and Judita Koreiviene. I thank you all biogas plants that answered my questionnaire about reject water management.

This thesis is dedicated to my lovely and supportive family:
Marcus, Meri, Rosa, Maria and Ron Merin.

Sara Merin

In Helsinki on the 2nd of November, 2016

TABLE OF CONTEST

LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations

Chl.	Chlorophyll
Chlorella sp.	*cf. Chlorella sp.*
D/N	Denitrification-nitrification
EDTA	Ethylenediaminetetraacetic acid
MAM	Modified Acid Medium
MWC	Modified WC Medium
N/D	Nitrification-denitrification
PS II	Photosystem II
RAS	Recycled activated sludge
RC	Reaction center
RW	Reject water
S. obliquus	*Scenedesmus obliquus*
S. quadricauda	*Scenedesmus quadricauda*
WW	wastewater
WWTP	Wastewater treatment plant

Chemical abbreviations and compounds

BOD	Biological oxygen demand
C/N	Ration between carbon and nitrogen content
CH_4	Methane
COD	Chemical oxygen demand
HAc	Acetic Acid
H_2S	Hydrogen sulfide
HCl	Hydrochloric acid
N/P	Ratio between nitrogen and phosphorus content
NaOH	Natrium hydroxide
NH_3	Ammonia
NH_4^+	Ammonium nitrogen ion
NH_4-N	Ammonium nitrogen
NO_2	Nitrite

NO_3	Nitrate
PO_4-P	Phosphate
TN	Total nitrogen
TP	Total phosphorus
TS	Total solids
TSS	Total suspended solids

Parameters

F_0	Initial fluorescence intensity
F_M	Maximal fluorescence intensity
F_V	Maximal variable fluorescence
N	Number of Organisms
NER	Positive net energy ratio
µ	growth rate

Units

A.U.	Arbitrary unit
°C	Celcius
GWh	Gigawatt hour
h	hour
ha	hectare
M	mol/l
mg/l	milligram per liter
mol	molar
RFU	Relative fluorescence unit
RPM	Revolutions per minute
V-%	Percentage by volume
µs	millisecond

1 INTRODUCTION

The main focus of this thesis was to consider the potential of sludge liquor effluent from a dewatered digestate generated by an anaerobic digestion, in other words reject water, as a microalgae culture medium and the uptake of nutrients followed by algal biomass growth. I examined microalgal physiology outline, typical characterizes of reject water generated at biogas plants focusing and consider reject water management at biogas plant especially in Finland. The aim of the experimental part of the thesis was to conclude how reject water as a culture medium affects to the growth of various microalgal species based on the demonstration of theoretical methods. In addition, I tested how to improve algal growth by various treatment methods on reject water medium. Finally, in the conclusions, it is discussed the profitability of microalgae cultivation system.

1.1. Opportunities of microalgae cultivation system

The utilization of microalgae constitutes an attractive field in the waste treatment and recycling processes since microalgae have been shown to remove impurities from various wastewaters and the chemical content of microalgal biomass is a potential source of bioenergy for production of biogas and biodiesel. (Rusten & Sahu 2011, Yuan et al. 2012, Ficara et al. 2014) Compared to conventional biofuel materials, such as oils crops and animal fats, microalgae can convert captured solar photons to biomass with higher efficiency in terms of biomass yields per hectare (Schenk et al. 2008). These advantages could possibly be used in a cycle system where microalgae cultivation in wastewater is combined with further utilization of its biomass for bioenergy production. Since microalgae tolerate various environmental conditions and its changes, reject water generated through anaerobic digestion processes could be a feasible culture medium. In addition, the cultivated biomass is potentially a co-feedstock for increasing biogas production at the existing digestion plant (Hermann et al. 2016).

The produced algal biomass in reject water can be utilized as oil-rich material. It can be refined e.g. to biodiesel or alternatively biogas through anaerobic digestion process. (Muñoz & Guieysse 2006) Also, sugars, biopolymers, lipids, proteins and antibiotics

constitute other potential products from algal biomass. The extraction of these valuable co-products could be a solution for economical algal biomass utilization. (Hannon et al. 2010, Lakaniemi 2012 p. 80, Larkum 2010) However, hygienic requirements and public acceptance often prevent biomass utilization for food or high value chemical production from biomass cultivated in wastewater. Therefore, production of biogas or biodiesel constitutes more attractive options. (Muñoz & Guieysse 2006)

Wastewaters typically include high concentrations of both nitrogen and phosphorus compounds, which may cause eutrophication in natural waters. Hence, the wastewaters should be purified before they are released to natural waterbodies to avoid this pollution effect (Ruiz-Marin et al. 2009). Especially sludge liquor effluent i.e. reject water from a dewatered digestate after an anaerobic digestion process at the biogas production industry typically possess extremely high concentrations of nitrogen in the form of ammonium and also other high concentrations of impurities (Wäger et al. 2010, Baunmann & Fuchs 2010, Pitman 1999, Kuglarz et al. 2015) Reject water can be difficult to be purify from the impurities (Vesitalous 1/2011 p. 16).

Microalgae cultivation installation connected to a biogas plant could be beneficial because it can: (1) operate as a treatment method for reduction of nutrients and other impurities from reject water, potentially reducing treatment costs; (2) increase biogas yield by co-digestion, producing biodiesel or other valuable algal biomass originated products; (3) increase renewable energy generation and nutrient recycling; (4) capture CO_2 emissions from energy production (Williams 2012, Yuan et al. 2012, Manninen et al. 2015). The system could potentially create an effective cycle of materials and energy based on circular economy (Figure 1). However, a positive net energy ratio (NER) may only be possible with the highest biogas production potential of algal biomass (Manninen et al. 2015).

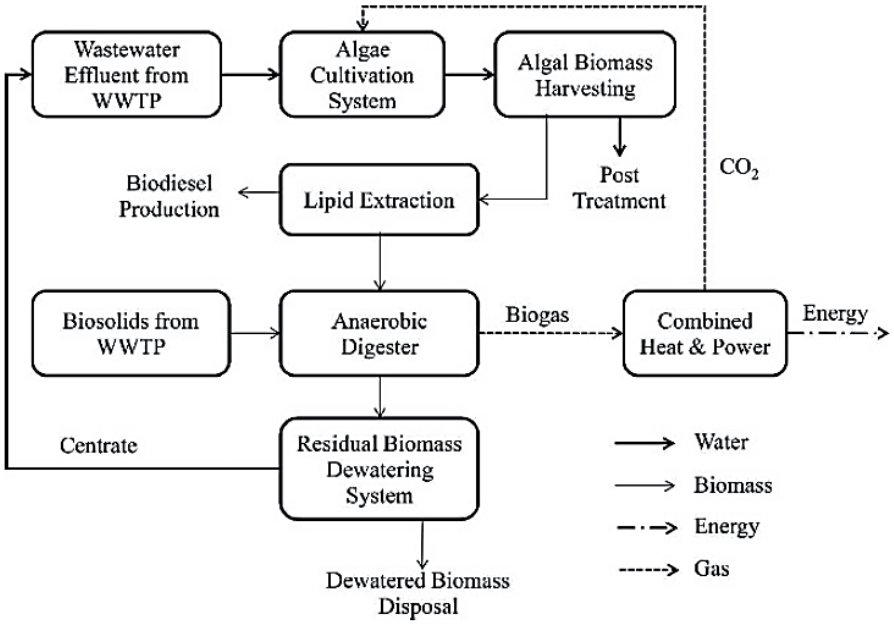


Figure 1. An example of algal cultivation process in a biogas plant which also utilizes algal lipids for biodiesel production. (Yuan et al. 2012)

1.2. Biogas production in Finland

This thesis considers reject waters generated at biogas plants in Finland. Only biogas installations with a digester reactor are considered since these digestion processes are highly controlled and they produce reject water streams with high concentration of nutrients. In Finland, the majority of generated biogas is collected from landfills that reject nutrient-rich wastewater called landfill leachate. The biogas is not generated by a controlled and compact digestion process such as in reactor installations. In terms of utilization of microalgal biomass as a co-feedstock, an existing digester reactor is for these reasons desirable. Therefore landfill leachate was omitted from the empirical part of this thesis.

In Finland in 2015, the total amount of produced biogas was 152,9 million m^3, which includes both biogas from reactor installations and collected landfill gas. Landfills generate the majority of the total biogas (Figure 2). The majority of the total biogas was utilized in heat and electricity production. The total produced energy was 630,4 GWh, which was approximatley 0,5 % of total produced renewable energy in Finland (Huttunen & Kuittinen 2016 p. 16).

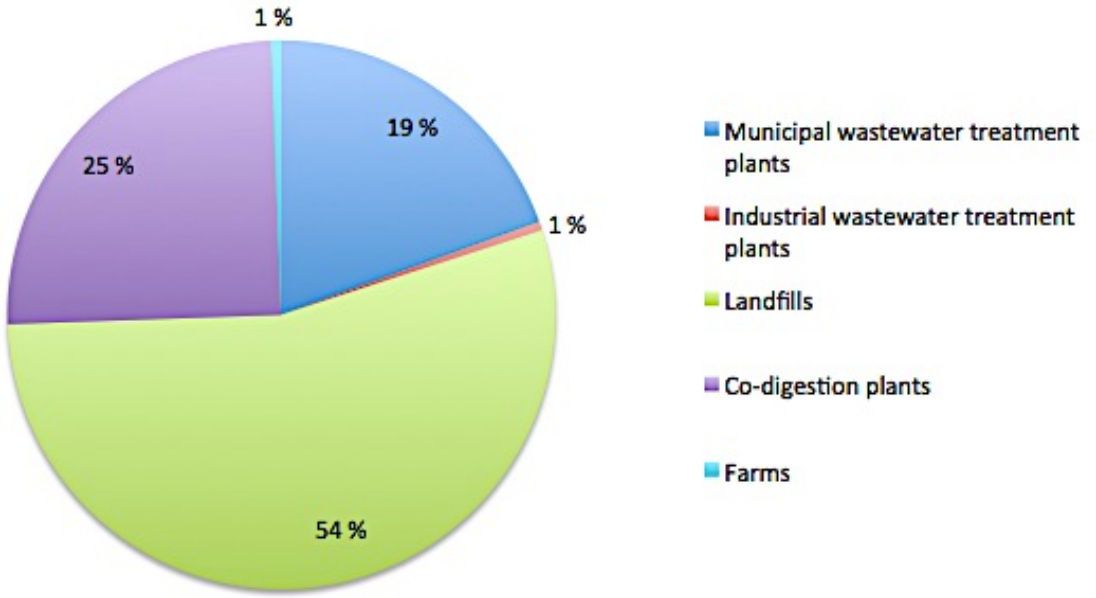


Figure 2. Shares of biogas (m^3/m^3_{tot}) generated by various biogas productions in Finland in 2015. (Based on data by Huttunen & Kuittinen 2016)

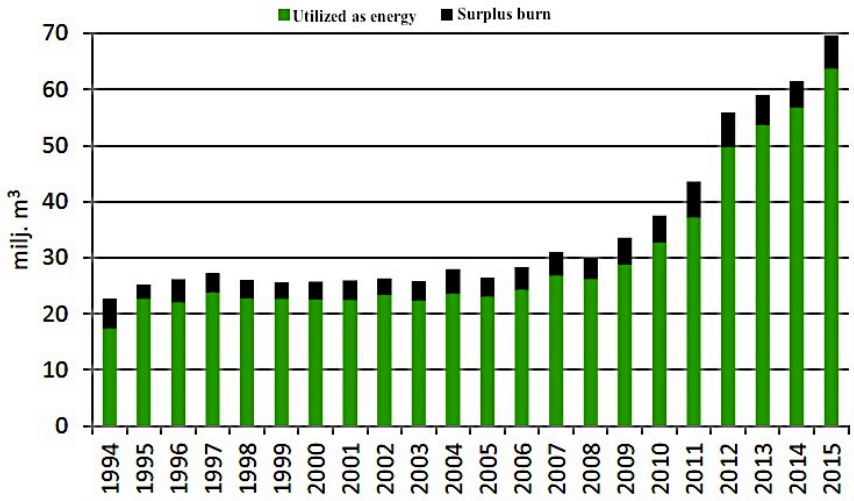


Figure 3. Total amount of biogas (CH_4+CO_2) produced by reactor installations in Finland has increased 3-fold since the 1990's. (Huttunen & Kuittinen 2016 p. 18)

The digestion processes in anaerobic reactors operate at wastewater treatment plants (WWTPs), farms, certain industries and biowaste treatment plants. Currently, the number of the operating reactors is 42, which includes biogas plants operating by municipal (15) and industrial (2) wastewater treatment plants, by various farms (11) and by co-digestion plants (14). The amount of the produced biogas by reactor installations has been increasing

3-fold from the early 1990s until 2015 (Figure 3). Figure 4 shows locations of municipal wastewater (WW) sludge and co-digestion plants.

Fourteen of the biogas plants constitutes a group of combined anaerobic digestion plants, i.e. co-digestion plants, which utilize various biodegradable matters such as manure, wastewater sludge, municipal and industrial biowaste. (Huttunen & Kuittinen 2016 p. 30) Each of these plants generate reject water with the exception of a plant owned by LABIO Oy in Lahti, which operates without any reject water streams since the digestion process is dry (Appendix 1).

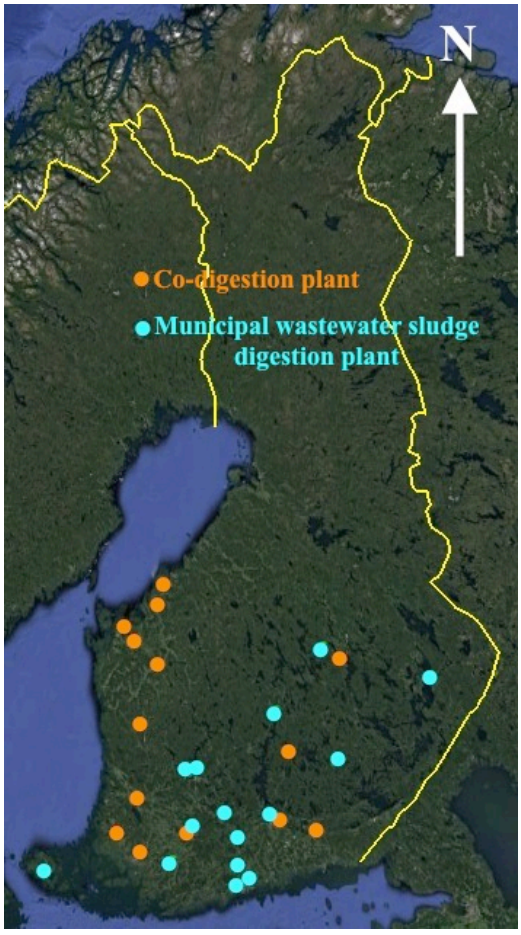


Figure 4. The locations of large-scale co-digestion plants operating by the utilization of biodegredable waste fractions and sewage sludges in Finland 2014. (Google Earth 2009, Huttunen & Kuittinen 2016 p. 21, 30)

In 2015 there were 24 public biogas refueling stations and nine (9) biogas refineries, most of them located in southern Finland. Biogas use for transportation has 1200-fold increased during the previous decade (Huttunen & Kuittinen 2016 pp. 13–14). The directive 2014/94/EU of the European Parliament and of the Council requires to produce at least 20 % of the total energy consumption to be from renewable energy sources and 10 % of the

renewable energy yield should use at the transport sector by 2020. One of the important developments to achieve the directive targets constitutes to construct an extensive biogas refueling network. Furthermore, it has been forbidden to dispose of waste with over 10 % share of biodegradable material to the landfill from 2016 in Finland (Finnish Government Decree on Landfills 331/2013). Due to these factors, the increase of biogas production by digestion process is a necessary step, which will also increase the amount of reject water streams. These larger volumes of reject water will cause a raised necessity for water treatment technologies since reject water has already been observed to cause difficulties at conventional wastewater treatment plants due to high concentrations of impurities especially in terms of nitrogen.

2 PHYSIOLOGY AND CULTIVATION OF MICROALGAE

Microalgae possess a unicellular or simple multicellular structure (Polprasert p. 219). They live under various environments and can adapt even challenging conditions, which makes them interesting microorganisms (Richmond & Hu 2013, Barsanti & Gualtieri 2010 p. 2). This chapter introduces to the general physiology of microalgae and main factors and compounds which effect to the growth of microalgae. In addition, the growth analysis of microalgae is discussed. Finally, bioenergy products generated from microalgal biomass are presented.

2.1. General physiology of microalgae

Microalgae are photosynthetic oxygen-releasing microorganisms that appear in a wide variety of shapes and forms (Figure 5). They can live in both salinity and fresh water conditions. Microalgae are often found in water but they can also live on rocks, snow, soils, plants and animals. (Barsanti & Gualtieri 2010 p. 2, Richmond & Hu 2013, Polprasert pp. 219–220) Photosynthesis operates as an essential light-driven reaction for metabolism and growth of microalgae, resulting to the production of oxygen and organic compounds with a presence of CO_2, water and appropriate nutrients. The commonly known microalgal groups are the diatoms (*Bacillariophyceae*), the green algae (*Chlorophyceae*) and the golden algae (*Chrysophyceae*). (Richmond & Hu 2013, Demirbas & Demirbas 2010) Probably more than 50 000 microalgal species exist and only a share is studied and analyzed (Mata et al. 2009)

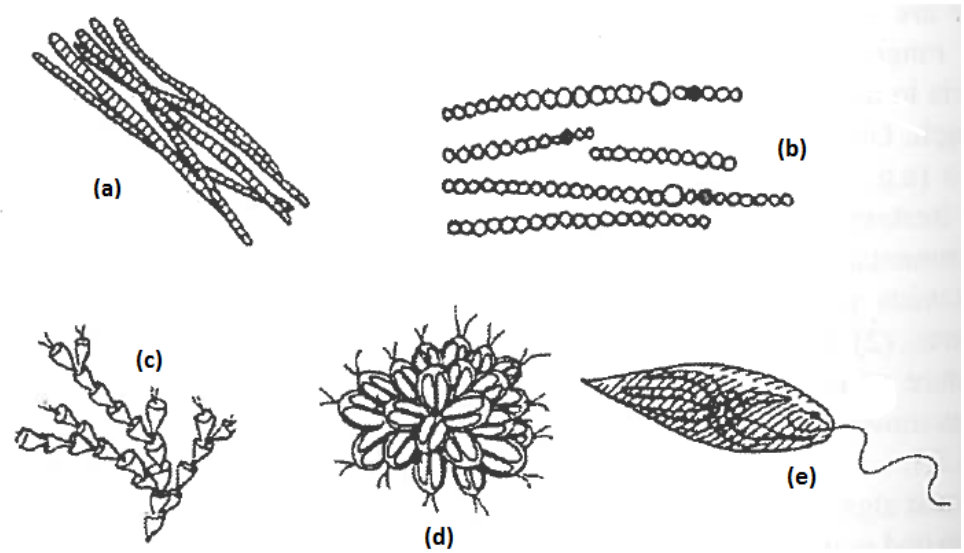


Figure 5. Various microalgae species. a) *Aphanizenom* b) *Anabaena* c) *Dinobyron* d) *Synura* e) *Euglena* (Polprasert 2007 p. 220)

2.1.1. Microalgal ultrastructure

Microalgae appear in various microscopic sizes and they mainly constitute unicellular organisms such as the microalgae species in Figure 6. The biological composition of the cell wall varies amongst species and determines several features of microalga. The properties of cell wall affect e.g. flexibility, genetic formation and tolerance of microalga. Also, the easiness to extract lipids or proteins from microalgal biomass and endurance to survive through e.g. pumps or strong mixing depends on the strength of the cell wall. However, some microalgae are lacking a cell wall e.g. *Euglena*. Thus, *Euglena* must live osmotically balanced in the surrounding conditions and therefore its tolerance can be high towards environmental changes. The extraction of valuable compounds e.g. lipids is usually easier from the species lacking of a cell wall. (Lee et al. 2012, Richmond 2004 p. 8, Richmond & Hu 2013) All microalgal cells include chroloplasts that function in the photosynthetic reactions. The chloroplast DNA, ribosomes, thylakoids and many enzymes are surrounded by stroma fluid inside a chloroplast. The thylakoid membranes trap the essential light energy for photosynthesis (Figure 7). (Richmond 2004 p. 9, Campbell & Reece 2008)

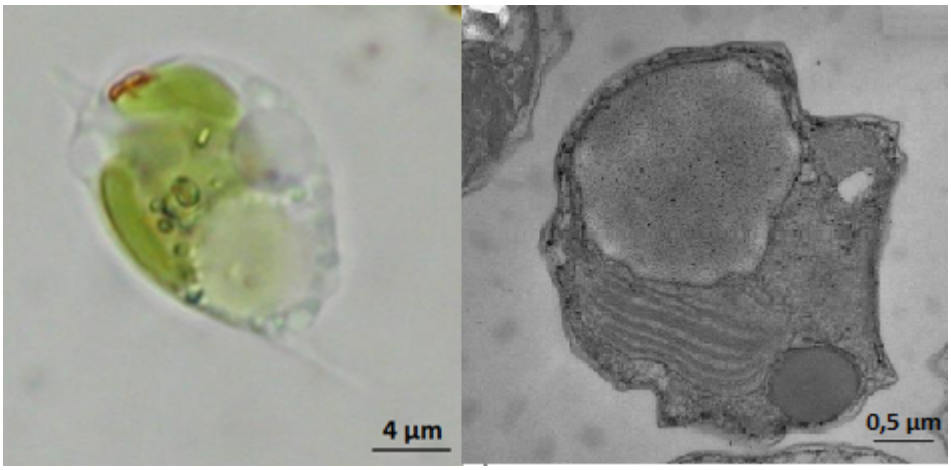


Figure 6. Two unicellular species: A) *Ochromonas* B) *Nannochloropsis* (Barsanti & Gualtieri 2010 p. 8)

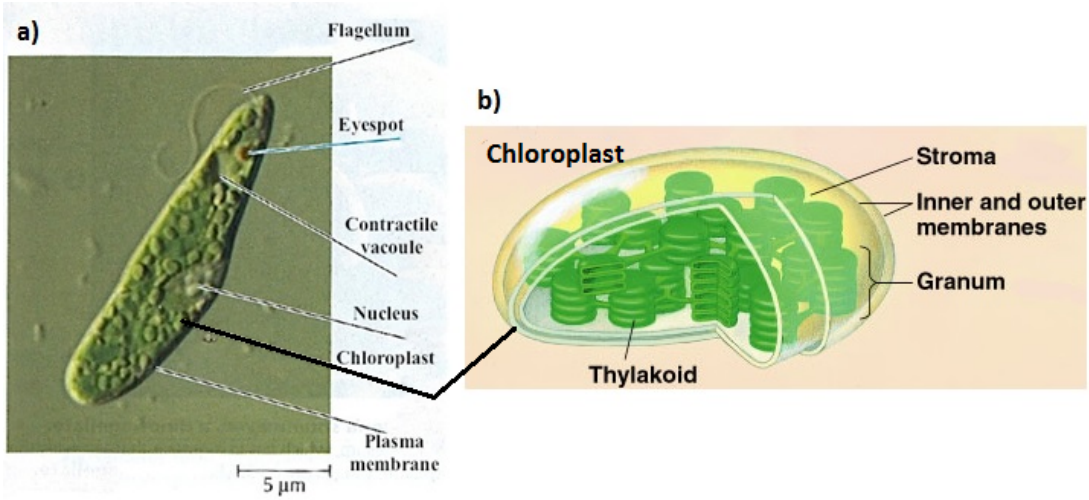


Figure 7. a) Structure of the unicellular specie *Euglena*. b) Structure of the chloroplast. (Campbell & Reece 2008)

2.1.2. Photosynthesis

Photosynthesis is a light-driven reaction that is directly or indirectly essential for the growth and metabolism of all forms of life on Earth. Light energy converts inorganic compounds to chemical energy and organic matter by photoautotrophs inside chloroplasts. The cells of alga transfer chemical energy to oils, carbohydrates, and proteins for the biomass production. The photosynthetic reactions result to two main end products: oxygen O_2 and sugars $C_6H_{12}O_6$. CO_2 operates as source of sugars and O_2 is evolved from water molecules. (Demirbas & Demirbas 2010 pp. 98, Richmond 2004 pp. 20–29) The overall photosynthetic reaction is simply presented with following equation (Bitton p. 56):

$$6\ CO_2 + 12\ H_2O \xrightarrow{Light\ energy} C_6H_{12}O_6 + H_2O + 6\ O_2 \qquad (1)$$

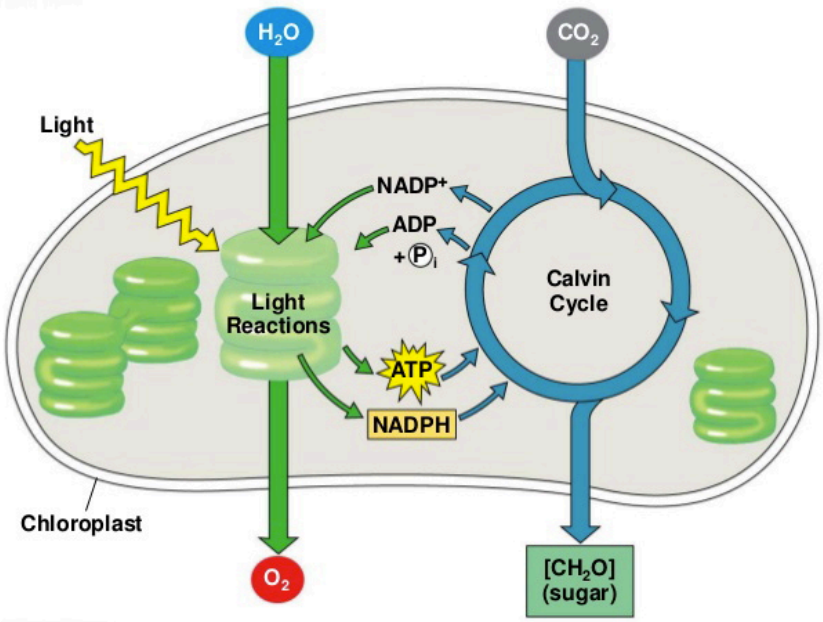


Figure 8. Photosynthesis includes light and dark reactions that occur in a chloroplast. The energy compounds NADPH, $NADP^+$, ATP and ADP are recycled between these reactions. (Campbell & Reece 2008)

Photosynthesis is divided into two stages: light and dark reactions. The light reactions are operated by light absorption, transfer of excitons and electron and proton translocation in thylakoid membranes of the chloroplast, which results to the formation of O_2 from water, nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP). (Richmond & Hu 2013) ATP operates as a source of chemical energy and NADPH as a source of electrons for the dark reactions in the so-called Calvin cycle (Figure 8). (Campbell & Reece 2008) The overall reaction is the following (McDonald 2003):

$$NADP + H_2O + ADP + P_i \xrightarrow{light\ energy} NADPH + ATP + O_2 + H^+ \qquad (2)$$

The conceptual phenomenon Calvin cycle performs the dark reactions that occur in the stroma of the chloroplast. The end products NADPH and ATP from the light reactions are utilized for the conversion of CO_2 to sugars, which is known as carbon fixation. The formation of $NADP^+$ and ADP occurs in the cycle, which operates as a source of energy for the light reactions. (Campbell & Reece 2008) The following equation presents the reaction (Richmond & Hu 2013):

$$CO_2 + 4\ H^+ + 4e^- \xrightarrow{2\ NADPH,\ 3\ ATP} [CH_2O]_n + H_2O \qquad (3)$$

2.1.3. Microalgal growth phases and the growth rate

Microalgae perform photosynthesis 10–50 times more efficiently than plants and therefore they constitute the fastest growing phototrophic organisms (Li et al. 2008). Microalgae multiply by a non-sexual cell division and mathematical analyses used in bacteriological research can be applied for estimating the growth of the algal culture. A batch culture denotes an algal culture that is transferred to a new growth medium. Thereafter the culture begins to grow and its total biomass increases. (Coombs et al. 1985 pp. 188–189)

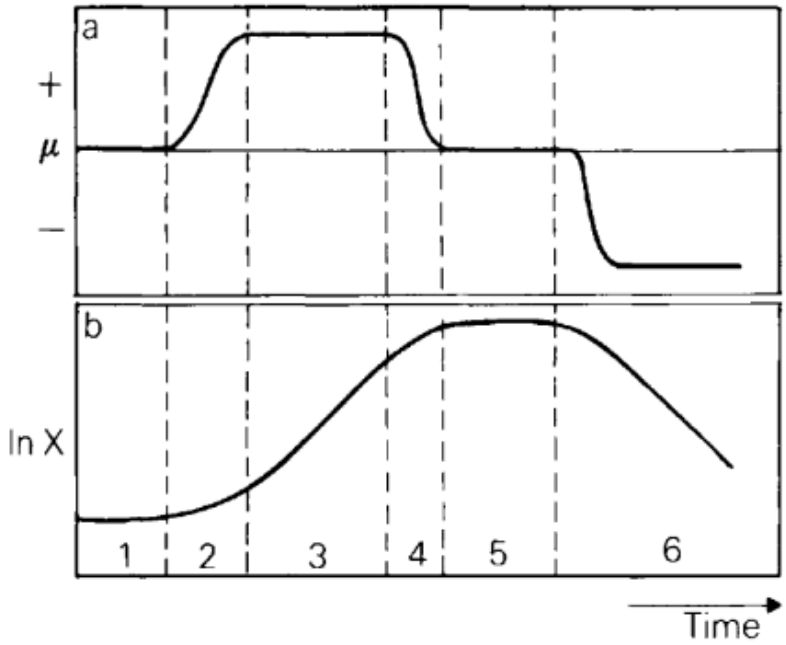


Figure 9. The growth rate μ and biomass X as the function of the time. (Coombs et al. 1985 p. 189)

The growth of the batch culture is divided into six phases: 1) lag phase, 2) accelerating phase, 3) exponential phase, 4) decelerating phase, 5) stationary phase and 6) death phase (Figure 9). Firstly, a freshly transferred batch culture has to adapt the environmental conditions in a new culture medium. Secondly, if the batch culture is taken from the stationary or death phase of the parent culture it may have been in a metabolically poor state. The lag phase (1) often exists due to these two reasons and therefore the growth rate remains momentarily in zero. In the following accelerating phase (2) the growth rate and the amount of biomass dry weight increases due to increase of proteins. The number of cells has slightly increased in this phase. In the next exponential phase (3), the maximum growth rate is achieved and the biomass grows exponentially. The algal cells react more sensitively to physical and chemical factors in the exponential growth phase than in the

following phases. (Coombs et al. 1985 p. 188–190, Aittomäki et al. 2002 p. 28) The growth rate can be presented with following equation (Bitton 2005 p. 63):

$$\mu = \frac{lnXt - lnXo}{t} \quad (4)$$

,in which μ = specific growth rate, X_t = numbers or biomass of algal cells after time t, X_o = initial numbers or biomass of algal cells.

In the decelerating phase (4), the amount of the biomass increases slower than in the exponential phase and therefore the growth rate decreases near to zero. The stationary phase (5) occurs for several reasons: exhaustion of essential nutrients, changes in pH and light limitation due to dense biomass in the culture. The amount of the biomass remains constant in this phase. Lastly, in the phase (6) the algal cells die and lyse due to ratio of respiration to photosynthesis is greater than one. (Bitton 2005 pp. 62–63, Coombs et al. 1985 p. 189)

2.1.4. Chemical composition of microalgae

The chemical content of the algal biomass varies both between species and individuals (Table 1). Typically, microalgae possess a high protein content that forms the largest share of the total dry weight of the biomass. Cellulose, pectin, glycoproteins and silica constitute typical compounds that can be found from cell walls. (Richmond & Hu 2013) Carbohydrates perform generally in form of starch, glucose, sugars and other polysaccharides. Microalgal biomass also contains various valuable vitamins such as A, B_1, B_2, B_6, B_{12}, C, E and biotin. (Priyadarshani & Rath 2012, Spolaore et al. 2012)

Table 1. Chemical composition of the microalgae species (% of dry matter).

Strain	*S. obliquus*	*Spirulina maxima*	*Chlorella Vulgaris*	*Euglena Gracialis*
Protein	50–56	60–71	51–58	39-61
Lipids	10–72	13–16	12–17	14-20
Carbohydrates	12–14	6–7	14–22	14-18
Reference	Spolaore et al. (2006)	Spolaore et al. (2006)	Spolaore et al. (2012), Demirbas & Demirbas (2011)	Singh & Gu (2010), Demirbas & Demirbas (2011)

2.2. Nutrient requirements

Sunlight, water and carbon dioxide (CO_2) as a source of carbon constitute three main elements that living microalgae require (Demirbas & Demirbas 2010 pp. 75). At least carbon, nitrogen, phosphorous, potassium, sulfur and magnesium are constitutes that are important for most microalgae. In addition, trace metals, minerals, nucleic acids, vitamins and EDTA assists the active algal growth. (Becker p. 10–11)

2.2.1. Carbon

Carbon constitutes an essential element for the formation of sugars in photosynthesis. Most microalgae species acquire carbon that is originated from CO_2. In the aquatic cultivation pond solute CO_2 exists mostly in form of bicarbonate (HCO_3^-) while the pH conditions ranges between 6,4 and 10,3. Microalgae trap bicarbonate and convert it to CO_2 and further to sugars, which increases the biomass (Chapter 2.1.2., Sayre 2010). However, certain algal groups e.g. cyanobacteria and chlorophyceae have also ability to trap gaseous CO_2 by the enzyme carbonic anhydrase or organic carbon sources e.g. sugars and amino acids (Markou & Georgakakis 2010). The estimation for the biomass production is approx. 1 g of biomass per 1,6–2 grams of captured CO_2 (Sayre 2010). In addition to atmospheric CO_2 industrial flue gases can be utilized as source of carbon for algal cultivation (Sayre 2010, Sonck 2010).

2.2.2. Nitrogen

The growth of microalga requires nitrogen (N) for structural and functional proteins for the production of the biomass and cell wall materials. The most microalgae utilize nitrogen fixed to ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) or urea (CH_4N_2O). (Markou & Georgakakis 2010) Certain cyanobacteria such as *Oscilatoria, Anabaena* and *Spirulina*, can convert gaseous N_2 to NH_4^+ for the utilization of nitrogen by the enzyme nitrogenase (Hannon et al. 2010, Markou & Georgakakis 2010). The available nitrogen source may effect significantly to the growth of microalgae. For example, NO_3^- or NH_4^+ can constitute a nitrogen source performing the highest growth rate depending on the algal specie. The growth rate can also remain around the same regardless of nitrogen source. (Lakaniemi 2012 p. 55)

2.2.3. Phosphorus

Although the total algal biomass often includes phosphorous (P) less than 1 % it is an essential element for the sustain growth of microalgae (Hannon et al. 2010). Microalgae have an absolute requirement for a minimum need for phosphorus per a cell (Correll 1999). Microalgae can utilize phosphorus from orthophosphates: PO_4^{3-}, $HPO_4^{2-,}$ $H_2PO_4^-$ and H_3PO_4 for their biological metabolism (Richmond 2004, Tchobanoglous et al. 2003 p. 63) Many inorganic salts operate as sources of phosphorus for algae due to P bounds easily to other ions and therefore a lack of ions such as K^+, Na^+ and Mg^{2+} decreases the availability of phosphate in a culture medium. Furthermore, pH conditions have an influence to the uptake of phosphorus. The phosphorus uptake by algae decreases under acidic and relatively alkaline conditions (Markou & Georgakakis 2010).

2.2.4. Other essential compounds

In addition to the most important nutrients carbon, N and P microalgae also require numerous other nutrients including e.g. potassium, sulfur, silicon and iron. For example, silicon occurs in cell walls and sulfur operates for protein synthesis and lipid metabolism. (Hannon et al. 2010) Furthermore, certain species require small quantities of organic compounds for the growth e.g. vitamins. Trace elements are constituents for the biosynthesis of vitamins e.g. cobalt is essential for vitamin B_{12} production. Moreover, ethylenediaminetetraacetic acid (EDTA) is a constituent for chelating potential. (Richmond 2004)

2.2.5. N/P ratio and culture medium recipes

The Redfield ratio of 106C:16N:1P quantifies atomic ratios of C, N and P for algae, which takes account of possible nutrient limitations (Correll 1999, Weber & Deutsch 2010). The optimal ratio is approximately estimation and it can vary depended on numerous other environmental conditions e.g. light intensity or light quality can affect to the beneficial N/P ratio of 16 by Redfield. Therefore, the optimal nutrient content is difficult determine for microalgae since the need vary depended on numerous factors. For instance, the stage in the algal cell division cycle will have an influence to the required P content of the cell. (Correll 1999) The beneficial element concentrations of the culture medium may need to be examined more carefully by various experiments instead of concentrating on chemical content of biomass of the microorganism (Pauli & Kaitala 1995). Human's knowledge and studies about physiology of microorganisms has been allowed to the ability to prepare artificial culture mediums. They generally include various vitamins, trace metals and EDTA. (Price et al. 1989) For instance, recipes Modifield Acidic Medium (MAM) and Modifield WC Medium (MWC) constitute nutrient recipes (Appendices 4, 5).

2.3. Optimal environmental conditions

Microalgae species can adapt into wide selection of environments even challenging ones. However, the maximal growth rate can be found under specific temperature, light and pH conditions. (Mata et al. 2009) This chapter represents these three main environmental factors that have an influence to the microalgal growth.

2.2.6. Light

Light energy constitutes the major factor for the sustainable growth of microalgae. It operates for the photosynthesis reactions in algal cells in order to form chemical energy and organic compounds e.g. sugars for the metabolism and growth of algae. The photosynthesis requires visible light radiation of wavelengths between 400 nm and 700 nm that is captured by chlrolophylls. (Campbell & Reece 2008, Bitton p. 56) The solar light intensity depends on several factors: location on the Earth, latitude, season and other meteorological effects (Polprasert 2007 p. 227). Therefore, the available light energy restricts outdoor microalgae cultivation in certain locations e.g. in Finland during dark and cold winter seasons. Microalgae strains can be cultivated also under artificially illuminated environments (Sakarika & Kornaros 2016). LED lights, optical fibers and multi-LEDs combined with a solar panel and wind turbine constitute innovative artificial light systems, which could be utilized for generating artificial light for microalgae. However, there exist

also microalgal strains that can live under dark conditions. These species utilizes organic carbon sources e.g. glucose, acetate, sucrose and lactose instead of light. These heterotrophic species are at least *Chlorella protothecoides* and *Chlorella vulgaris.* (Chen et al. 2010)

2.3.2. Temperature

The surrounding temperature constitutes another important factor that affects to the growth of microalgae (Mata et al. 2009). The favorable temperature for the most species varies in the range of 20–30 ℃ (Demirbas & Demirbas 2010). If the temperature increases excessively high it can denature membrane structure and under low temperature condition the enzymatic reaction rates in the cells of microorganisms decline. (Bitton 2005 p. 68) Furthermore, temperature conditions affect to the solubility of CO_2 that constitutes to the main compounds for microalgae (Tchobanoglous p. 65). However, certain species can grow above 40 °C or below 10 °C. Algal communities has been collected from the Antarctic at 1 °C and also from hot springs at 65 °C. (Richmond 2004) If algae possess a high content of unsaturated fatty acids in their cell membrane it helps they grow at low temperatures, whereas algae with a high content of saturated fatty acids can grow under high temperatures (Bitton 2005 pp. 68).

2.3.3. pH

The pH optimization of the algal culture is important since pH conditions affect strongly to the biomass growth yield. An optimal pH for microalgae may be difficult to find since it is depended on specie and other characterizes in the culture medium. The beneficial conditions for most of species occur at around pH of 7. However, for example, *Dunaliella salina* prefers pH of 11,5 whereas *Dunaliella acidophila* prefers acidic pH conditions below 3. *Chlorella* has shown to to adapt a wide pH in the range of 5–9. Nevertheless, an aggregation effect of microalgal cells followed by flocculation has been observed at above 9 pH conditions (Sakarika & Kornaros 2016, Spilling et al. 2011) Microalgae may also change the pH value by themselves in the surrounding environment since the CO_2 uptake by microalgae often raises pH conditions in the medium ((Bitton 2005 p. 68, Muñoz & Guieysse 2006). Nevertheless, Tam & Wong (1996) noted that the growth of *Chlorella sp.* rose pH to the acidic level.

2.4. Microalgae growth analysis

The photosynthetic activity increases by increased algal cell density under the aquatic environment. The variability of fluorescence value constitutes a useful method for considering changes of the photosynthesis activity. (So & Dong 2002, Strasser & Govindjee 1991)

2.4.1. Fluorescence and its measurement instruments

Fluorescence probes the photochemical activity that occurs mostly in a photosystem II (PSII) that is a protein complex in in a chloroplast (Murchie & Lawson 2013, Strasser & Govindjee 1991). Chlorophylls (Chl) are chemically active pigment molecules within the light-harvesting complexes (LHCs) (Murchie & Lawson 2013). They capture light energy and transport it forward for light reactions performing in microalgal cells (Strasser & Govindjee 1991). When the charged electrons transfer to the lower energy level, an atom emits a photon that performs as light with a specific wavelength. The emitted wavelength, emission spectra i.e. chlorophyll fluorescence wavelength can be used as a measurement for cellular growth and metabolism. It constitutes a measure of re-emitted light in the red wavelengths. (Lakowicz 2006 p. 27, Murchie & Lawson 2013)

In order to measure fluorescence, a source of light, an emission measuring sensor and a fluorescent detector are required (Aittomäki et al. 2002 pp. 240–241). Fluorometers are commonly used instruments for measuring fluorescence spectrum. The excitation spectrum determines the fluorescent intensity that is measured as a function of excitation wavelength at the constant emission wavelength. Fluorometers have been developed also for the purpose to measure a lifetime of fluorescence intensity. (So & Dong 2002) Fluorescence instruments often measure samples in the darkness and highly controlled light environments since any presence of light can interfere with the measurement of fluorescence (Murchie & Lawson 2013).

2.4.2. O-J-I-P kinetic steps

O-J-I-P is accounted an important biophysical phenomenon that reflects the time course of the photosynthesis reactions (Equipements Scientifiques SA 2008). This describes the induction and raise of fluorescence as the function of time performing in the PSII. O-J-I-P is divided into three steps: O-J, J-I and I-P (Figure 10). The phase O-J is the most important phase since it raises the initial fluorescence F_0 exponentially the largest amount and it forms the greatest share of the total maximal fluorescence. (Boisvert et al. 2006) At

the fluorescence level of F_0, all the reaction centers (RCs) are open and the first quinone electron acceptor of PSII is oxidized whereas the maximal fluorescence F_M is the fluorescence when all the quinone electron acceptors are oxidized (Lazár 2006). In the following J-I phase the increase of fluorescence is slowing down and during the phase I-P fluorescence increase will be expired. The F_M is achieved after the phase I-P at the final spot P where all the RCs are closed. The overall mechanism of the O-J-I-P mechanism, however, is partly unexplained. (Boisvert et al. 2006, Lazár 2006, Beneragama & Goto 2010) The examination of formed O-J-I-P curve by specific instruments has been discovered to be a useful tool for considering changes in thylakoid membranes, which can be utilized for considering photochemical activity in an algal culture (Boisvert et al. 2006, Strasser & Govindjee 1992). The shape of the curve is strongly depended on the stress position of the algal suspension. For example, changes in environmental conditions, e.g. light intensity, temperature, drought, atmospheric CO_2 or ozone elevation and chemical influences may cause stress for microalgae. (Strasser et al. 2004)

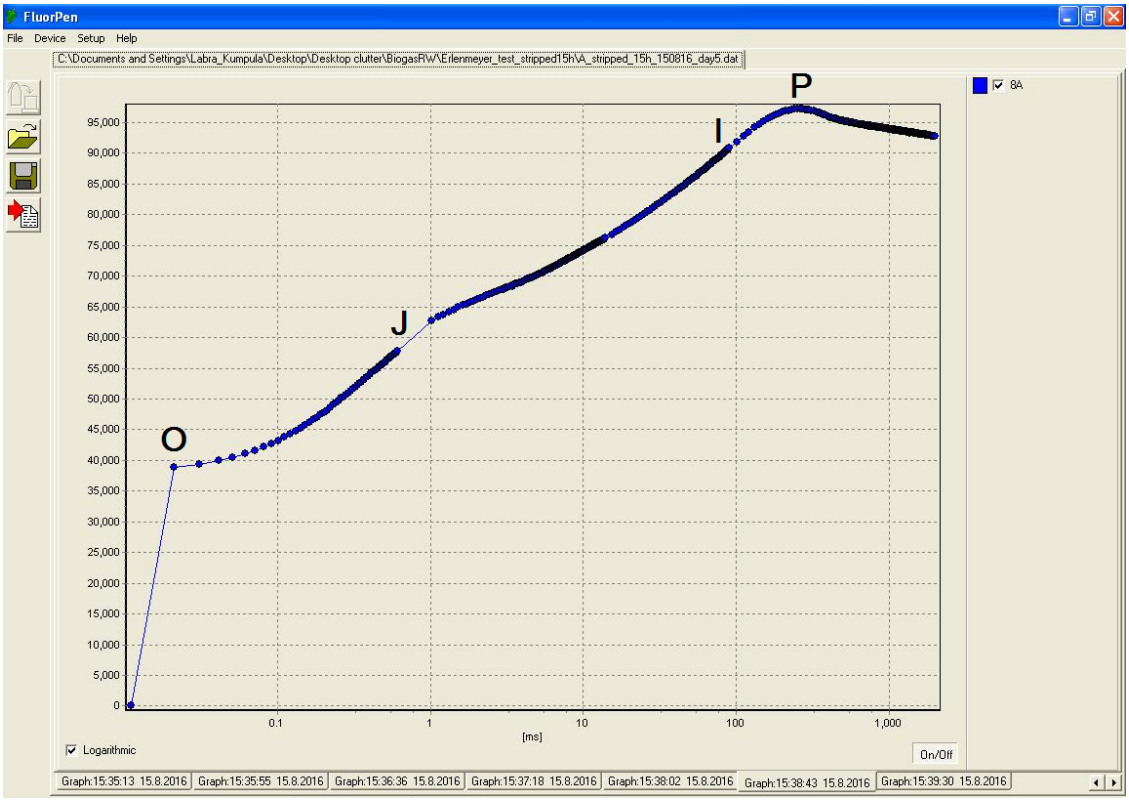


Figure 10. O-J-I-P curve of the microalgal culture in reject water. The curve was drawn with the FluorPen program. The fluorescence is measured by the fluorometer AquaPen-C AP-C 100 at the Finnish Environment Institute. The unit of the x-axel is milliseconds (ms) and the unit of the y-axel is arbitrary unit (A.U.). The x-axel (time) is on a logarithmic scale.

2.5. Bioenergy production from algal biomass

High oil (lipid) content and relatively high growth rate constitute excellent features of microalgae. Therefore investments to algal biofuel production have potential to respond the demand for alternative energy sources due to increasing population and expanding economy. (Hannon et al. 2010) Microalgal biomass can substitute current bioenergy sources such as agricultural crops and biowaste material for biofuels (Figure 11).

Algal cells produce oil-rich biomass in the natural photosynthesis. However, the energy efficiency of the conversion of solar photons to biomass (1–2 %) in algae and plants is significantly lower compared to the efficiency (of other solar energy capture technologies such as photovoltaic (PV) technologies and thermal collectors. For example, a multicrystalline silicon solar cell (mc-Si) can achieve solar energy conversion efficiency of approx. 20 % (Schultz et al. 2004). In addition, the bioenergy production from algae may be energy and carbon intensive. (Larkum 2010) Nevertheless, compared to conventional biofuel materials such as oils crops and animal fats microalgae can convert captured solar photons to oil with higher efficiency in terms of liter biomass yields per a hectare. For example, rapeseed, oil palm and algae can produce biodiesel 1190, 5959 and 12–98 500 l/ha/a, respectively (Schenk et al. 2008). Also, an algal biomass production installation can be placed in the presence of the existing energy plant station. Then the waste heat, wastewater and CO_2 can be utilized to offset the energy, nutrient and CO_2 demand of the algal culture installations. (Larkum 2010. The installations for large-scale algal biomass cultivation are currently divided to closed photobioreactors (PBRs) and open systems. The advantages of PBRs are high biomass productivity and low risk for contamination. Open systems are typically ponds: e.g. raceway and circular ponds, in which contamination risk is higher and biomass productivity lower. In addition, they are less costly compared to PBRs. The cultivation installations can utilize both natural light or artificial light sources. (Lakaniemi pp. 13–16)

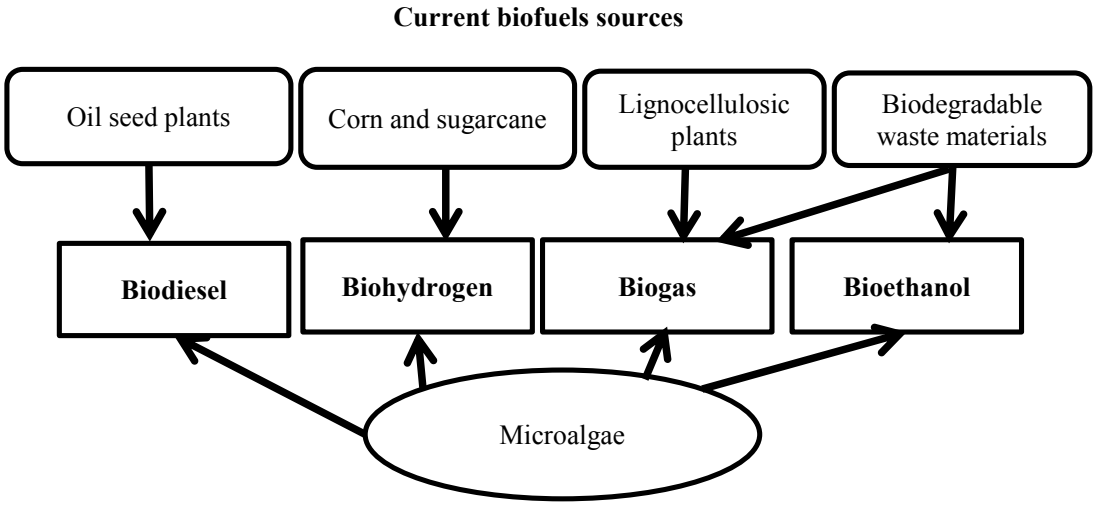


Figure 11. Microalgae have potential to substitute current biofuel sources. (Based on Jones & Mayfield 2012)

2.6.1. Biodiesel

Due to high lipid content, microalgal biomass constitutes an excellent source of oil that can be refined further to biodiesel for replacing fossil fuels and increasing security of energy supply. Moreover, the oil yield from algal biomass is higher compared to oil productivity from vegetable oil crops in terms of land use. The algal cell structure should be broken first for efficient exctraction of lipids and further biodiesel production. (Mata et al. 2009, Singh & Gu 2010)

2.6.2. Bioethanol

Many microalgae species possess a high carbohydrate content (>40 % of the dry weight) that is feasible to convert to ethanol through fermentation. Since carbohydrates are mainly in form of polysaccharides starch and cellulose in algal biomass they can be processed to monosaccharides and further to bioethanol. (Ho et al. 2012) Current bioethanol feedstocks e.g. agricultural crops or waste includes lignin are more complex to process compared to the bioethanol production from lignin-free microalgal biomass. (Sun & Cheng 2002). Based on the studies by Ho et al. (2012) and Nguyen et al. (2009) a large-scale bioethanol production is feasible by the cultivation of carbohydrate-rich microalgae. However, microalgae also possess a high content of lipids and therefore biodiesel is often preferred as an ideal main product. One solution for this is to extract lipids first and thereafter the residual biomass can be utilized for bioethanol production. (Li et al. 2014)

2.6.3. Biohydrogen

The demand for sustainable energy sources has gained increased attention to the production of biohydrogen. Hydrogen constitutes a gas with a remarkably high energy content and its combustion generates only water in addition to energy. Direct photolysis, indirect photolysis, photo-fermentations and dark fermentation constitute current techniques for the biohydrogen production. However, photo-fermentations, direct and indirect photolysis require high light energy and suitable warm temperature to produce effectively hydrogen, which constitutes issues in terms of large scale biohydrogen production in areas with cold and dark seasons. (Levin et al. 2004) The metabolic processes performing in algal cells need to be understood more detailed for the development of algal biohydrogen production. The viability for commercial algae biohydrogen still seems to be far in future. (Jones & Mayfield 2012)

2.6.4. Biogas

An anaerobic digestion is a promising technique for utilizing microalgal biomass for biogas production and nutrients for recycling (Hannon et al. 2010). Algal biomass can produce energy-rich methane CH_4 through an anaerobic digestion process, which has been proven in numerous studies (Ward et al. 2014). Furthermore, the co-digestion with other feedstocks can be feasible. Carbon-rich co-feedstocks are probably most suitable with microalgal biomass since they may prevent the possible inhibition in the digestion process followed by increased C/N ratio of the input material. (Herrmann et al. 2016) A challenge is the hard cell wall of the algal structure that may affect negatively to the biogas yields due to incomplete degradation. Thus, a lipid extraction for e.g. biodiesel production before anaerobic digestion may be an economical solution since it breaks the algal structure including cell walls. (Neves et al. 2016)

3 REJECT WATER FROM AN ANAEROBIC DIGESTION

This chapter introduces to the formation of reject water from anaerobically digested organic matter and considers the main quality parameters of reject water. First, the principle of anaerobic digestion process is represented. Secondly, the formation of reject water from a digestate is explained. Lastly, the factors that affects to the quality of reject water and typical chemical composition of reject water are examined.

3.1. Principle of anaerobic digestion

An anaerobic digestion process reduces organic matter and the volume of the feedstock waste by microbes under oxygenless and high temperature conditions, which produces energy-rich biogas CH_4. Animal waste, leftover food, garden waste and WW sludges constitute the examples of biodegradable waste fractions that can be treated through an anaerobic digestion. The residual digestate from the digested material includes valuable nutrients that can be utilized further. (Christensen 2011 pp. 601–603, Liu 2007, Park et al. 2010) An anaerobic digestion can be operating under mesophilic or thermophilic conditions: optimal temperature occurs respectively in the range of 30–38 °C or 49–57 °C (Karttunen 2004 p. 205).

Carbohydrates, proteins and lipids constitute compounds that fermentative microbes convert to the end products of the digestion. An anaerobic digestion includes hundreds of possible intermediate compounds and complex reactions. Furthermore, the feedstock material into the digestion reactor is often very heterogeneous and its quality can vary widely depending on the waste fractions in the batch loads. Therefore the biogas potential and other parameters e.g. the volume of the generated digestive, reject water flow and their chemical compositions are difficult to determine by theoretical calculations. (Christensen 2011 pp. 586–592) However, the simplified digestion reaction can be presented with the following reaction (Bitton 2005 p. 349, Polprasert 2007 pp. 151):

$$\text{Organic matter} \xrightarrow{Anaerobic\ digestion} CH_4 + CO_2 + H_2 + NH_3 + H_2S$$

The anaerobic digestion generates two main outputs: biogas that mainly contains energy-rich CH_4 and a residue called a digestate. In addition to CH_4 the generated biogas includes CO_2 in the range of 30-47 V-% and other volatile organic compounds such as ammonium (NH_3) and H_2S but they form less than 1 V-% of the total biogas volume. The biogas yield is depended on many environmental factors e.g. nutrient balance, temperature, pH, alkalinity and toxic compounds in the digestion reactor (Bitton 2005 pp. 354–357). The biogas can be utilized to heat and electricity production at a combined heat and power (CHP) plant or as gaseous fuel for vehicles. (Christensen 2011 pp. 583, 612, 620–621)

3.1.1. Wet and dry digestion

An anaerobic digestion process can be either wet or dry. The division is based on the water content of the feedstock material (Table 2). Wastewater sludge, biowaste and agricultural waste possess a high water content and therefore they are typically digested through a wet process with the share of less than 15 % of total solids in the input material. The total solids content of the input material occur around 20 % or higher in a dry digestion process. (Latvala 2009 p. 33, Tchobanoglous et al. 1993 pp. 697, 701) The biogas production generally varies from 0,5 to 0,75 m^3/kg of biodegradable volatile solids destroyed in wet digestion. The dry digestion generally produces more biogas up to 1 m^3/kg of biodegradable volatile solids destroyed. (Tchobanoglous et al. 1993 pp. 681, 701–702)

Table 2. Wet and dry digestion quantities. (Tchobanoglous et al. 1993 pp. 701–703)

Quantity	Wet digestion	Dry digestion
Water content of the feedstock	> 85 %	< 80 %
Total solids destroyed	40–60 %	Depending on the lignin content
Destruction of volatile solids waste	60–80 %	90–98 %
Biogas production per kg of volatile solids destroyed	0,5–0,75 m^3/kg	0,625–1 m^3/kg
Temperature (mesophilic)	30–38 °C	30–38 °C
Temperature (thermophilic)	55–60 °C	55–60 °C

3.1.2. Digestive

A digestate is a residue after the conversion of biodegradable volatile compounds to biogas through anaerobic digestion. A digestate is a mixture of organic and inorganic compounds including nutrients. Also, e.g. heavy metals can be founded in a digestate. The water content varies generally in the range of 75–96 % depending on the content of feedstock materials, digestion technique and conditions in the digester reactor. (Christensen 2011 p. 618, Latvala 2009) Table 3 represents a typical digestate composition generated from digested wastewater sludge.

Due to biotransformation of proteins during the anaerobic digestion the digestate contains soluble inorganic nitrogen (NH_4-N) and phosphorus (PO_4-P) (Othman et al. 2009). Approx. a half of the total nitrogen is inorganic ammonium (NH_4-N) and the other half is organic

nitrogen. The nutrient NH_4-N is easily available for plants and also for microalgae. (Christensen 2011 p. 620, Chapter 2.2.2)

The nutrient content of the generated digestate is often utilized after dewatering. Additionally, an untreated digestate can be spread straight on the yields or it can be composted or incinerated. However, the hygienization requirements must be noted before the utilization as a fertilizer. (Christensen pp. 604, 612) For example, in Finland a digestate must be hygienized after mesophilic digestion or alternatively input feedstock must be hygienized before mesophilic digestion to exterminate possible harmful bacteria (Ministry of Agriculture and Forestry Decree 24/11).

Table 3. Various character values of the digestate from digested waste water sludge. (Karttunen p. 558)

Character	**Value range**	**Unit**
Alkalinity $CaCO_3$	2500–3500	mg/l $CaCO_3$
Dry solids (TS)	6–12	%
Energy content	1720–2580	kJ/kg
Fats	5–20	% of dry solids
Iron	3,0–8,0	% of dry solids
Kalium K_2O	0,0–3	% of dry solids
Nitrogen	1,6–6	% of dry solids
Organic acids	100–600	mg/HAc
pH	6,5–7,5	-
Phosphorus P_2O_5	1,5–4	% of dry solids
Protein	15–20	% of dry solids
Sellulose	8–15	% of dry solids
Silicon SiO_2	10–20	% of dry solids
Volatile solids	30–60	% of dry solids

3.2. Reject water and its characterizes

A nutrient-rich effluent digestate is usually dewatered to raise its total solid content for further utilization e.g. as a fertilizer. The separated liquid is called reject water (RW) that contains valuable nutrients and several other compounds. (Constantine 2006, Karttunen 2004 p. 555) In addition, reject water can be originated from the removed liquid from a digester reactor or biogas washing process (Lehto 2010 p. 10). The estimated amount of the generated reject water varies between 75 to 90 % of the mass of a digestate or 1,3–2,9 m^3 per ton of input waste (Latvala 2009 p. 55, Lehto 2010 p. 33).

3.2.1. Effect of the dewatering technique to the content of reject water

The temperature range of the digestion process (mesophilic or thermophilic) affects to the composition of generated reject water (Vesitalous 1/2011 p. 32). Also, the selected dewatering technique has an influence to the separation efficiency of the liquid and solid phase from the digestate. This has an effect especially to the final solid content but also to other chemical contents in the generated reject water. Spin driers are commonly used dewatering equipments. Other dewatering techniques constitute e.g. belt filter presses and vacuum-assisted drying beds. Generally, digestate dewatering requires addition of a chemical for achieving efficient dewaterability. The selection of added chemical is an important factor since that affects to the final solid content and volume of the separated reject water. Anionic and cationic polymers are commonly used dewatering chemicals since the addition of them increases only slightly the amount of the total dewatered digestate sludge volume. The most effective polymer for the dewatering has to be determined experimentally. Ferric chloride and lime can be also used for improving dewaterability but they increase the total digestate volume significantly. (Tchobanoglous et al. 2003 p. 1559, Karttunen 2004 p. 578) Also, the increase of pH with the addition of hydrogen peroxide constitutes a functional digestate processing method resulting the generation of reject water with a greater quality (Lehtovuori 2016). Moreover, the persistent optimization and adjustment of the dewatering process has been resulted to a decreased amount of solid matter in the generated reject water. Especially routine measurements and optimization programs of solid matter have showed to prevent external water in the dewatered digestate. In addition, online measurements have been examined to increase energy efficiency of the total dewatering process since e.g. functionality of pumps and bacteria are improved. (Tekniikka ja Talous 2.9.2016)

3.2.2. General composition of reject water from literature

Reject water from an anaerobic digestion process contains typically high concentrations of dissolved ammonium nitrogen (NH_4^+-N), phosphorus (P); and suspended and colloidal solids. (Pitman 1999, Wäger-Baumann & Fuchs 2011) The features of the reject water composition and their variation scale between biogas plants are presented in Tables 4 and 5. In general, all the presented concentrations are higher in reject water compared to the conventional municipal WW. Total nitrogen (TN) load that includes nitrogen forms of organic N, NH_3, NH_4^+, NO_2^- and NO_3^- is 40–200-fold higher compared to TN in conventional WW. In terms of total phosphorus (TP) and chemical oxygen demand (COD)

concentrations, they can be maximally 10–100- and 40–250-fold respectively compared to the concentrations in conventional municipal WW.

Table 4. Reject water compositions from singular biogas plants in Europe and Japan.

Country	Austria	Finland	Nordic Country (North Europe)	Nordic Country (North Europe)	Norway	Japan
Feedstocks	Kitchen garbage, spoilt food, lop material from, grease separators	Biowaste	Biowaste (70 %), sewage sludge (30 %)	Biowaste (25 %), sewage sludge (75 %)	Sewage sludge	Pig manure, kitchen garbage
pH	8,1–8,8	-	-	-	-	7,5
Total solids	17,0–21,2 g/l	3,9 g/l	0,45 %	0,4 %	3,7 mg/l	-
COD (mg/l)	10 478–14 988	6 550	5252	6000	7525	2 290
NH_4-N (mg/l)	3 240–3 690	642	-	-	-	1 510
TN (mg/l)	3 610–4 120	1 003	1025	3000	1655	1 770
TP (mg/l)	58–167	82	77	75	-	432
Reference	Wäger et al. (2010)	Latvala (2009)	Lehto (2010)	Lehto (2010)	Rusten & Sahu (2011)	Lei et al. (2006)

Table 5. Chemical parameters of reject water from various biogas plants and the composition of the typical municipal wastewater for the comparison. The collected data by Wäger-Baumann (2011) is based on the data from the author's own investigations and various references from the literature. The data by Lehto (2010) is based on the interviews of six biogas plants that use co-digestion process in Finland and Sweden. The data by Karttunen (2004) is measured from various municipal wastewaters.

	Reject water Reference (Wäger-Baumann 2011)	**Reject water Reference: Lehto (2010)**	**Typical municipal wastewater Reference: Karttunen (2004, pp. 494)**
Parameter	**Quantity**	**Quantity**	**Quantity**
COD [mg/l]	15000–80000	3770–11500	300–450
BOD_5/BOD/BOD_7 [mg/l]	1000–1500	1270–3600	125–175
TN [mg/l]	3000–8500	1025–3000	25–40
NH_4-N [mg/l]	2500–7500	-	15–25
TP [mg/l]	100–1000	5–111	6–8
PO_4-P [mg/l]	50–800	-	-
Dry matter	1,5–7 %	0,20–0,45 %	350–600 mg/l
SS	0,6–6 %	-	150–200 mg/l

3.2.3. Physical parameters

The separated reject water possesses a relatively high pH. The value of pH typically varies in the range of 7,5–8. (Kymäläinen & Pakarinen 2015 p. 104) Moreover, if the dewaterbility of the digestate is improved by addition of lime the pH remains even higher (Wett et al. 1998). An extremely high alkalinity and buffering capacity are typical characteristics of the reject water that can cause difficulties in terms of its purification (Lehtovuori 2016). The temperature of reject water is often 25–35 °C since the digestion process operates under quite high temperatures leaving the generated digestate warm. The final temperature of reject water depends on the digestion process temperature and dewatering technology. (Gustavsson 2010)

Generally, the transmittance of light is notable low and the color is extremely dark in reject water generated at a biogas plant (Rusten & Sahu 2011). The color of reject water from thermophilic digestion is usually darker compared to reject water from a mesophilic process (Vesitalous 1/2011 p. 16). Conventionally, the dark color is due to presence of metallic sulfides. The formation of metallic sulfides in followed by the production of sulfides by anaerobic digestion reactions. The sulfides react with metals in the feedstock material forming metallic sulfides. (Tchobanoglous et al. 2003 p. 52)

3.2.4. Nitrogen content

Conventionally, reject water contains high concentrations (1025–8500 mg/l) of dissolved ammonium nitrogen (NH_4-N) (Table 5). It can form up to 80 % of the TN. Thus, the share of soluble organic nitrogen and stable organic nitrogen is only 20 % of the TN. (Marttinen et al. 2013 p. 17) The proteins of the input feedstock cause the majority of released NH_4-N. A co-digestion often results to a higher NH_4^+-N concentration. Furthermore, NH_4-N concentration increases if the feedstock sludge is thickened effectively. The nitrogen load in reject water can be lowered selecting input feedstocks that contain mostly of carbohydrates or fats. (Gustavsson 2010) The reject water generated after thermophilic digestion contains typically 30 % more of NH_4-N compared to the reject water from a mesophilic process (1 300 mg/l vs. 1 000 mg/l) (Vesitalous 1/2011 p. 16). Also, higher nitrogen content of the feedstock material probably increases the concentration of nitrogen in the generated reject water. If the majority of the feedstock material is municipal WW sludge it perhaps result to higher concentration of nitrogen in the reject water. Regardless, the content of reject water can vary unpredictably since the quantity of the nitrogen concentration is depended on the origin and quality of the WW sludge. (Lehto 2010 pp. 35–36)

3.2.5. Phosphorus content

Phosphorus concentration is significantly lower than nitrogen concentration in reject water (Table 5). The majority of the phosphorus is in a water-soluble form (PO_4-P) (Marttinen et al. 2013 p. 17). According to the Table 4, TP concentration is 100 mg/l and below it in reject waters generated at digestion plants in Europe. However, the TP content can vary significantly between biogas plants. For example, the TP concentration of 432 mg/l measured in Japan was remarkable higher compared to other TP concentration (<170 mg/l) from the considered biogas plants in Table 4, which is probably due to the feedstock material in Japan that mostly consisted of pig manure that typically has a higher P concentration (1,8–2,8 % of TS) compared to e.g. biowaste from the households (0,2–1,0 % of TS) (Christensen p. 667). The majority share of municipal WW sludge in the feedstock material will result to lower concentration of phosphorus in reject water. The used chemicals at the WWT process affect to the function of phosphorus ions, which have an influence to the amount of bound phosphorus in the solid matter during the digestion process and to the amount of phosphorus in reject water. (Lehto 2010 p. 37) Also, a co-

digestion with a rapeseed has observed to increase the phosphorus content in reject water (Kuglarz et al. 2015).

3.2.6. Other quality factors

The quality and features of the input feedstock material to the digester reactor has a remarkable influence to the final composition and quality of reject water. Commonly, reject water from a digested WW sludge includes diversely essential micronutrients and trace elements such as sulfur, iron and cobalt that are preferable for the growth of anaerobic bacteria. The growth of anaerobic bacteria degrades impurities e.g. organic matter and solid particles effectively which leads to that reject water is easier treatable and its color is less dark. If the input feedstock material consists of e.g. only agricultural waste alone it probably generates nutritionally unilateral conditions for anaerobic bacteria, which affects negatively to reject water quality. (Lehtovuori 2016) The diverse content of the feedstock material has also an influence to the biogas yield. The content should be balanced in terms of nutrients and other essential compounds for anaerobic bacteria to maximize the biogas yield. (Bitton 2005 pp. 354–356)

A high solid concentration of reject water is interrelated to high biological oxygen demand (BOD) (Lehto 2010 pp. 39, 56). In other words, a high BOD indicates about a high organic pollution concentration in wastewater (Tchobanoglous 2003 p. 81). Also, simultaneously high BOD and high chemical oxygen demand (COD) are connected with eachother in reject water. The BOD/COD ratio ranges between 0,25 and 0,35 in generated reject water at Finnish and Swedish co-digestion plants. (Lehto 2010 p. 40) The ratio is relatively low compared to the ratio of conventional WW that varies between 0,3 and 0,8. The BOD/COD ratio determines the biological treatability for the considered WW: greater than 0,5 indicates that it is easily treatable whereas a low (< 0,5) indicates that the WW may include inhibiting compounds or concentrations for microorganisms. (Tchobanoglous 2003 pp. 97–98)

3.2.7. Reject water recycling and utilization

Reject water can be recycled back to the digester reactor to increase the total water content of the input waste to the required range of higher than 85 % in terms of wet digestion process (Christensen p. 611, Table 2). The reject water is generally leaded to the beginning of the water treatment process in WWTPs that digest WW sludge (Karttunen 2004 p. 572). Also, various separate treatment methods for decreasing impurities or separating valuable

nutrients from reject water are used worldwide (Constantine 2006). The commonly used separate treatment technologies are considered in the Chapter 4.

Reject water can be utilized as a fertilizer product due to nitrogen and phosphorus content Nevertheless, if untreated reject water is speard straight on agricultural fields, high ammonium concentrations can lead to undesirable nitrogen pollution. Thus, European Communities restricts the use of reject water as fertilizers causing reject water disposal issues at biogas plants. (Wäger-Baumann & Fuchs 2010) The utilization of aquacultures such as water plants and algae is attractive since they can bind the nutrients in the reject water resulting outcome of a treated effluent liquid (Christensen 2011 p. 617).

4 REJECT WATER MANAGEMENT

High nitrogen concentration constitutes the largest issue of the reject water quality. Although reject water flows are relatively small (0,5–1 % of the total flow) they usually are involved 15–20 % of total nitrogen load in the total WW flow at the WWTP. When the nitrogen-rich reject water is leaded straight to the WWTP for its purification it overloads the process causing additional costs for the WWTP. Reject water streams possess a relatively low flow rate, high ammonium nitrogen concentration and warm temperature. Due to these characterizes a separate reject water treatment cam be a cost-effective. (Constantine 2006, Lehto 2010, Singer & Lawler 1982)

A separate treatment of reject water can be a solution for high nitrogen loads and more stringent discharge limits of nitrogen. Physical and chemical methods for nitrogen removal cause higher costs compared to biological methods e.g. nitrification-denitrification reaction. Generally, the nutrients are perceived valuable as fertilizers for agriculture. Due to that physical and chemical removal methods are also applied. (Gustavsson 2010) Figure 12 represents certain existing separate treatment methods for reject water.

Various treatment options and combinations must be tested by laboratory tests since the function of a specific treatment technology operates differently in various reject waters. The quality of the specific reject water is strongly depended on the dewatering technology and the quality of the feedstock material. Hence, the most functional treatment for reject waters is difficult to be determined in general (Chapter 3.2).

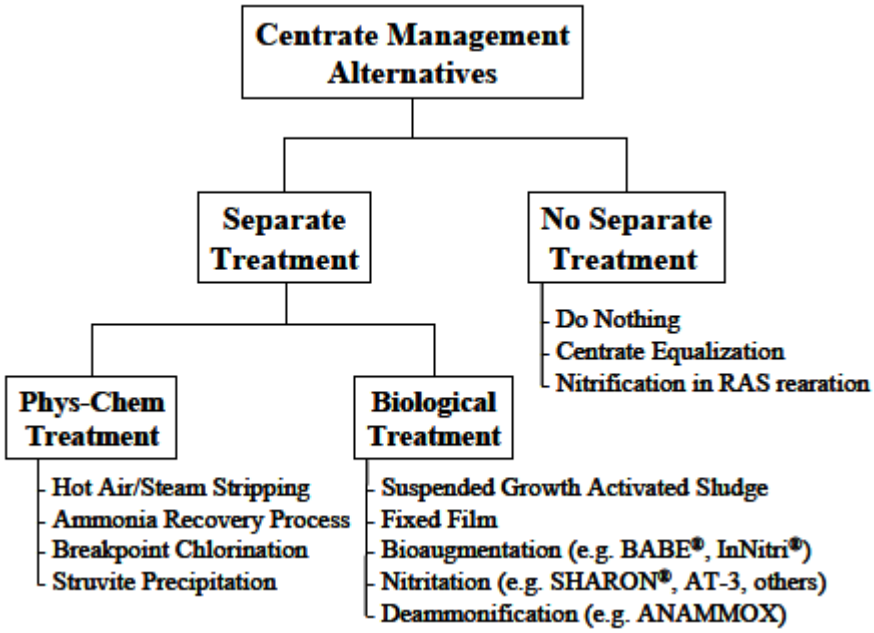


Figure 12. Various separate reject water treatments sorted by Constantine (2006). The treatments have been investigated, tested or they are operating at the installations around the world.

4.1. Physical-chemical treatment methods

The main advantage of the physical-chemical treatments is that they function immediately without a need for e.g. a growth of bacterial biofilm. The weaknesses are typically energy-intensity, a need for the addition of chemical and the formation of undesirable co-products. Hence, these methods are often more costly compared to biological methods. (Ruissalo 2006 pp. 32–35)

4.1.1. Ammonia stripping

Ammonia stripping constitutes a wastewater treatment converting ammonium nitrogen (NH_4-N) from the liquid phase to the gaseous phase of ammonia NH_3. WW streams as well as reject waters include both dissolved ammonium nitrogen ions (NH_4^+) and gaseous ammonia (NH_3) remaining in the equilibrium depending on the pH conditions (Figure 13). (Tchobanoglous pp. 60–61) The following equation illustrates the balance (Tchobanoglous p. 61):

$$NH_4^+ \leftrightarrow NH_3 + H^+ \quad (5)$$

The pH conditions must be raised above 7 to shift the above-mentioned balance to the right of the equation for the formation of NH_3. The addition of lye (NaOH) or lime ($Ca(OH)_2$) is generally used for the pH adjustment. The alkalinity of the reject water and the temperature of the presence air determine the required amount of alkali compound to increase the pH to the optimal stripping level. In order to the maximal conversion of ammonia the process requires precense of air and high temperature that decrease the partial pressure of the NH_3 resulting the formation of gaseous ammonia. This conversion is based on the Henry's law. (Kymäläinen & Pakarinen 2015 pp. 103–104, Tchobanoglous 2003 p. 1163)

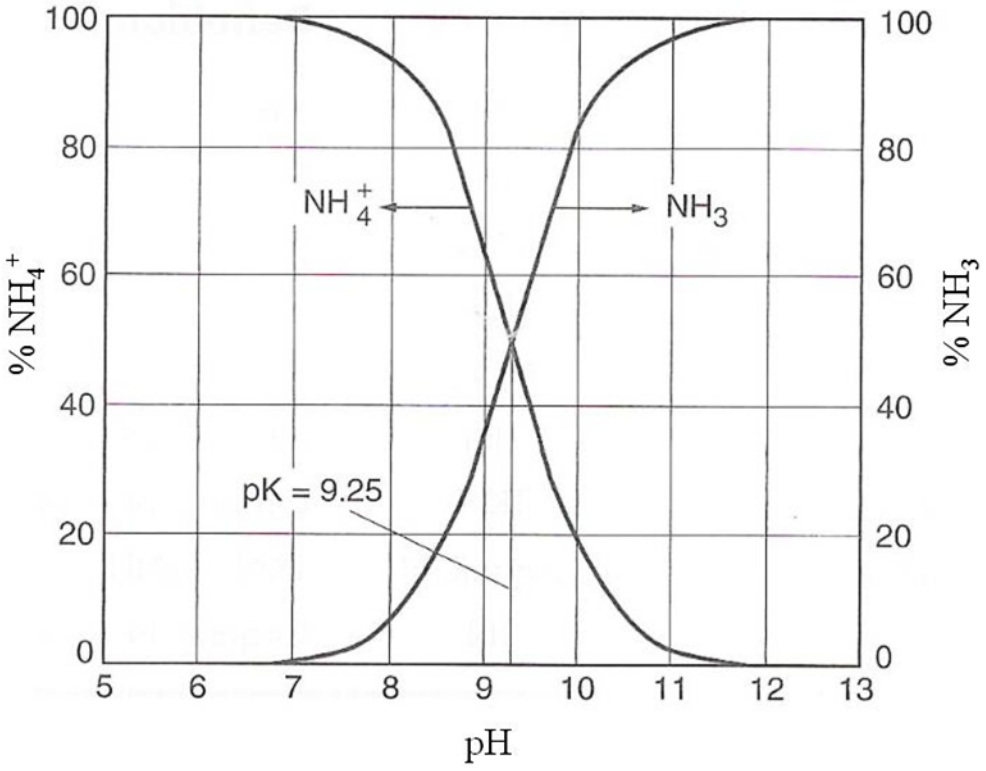


Figure 13. Percentual shares of NH_4^+ and NH_3 in a water dilution as a function of pH. (Tchobanoglous 2003 p. 62)

Ammonia stripping can remove over 95 % of the ammonia depending on pH, temperature and operational time (Figure 14). Also available airflow has an influence on the ammonium reduction rate (Campos et al. 2013). The stripped gas can include odorous gases and volatile organic compounds along with NH_3. Hence, the stripped gas is often treated with scrubbing that dilutes NH_3 into the water or acidic solution e.g. sulfuric acid. The released NH_3 can be utilized for the production of fertilizers or in the industry for replacing urea. (Kymäläinen & Pakarinen 2015 p. 104, Tchobanoglous 2003 p. 1162) After

the stripping, pH conditions in the reject water remains high, which may be an issue for further utilization or treatment (Lei et al. 2006).

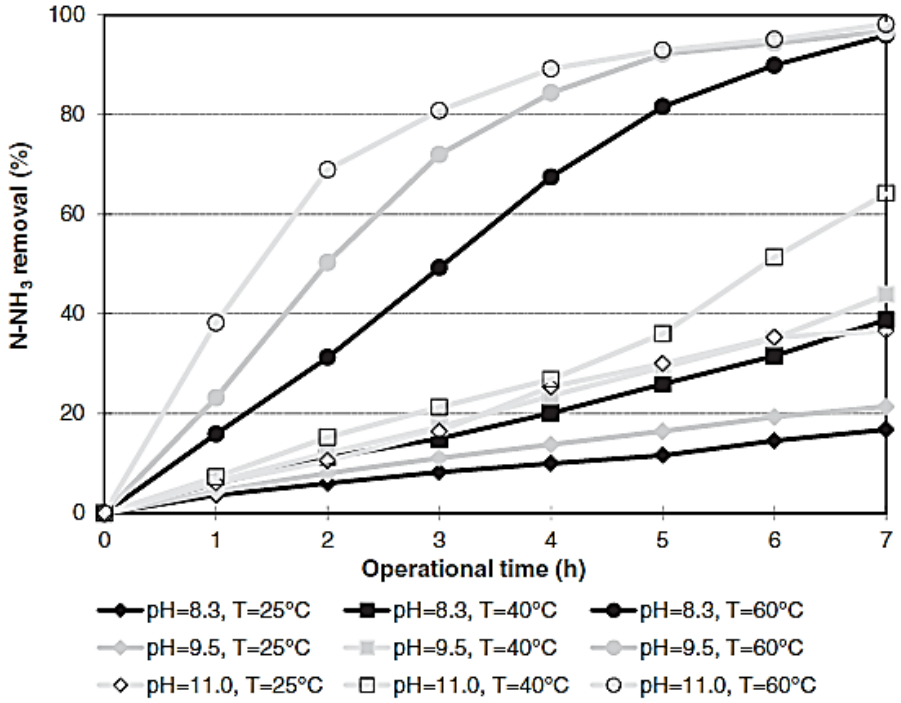


Figure 14. Ammonia gas removal efficiency is strongly depended on the pH, temperature and operational time. Removal efficiency of 96,7 % can be achieved under the pH of 11 and the temperature conditions of 60 °C with 7 hours operational time. (Campos et al. 2013)

Ammonia stripping has several operating challenges and weaknesses: 1) maintaining the high pH conditions 2) maintaining the favorable temperature especially on cold winter conditions 3) fouling due to the presence of organic compounds 4) high investment and operating costs compared to biological treatment technologies. (Partanen 2010 p. 23, van Kempen et al. 2001, Tchobanoglous 2003 pp. 1179–1180)

CO_2 injection can decrease the high pH in the ammonia stripped reject water. Lei et al. (2006) neutralized pH from greater than 11 to around 7 by addition of CO_2 that was extracted from generated biogas. In the experiment the methane concentration of the biogas increased from 60 % to 73 %. Te CO_2 utilization constitutes noteworthy application for CO_2 capture, improvement of quality of reject water and biogas purification. (Lei et al. 2006) The following reaction equations represent the solution of CO_2 into water (Lei et al. 2006):

$$CO_2\,(g) \leftrightarrow CO_2\,(aq) \quad (6)$$

$$CO_2\,(aq) + H_2O\,(l) \leftrightarrow H^+\,(aq) + HCO_3^-\,(aq) \quad (7)$$

$$HCO_3^-\,(aq) \leftrightarrow H^+\,(aq) + CO_3^{2-}\,(aq) \quad (8)$$

The effect of decreased pH is due to the increase of acidic H^+ ions that are formed by the previous reaction (7).

4.1.2. Chemical flocculation and coagulation

Typically, reject water from a biogas plant contains high concentrations of suspended solids and the color is extremely dark (Rusten & Sahu 2011). In order to resemble conventional wastewater, a chemical flocculation or coagulation could be a solution to improve the separation of solid and colloidal particles. (Karttunen 2004 p. 133) There exist coagulants and flocculants with a cationic (positive), anionic (negative) and nonionic (neutral) charge (Tchobanoglous p. 485).

A chemical flocculation or coagulation is generally used for removing solid compounds by growing the size of the solid particles in the WW or sludge. Concurrently, when the sludge is being mixed a coagulant e.g. cationic polymer or hydrolyzed metal ions e.g. ferric sulfate neutralizes the charges between solid particles resulting in the formation of flocs. Ferric sulfate precipitates suspended solids, especially phosphorus. A flocculant e.g. an anionic or nonionic organic polymer forms flocs by an adsorption of separate solid particles. (Tchobanoglous 2003 pp. 478–502) A sand filter, for example, constitutes one of the suitable options for the separation of the flocs from the liquid phase after flocculation (Rusten & Sahu 2011). Other solid separation techniques are e.g. flotation tanks and technologies, centrifugation, athtracite filter, membrane filtration (MF) and reverse osmosis. (Karttunen 2004 pp. 97–102, Tchobanoglous 2003 pp. 1087, 1104–1106, 1159)

Organic polymers are commonly used for digestate dewatering since the addition of polymer improves the reject water separation efficiency from the digestate and decreases the solid content in the generated reject water (Karttunen 2004 p. 578, Tchobanoglous et al. 2003 p. 1555–1556). Thus, the addition of the polymer can be applied to treat also reject water to remove solid particles. Especially, the addition of cationic polymer may be beneficial for improving reject water quality since it is applied for the removal of both solid particles and color (Figure 15). A flocculation with a cationic polymer Zetag 8125

was tested by Rusten & Sahu (2011) whom investigated that the combination of the polymer addition, flocculation and filtration resulted to the 85 % improvement of the light illuminance in the reject water.

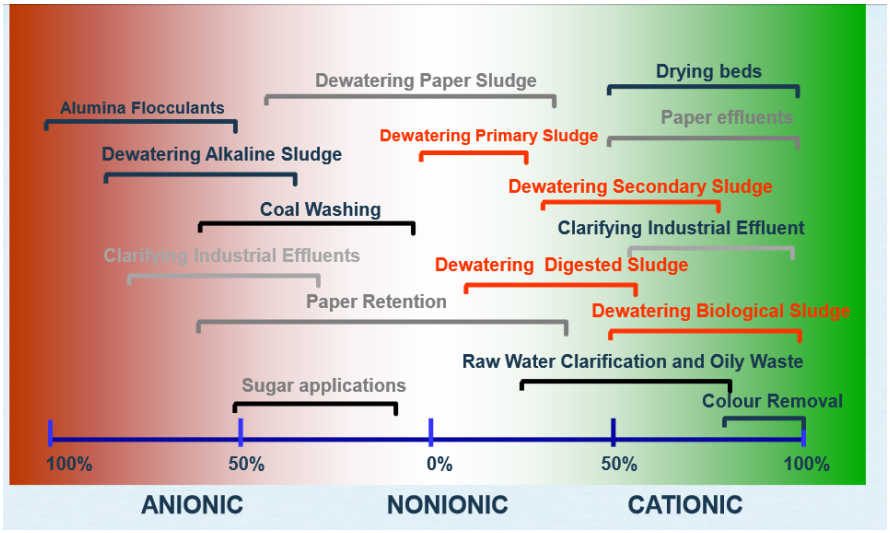


Figure 15. Addition of cationic polymer constitutes a suitable treatment for removal of solid particles from high-solid reject water. It also can remove color. (Normington 2012)

An organic polymer chitosan constitutes an attractive potential compound for the purification of wastewater effluents (Manninen et al. 2015, Spilling et al. 2011). It possesses a high cationic charge density and long polymer chains and therefore it can be capable to remove solid particles and dissolved substances from WW (Renault et al. 2009). However, its functionality, energy- and cost-effectiveness are unsolved and debatable (Lehtovuori 2016, Spilling et al. 2011). Various flocculants e.g. cationic polymers, ferric sulfate as well as chitosan can be also utilized for forming algal flocs for harvesting the biomass (Lakaniemi p. 18, Shelef & Sukenik 1988).

Lehtovuori (2016) reported that the precipitation of the solid particles is difficult from reject water with the injection of the polymer alone. Therefore, the cotemporally addition of both coagulant and flocculant can be a solution for the purification of the reject water since it may lead to more effective removal of solid particles (Figure 16). In addition, the combination of ferric sulfate and polymer can be more workable for improving reject water quality. Nevertheless, since reject water typically possesses a high solid content and alkalinity the amount of added chemicals is often abundant, which causes external costs. (Lehtovuori 2016)

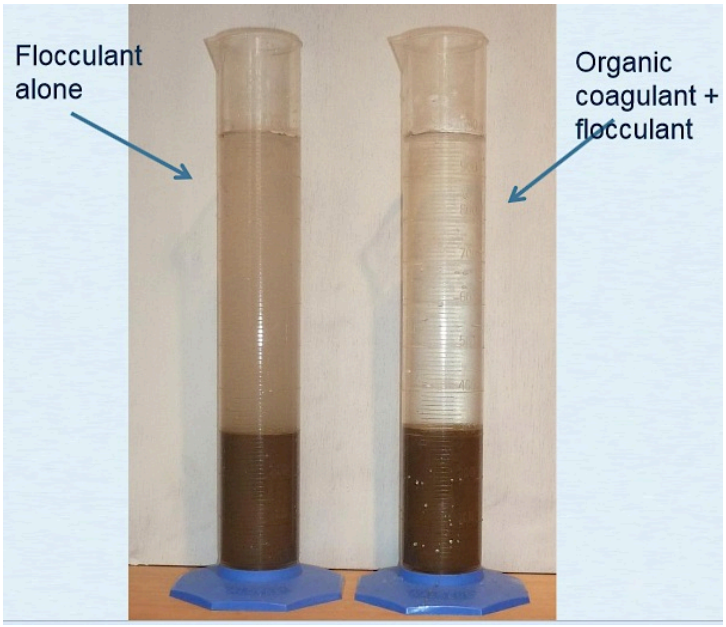


Figure 16. Combination of addition of both coagulant and flocculant can result a greater WW purification. (Normington 2012)

4.1.3. Membrane filtration

Membrane filtration (MF) technologies can purify WW efficiently. Especially microfiltration and ultrafiltration are suitable for the removal of suspended solids, microorganisms and macromolecules. (Wäger et al. 2010) Reject water from a co-digestion plant has been purified successfully by the ceramic MF in the studies by Wäger et al. (2010) and Wäger-Baunmann & Fuchs (2011). The removal of phosphorus was 67–78 %. However, after the MF reject water still included a high concentration of ammonium nitrogen since the nitrogen removal was only 5–18 %. Moreover, the formation of filter cake on the membrane was an issue. Therefore, a flocculation treatment can be solution to enlarge size of particles before MF, which has been investigated to be workable in the study by Wäger et al. (2010).

Membrane bioreactor (MBR) constitutes a technology that combines MF and conventional biological activated sludge. MBR has been tested successfully for reject water in the study by Wäger-Baumann & Fuchs (2011). They investigated that the activated sludge process removed efficiently nitrogen and it improved the performance of the membranes. The nitrogen removal of 98 % and low ammonium concentration was established after the MBR treatment. The activated sludge, however, required an external carbon source for the

performance of the denitrification reaction. Furthermore, membranes may require continous cleaning due to te hformation of the filter cake.

Reverse osmosis (RO) removes extremely small-size compounds such as ions and it is known as the most effective membrane technique. It has capable to remove even bacteria and color. (Ek et al. 2006, Kieniewicz 2006, Liu 2007 p. 88, Wäger-Baumann & Fuchs 2010) RO filtration removed over 98 % of both NH_4-N and PO_4-P from reject water after the sand filtration in the study by Kieniewicz (2006).

4.1.4. MAP/Chemical precipitation of struvite

The MAP process (magnesium-ammonium-phosphate) includes the chemical precipitation of struvite ($MgNH_4PO_4$) that removes both phosphorus and ammonium nitrogen under high (9–11) pH conditions (Equation 9, Saidou et al. 2009) The treatment requires relatively high phosphorus and magnesium concentrations that generally are low in reject water. Therefore the addition of magnesium and phosphorus may be essential, which increases the costs significantly. However, Mg and P could be recycled but it may encounter grave difficulties since then the ammonium nitrogen is also recycled back into the process. (Ruissalo 2006, Solon 2013) Alternatively, the formed struvite matter can be utilized as a mineral fertilizer (Saidou et al. 2009).

$$Mg^{2+} + PO_4^{3-} + NH_4^{+} \leftrightarrow MgNH_4PO_4 * H_2O\ (x) \quad (9)$$

4.2. Separate biological treatments for high nitrogen concentrations

Biological treatments are usually less costly and it operates without producing co-products compared to physical-chemical treatment methods (Ruissalo 2006 p. 35). The conventional biological nitrogen removal process with nitrification/denitrification (N/D) reaction is an unattractive treatment method for reject water due to unfavorable COD:N ratio for heterotrophic denitrification (Fux & Siegrist 2004). An efficient denitrification reaction requires addition of external carbon for high nitrogen loads in reject water, which increases the operational costs of the conventional N/D process (Fux & Siegrist 2004, Schmidt et al. 2003). This conventional activated sludge treatment is shown to be energy-negative and it only operates efficiently in WWs with low nitrogen content. Also, the required aeration is energy-intensive: the consumed energy is approx. 60–70 % of the total energy

consumption. (Clippeleir et al. 2011) Therefore, more cost-effective biological nitrogen removal treatment options that are specialized for high nitrogen loads have been developed. Table 6 shows the commonly known separate biological nitrogen removal techniques: conventional N/D process and treatments for high nitrogen loads and their efficiencies.

Table 6. Total nitrogen removal efficiencies of various biological nitrogen removal treatments in literature. *a treatment for high nitrogen loads

Treatment	Conventional N/D	SHARON*	Anammox*	ANITA^{TM}Mox*	DEMON*	CANON*
TN reduction	90–95 %	85 / 90 %	89 %	90 % (NH_4-N)	98 %	81%/92%
Reference	Gustavsson (2010)	Hellinga (1998) / Mulder et al. (2006)	Gustavsson (2010)	Christensson et al. (2013)	Bäcklund (2016), Appendix 1	Li et. al (2008) / Sliekers (2003 pp. 34)

The SHARON process utilizes ammonia oxidizer bacteria *Nitrosomas* and *Nitrobacter* (Parades et al. 2007). The nitrogen uptake by *Anammox bacteria* is utilized in Anammox, ANITA^{TM}Mox, DEMON and CANON processes (Christensson et al. 2013, Masłoń & Tomaszek 2007, Parades et al. 2007, Strous et al. 1998) Figure 17 shows the *Anammox bacteria* cell and Figure 18 shows an ANITA^{TM}Mox pilot at Viikinmäki WWTP.

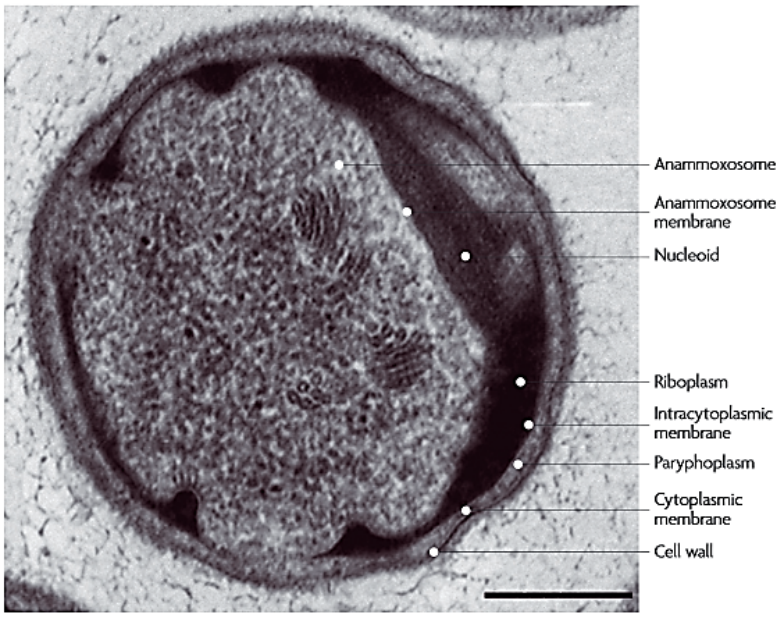


Figure 17. A cell of *Anammox* specie *Candidatus Kuenenia Stuttgartiensis*. *Anammox bacteria* possess a separate, anammox-specific membrane-bound compartment, which is termed as *Anammoxosome* Photographed by L. van Niftrik, Radboud University, Nijmegen, The Netherlands. (Kuenen 2008)

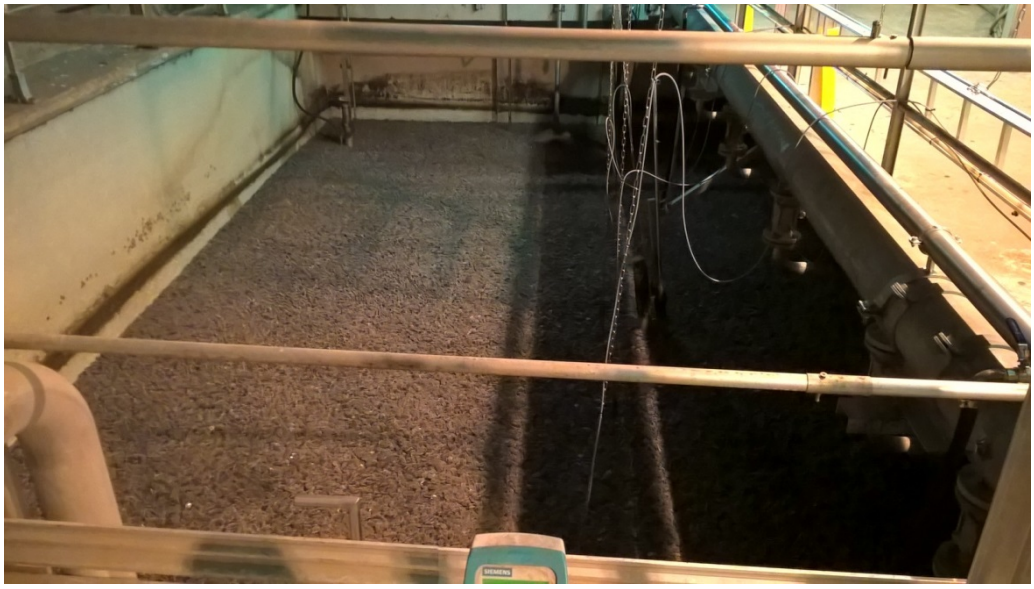


Figure 18. Photograph of the ANITA™ Mox pilot at Viikinmäki WWTP, Helsinki, Finland. Photographed by Sara Merin. ANITA™Mox is a process that utilizes the nitrogen uptake by *Anammox bacteria* biofilm. The process is based on the partial nitrification and autotrophic N-removal by *Anammox bacteria* under both aerobic and anaerobic environments. (Christensson et al. 2013) The beneficial pH and temperature for *Anammox* vary in the ranges of 6,7–8,3 and 20–43 °C, respectively (Strous et al. 1999). The ANITA™ Mox is often operated at a one-stage moving bed biofilm reactor (MBBR). The process is a cost- and energy effective process for high nitrogen loads. The process has been treating RWs at least in Sweden at Sjölunda WWTP in Malmö and Sundet WWTP in Växjö. (Christensson et al. 2013).

4.3. Biological phosphorus removal

Biological phosphorus removal has been regarded as an interesting alternative for typical chemical precipitation of phosphorus. Phosphorus Accumulating Organisms (PAOs) are aerobic heterotrophs that have a capability to utilize excess phosphates as polyphosphates on wastewater. (Jeyanayagam 2005, Pauli & Kaitala 1995) Pauli & Kaitala (1996) proved that a PAO *Acinetobacter* can uptake rapidly phosphates during its growth by decreasing P concentration from around 8 mg/l close to 0 on the culture medium. Figure 19 shows the principle of biological phosphorus uptake by *Acinetobacter*. Certain PAOs are also feasible to denitrify nitrogen. The presence of volatile fatty acids (VCAs) is important for the accumulation of phosphorus by the uptake of PAOs and denitrification rates. (Jeyanayagam 2005)

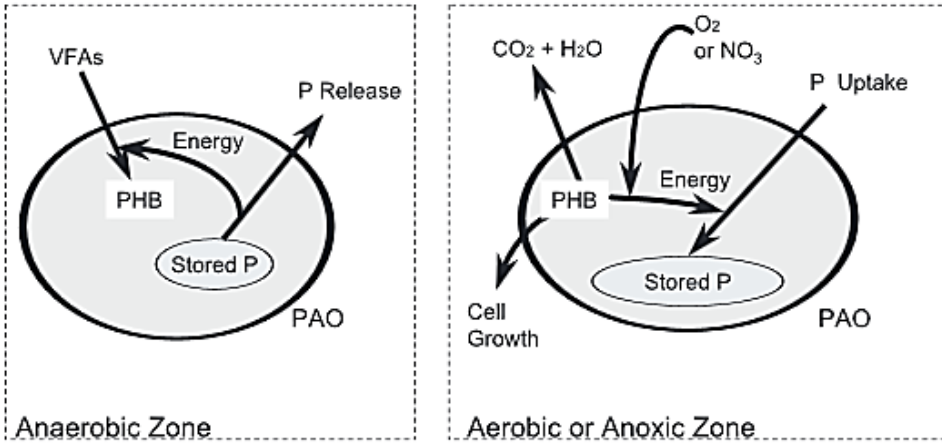


Figure 19. *Acinetobacter* requires VFAs for the uptake of phosphates. Poly-b-hydroxybutyrate (PHB) operates as carbon storage that is formed from VFA under anaerobic conditions. PAO utilizes PHBs as energy for phosphate uptake. (Jeyanayagam 2005).

4.4. Reject water treatments in Sweden

Together 23 WWTPs treats reject water from digested WW sludge in Sweden. A sequencing batch reactor (SBR) is the most commonly applied purification technique (Figure 20). Especially, the low electricity consumption and short start-up period of the SBR system constitute probably main features for selecting SBR system at WWTPs in Sweden. Also, a bioaugmentation technology is commonly applied in the biological ScanDeNi® processes (Figure 21). The ScanDeNi® process is developed in Sweden and it is often located at WWTPs with an activated sludge process. The technology is suitable for WWTPs with a presence of anaerobic digestion reactor since it utilizes ammonia consuming microorganisms. It removes nitrogen through N/D reaction from the mixture of the recycled activated sludge (RAS) and reject water generated by digested sludge. (Rosén & Huijbregsen 2003) In addition to the separate reject water treatments in Figure 20, Sjölunda WWTP in Malmö and Sundet WWTP in Växjö treated their reject waters by an ANITA™Mox process in 2013 (Christensson et al. 2013). The most common nitrogen removal system in Sweden is a conventional N/D process (Figure 20). The secondly often selected process is a nitrification-denitritation reaction that is applied in SBRs and the SHARON process. Ammonia stripping has been operating at a certain WWTP in Sweden but its operation has been discontinued. (Gustavsson 2010)

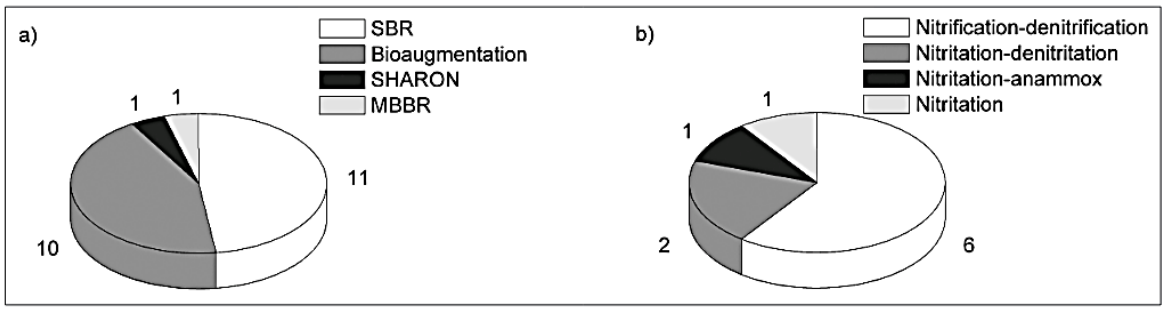


Figure 20. a) Reject water treatment technologies operating at biogas plants in WWTPs in Sweden. b) Separate nitrogen removal systems at biogas plants in WWTP in Sweden. Conventional N/D system is the most commonly applied as a nitrogen removal technology. (Gustavsson 2010)

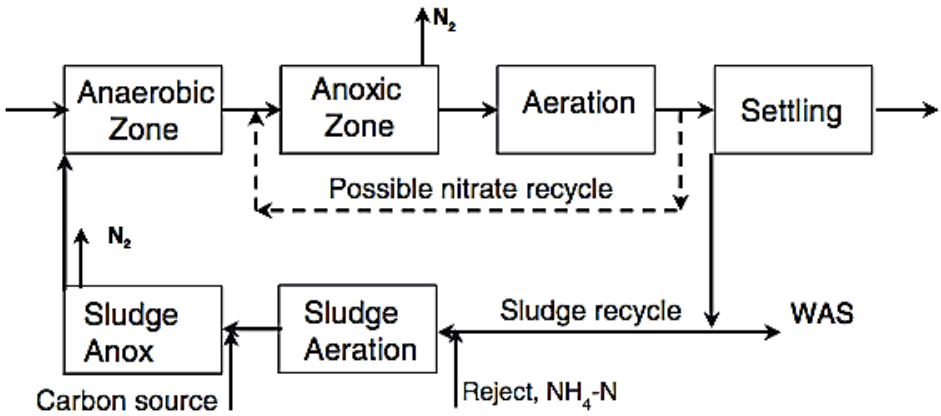


Figure 21. The ScanDeNi® process scheme. The sludge flow from the settling tank is RAS = recycled activated sludge. The reject flow is from dewatered digested sludge. (Rosén & Huijbregsen 2003)

4.5. Reject water management in Finland

This chapter is mostly based on the interviews by the questionnaire that was sent co-digestion plants. In addition to the questionnaire, the reject management information was collected from private phone and e-mail interviews. Appendix 1 shows the interview results. The answer rate for the questionnaire was 70 %. The inverviewed co-digestion plants digest mainly biowaste, sewage sludge and agricultural waste to produce biogas (Table 7). The majority of these digestion processes operates under mesophilic temperature.

Table 7. Feedstocks at the interviewed co-digestion plants.

Biogas plant	Digestion feedstocks	Reference
BioKymppi Oy	Biowaste, groceries waste, manure, plant based waste	BioKymppi Oy (2016)
Biotehdas Oy	Manure, sewage sludge, biodegradable co-products from industry	Biotehdas Oy (2016)
Biovakka Suomi Oy, Vehmaa	Groceries side streams, swine sludges	Biovakka Oy (2016)
Envor Biotech Oy	Food waste, manure, groceries	Envor Group Oy (2016)
Jeppo Biogas Ab,	Biowaste, fodder sludges, groceries manure, plant waste	Jeppo Biogas Ab (2016)
Joutsan Ekokaasu Oy, Joutsa	Biowaste, fats, manure, oils	Joutsan Ekokaasu Oy (2016)
Kouvolan Vesi Oy	Biowaste, sewage sludge	Kouvolan Vesi Oy (2016)
LABIO Oy	Biowaste, plant waste, sewage sludge	Päijät-Hämeen jätehuolto (2016)
Laihian kunta	Biowaste, malt and grain waste, sewage sludge	Lehtomäki et al. 2007
Lakeuden Etappi	Biowaste, sewage sludge	Lakeuden Etappi (2014)
Stormossen	Biowaste, landfill leachate sludge, sewage sludge	Kuosma (2016)

4.5.1. Characterizes of the reject water

Table 8 represents nutrition and impurity concentrations in untreated reject water at the co-digestion plants in Finland. TN concentrations are 20–100-fold compared to TP concentrations. COD concentrations are over 4000 mg/l. The variation of the concentrations between plants is a remarkable circumstance. For example, the highest TP of 600 mg/l (Biovakka Oy) is 60-fold compared to the lowest TP of 13 mg/l (Biotehdas Oy). Biovakka Oy utilizes mainly groceries waste and swine sludge whereas Biotehdas Oy utilizes animal manure, biodegradable waste from industry and sewage sludge (Table 8). A

connection between digestion feedstock and reject water content is difficult to interpret since the detailed feedstock contents are unknown when the analyzed reject water samples of Table 8 are generated. Also, the water contents of the feedstocks are unknown. The value of the pH is the only characterize that is approx. at the same level of 8 in each reject water.

Table 8. Characterizes of raw reject water from co-digestion plants in Finland. All the data are based on the interviews in 2016 (Appendix 1).

Biogas plant	1	2	3	4	5	6	7
BOD [mg/l]	1 000	-	367*	-	2000	-	-
TS [mg/l]	2 300	-	-	-	1700	-	-
COD [mg/l]	4 200	-	5900	7400	7500	4916	-
TN [mg/l]	949	5000	640*	3100	2100	844	6000
TP [mg/l]	45	13	9,2*	100	23	64	800
NH_4-N [mg/l]	-	-	-	-	1800	-	
pH	-	-	8,3	8,4	8,3	-	8
Alkalinity [mmol/l]	-	-	-	-	-	54	-

* unit: kg/d

4.5.2. Reject water treatments and utilization

High nitrogen load of reject water strains the WW treatment process if it is leaded straight to the WWTP. Therefore a separate reject water treatment is operating at several co-digestion plants in Finland. High nitrogen load and other high concentrations e.g. total solids are the main issue in reject water processing (Saarela 2016, Paturi 2016, Kuosma 2016, Nyysti 2016). Kuosma (2016) from Stormossen reported that high total solids and nitrogen concentrations have disturbed their processwater treatment plant (PWTP). At the thermophilic biogas plant of Loimi-Hämeen jätehuolto high total nitrogen load has caused failures in the biogas production when the generated reject water was recycled back to the digester reactor. For preventing disturbances, they are planning a nitrogen removal treatment. (Paturi 2016) Nevertheless, Juura (2016) from Biotehdas Oy notified that the recycled reject water back to the reactor can operate trouble-free in a mesophilic process even with a 5000 mg/l nitrogen concentration. However, reject waters from dewatered digestate from a thermophilic process such as at the Loimi-Hämeen jätehuolto probably contain more nitrogen compared to reject water from a mesophilic process. Also, the digestion reactions function differently under higher temperature.

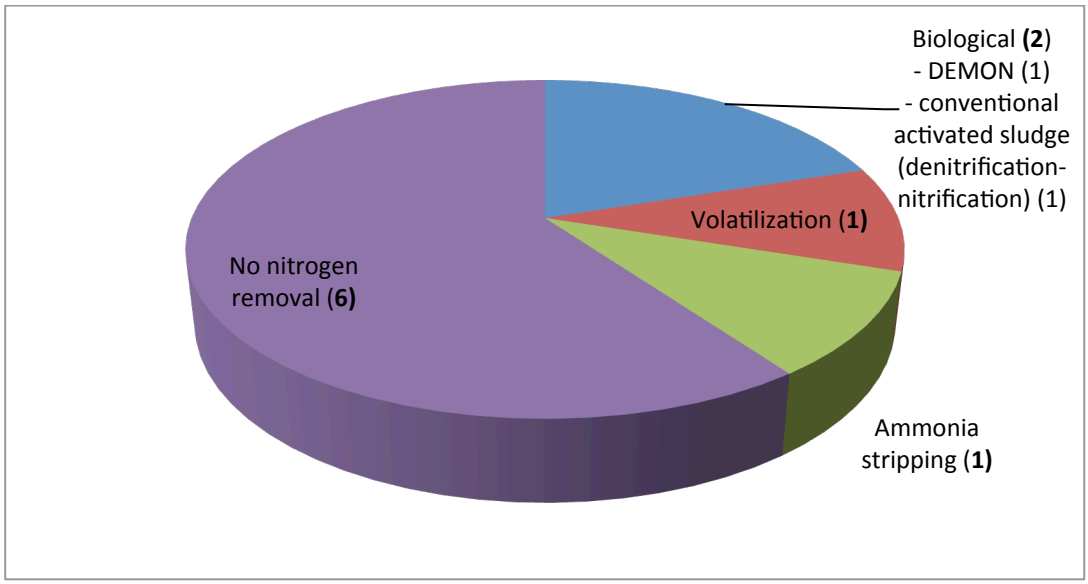


Figure 22. Nitrogen removal treatments at co-digestion plants in Finland 2016. Based on interviews. (Appendix 1, Paajanen 2016)

A separate nitrogen removal reject water treatment is operating at four of ten interviewed co-digestion plants: a biological nitrogen removal treatment DEMON at Lakeuden Etappi, a conventional activated sludge process with a denitrification-nitrification (D/N) process at Stormossen, ammonia stripping at Envor Group Oy and volatilization technique at Biovakka Oy (Figure 22). Table 9 shows the nitrogen reduction rates of these operating separate treatments.

Although the DEMON technology is commonly used in central Europe, Lakeuden Etappi constitutes the only co-digestion plant in Finland that utilizes the bacterial DEMON for nitrogen removal from reject water. A nitrogen removal efficiency of 98 % is achieved. (Appendix 1, Table 9) However, the DEMON-process has been observed to cause failures and its maintenance has been reported to encounter difficulties (Bäcklund 2016). The DEMON process has been piloted at Viikinmäki WWTP and it has been operating at Oy Pohjanmaan Biokaasu. At the Oy Pohjanmaan Biokaasu the DEMON encountered various failures during the year 2013, and therefore the untreated reject water was leaded to the local WWTP (Virtanen & Aaltonen 2013). Lindell (2012) reported that the high total solids concentration constituted to the inhibiting factor damaging the activity of *Anammox bacteria* based on the pilot experiment at the Viikinmäki WWTP

Stormossen biogas plant reduces systematically SS, N, P and BOD concentrations in reject water through a separate PWTP. The target is to decrease the impurity concentrations to

the level of typical municipal WW before it is leaded to the local WWTP. Lime is added before the D/N reactions in the activated sludge tank to raise the alkalinity and pH. In addition, a chemical ferric sulfate ($Fe_2(SO_4)^3$) (PIX-105) is added to improve the cleaning result in the last secondary clarifier. (Kuosma 2016, Saarela 2016) The WW sludges that are generated at the PWTP process are leaded to the dewatering where they are spin-dried. The dried sludge will be digested under thermophilic conditions with municipal WW from local WWTPs and landfill leachate sludge from Stormossen's leachate treatment plant. The filtrate is recycled back to the PWTP process. Appendix 2 represents the flows into the PWTP process at the Stormossen. (Kuosma 2016, Saarela 2016)

Envor Group Oy treats reject water separately by ammonia stripping that achieves NH_4-N reduction of 90–95 %. The stripping operates under the heat generated after mesophilic digestion process. Thus, no external heating is required for ammonia stripping. The pH is increased by addition of NaOH. (Paajanen 2016) After the stripping, ferric sulfate (PIX-105) is added and reject water is settled in a separate flotation tank. The settled phosphorus-rich solid matter is utilized as a fertilizer. (Juhe 2016)

Biovakka Oy in Vehmaa treats reject water by volatilization that results to a nitrogen- and phosphorus-rich concentrate. The concentrated liquid is utilized as a fertilizer in agriculture or in the industry. The N and P reduction rates are the highest of the separate reject water treatments in Finland (Appendix 1). TN reduction efficiency of 99 % can be achieved with this technique. The company also sells untreated reject water as a fertilizer for agriculture. (Haapanala 2016)

Table 9. TN reduction efficiency by various separate RW treatments in Finland. (Appendix 1) *Reference: Paajanen (2016)

Biogas plant / Separate nitrogen removal treatment	Envor Group Oy, Ammonia stripping	Lakeuden Etappi, DEMON	Biovakka Vehmaa Volatilization	Stormossen process water treatment plant
TN of the raw RW	-	640 kg/d	6000 mg/l	844 mg/l
TN after the treatment	-	12 kg/d	3–15 mg/l	321 mg/l
TN remove efficiency	-	98 %	99,75–99,95 %	62 %
NH_4-N reduction	90–95 %*	-	-	-

Five digestion plants of ten lead generated reject water straight to the local WWTP for its purification. A single plant treats reject water separately and then leads it to the local WWTP without utilization. The rest 5 co-digestion plants utilize either digestate or reject as a fertilizer (Figure 23). Both Jeppo Biogas Ab and Envor Group Oy utilize the total volume of the generated digestate without dewatering as a nutrient-rich fertilizer. Envor Group Oy, however, sometimes dewaters the digestate and treats the reject water through ammonia stripping and PIX-105 addition as previously mentioned (Paajanen 2016). Stenvall (2016) (from Jeppo Biogas Ab) informed that the delivery of the undewatered digestate to the fields is extremely costly. However, both Stenvall and Paajanen (2016) mentioned that separate reject water treatments also cause remarkable costs. In addition, Paananen (2016) reported that customers have been extremely satisfied for the digestate fertilizer product and the product is easily spreadable on fields. BioKymppi Oy dewaters the digestate and utilizes the reject water as a liquid fertilizer product.

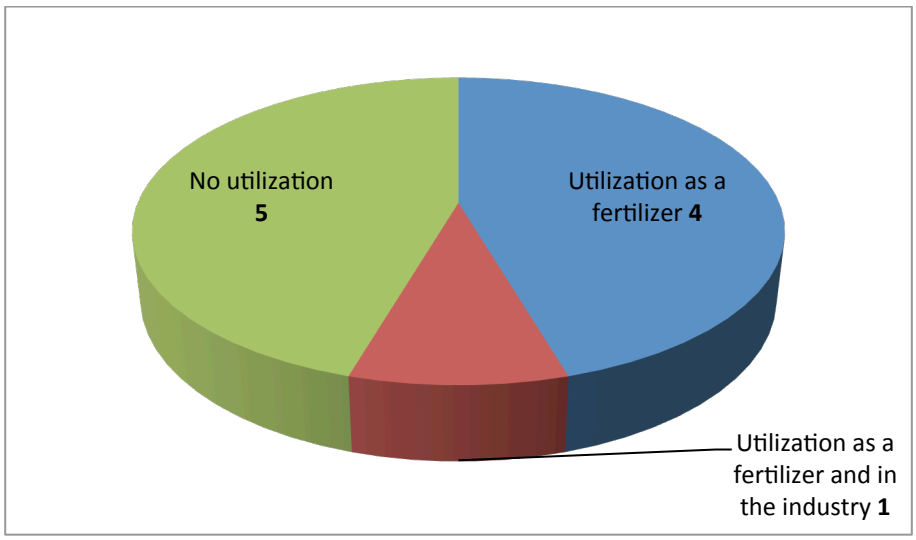


Figure 23. Utilization of nutrient concentrations the reject water at the Finnish co-digestion plants. (Appendix 1)

4.5.3. Prospects and new technologies in reject water management in Finland

High nitrogen load constitutes a topical issue in reject water management in Finland. At least two co-digestion plants (Loimi-Hämeen jätehuolto Oy and Laihialan Kunta) without a separate treatment for reject water are planning a nitrogen removal treatment (Paturi 2016, Nyysti 2016). Also, new reject management technologies are under development. For example, Jeppo Biogas Ab is piloting RO purification system and Viikinmäki WWTP is piloting an ANITA™ Mox process. Lehtovuori (2016) reported about a functional digestate processing method that improves the quality of the reject water. The method

includes injection of hydrogen peroxide (H_2O_2) under low pH conditions. The method was tested in the reject water from a co-digestion plant owned by Envor Group Oy but it is not operating currently probably due to strong foaming and high costs. New technologies should be adapted and developed since the demand for cost- and energy-effective solutions for improving reject water quality exists. The focus should be also concentrated to dewatering technology before reject water is even generated.

Since biogas production has increased 3-fold since the 1990's the volume of the reject water flows is also increased. Therefore, it is important to purify reject water or process it to products for utilization e.g. fertilizers. Juura (2016) mentioned that there is demand for reject water fertilizers especially for fields with high phosphorus but low nitrogen content. However, the NPK (nitrogen, phosphorus, potassium) nutrient content must be 1 % in raw reject water for the utilization as a fertilizer product (Act of Fertiliser products 539/2006, Finnish Food Safety Authority Evira 2016). A solution for preventing the generation of difficulty treatable reject water is to utilize dry digestion technology instread of wet as the LABIO Oy co-digestion plant.

Based on the interviews, Juura, Stenvall and Saarela (2016) expressed positive attitudes towards cultivation of microalgae in reject water. Hence, a potential for microalgae cultivation installation in the presence of biogas plant perhaps be more realistic and achievable in Finland.

4.6. Reject water as a culture medium of microalgae

Microalgae cultivation is feasible in various effluent waters after anaerobic digestion, which has been proven in several studies (Table 10). The reject water from a digestion reactor possesses conventionally a high temperature, high nitrogen concentration and low COD:N ratio that create attractive conditions for autotrophic organisms (Gustavsson 2010). However, the weaknesses are a low light transmittance, high turbidity, low carbon content and possible bacteria (Cai et al. 2013, Rusten & Sahu 2011). The features of reject water may be challenging for microalgae due to risks for inhibition. Also, the available nutrients for algae in WW have a strong influence to the production of algal biomass (Cai et al. 2013). Also, the nutrient content varies depended on the quality of the input feedstocks and the functionality of the digestion process (Chapter 3.2.). Both the benefits and weaknesses of the reject water quality are represented in this section.

4.6.1. Existing experiments

There exist several studies in which microalgae cultivation in reject water has been experienced (Table 10). However, reject water from a co-digestion process is not experienced, whereas reject water from digested municipal WW sludge is the most commonly experienced medium for microalgae cultivation. All the experiments that are collected in Tanle 10 have achieved positive microalgal growth results. *Scenedesmus* and *Chlorella sp.* constitute the mostly selected microalgal species for the experiments. The details of the studies e.g. inhibiting factors and pre-treatments for the cultivation medium are considered next.

Table 10. Existing experiments about the cultivation of microalgae in reject water or digested sludge from the literature.

Tested microalgal specie	Tested culture medium (RW)	Accorded study
Chlorella sp.	RW from a digested WW sludge	Rusten & Sahu (2011)
Chlorella sp., *Spirulina platensis*	Digested waste activated sludge (WAS) from a laboratory scale anaerobic digester	Yuan et al. (2012)
Chlorella sp., Scenedesmus obliquus *Botryococcus brauniiand*	RW from digested WW sludge	Ficara et al. (2014)
Scenedesmus	RW from digested piggery waste	Park et al. (2010)

4.6.2. Light transmittance

Generally, the transmittance of light is notable low and the color is dark in reject water generated at a biogas plant, which constitutes a challenging issue in terms of cultivation of microalgae. Reject water typically contains plenty of fine solids particles and has a high turbidity. Due to that, the availability of light decreases in a reject water culture medium and reduces the algal photosynthetic activity. (Markou & Georgakakis 2010, Rusten & Sahu 2011) For instance Yuan et al (2012) observed that the light availability limited the growth of microalga *Chlorella*. However, in several studies species *Chlamydomonas*, *Scenedesmus* and *Chlorella* has shown to be capable for heterotrophic growth by utilizing carbon from acetate, glucose and organic acids even in the dark (Tam & Wong 1996). Moreover, mixotrophic cultures may be more advantageous for cultivation under poor light conditions since they require less light for the growth and have higher metabolic activity (Markou & Georgakakis 2010). Therefore, a multicultural culture medium should be examined to clarify the benefits. The color of the reject water from thermophilic digestion

is usually darker compared to the reject water from a mesophilic process (Chapter 3.2). Thus, the reject water after mehophilic digestion can be more easily adapted by microalgae.

According to the previous, a separate treatment for improving light conditions in reject water is probably essential for a high-rate growth of microalgae. A suitable treatment must extract solid particles to improve light illuminance without a significant extraction of essential nutrients. (Rusten & Sahu 2011) Cho et al. (2011) founded that the filtration by filters with a pore-size of 0.45 μm or less resulted greater growth of *Chlorella sp. 227* in municipal wastewater from aeration tank. A combination of polymer addition, flocculation and filtration constitutes a practical solution as a separate treatment for the removal of suspended solids from reject water, which is tested in the study by Rusten & Sahu (2011). They reported that the specie *Chlorella sp.* grew efficiently in the previously mentioned undiluted reject water at a pilot-scale cultivation system in a greenhouse inside a WWTP.

Glass fiber filters (GF/C) have been successfully tested for reduction of TSS from reject water before its utilization as a culture medium (Park et al. 2010, Yuan et al. 2012). Also, membrane filtration (MF) has reduced effectively suspended solids from reject water (Wäger et al. 2010). Since a separate treatment for reject water requires investments e.g. polymer dosing equipment or filtrates the total costs for algae cultivation installation in reject water increase. A solution to improve light illuminance is a dilution of reject water with other nutrient-rich WW e.g. untreated municipal WW from the secondary treatment of the process (Ficara et al. 2014).

The reject water from digested sewage sludge at a WWTP may be more attractive compared to the reject water from a co-digestion plant due to more succesfull function of digestion process that leads to greater quality of reject water (Chapter 3.2). Furthermore, there exist studies in which the microalgae cultivation in reject water from a WWTP is succeeded without any separate treatments (Table 10). For example, Ficara et al. (2014) cultivated *Botryococcus brauniiand, Chlorella sp.* and *S. obliquus* in undiluted reject water from the belt-pressed WW sludge digestate and the microalgae reached efficient biomass growth. The reject water included smaller concentrations of nutrients and organic matter compared to the typical reject water from a co-digestion: e.g. COD was 470±127 mg/l that

is 8–20-fold smaller compared to the typical concentrations of reject waters from co-digestion biogas plants (Table 5 and 6).

The availability of light can also be challenging depended on season of the year and the generation of artificial light may cause remarkable costs (Manninen et al. 2015). A cultivation system with a shallow medium layer may be a functional solution for improvement of light availability in the reject water since the light intensity decreases with an increasing cultivation pond depth (Equation 10). However, shallower medium layer leads to increased culture medium area. The increased concentration of algae biomass also decreases the light availability in the culture medium. Thus, culture ponds and reactors must be designed for the maximal light demand conditions. (Polprasert 2007 p. 231) Certain functional cultivation installation is an algal turf scrubber i.e. immobilized algal system that is beneficial for species that are able to grow under environments lacking of surrounding water (Spilling 2016).

The light intensity at the depth of L is presented in the following reaction (Markou & Georgakakis 2010):

$$I_L = I_0 * e^{-\gamma L} \quad (10)$$

I_L is the light intensity at depth of L, L the depth of the pond, γ turbidity, I_0 the initial light intensity.

4.6.3. Temperature and pH

After the dewatering, the temperature of reject water can be high especially if the digestate is thermally hygienized after the mesophilic digestion process (Chapter 3.1.). Thus, reject water can offer a favorable temperature as a culture medium for microalgae at the facilities of the plant. Reject water possesses relatively high pH. If the reject water is ammonia stripped the pH will remain even higher. Since the optimal pH for microalgae occur around 7, reject water probably requires pH adjustment before the cultivation of microalgae in it. However, the beneficial pH value varies between microalgal species. A potential and economical method for lowering high pH could be CO_2 injection from flue gases or biogas (Lei et al. 2006). However, e.g. *Spirulina platensis* has shown to grow under high pH conditions (Yuan et al. 2012).

4.6.4. Ammonium and phosphorus

Typically, reject water contains ammonium nitrogen and phosphorus but the concentration varies depended on the quality of the feedstocks and the functionality of the process (Chapter 3.2.). The nutrient concentrations vary between various plants but also between digestion batches at an individual plant. In terms of microalgae cultivation, the variation of N and P concentrations can have a considerable influence to the cellular growth including the chemical content of the biomass since the availability of these nutrients is essential for microalgae.

Nitrogen is typically in a form of ammonium NH_4^+ in reject water and it constitutes a preferable source of nitrogen for many microalgae species (Chapter 2.2.2. and 3.2.4.). Furthermore, an increased ammonium nitrogen concentration has been noted to be connected increased biomass growth in the medium and protein content in the biomass. (Tam & Wong 1996) However, an excessively high NH_4-N concentration can be an inhibiting factor for algal growth (Tam & Wong 1996). Furthermore, a high pH level combined with an affluent amount of NH_4-N constitutes especially an inhibiting combination in the culture medium since then NH_4^+ exist mostly in form of free ammonia in the reject water based on the Henry's law (Equation 5, González et al. 2008, Tam & Wong 1996). However, the tolerance towards high NH_4-N or NH_3 concentrations is strongly depended on the characterizes of the individual algal specie and the culture conditions and therefore it can vary substantially (Tam & Wong 1996). *Chlorella vulgaris*, *Scenedesmus sp.* and *Euglena* have been reported to tolerate high ammonia concentrations (Park et al. 2010, Tam & Wong 1996, Yuan et al. 2012). Tam & Wong (1996) reported that pH changed acidic during the exponential phase of cultivation of *Chlorella sp*. in the NH_4-N concentrated medium. This perhaps indicates about the capability of *Chlorella sp.* to avoid the formation of the inhibiting amount of free ammonia by itself.

A reject water dilution with other WW can be a solution for decreasing inhibiting amount of NH_4-N concentration. For example, a dilution with an effluent from nitrification phase in a WW treatment process may lead to more adaptable NH_4-N concentration for algae (Yuan et al. 2012). In addition, a dilution can balance an unbalanced N/P ratio and decrease the total solid concentration in reject water. Nevertheless, an unbalanced N/P ratio may have a negligible effect to nitrogen or phosphorus removal by microalgae (Wang et al. 2010). In the experiment by Ficara et al. (2014) *Chlorella* and *Scenedesmus* showed to

grow greatly in the mixture of reject water from digested WW sludge and sand-filtered wastewater from a WWTP.

The importance of the presence of both N and P is essential but the optimal relative ratio (e.g. by The Redfield ratio) has been founded to be inconsequential (Chapter 2.2.5.). However, a lack of P has been reported to cause lower cell densities (Markou & Georgakakis 2011). Therefore, the presence of sufficient amount of P is essential for the maximal biomass productivity and further biogas production. Neverthless, e.g. *Anabaena variabilis* can adapt phosphorus-weak conditions by increasing its carbohydrate content and decreasing its protein content (Markou & Georgakakis 2011). This can be beneficial in terms of co-digestion with microalgal biomass since a co-feedstock with high protein content is unattractive due to risk of nitrogen inhibition (Yen & Brune 2007).

4.6.5. Contamination and competition

Reject water from a digestate from thermophilic conditions usually contains less pathogens compared to reject water originated from a mesophilic process (Chapter 4.5.). For example, unhygienized reject water originated from municipal WW sludge in Viikinmäki WWTP with a mesophilic digestion reactor may include *Legionella bacteria* (Kuokkanen 2016). Reject water can be hygienized by various physical or chemical methods but they are often expensive for small plants since disinfection technologies are energy intensive or require a large amount of chemicals (Schumacher et al. 2003).

Wastewaters generally contain pathogens and other bacteria that can be harmful for living organisms such as human and algae (Polprasert 2007 p. 222). A presence of them can reduce significantly the potential biomass productivity of certain species. For example, *Chlorella sp. 227* reduced its biomass production significantly when the wastewater from aeration tank included bacteria and protozoa in the experiment by Cho et al. (2011). The growth was more rapid after the tested WW was radiated by a strong UV-B dose.

Wastewaters typically include organic compounds that create a beneficial medium for other microorganisms such as heterotrophic bacteria and fungi, which may cause contamination issues for algae. Therefore, it might be infeasible to achieve maximal algal biomass productivity by treating reject water with algae. (Cho et al. 2011, Muñoz & Guieysse 2006) Furthermore, in the experiment by Cho et al. (2011) the TOC

concentration was increased due to extracellular organic compounds released after the cultivation of *Chlorella sp. 227*.

Li et al. (2011) compared the algal growth in an autoclaved centrate from an activated sludge process and in a raw centrate. In the study microalgal biomass increased more effectively in the autoclaved centrate, which was probably due to the extermination of the competitive bacteria. The injection of CO_2 has shown to decrease bacteria, which could prevent injections and increase stableness in the reject water (Min et al. 2011). Nevertheless, so called algal-bacterial symbiosis that is presented in Figure 24 can have also advantages since by-products of both bacteria and algae are utilized creating a cycle system between these organisms (Polprasert 2007 p. 226).

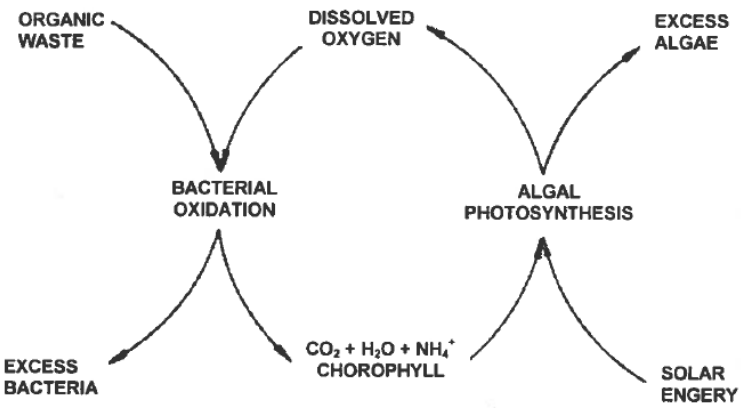


Figure 24. The cycle of the "algal-bacterial symbiosis" (Oswald and Gotaas 1957).

A microalgal culture can also destruct pathogens by itself in WW. Firstly, due to photosynthetic reactions pH can vary diurnal and create toxic conditions for pathogens. Secondly, some algae species can produce toxic compounds in their cells. Thirdly, the growth of microalgae increases the dissolved oxygen concentration (DOC), which can inhibit some bacteria. (Muñoz & Guieysse 2006, Polprasert 2007 pp. 222–223)

Diverse microalgal communities have potential to be more stable than monocultural communities in unsterile conditions (Sambusiti et al. 2015). A multicultural community can be more resilient to e.g. fungal infections and pathogens. Moreover, some microalgal species can create mutualistic relationships between each other and produce essential compounds to each other. (Stockenreiter et al. 2016) Moreover, since various species possess different nutrient requirements, a multiculture can reduce nutrients efficiently from

wastewaters (Ogbonna et al. 2000). Schumacher et al. (2003) reported that the concentration of pathogen *E.coli* was reduced by an algal biofilm that included various algal species such as *Ulothrix*, *Stigeoclonium* and *Chlamydomonas* and *Oscillatoria*. The high pH was observed to improve the reduction. According to the study, the cultivation of microalgae in WW can also be a cost-effective option as a biological disinfection treatment.

According to the previous the tolerance of algae towards to bacteria and pathogens is specie-depended. The advantages of the multicultural cultivation should be examined to solve a beneficial combination of various species for the maximal stableness and tolerance towards injurious organisms.

4.6.6. Positive effect of aeration and injection of CO_2 into reject water medium

Aeration has shown to improve the cell density of *Scenedesmus sp.* in a NH_4-N-rich culture medium in the study by Park et al. (2010) (Figure 25). Both dissolved oxygen (DOC) and NH_4-N concentration were decreased in the medium due to a stripping effect followed by aeration. However, energy-intensive aeration constitutes one of the most costly device installations in WW processes (Clippeleir et al. 2011).

An exessively high DOC in the culture medium can inhibit regular photosynthesis reactions and carbon fixation. Also, a high DOC can generate photo oxidative damage on algal cell and decrease growth rate as well as the uptake of nutrients by algae. (Nguyen 2009, Muñoz & Guieysse 2006) Heterotrophic bacteria consume O_2 continuously and therefore the supersaturation of the DOC might be unreachable in reject water. However, the DOC has been increased after the pollutants have been depleted in a culture medium, which can be an useful indicator in continuous treatment processes. (Muñoz & Guieysse 2006)

Microalgae require inorganic carbon typically in a form of CO_2 for the growth and therefore the cultivation can operate as a carbon sink and decrease the amount of greenhouse gas emissions. CO_2 can be purchased from e.g. produced biogas from an anaerobic digestion plant or flue gases from the energy production in a CHP-plant. The CO_2 capture from biogas also purifies the generated biogas to purer methane. (Sialve et al. 2009) The injection of CO_2 from flue gases to a microalgae cultivation installation is

feasible based on studies e.g. by Chinnasamy et al. (2009), González-López et al. 2012 and Sonck (2010). As high as 80% absorption rate of CO_2 from the flue gases was achieved with the biomass production of *Anabaena sp.* (González-López et al. 2012).

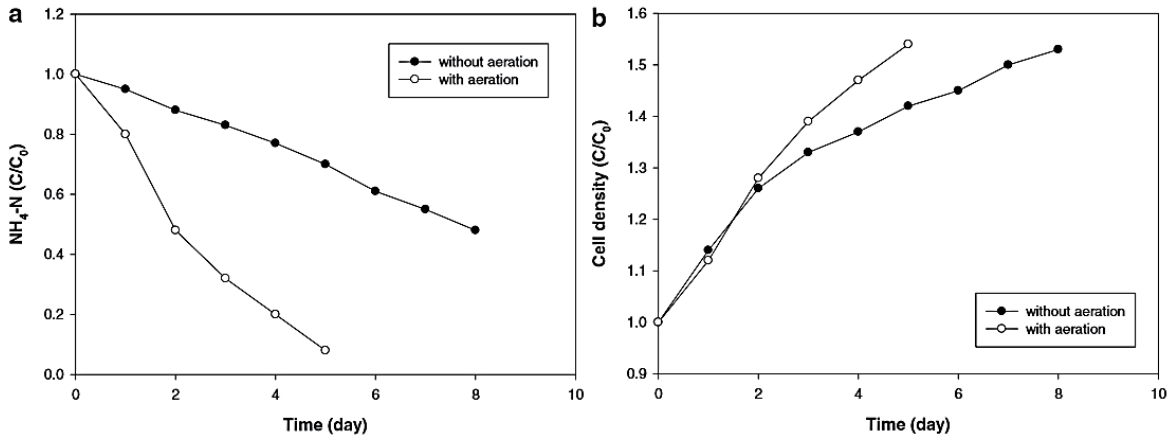


Figure 25. Effect of the aeration to (a) the ammonia uptake and (b) the cell density of *Scenedesmus accuminatus* in wastewater from a digestate effluent from a piggery. The microalgae were cultivated in a cylindrical glass reactor. (Park et al. 2010)

Chinnasamy et al. (2009) observed that the biomass dry weight of *Chlorella vulgaris* was 20-fold after the CO_2 injection compared to a dry weight under ambient CO_2 concentration. The CO_2 concentration in which microalgae can achieve the highest growth rate is specie depended. The injection of CO_2 had a negligible effect on the biomass productivity and nutrient removal of *Chlorella sp.* in undigested wastewater sludge from a WWTP, which was observed by Min et al. 2011. The possible positive effect of CO_2 injection according to the study is that it suppresses the bacterial growth. Due to that, the CO_2 injection can help to avoid infections and increase the stableness of the culture.

Lei et al. (2006) showed the injected CO_2 decreased pH value from 11 to around 7 in the ammonia stripped reject water. Thus, the reject water can be more attractive as a culture medium for microalgae after the injection of CO_2. Furthermore, a continuous CO_2 supply can prevent a possible increase of pH after photosynthesis reactions, which will prevent the formation of inhibiting amount of free ammonia in reject water.

4.6.7. Effect to new reject water after co-digestion of algal biomass

The culture growth will be affected in a new reject water medium if the algal biomass is utilized as a co-feedstock in a digestion process (Sahu et al. 2013). The effects to the composition of the new reject water should be investigated by a pilot project in order to

determine possible changes in the nutrient concentrations and other quality components. In the literature there exist poorly investigations about the topic. However, Yuan et al. (2012) reported that the co-digestion of *Spirulina platensis* biomass improved dewaterability of digestate when the algal biomass share was 5 or 15 % of the total feedstocks, which was shown by decreased capillary suction time (CST) of the digested mass compared to the CST of the digested WAS alone. However, the co-digestion with *Chlorella* affected negatively to the performance of the sludge dewatering. Hence, the specie selection must be done carefully since the microalgal biomass digestion may have an influence both to the amount and quality of the generated reject water. Furthermore, the possible chemical for improving dewaterability must be choosen carefully since the function of the chemical may depend on the chemical content of the microalgal biomass.

4.6.8. Removal of nutrients and other impurities by microalgae

The potential of the nitrogen removal by microalgae is attractive since the N concentrations are discovered problematic in reject waters (Chapter 4). However, an excessively high NH_4-N concentration probably leads to the collapsion of the algal culture. Also, a phosphorus removal by biological applications has been an interest at the municipal wastewater treatment sector (Pauli & Kaitala 1995). The nutrient uptake by the microalgal culture can be an alternative for both existing biological nitrogen removal treatment and chemical precipitation of phosphorus at WWTPs. Its functionality, however, requires plenty of investigations since e.g. especially algal biomass harvesting has been encountered challenges at the end of the treatment process (Ruiz-Marin et al. 2009). Based on the nutrient reduction rates from literature (Table 11) microalgae utilize more efficiently of the TN (77–90 %) than the TP (63–81 %), which supports the fact that the algal biomass contains narrowly phosphorus (Chapter 2.2.3). *Chlorella vulgaris*, *Chlorella sp*. and *Scenedesmus* have shown to remove NH_4-N efficiently (76–95 %), which indicates that NH_4-N is a pleasant source of nitrogen for these species. Low nitrite and nitrite concentration indicates that atleast a nitrification reaction is not the reason for a NH_4-N decrease in a culture medium, which proved that *Chlorella vulgaris* decreased the concentration in the study by Tam & Wong (1996).

Table 11. Nutrient removal efficiencies by microalgae cultivation in various wastewaters in the literature.

Strain	Culture medium			Removal of:				Reference
	Waste water	Treatment	Cultivation days	NH_4-N	PO_4^{3-}-P	Total N	Total P	
Chlorella vulgaris	Primary effluent from WWTP	A vacuum filtered with 0,45 µm filter paper	14	95 %	61 %	-	80,9 %	Williams (2012)
Chlorella sp.	Effluent from activated sludge thickening process	Settled, filtered and autoclaved	14	93,9 %	-	89,1 %	80,9 %	Li et al. (2011)
Scenedesmus and Chlorella (mixture)	reject water from dewatered digested wastewater sludge	Raw	10	-	-	77–82%	-	Ficara et al. (2014)
Chlorella sp.	Digested dairy manures	Diluted and filtered by glass microfiber filters	7	75.7–82.5 %	-	-	62.5–74.7%	Wang et al. (2010)
Scenedesmus	Anaerobic digestion effluent from piggery	GF/C filtered and autoclaved	5	89 %	-	-	-	Park et al. (2010)
Chlorella sp.	RW from digested activated sludge	Polymer addition and filtration	4	80–90 g kg TSS	3,83 g/ kg TSS of grown biomass	-	-	Rusten & Sahu (2011)

The microalgal growth releases oxygen that may oxidizes BOD followed by the growth of heterotrophic bacteria. The oxygenation rates of 0.48–1.85 kg O_2 $m^{-3}d^{-1}$ have been achieved in a pilot-scale pond and laboratory-scale photobioreactor in which microalgae have been treating various wastewaters. Hence, the microalgae cultivation also potentially decreases the BOD concentration. (Abdel-Raouf et al. 2012, Gonzáles et al. 2008)

In the experiment by Wang et al. (2009) *Chlorella sp.* removed metal ions, especially Al, Ca, Fe, Mg, and Mn in WW from an activated sludge process. Certain algae are also capable to remove synthetic dyes that can be toxicity, carcinogenicity and harmful for the ecosystem. For example, textiles, food, cosmetics and paper printing constitute sources of synthetic dyes. (Mostafa et al 2009) Also, algae have potential to remove other harmful compounds such as drug residuals and heavy metals from WWs, which can solve current

issues with them in the WW technology. For example, strains *Scenedesmus sp.*, *Chlorococcum sp.*, *Chlorella vulgaris var. vulgaris* and *Fischerella sp.* have achieved over 90 % uptake rate of mercury (Hg). Also removal efficiencies of 89 % and 88 % of cadmium (Cd) and lead (Pb), respectively, can be achieved by *Chlorella vulgaris var. vulgaris*. (Inthorn et al. 2002) Moreover, microalgae can be feasible to remove antibiotic substrances due to their catabolic systems, capability for carbon fixation and heterotrophic growth (Xiong et al. 2016).

5 METHODS OF THE LABORATORY EXPERIMENTS

This section explains the objects and used methods in the laboratory experiments that were operating at the Marine Research Centre at the Finnish Environment Institute in Helsinki, Finland at the time period from May 2016 to September 2016.

5.1. Objects

Firstly, the purpose of the experimental part was to clarify if microalgae can grow in reject water, which was executed by cultivating various microalgal cultures in reject water and discovering the most adabtable species. Secondly, the other object was to clarify inhibiting or limiting factors that possibly prevent the effective microalgal growth in reject water. Thirdly, separate treatments for improving the quality of reject water to a more beneficial culture medium for microalgae were investigated. Finally, the potential of microalgae to remove nutrients from reject water was briefly considered.

5.2. Sampling description and sample analysis

The first reject water sample was purchased from a co-digestion plant owned by Envor Group Oy. The digestion process operates under a mesophilic temperature and it utilizes various biowastes and agricultural wastes for the biogas production. The total solid content of the feedstock material is approx. 13%. The generated digestate is hygienized under 70 °C conditions for an hour before the dewatering. The digestate is dewatered by spin driers, which generates a liquid effluent i.e. reject water. The reject water or alternatively the overall digestate is utilized as a fertilizer for agricultural fields. However, when the cropping period is over in Finland the generated reject water is leaded to the ammonia stripping and flotation tank in which ferric sulfate (PIX-105) is added. After the flotation tank, the settled reject water sludge is utilized as a phosphorus-rich fertilizer. (Juhe 2016, Paajanen 2016)

The reject water sample for the experiments was ammonia stripped and it was collected immediately after stripping. The color of the sample was extremely dark, close to black. The pH, nutrient and other impurity concentrations were analyzed at Häme University of Applied Sciences (HAMK) (Table 12).

The other reject water sample was purchased from a Viikinmäki WWTP owned by Helsinki Region Environment (HSY). The WWTP produces biogas from municipal WW sludges from their WW treatment process. In addition to WW sludge, Viikinmäki digests coincidentally fat wastes from restaurants and other food waste sludges from the food industry. (Kuokkanen 2016) The total solid content is approx. 3,4 % of the total feedstock mass (Fred 2013). The digestion process operates under mesophilic temperature conditions. Reject water is generated in the dewatering process of the digestate by spin driers. Thereafter, reject is leaded to the settling tank. The produced reject is recycled back to the WW treatment process after the settling. The dewatered digestate is utilized as a fertilizer after it is composted. Currently, Viikinmäki WWTP is piloting an ANITA™ Mox process as a separate reject water treatment (Figure 18). (Kuokkanen 2016)

The reject water sample for the experiments was collected from the settling tank (Figure 26). MetropoliLab analyzed the pH, nutrient and other impurity concentrations in the sample (Table 12). The reject water was dark but the color was lighter compared to the sample from Envor Group Oy. NH_4-N concentration was 5,7-folded and PO_4-P was only a tenth of the concentration in reject water from Envor Group Oy. The low PO_4-P is probably due to that Viikinmäki removes phosphorus by addition of ferrous sulfate in their WW treatment process. Appendix 3 represents seven reject water composition analyses on Juny 2016. The variation of the concentrations can be remarked.

Figure 26. The settling tank where the RW sample was collected at the Viikinmäki WWTP.

Table 12. Data of the RW samples from Envor Biotech biogas plant and Viikinmäki WWTP.

Digestion process type	Name of the plant	Location (City, country)	Digestion process type	Name of the plant	Location (City, country)
co-digestion	Envor Group Oy a co-digestion plant	Forssa, Finland	wastewater sludge digestion	Viikinmäki wastewater treatment plant	Helsinki, Finland
Sample 1			**Sample 2**		
Measurement	Value	Analysis method	Value	Analysis method	Uncertainty-% (only for the measurement of Sample 2)
$COD_{Cr,soluble}$ (mg/l)	-	-	1500	ISO 15705:2002	± 15
NH_4-N (mg/l)	120	Kjehdahl/Foss Kjeltec 2300	679,578	ISO 7150: 1984	± 15
PO_4-P (mg/l)	8	Hach Langen LCK 349 cuvettes	0,846	SFS-EN ISO 6878: 2004	± 15
TN (mg/l)	-	-	1003,555	SFS-EN ISO 11905-1	± 15
TP (mg/l)	25,50	Hach Langen LCK 349 cuvettes	11,461	SFS 3026 mod. DA	± 15
Suspended solids (SS) (mg/l)	-	-	980	SFS-EN 872:2005	± 10
Alkalinity (mmol/l)	-	-	57,41	SFS-EN ISO 9963-1:1996	± 10
pH	9	Hanna Instruments pH meter	8,04	SFS 3021:1979	± 3
N/P ratio	33	Calculated	1776	Calculated	-

5.3. Microalgal strain inoculants

Specie inoculants were cultivated in the artificial MWC (Modified WC Medium) culture medium except inoculants of *Euglena Gracialis* and *Coccomyxa* that were cultivated in the artificial MAM (Modified Acid Medium) culture medium. The compositions of these artificial mediums are represented in Appendix 4 and 5. The inoculant cultures were cultivated in plastic cell culture flasks. The diurnal light supply during cultivation was the following: 16 hours under light and 8 hours in the dark. The surrounding temperature was approx. 23 °C. The microalgae species were pipetted from the inoculant flasks into the containers during the experiments: either Erlenmeyer flasks or microplate wells. Table 13 represents all the tested microalgae strains in the experiments.

Table 13. Thirteen tested microalgae strains in the experiments. The algal cultures were axenic.

Label	***Strain name* / Code**	**Origin**
EG.	*Euglena gracialis* / CCAP 1224/5Z	Culture Collection of Algae and Protozoa (CCAP)/ Scottish Marine institute/
CC.	*Coccomyxa* / CPCC 508	Canadian Phycological Culture Centre (CPCC) / University of Waterloo in the Department of Biology
A	*Scenedesmus obliquus Kützing (S. obliquus)* / 276-10; SAG	Culture collection of Algae /SAG)/Göttingen/Germany
B	*Selenastrum capricornutum Printz* / 37; UTCC	University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC)/Canada
C	*Golenkinia brevispicula Hegewald et Schnepf* /4.81; SAG	SAG/Göttingen/Germany
D	*Haematococcus pluvialis Flotow em. Wille* / 34-1l; SAG	SAG/Göttingen/Germany
E	*Pediastrum simplex Meyen* / 21.85; SAG	SAG/Göttingen/Germany
4	*Monoraphidium contortum (Thuret)* / 2012/NATV1/C2	Judita Koreiviene/ Nature Research Centre/Lithuania
6	*Sorastrum spinulosum Nägeli* / 2012/ZAD1/E8	Judita Koreiviene/Nature Research Centre /Lithuania
11	*Crucigeniella apiculata (Lemmermann*) / 2013/SPE1/B7	Judita Koreiviene/Nature Research Centre /Lithuania
14	*Crucigenia tetrapedia W. et G.S. West* / 218-3; SAG	SAG/Göttingen/Germany
20	*Scenedesmus quadricauda* / 2014/KDTV1/C2	Judita Koreiviene/Nature Research Centre /Lithuania
25	*cf. Chlorella sp.*/ 2014/KDTV1/B5	Judita Koreiviene/Nature Research Centre/Lithuania

5.4. Hygienization

Reject water sample from the Viikinmäki WWTP was hygienized thermally on a hotplate heater (Heidolph MR3001K, Germany) with magnet stirring at the temperature of 70 °C for an hour to exterminate possibly presenting *Legionella bacteria* before handling it in the laboratory in the cultivation experiments. *Legionella bacteria* dies in 32 minutes at a minimum temperature of 60 °C (Courtesy of Ron George 2013). However, the hygienization time of one hour was chosen since digested sludge is often thermally hygienized at temperature of 70°C for an hour to exterminate possible pathogens at Finnish mesophilic biogas plants (Partanen p. 18).

5.5. Separation of solid particles by a centrifuge

A conventional GF/C filtration was unworkable for the reject water of Envor Group Oy since the membranes blocked instantly. Thus, a centrifuge was used for separation of solid particles in order to remove solid particles and improve the light illuminance in both reject water samples during a part of microplate tests. The used centrifuge model was Rotanta 460R (Germany). The samples were centrifuged inside centrifuge tubes of 50 ml. A reject water sample volume of 45–50 ml were centrifuged for an hour with a rotational speed of

11 500 RPM. The used input program was 48 and the temperature was set to 22 °C. Both input of acceleration level and input of braking level were set to 9. The used rotor was a 6-place 45° angle rotor. The separated liquid was poured into the other container after the centrifugation.

5.6. Increase of N/P ratio

Since the N/P ratio was extremely high in the reject water from Viikinmäki WWTP external phosphorus was added to raise the N/P ratio to the beneficial of 16 following the Redfield ratio (Chapter 2.2.5.). Solid $NaH_2PO_4 \cdot H_2O$ was used as a source of external PO_4-P. In terms of ammonia stripped samples, a dilution of $NaH_2PO_4 \cdot H_2O$ was used as a source of external P. The dilution was prepared diluting solid $NaH_2PO_4 \cdot H_2O$ into MQ-water. The final concentration of the dilution was 0,182 $mmol_{NaH2PO4}/l$.

5.7. Ammonia stripping

The purpose of the ammonia stripping experiment was to clarify if the high ammonium nitrogen concentration constitutes the inhibiting factor in the reject water from the Viikinmäki WWTP. Since ammonia stripping requires only a high pH with a presence of high temperature, the operation was simple to execute at the laboratory scale.

The ammonia stripping experiment began with the pH adjustment. The pH of the hygienized samples was adjusted to around 11 since then approx. 98 % of the NH_4-N was supposed to be in the gaseous form of ammonia NH_3 along with Henry's law (Equation 5, Figure 13). A strong NaOH dilution of 2M was used as an alkaline for increasing pH value. Stripping experiments were operated under a temperature of 60 °C and with operational times of four (4), six (6), eight (8) and fifteen (15) hours. The used hotplate for heating samples in to 60 °C was Heidolph MR3001K (Germany) that included also a feature for magnetic stirring. After stripping experiments the pH of each samples were adjusted to approx. 7,1–7,2 by the addition of hydrochloric acid HCl if the samples were used for microalgae cultivation.

5.8. pH instrument and adjustment

The 780 pH meter by Metrohm (Switzerland) with a program version 5.780.0020 was used for measuring pH from the reject water samples and culture mediums during the experiments. The pH values were raised by the addition of strong 2M NaOH whereas they were lowered by the addition of strong 2M HCl. The pH conditions in the reject water

culture mediums were adjusted into 7,1–7,2 before filling them into the Erlenmeyer flasks or pipetting into the microplate wells. However, the pH conditions were perhaps higher or lower in the various reject water dilutions compared to the adjusted pH in the undiluted reject water sample.

5.9. Instruments for measuring algal growth

AquaPen-C AP-C 100 made by Photon Systems Instruments (Germany) was used for measuring photosynthetic parameters for estimating the microalgal growth (Figure 27). This instrument is a fluorometer which measures photosynthetic parameters in algal or cyanobacterial cultures by applying blue light with the excitation wavelength of 455 nm and red-orange light with the emission wavelength of 620 nm (Photon Systems Instruments 2016, Equipements Scientifigues SA 2008). The light pulse intensity was 3000 µmol m^{-2} s^{-1}. The measured data was managed by FluorPen 1.0 software. The used measurement was OJIP in which following measured parameters were under the scope: F_0, F_V and F_M (Table 14). The principle of the O-J-I-P reaction is explained in the Chapter 2.4. However, the main indicator for the monitoring microalgae growth was F_V/F_M ratio. It determines the photosynthetic capability in the culture medium by determining the efficiency of the PSII (Bowker et al. 2002). It also illustrates the stress position and follows the intensity of microalgal cell reproduction in the culture. A high F_V/F_M ratio (>0,3) indicates about the effective growth. (Piiparinen 2016) The growth rate can be calculated with the following equation (Zwietering et al. 1990):

$$\mu = \frac{lnNt - lnN0}{t - t0} = \frac{lnFMt - lnFM0}{t - t0} \quad (10)$$

,in which µ = growth rate, N_t = number of organisms at a time t, N_0 = initial number of organisms, F_{Mt} = maximal fluorescence intensity at a time t, F_{M0} = initial maximal fluorescence intensity at the time 0. The growth rate can be clarified from the plot of lnF_M/lnF_{M0} values as a function of time. The growth rate is the slope of the trend line during the exponential growth phase.

AquaPen measurements were used for considering the algal growth in the cultures that were growing in Erlenmeyer flasks. AquaPen can measure liquid samples from cuvettes of 4 ml. Approximately 3,5 ml of the microalgal culture medium was poured into a cuvette on each measurement time. The cuvette samples were dark-adapted 10 minutes before the

fluorescence measuring since then the RCs of the PS II were completely oxidized (Strasser et al. 2004).

Table 14. AquaPen-C AP-C 100 measurements which were under the scope during the experiment. RFU is the relative fluorescence unit. The Fv/Fm ratio is unitless. (Equipements Scientifigues SA 2008)

Parameter	**Description**
F_0 [RFU]	Initial fluorescence, fluorescence intensity at 50 µs
F_M [RFU]	Maximal fluorescence intensity, unit
Fv [RFU]	F_M-F_0, maximal variable fluorescence
F_V/F_M	Determines photosynthetic capability that performs the quantum efficiency of the PSII. The parameter estimates the dark adaption quantum yield. (Bowker et al. 2002) It also illustrates the stress position and an increasing ratio indicates about cellular growth in a culture medium (Piiparinen 2016).

The fluorescence spectrophotometer for measuring algal growth in culture mediums growing on 96-well microplates was Cary Eclipse 6257 by Varian Inc. (USA) (Figure 27). The instrument scanned wells by a microplate reader. The mode of the operation was fluorescence intensity that measured the parameter for determining the maximal fluorescence F_M. The set excitation and emission wavelengths were 450 nm and 680 nm, respectively. Both excitation and emission slits were 5 nm. The PMT Voltage was set to medium (600 volts) and the average measurement time was 0,1 seconds.

Figure 27. Used instruments for estimating fluorescence in the algal cultures. AquaPen-C AP-C 100 by Photon Systems Instruments (on the left) and Cary Eclipse 6257 by Varian Inc (on the right). Photographed by Sara Merin.

5.10. Erlenmeyer flask setups

The Erlenmeyer flasks (250 ml) were filled with a volume of 200 ml reject water. Various dilution mediums were prepared by mixing Milli-RO water with the reject water. The inoculum volumes that were pipetted into each Erlenmeyer flasks varied between 1–5 ml depending on the specie. Also a multicultural medium of five species was tested in which the overall inoculum volume was around 5 ml. In addition, an Erlenmeyer flask was filled with the reject water without any specie inoculum for operating as a blank sample for a comparison. After the Erlenmeyer flasks were filled, cotton wool was added as a flask cap to reduce the risk of the contamination and possible odors. The Erlenmeyer flasks were set on a glass bond (length: 120 cm, width: 36 cm) that was lit by a fluorescent lamp (OSRAM L58W/965, Germany) during the cultivation period (Figure 28). The surrounding temperature was approx. 23 °C. The Erlenmeyer flask cultivation setups were operated in a laboratory room on the second floor of the Marine Research Centre building.

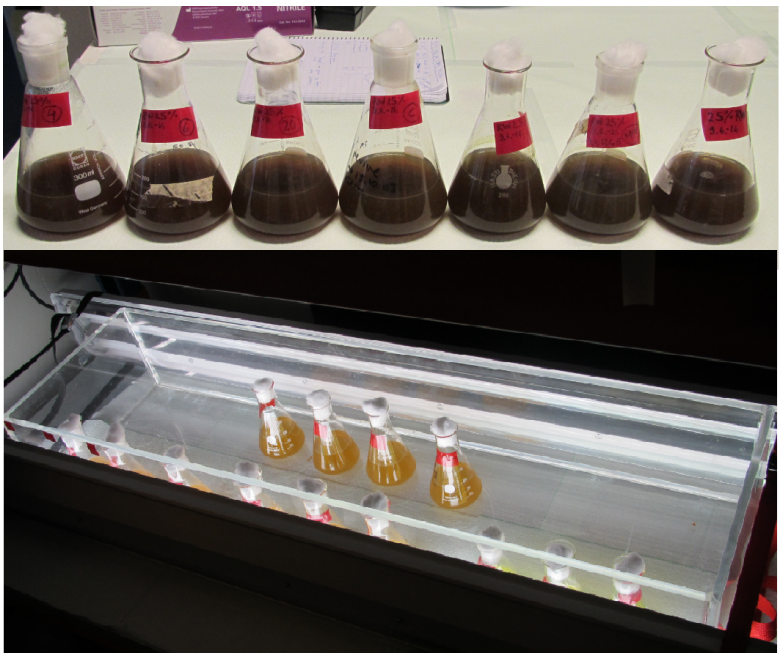


Figure 28. Cultivation setup was simple: an Erlenmeyer flask filled with the 25 % RW and strain inoculum. Cultivation was operating under on a lit glass bond without surrounding water (below). Above cultures in the RW from Envor Group Oy and below various experiments in RW from Viikinmäki. Photographed by Sara Merin.

5.11. 96-well microplate setups

The microplate setups were operating on 96-well (8 x 12) microplates with flat bottoms. The well rows were filled in various reject water dilutions (10–100%), Milli-RO-water or

artificial culture medium. The Milli-RO well (11th column) operated as a blank sample column. The 12th well column was filled with the artificial culture medium (MWC or MAM) with a pipetted specie inoculant. The volume of 20 µl of each microalgal inoculant was pipetted into each well except into the blank Milli-RO well. An overall culture volume in a well was 370 µl except the blank ones that were 350 µl. The well order with the various fillings is represented in the Table 15. The order was applied to all microplate experiments.

Each microplate cultivation test was operating similarly in a windowless climate room at the basement floor inside the Marine Research Centre building. The temperature was set to 20 °C during a cultivation period in the climate room. The diurnal artificial light supply cycle was the following: 16 hours under the light and 8 hours in the darkness. The light conditions were organized for the maximal light supply for the microalgae by setting microplates right underneath above the light. This setup enabled to consider microalgal growth in various reject water dilutions simply due to minimized culture medium volume. Also, fluorescence measuring was simplier and faster with the microplate reader than from Erlenmeyer flasks.

Table 15. Various RW dilutions in the microplate wells. 11th and 12th columns includes blank and medium samples.

Column	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
RW Dilution	**10 %**	**20 %**	**30 %**	**40 %**	**50 %**	**60 %**	**70 %**	**80 %**	**90 %**	**100 %**	**Blank**	**Medium**
RW (µm)	35	70	105	140	175	210	245	280	315	350	-	-
RO-water (µm)	315	280	245	210	175	140	105	70	35	0	350	-
Inoculum (µm)	20	20	20	20	20	20	20	20	20	20	0	20
MAM/ MWC	-	-	-	-	-	-	-	-	-	-	-	350
Total volume (µm)	370	370	370	370	370	370	370	370	370	370	350	370

5.12. Nutrient analysis

Nutrient concentrations were analyzed for the clearance of both ammonia stripping efficiency and the possible uptake of nutrients by *S.oblicuus* in the ammonia stripped reject water from Viikinmäki WWTP. The analyzed concentrations were NH_4-N, PO_4-P, TP, TN and NO_3+NO_2. The analyses were executed at the Marine Research Centre of the Finnish Environment Institute in Helsinki, Finland. Table 16 represents the applied analysis methods for the determination of the nutrient concentrations. The methods are developed for analyzing escpecially seawater and therefore the results may are uncertain

Table 16. Applied nutrient concentration analysis methods.

Nutrient	Analysis method
NH_4	A method developed by Koroleff (1983). It is based on the standard SFS 3032. The used method differs slightly from the original: threonine operates as a donator of active chlorine instead of hypochlorite.
NO_3+NO_2	A method (QuikChem Method 31-107-04-1-A) that has been developed for Lachat device by Lachat Instruments (1997).
TN	A co-boil with peroxomonosulfate in an alkaline solution (Koroleff, 1977 ja Grasshoff et al., 1999). Analyzed with FIA device.
PO_4	A method (QuikChem Method 31-115-01-3-A, 1998) that has been developed for FIA device by Lachat Instruments. Determination of phosphorus by flow injection analysis colorimetry.
TP	Co-boiling with peroxomonosulfate in an alkaline solution (Koroleff, 1977 ja Grasshoff et al., 1999). Analyzed with a FIA device.

Ammonia stripping efficiencies were determined from a sample that was stripped with a volume of 200 ml under temperature conditions of 60 °C. A sample was pipetted at an operation time of 2, 4, 6, 8 and 15 h from the sample during the stripping time. The NH_4-N concentration in each sample of the operational time was under the scope.

The nutrient uptake was analyzed from three culture mediums of *S. obliquus* that were cultivated in Erlenmeyer flasks. The cultivation period was 27 days. The mediums were stripped for fifteen (15) hours. The first sample was undiluted. The second sample was also undiluted but it included addition of external phosphorus. The third sample was diluted into reject water concentration of 50 %. The growth results of *S. obliquus* during this setup

are considered in Chapter 6.2.6. The culture mediums were centrifuged for removal of algal biomass and other solid particles before nutrient analyses since they can disturb the used nutrient analysis methods.

5.13. Flocculation

The flocculation setups with polymers were based on the sludge flocculation method by Aaltonen (2013). All the tested flocculant and coagulant chemicals were acquired from Kemira that is a global chemical company. The polymer chemicals were sample-size of 0,1 l. 0,1 g of each solid polymer was diluted in 100 ml of Milli-Q water. Hence, the final concentration of each dilution was 1000 ppm. The polymer dilution containers were DURAN glass flasks (100 ml). The dilutions were stirred for 3 hours to ensure that all the added polymer particles were diluted. Magnetic stirrers Heidoplh MR Hei-Mix S or Heidolph MR3001K were used for stirring the chemical dilutions. The coagulant C-577 was ready in a diluted water form with a polymer concentration of 48–52 %. Also it was diluted into the 1000 ppm for the experiments. Ferric sulfate PIX-105 was diluted to the concentration of 25 V-%. Table 17 represents tested chemicals and information about the prepared dilutions.

The first flocculation setup was the following: four graduated cylinders of 50 ml were filled with diluted or undiluted reject water. The prepared polymer dilutions were pipetted into these graduated cylinders samples. A flocculation dosage of 16 ppm was adapted according to Aaltonen (2013) since this dosage in her thesis resulted to great flocculation efficiency in the sludge. After this, the graduated cylinders were mixed turning them upside-down 3 times.

The other setup was following: decanter glasses were filled with diluted or undiluted reject water. The samples were stirred strongly by magnet stirrers Heidoplh MR Hei-Mix S. The PIX-105 (ferric sulfate) dilution of 25 % was added with various dosages into the decanter glasses with simultaneously stirring. After the addition, the stirring was interrupted and the samples were settled for an hour for considering the possible settling effect of the formed solid particles. This setup was not adabted from Aaltonen (2013)

Table 17. Tested flocculants produced by Kemira.

Composition	Commercial product name by Kemira	Type	Relative charge	Density
anionic polyacrylamide	SUPERFLOC A-100	Flocculant	Low	650–850 kg/m^3 (bulk)
anionic polyacrylamide	SUPERFLOC A-120V	Flocculant	Medium	650–850 kg/m^3 (bulk)
anionic polyacrylamide	SUPERFLOC A-150	Flocculant	High	650–850 kg/m^3 (bulk)
cationic polyacrylamide	SUPERFLOC C-491 VP	Flocculant	very low	750 kg/m^3 (bulk)
cationic polyacrylamide	SUPERFLOC C-492HMW	Flocculant	very low	750 g/l (bulk
cationic polyacrylamide	SUPERFLOC C-494HMW	Flocculant	medium	750 g/l (bulk)
cationic polyacrylamide	SUPERFLOC C-496HMW	Flocculant	high	650–850 kg/m^3 (bulk)
cationic polyacrylamide	SUPERFLOC C-498HMW	Flocculant	very high	750 kg/m^3 (bulk)
aquenous solution of polymeric quarternary amine (48–52 %)	SUPERFLOC C-577	Coagulant	-	1100–1200 kg/m^3
ferric sulfate 35–45 %, ferrous sulfate 0,1–1,5 %, manganese sulfate 0,1–0,25 %, sulfuric acid 0,1–1,5 %	KEMIRA PIX-105	Flocculant	-	1,45-1,55 g/cm³

6 RESULTS

The section presents the relevant results of the experiments at the laboratory. The results mainly discuss microalgae growth efficiency that is based on both fluorescence measurements and photographs. Finally, the final discussions concluse connected and logical results based on singular observations during the experiments.

6.1. Erlenmeyer flask cultivation (Envor Group Oy)

Based on the measurements of the F_M and F_V/F_M ratios, five species showed effective growth on 12,5 % reject water mediums during the cultivation period of fourteen days. These species were: 1) *Golenkinia brevispicula Hegewald et Schnepf* (C), 2) *S. quadricauda* (20), 3) *Pediastrum simplex Meyen (E)*, 4) *Monoraphidium contortum (Thuret)* and 5) *Sorastrum spinulosum Nägeli*. C, 20 and E reached nearly same or higher F_M measurements in the 12,5 % reject water mediums on the day 12 as the initial healthy inoculum culture of the day 0 (Figure 29). These species also showed a clear exponential growth phase in the F_V/F_M ratio plot and exceeded the value of 0,3 that indicates about a healthy culture (Figure 30). The growth rates were calculated from the exponential phase of the growth using (Figure 31) The lagging time was strongly depended on specie: it was about 2–3 days for C and E, seven days for 4 and 20; and ten days for 6. The lagging time can be observed from the decreasing, invariable or poorly increasing F_V/F_M ratio in the beginning of the cultivation period (Figure 30). In the end of the experiment, the color of the culture mediums was brightened slightly and also the odor of reject water was weaker. Figure 32 represents the color difference between cultivation days 0 and 13.

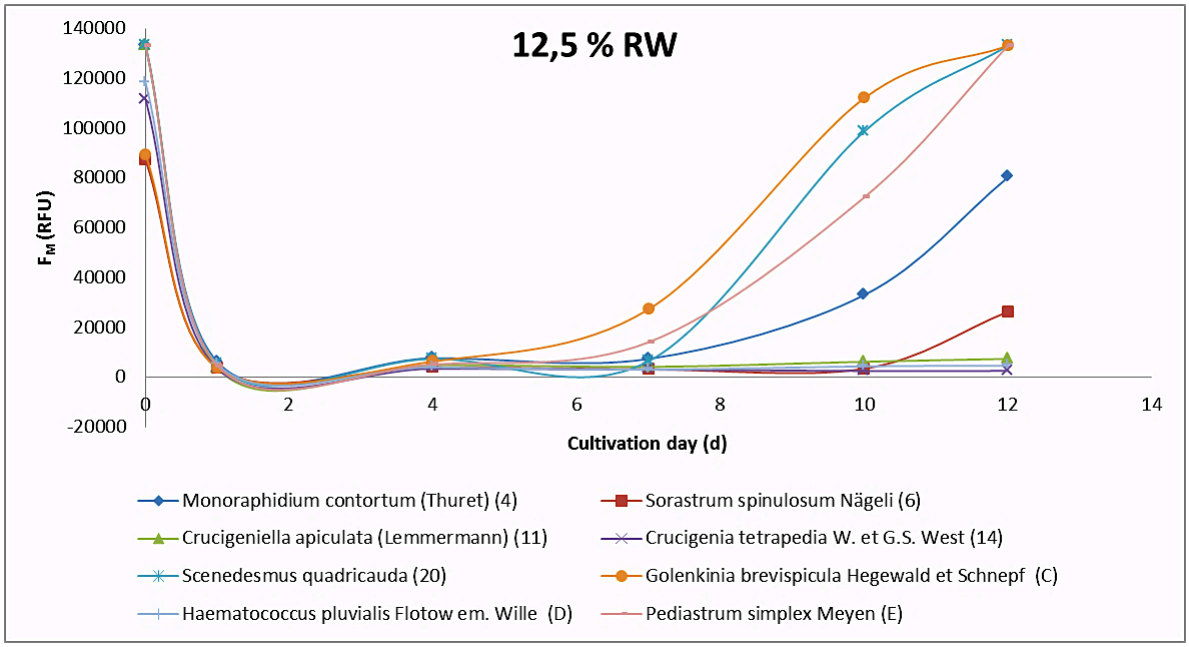


Figure 29. Maximal fluorescence F_M measurements during the 12 days cultivation period in the 12,5 % RW. The measurement of the day 0 was from the strain inoculum before it was pipetted into the Erlenmeyer flasks.

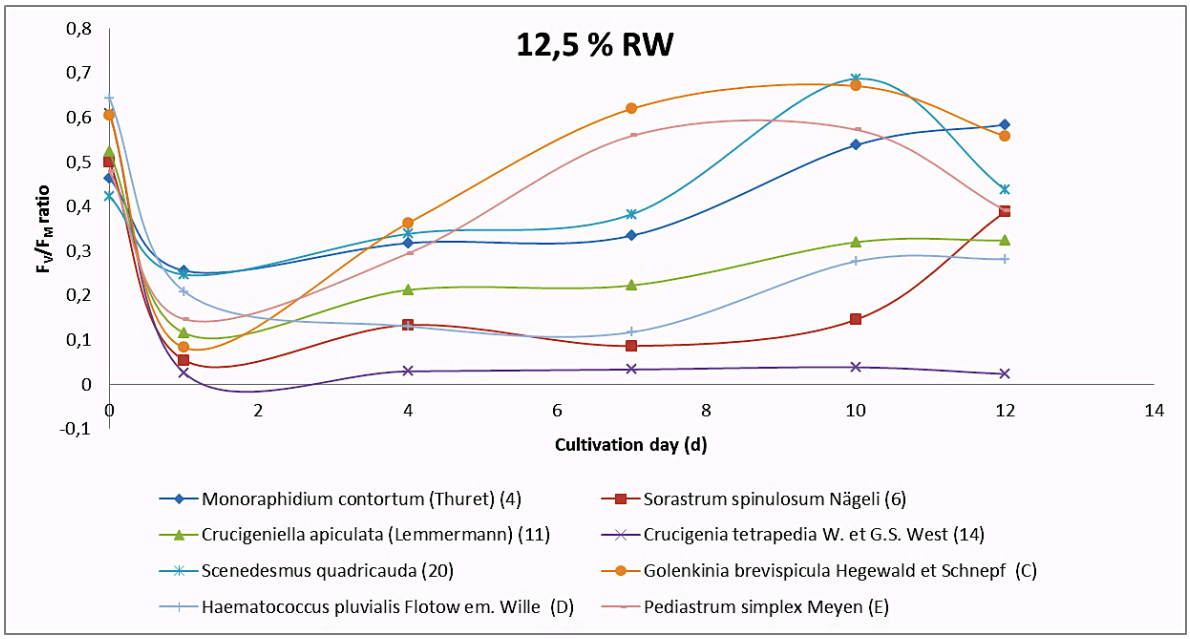


Figure 30. F_V/F_M ratio measurements during the 12 days cultivation period in 12,5 % RW. The measurement of the day 0 is from strain inoculum before it was pipetted into the Erlenmeyer flasks.

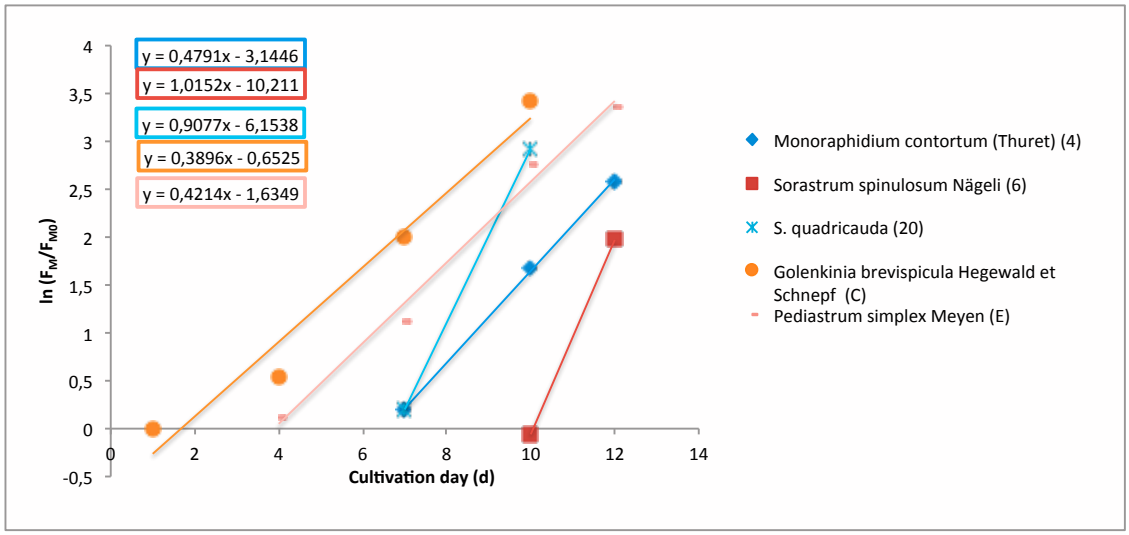


Figure 31. ln(F_M/FM_0) lines of five species during the exponential growth phase in 12,5 % reject water. The growth rates are the slope of the lines. Thus, the *Sorastrum spinulosum Nägeli* reached the highest growth rate of 1,01. However, its exponential phase is significantly shorter than e.g. in the culture of *Golenkinia brevispicula Hegewald et Schnepf.*

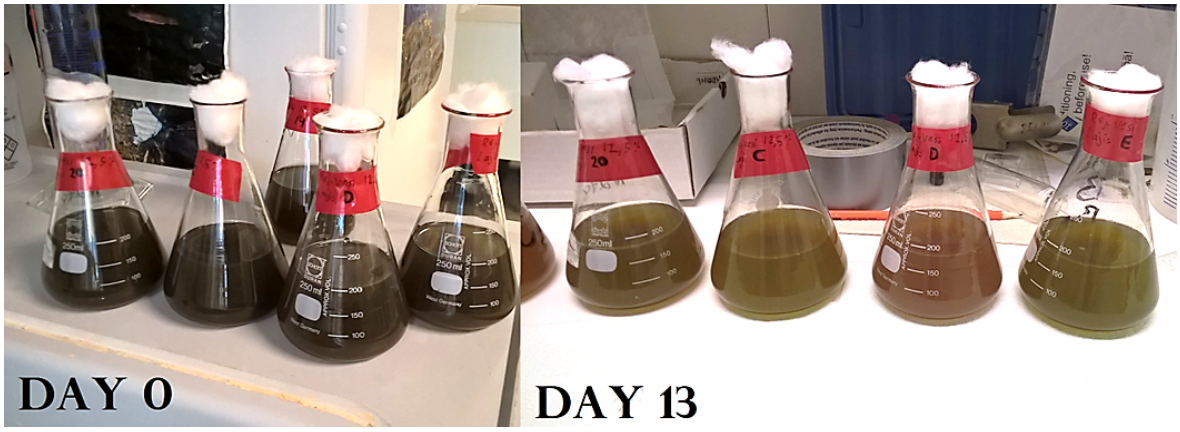


Figure 32. On the first day of the experiment (day 0) the color of the culture mediums of 12,5 % RW were dark. After 13 cultivation days, the color of the mediums was slightly lighter and a part of cultures was growing effectively turning the mediums into green. On the picture of the day 13 the three (3) green cultures from the left are *S. quadricauda* (20), *Golenkinia brevispicula Hegewald et Schnepf* (C), *Haematococcus pluvialis Flotow em. Wille* (D) and *Pediastrum simplex Meyen* (E). *Haematococcus pluvialis Flotow em. Wille* (D) showed no growth during the experiment. Photographed by Sara Merin.

In terms of cultivation on 25 % reject water mediums, singular species *Golenkinia brevispicula Hegewald et Schnepf* (C) and *Pediastrum simplex Meyen* (E) showed increased biomass yield during cultivation period according to F_M values and F_V/F_M ratios (Figures 33, 34). An exponential growth phase can be perceived from the F_V/F_M curves of these three culture mediums. *Golenkinia brevispicula Hegewald et Schnepf* (C) showed 4-fold F_M measurement compared to the F_M of *Pediastrum simplex Meyen* (E) in the last day of the cultivation period. Nevertheless, the combination of all singularly tested species

resulted to the highest F_M measurement (approx. 80 000 RFU) in the end of the cultivation period. These three cultures exceeded F_V/F_M ratio of 0,3, which indicates about a healthy culture. The lagging time was six days for C, eleven days or the combination and for fourteen days for E (Figure 34). A slight color change was observed in the culture mediums after thirteen cultivation days (Figure 35).

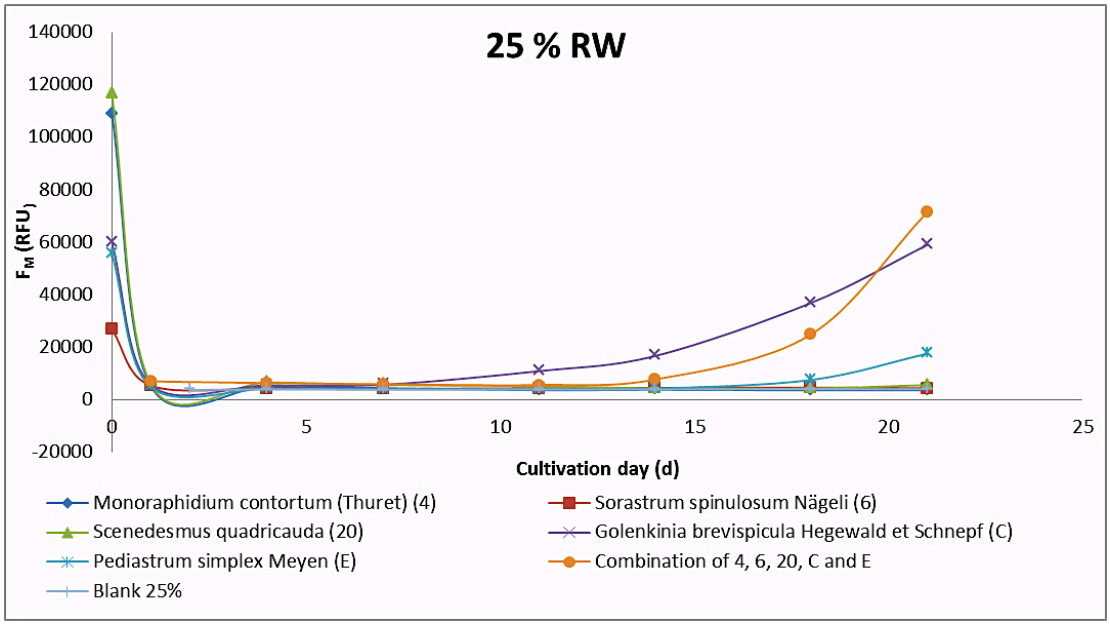


Figure 33. Maximal fluorescence F_M intensities of the cultures on 25 % RW after the cultivation period of 21 days.

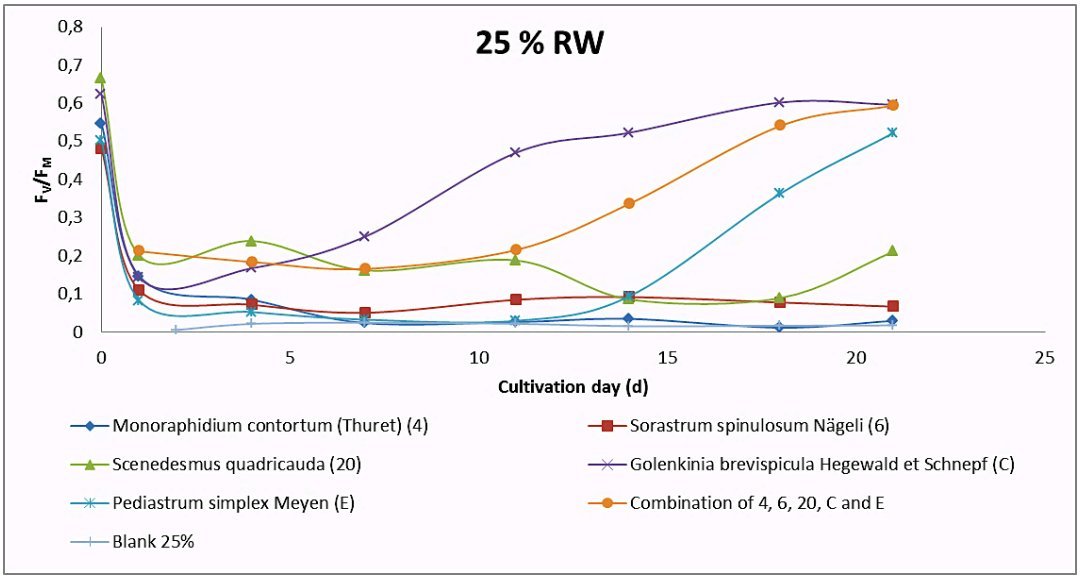


Figure 34. F_V/F_M ratio variation of the cultures on 25 % RW after cultivation period of 21 days. The mediums of *Golenkinia brevispicula Hegewald et Schnepft*, *Pediastrum simplex Meyen* and the combination of 4,6, 20, C and E were the only that showed increased F_V/F_M ratio.

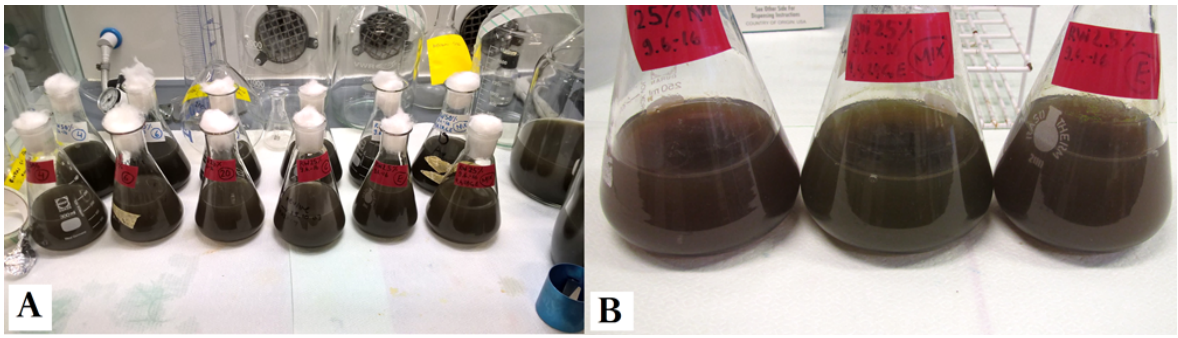


Figure 35. A) Microalgae cultivation samples in the RW mediums of 25 % and 50 % concentrations in the beginning of the experiment (day 0) B) From the left: The blank, culture combination and *Pediastrum simplex Meyen* (E) on the 25% RW on the cultivation day 13. The combination showed growth probably due to growth of *Golenkinia brevispicula Hegewald et Schnepf* (C) and the color of this medium was slightly greenish compared to the blank sample. Photographed by Sara Merin.

Both F_M and F_V/F_M values changed negligibly in 50 % reject water culture mediums (Figures 36, 37). This probably indicates that the culture mediums collapsed. *Pediastrum simplex Meyen* (E), however, grew on the walls of the Erlenmeyer flask probably due to the low light availability in the medium (Figure 38). Thus, this culture avoided the total collapse.

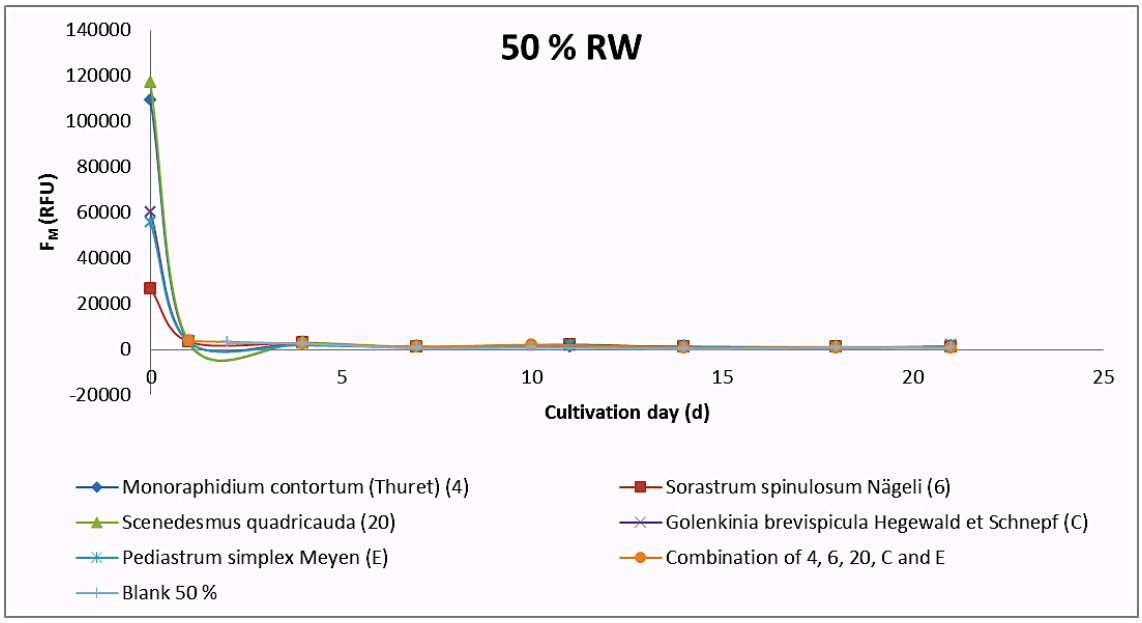


Figure 36. F_M measurements of the cultures in the RW mediums of 50 % concentration during the cultivation period of 21 days. The F_M values were negligible compared to the values of a healthy inoculum medium on the cultivation day 0. This indicates that 50 % concentration was overly strong to be adapted by microalgae and the cultures collapsed.

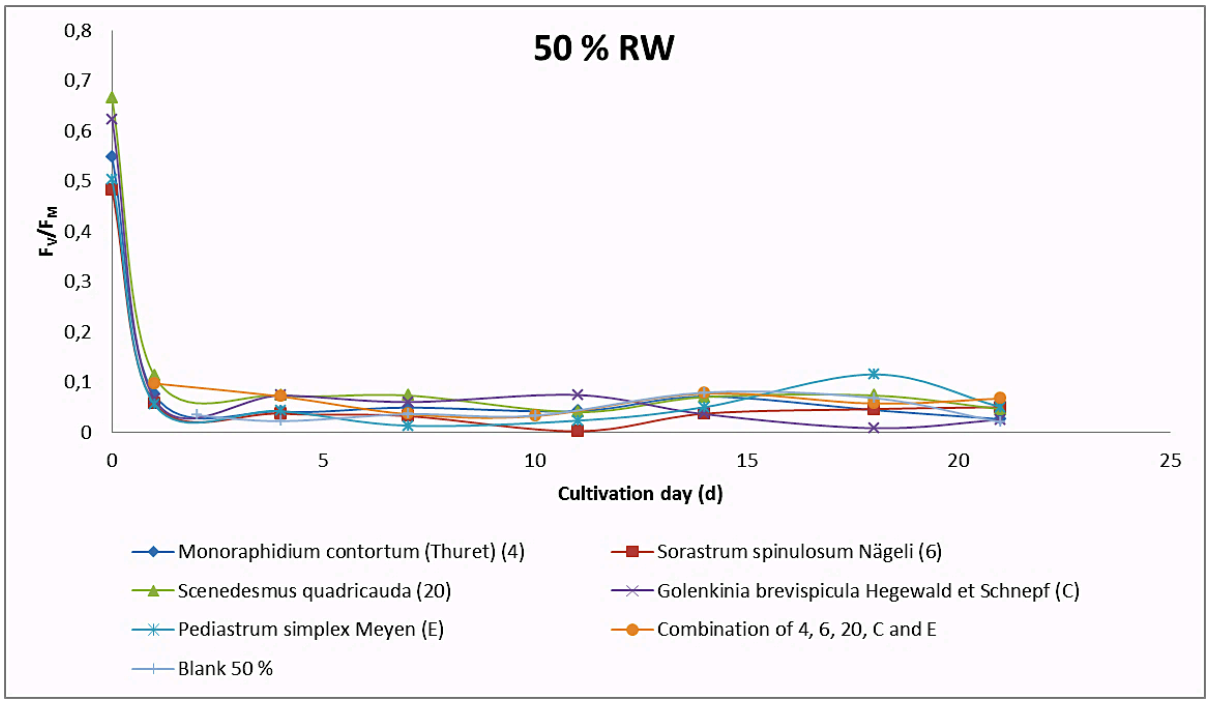


Figure 37. None of the cultures reached the level of a healthy culture (>0,3) in the mediums of 50 % RW dilutions.

Figure 38. *Pediastrum simplex Meyen* (E) showed growth on the walls of the Erlenmeyer flasks both in 50% (left) and 25% RW (right). Photographed by Sara Merin.

6.2. Microplate cultivation (Envor Group Oy)

Microalgae species showed growth in filtered reject water dilutions of 30 % or below in microplate wells (Figure 39). Species 1) *Monoraphidium contortum (Thuret),* 2) *S. obliquus,* 3) *Selenastrum capricornutum Printz*, 4) *Chlorella sp.* and 5) *Euglena Gracialis* were probably the most adaptable for various reject water dilution based on F_M

measurements (Figures 40, 41). Microalgal growth increased slightly at the cultivation days between seven and ten. This indicates about an extremely long lag period, i.e. the adaptation was poor and slow. The growth of each species remained negligible at the higher concentrations. However, the estimation of the algal growth was difficult since the wells seemed green although the F_M measurements were significantly lower compared to the MAM or MWC culture mediums. The green color in the 30 % reject water wells after the cultivation period indicates about growth although F_M values measured from blank wells are higher compared to the intensities in 30 % wells.

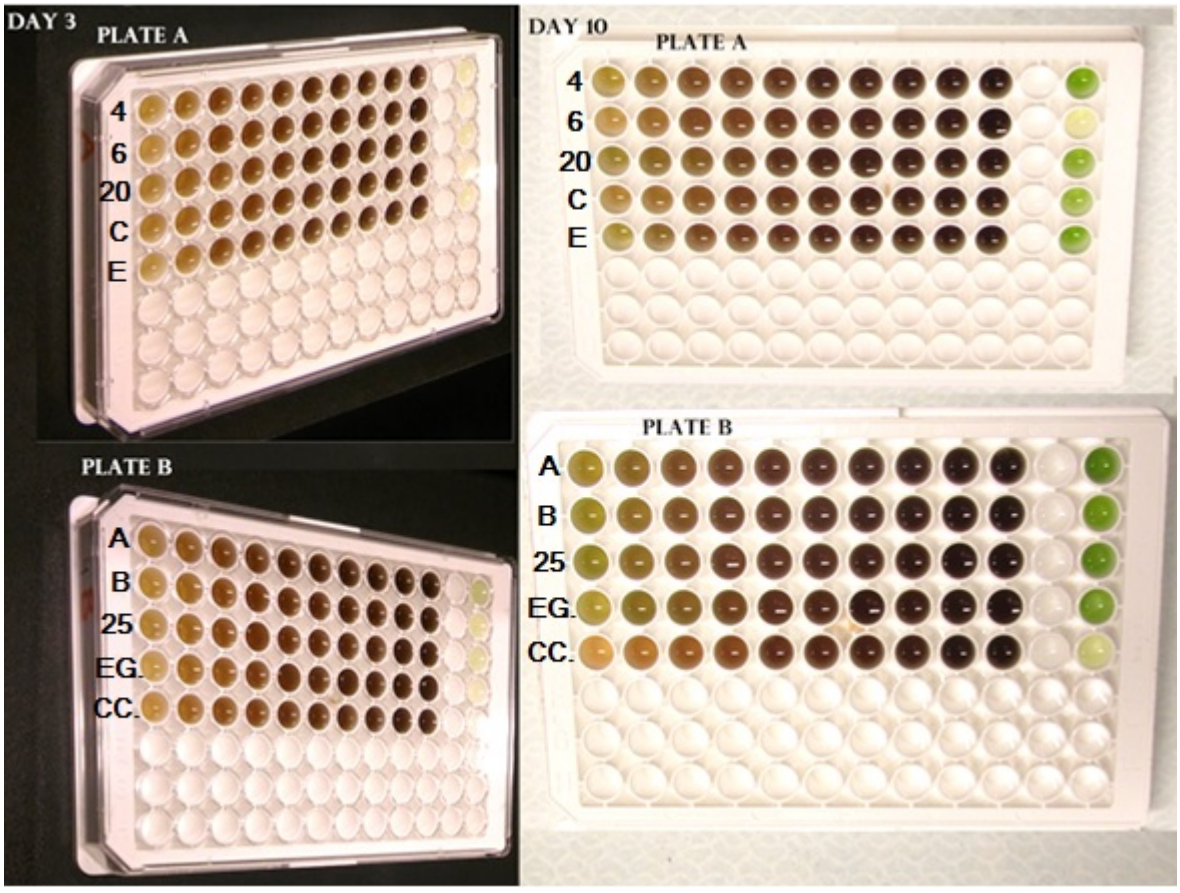


Figure 39. On the day 3, the algal growth was still negligible in the Envor Group Oy RW mediums. After 10 days, the colors of 10–30 % dilutions changed slightly green. *Coccomyxa* (CC.) seemed totally inhibited. The order of the plate rows followed Table 15. Microplates A and B on the left are covered with a plastic cover in the photograph. Photographed by Sara Merin.

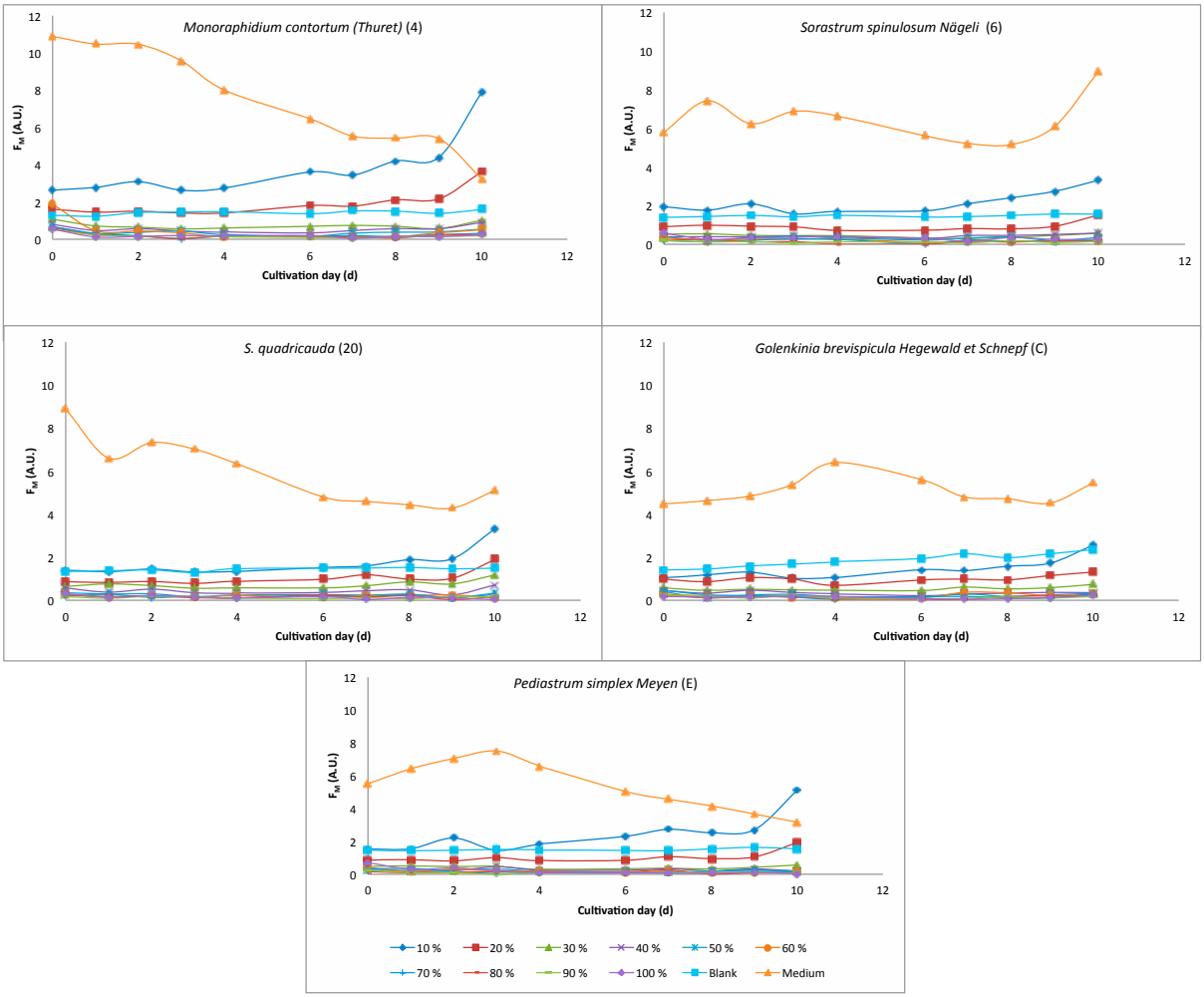


Figure 40. Fluorescence intensity F_M curves of *Monoraphidium contortum (Thuret)* (4), *Sorastrum spinulosum Nägeli* (6), *S. quadricauda*, *Golenkinia brevispicula Hegewald et Schnepf* (C) and *Pediastrum simplex Meyen* (E) during the cultivation period of 10 days. Microalgal growth remained poor compared to the growth in the artificial medium.

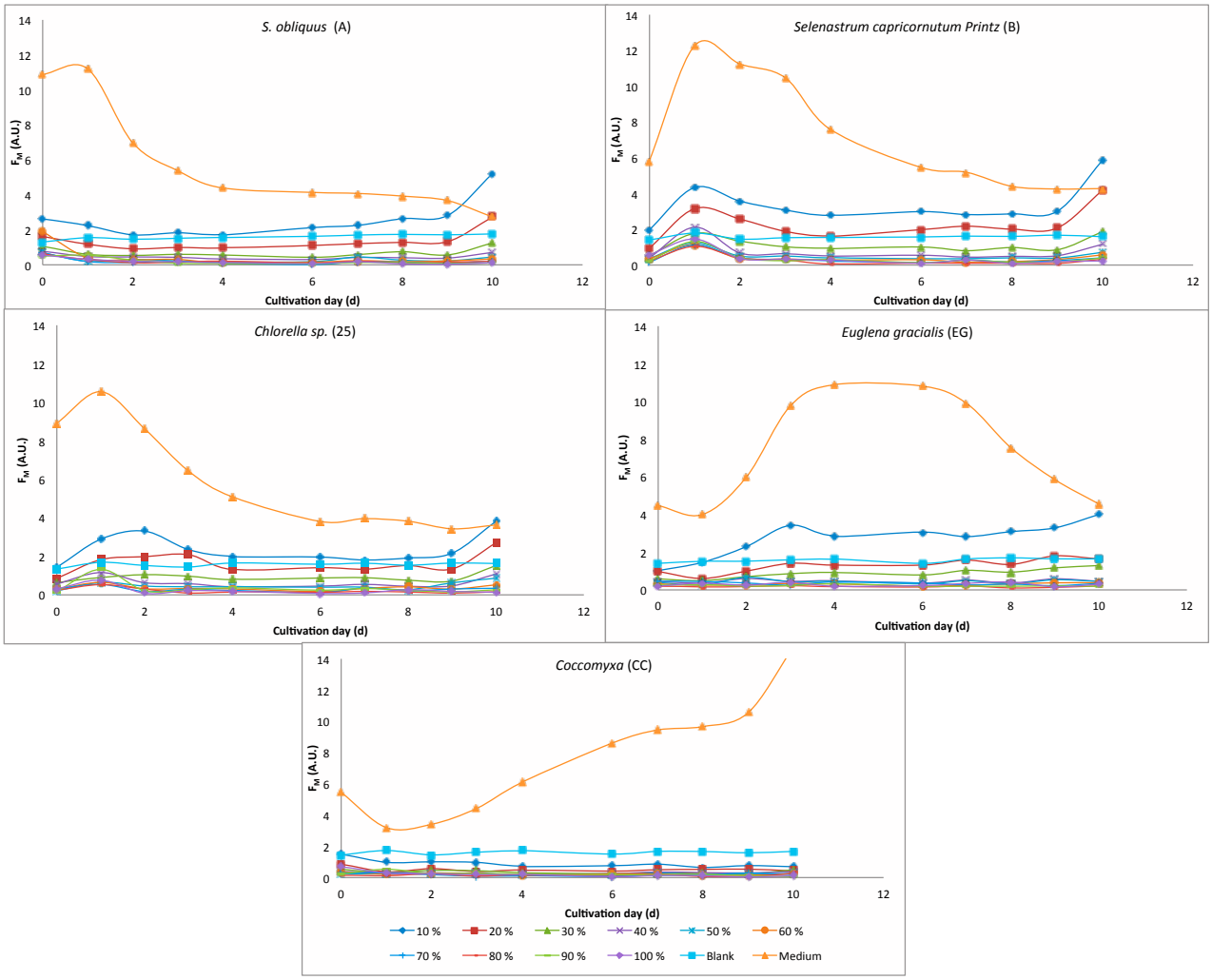


Figure 41. Fluorescence intensity F_M curves of strains *S. Obliquus* (A), *Selenastrum capricornutum Printz* (B), *Chlorella sp.* (25), *Euglena Gracialis* (EG.) and *Coccomyxa* (CC.) in various RW dilutions after 10 days cultivation period. Microalgal growth remained poor compared to the growth in the artificial medium.

6.3. Ammonia stripping efficiency (Viikinmäki)

When ammonia stripping was operated with a presence of stirring (100 rpm) it caused precipitation. Especially when the sample was unfiltered the formation of the precipitation was easily observed. The color of the formed precipitate was gray and white (Figure 42). According to Lehtovuori (2016), the formation of limestone ($CaCO_3$), struvite ($NH_4MgPO_4 \cdot 6\ H_2O$) or ferric phosphate ($FePO_4$) can constitute the possible substitutes of the precipitation. A formation of foam was also observed. A stripping experiment without stirring was also tested and then the formation of the precipitation was milder. The ammonia stripping changed significantly the color of the reject water, which probably leaded to a more beneficial culture medium for algae due to the improved availability of light (Figure 43). The cause for the color change is unexplained but the reddish color can be due to high iron concentration.

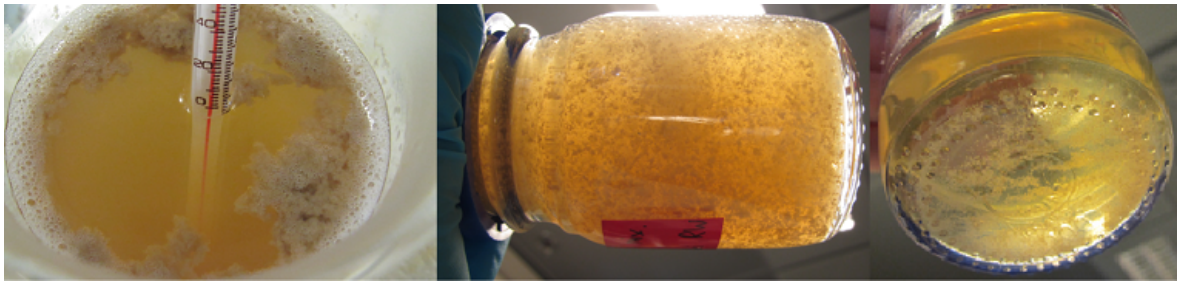


Figure 42. Ammonia stripping caused formation of foam and precipitate. Especially with simultaneous magnetic stirring the formation of precipitate was significant. On the left unfiltered sample during ammonia stripping, in the middle unfiltered sample after the stripping and on the right stripped centrifuged sample. Photographed by Sara Merin.

Figure 43. The color of the RW changed significantly after ammonia stripping. On the left RW before stripping experiment and on the right RW after 15 h stripping under temperature of 60 °C. This stripped sample was used for Erlenmeyer flask culture tests in the Chapter 6.2.6. Photographed by Sara Merin.

Based on the nutrient analysis results (Table 18), ammonia stripping reduced significantly NH_4-N concentration in the reject water from Viikinmäki WWTP (Figure 44). The initial NH_4-N concentration in the sample was 745 mg/l and final concentration was 9,5 mg/l after the stripping with fifteen hours operational time. This resulted to a 99,3 % reduction rate of NH_4-N (Table 17). Reduction rate of 72,2 % was achieved with an operation time of 2 hours. The results of an operational time of six (90,7 %) and eight (90, 3 %) are probably incorrect since an increased NH_4-N concentration is incoherent. Firstly, the applied method for the analyses has been developed for seawater samples and therefore uncertainty may be higher. Secondly, the mild dilutions (1:10 000 and 1:5000) of the analysis samples probably had an influence for possible uncertainties. Thirdly, the evaporation was notable during the stripping, which leaded to more concentrated reject water. Nevertheless, for instance the initial concentration before stripping is at the same level with the concentration analyzed by MetropoliLab WWTP (680 mg/l) (Table 12). An important observation constitutes the significantly lowered N/P ratio that followed the lowered NH_4-N concentration after stripping (Figure 44). The initial N/P ratio was 1238 and it dropped to 13 after 15 hours stripping experiment. The PO_4-P concentration increased probably due to evaporation during stripping under the high temperature (60 °C) (Table 18).

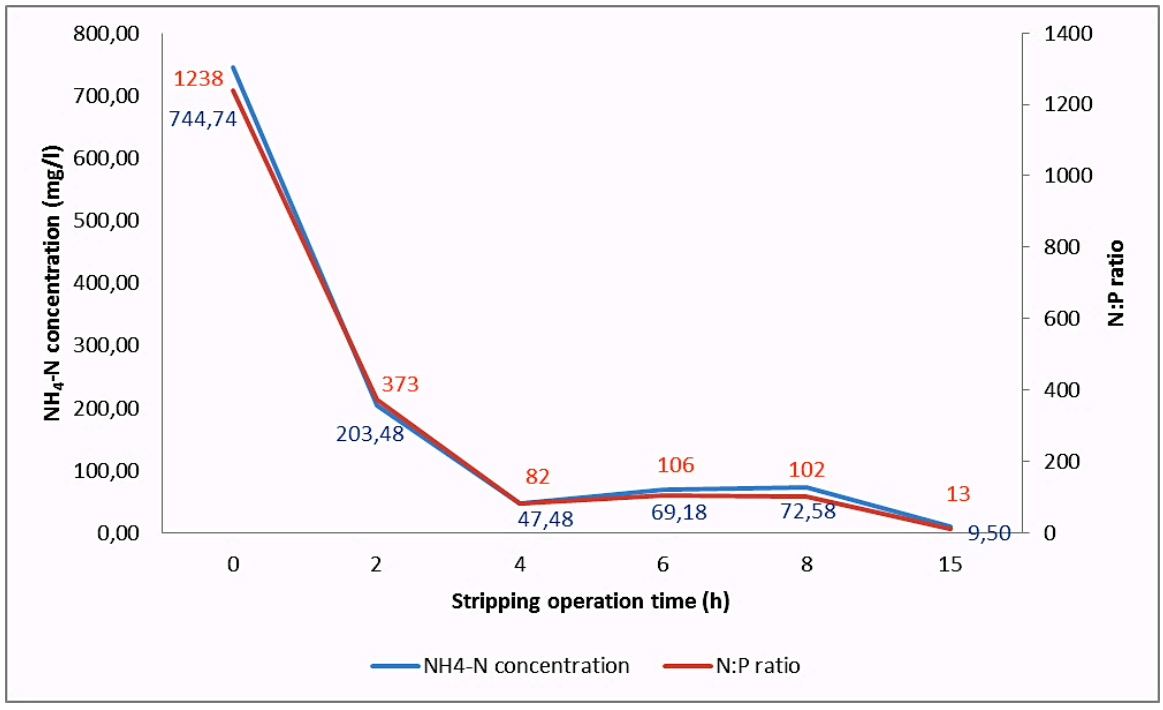


Figure 44. Ammonia stripping lowered significantly the high ammonium nitrogen concentration and N:P ratio in the RW sample from Viikinmäki WWTP.

Table 17. Ammonia stripping efficiency results with various operation times.

Operation time 2h	72,7 %
Operation time 4h	93,6 %
Operation time 6h	90,7 %
Operation time 8h	90,3 %
Operation time 15h	98,7 %

6.4. Microplate cultivation on unfiltered and centrifuged mediums (Viikinmäki)

The microplate experiments were operated both with unfiltered and centrifuged samples (Figure 45). Figure 46 demonstrates the microalgal growth on the unfiltered and centrifuged reject water mediums after six cultivation days. The highest F_M measurements were observed on the mediums of *Monoraphidium contortum* (4), *S. obliquus* (A) and *Chlorella* sp. (25) (Figures 47, 48 and 49) during the cultivation period. The wells of *S. quadricauda* were green but the F_M values were relatively low (Figure 50). *Monoraphidium contortum* and *S. obliquus* showed a separable exponential growth phase on the light dilutions (< 30–40 %) of the reject water whereas it was unclear on culture mediums of *Chlorella sp*. The cultures showed greater F_M values in the centrifuged mediums compared to the unfiltered mediums. The *S. obliquus* culture seemed to grow greater on the centrifuged dilutions between 30–50 % compared to the unfiltered mediums. However, the growth of *Monoraphidium contortum* and *Chlorella sp.* cultures did not achieve radical increases of F_M intensities in dilution above 30 %. The effect of the addition of external phosphorus had only a slight influence to the F_M intensities of *S. obliquus* and *Chlorella sp*. The P addition affected raisingly to the F_M values on centrifuged mediums. On the contrary, the effect of P addition was negligible or negative on the unfiltered mediums. Hence, the observation probably indicates that the centrifugation lowered the P content in the reject water.

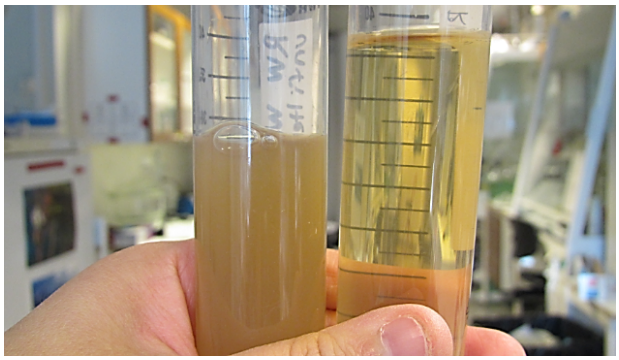


Figure 45. Centrifugation improved the light illuminance significantly in reject water from Viikinmäki WWTP. On the left an unfiltered sample. On the right a centrifuged sample. Both samples were hygienized before the photographing. Photographed by Sara Merin.

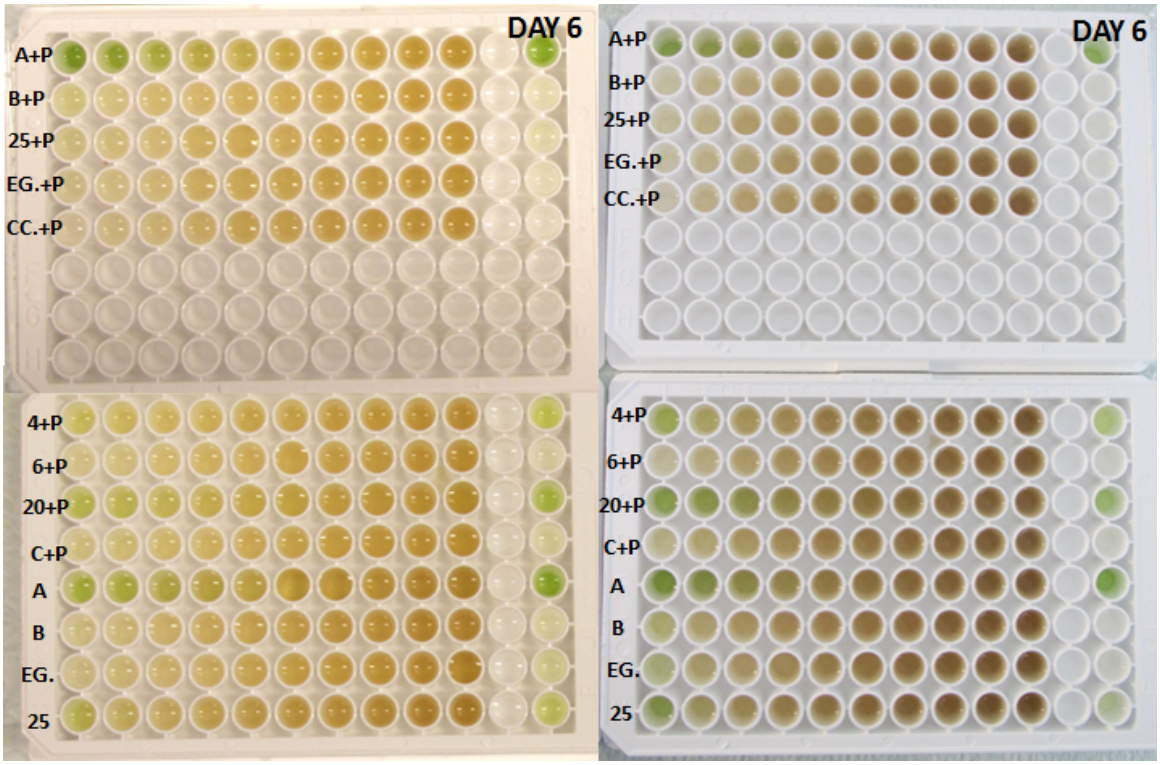


Figure 46. On the left centrifuged RW mediums and on the right unfiltered RW mediums after six days cultivation period. +P means that the mediums included addition of external P. The cultures probably collapsed in the dilutions above 40–50 % of RW.

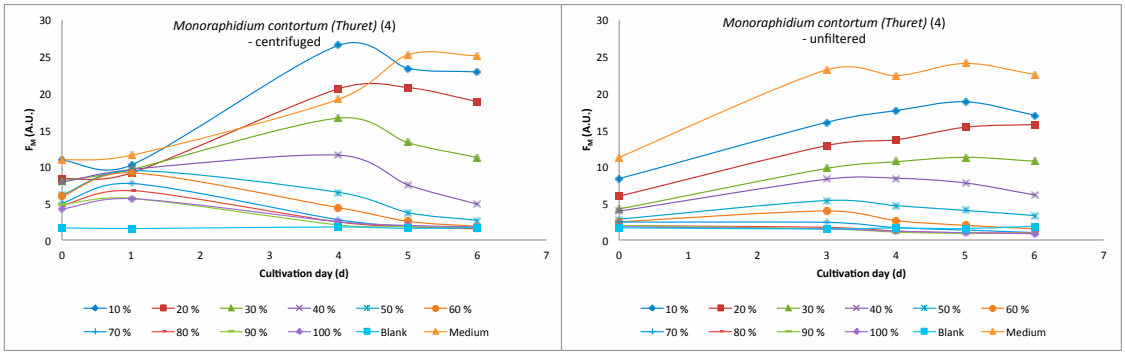


Figure 47. The culture of *Monoraphidium contortum* achieved increased F_M intensities in the centrigured mediums of 10–30 %.

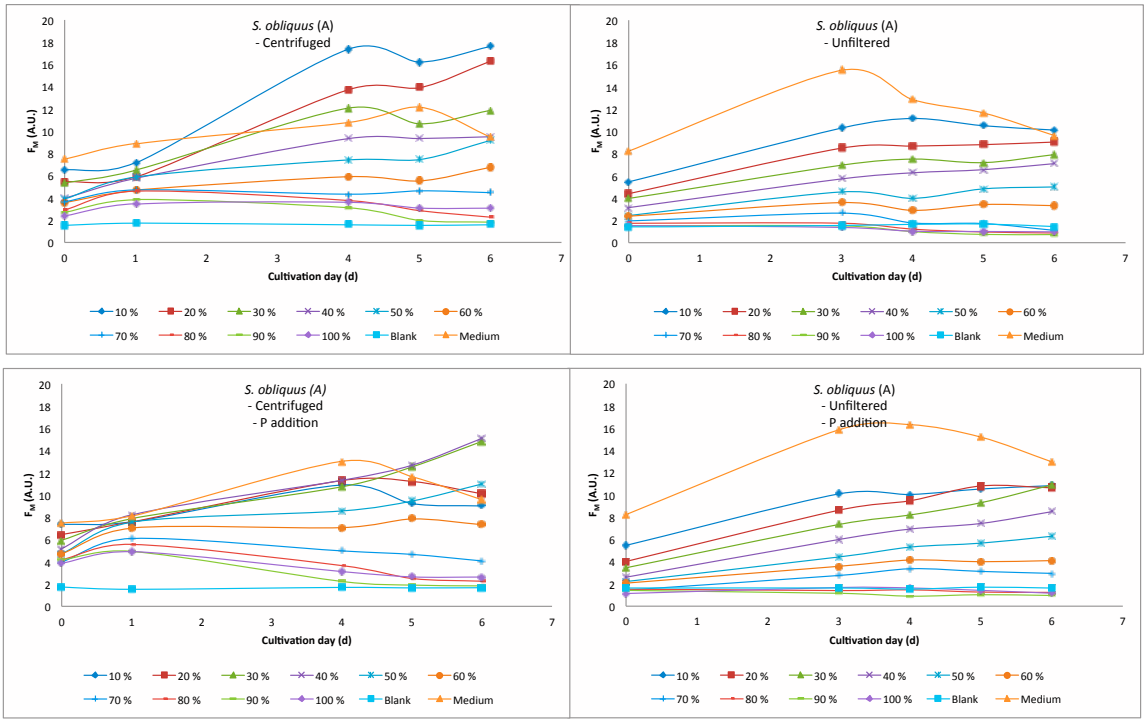


Figure 48. *S. obliquus* showed increased F_M values in the centrifuged mediums. The growth was slightly rapider when the external P was added. However, the F_M intensities were higher in the samples without P addition on the last cultivation day. In terms of ulfiltered samples, the P addition had only a slight positive influence to the growth.

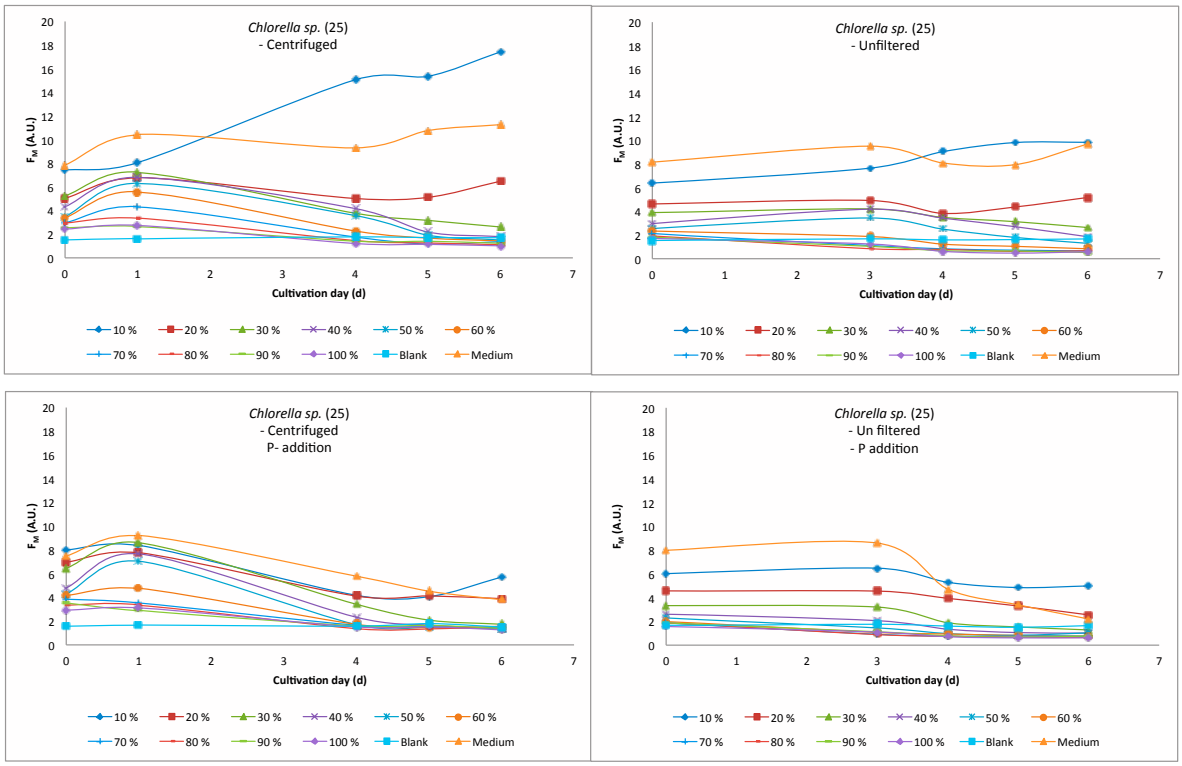


Figure 49. *Chlorella sp.* showed to grow more rapidly in the centrifuged sample. The culture achieved higher F_M intensities in the concentrations between 10–20 % when mediums were centrifuged. The F_M values were lower in the mediums when the external P was added both in the centrifuged and unfiltered mediums.

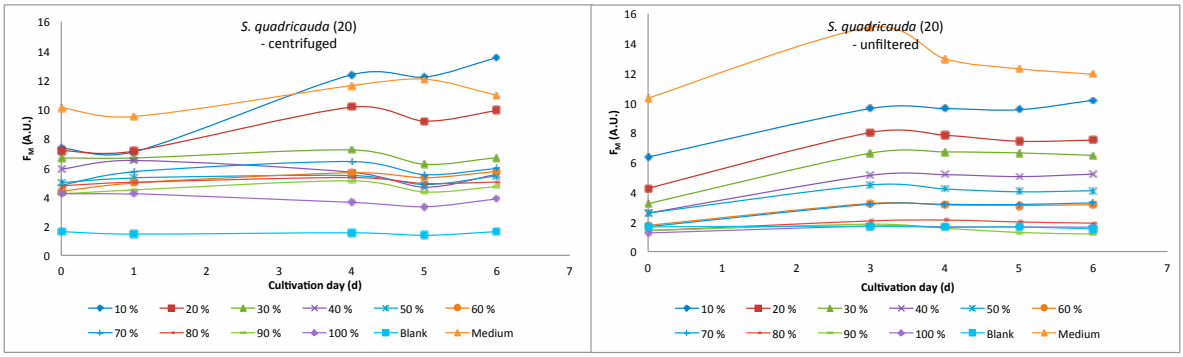


Figure 50. The F_M intensities showed poor rise in the culture mediums of *S. quadricauda* (20).

6.5. Microplate cultivation in ammonia stripped mediums (Viikinmäki)

The continuos stirring during ammonia stripping resulted to more beneficial culture mediums. After the observation, each stripping experiment was operated with continuous magnet stirring.

The growth of microalgae was more effective in the ammonia stripped reject water mediums compared to the unstripped mediums based on photographs of the microplates (Figures 46, 51) and to the previously considered F_M intensities (Figures 47, 48, 49, 50). Microalgae grew also on the dilutions above 50 % of the reject water, which was observed especially from the green color of the microplate wells (Figure 51). The biomass of *S. obliquus* (A) turned the well mediums into deep green color even in the 100% reject water. In addition, *Monoraphidium contortum* (4)*, Chlorella sp.* (25) *and S. quadricauda* (20) turned microplate wells green.

The highest F_M intensities were measured from the mediums of same cultures (4, A and 25) as in the previous microplate tests (Chapter 6.2.4.) (Figures 52, 53, 54). The F_M intensities in the mediums stripped for six hours of *Monoraphidium contortum* were higher in the dilutions of 50 % and under compared to the mediums that were stripped for four hours. The dilutions of 60% or stronger resulted to approximately same level F_M measurements as in the culture mediums of *Monoraphidium contortum* that were stripped only for four hours. The same observation was noted in the cultures of *Chlorella sp.* but the F_M values were at the same level in the dilutions of 70 % or above. In terms of the cultures of *S. obliquus*, the F_M measurements were higher in each dilution and also in the 100 % reject water medium in the comparison of the stripped reject water with four and six hour operation times.

The F_M intensities were higher in the stripped (6 h) 10–80 % reject water mediums with the external P of *S. obliquus* compared to the artificial medium on the last day of the cultivation period. The addition of external P had a negligible or unclear effect to the F_M measurements of *Monoraphidium contortum* (4) and *Chlorella sp.* (25). However, *S. quadricauda* showed higher F_M intensities on reject water dilutions below 50 % when external P was added, which probably indicates that highly diluted mediums included too less P for the demand of *S. quadricauda* (Figure 55).

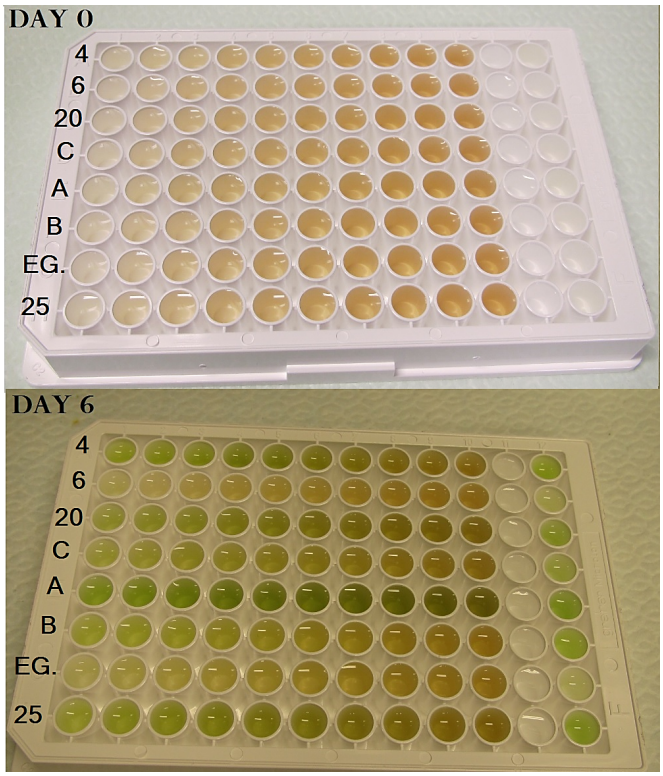


Figure 42. Ammonia stripping with four hours operation time and particle separation by a centrifuge resulted to a more beneficial culture medium compared to the unstripped reject water. The mediums of *Monoraphidium contortum* (4), *S. quandricauda* (20), *S. obliquus (A)* and *Chlorella sp.* (25) were the most green after six days cultivation period on the microplate. Photographed by Sara Merin.

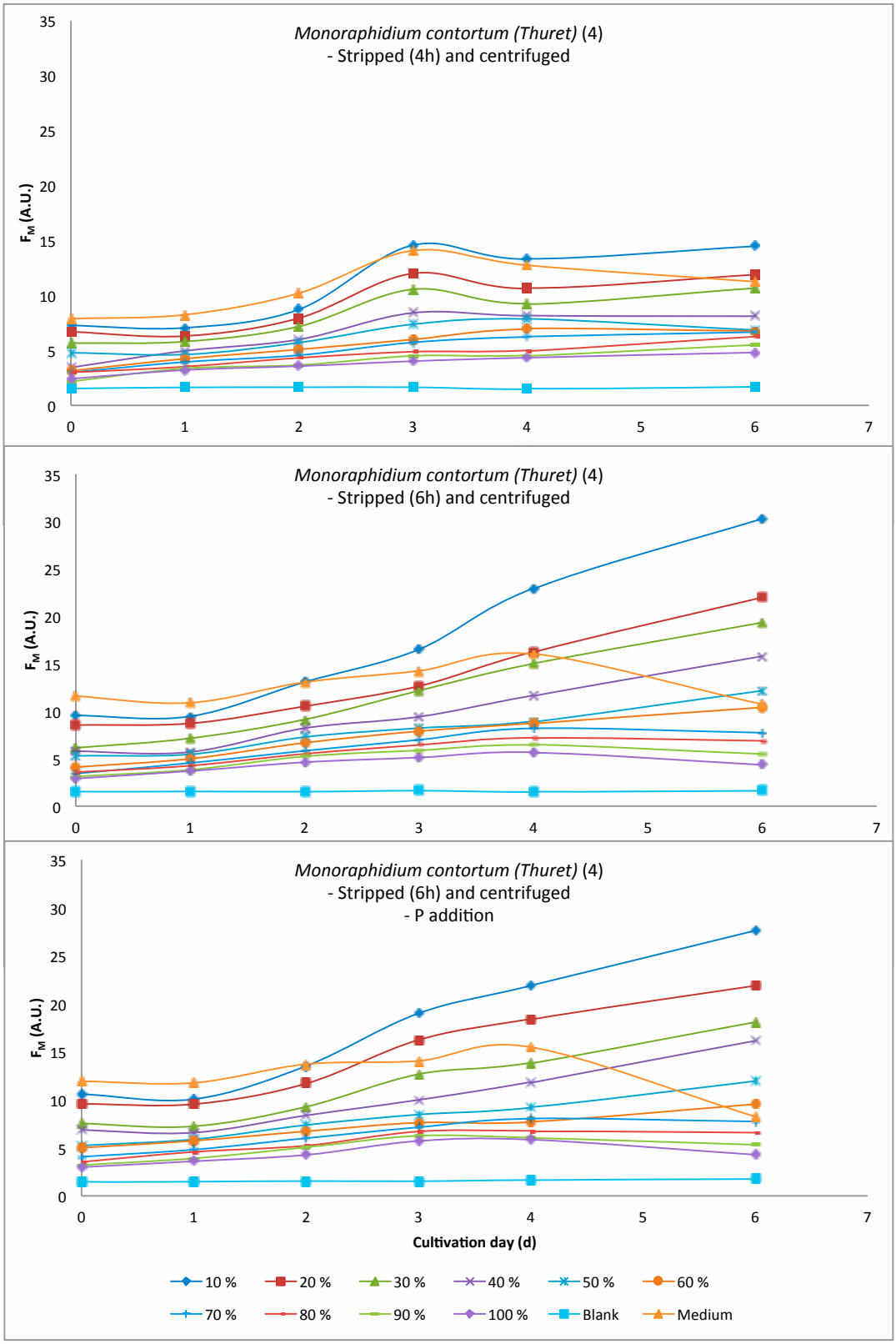


Figure 43. *Monoraphidium contortum (Thuret)* (4) showed the highest F_M measurements (the highest nearly 30 A.U.) of the tested cultures from the Viikinmäki WWTP. The longer stripping time increased the intensities. However, the longer stripping time (6 h) had a negligible influence to F_M values in the mediums of 60–100 %. The addition of external P had only a slight or negligible effect to the growth.

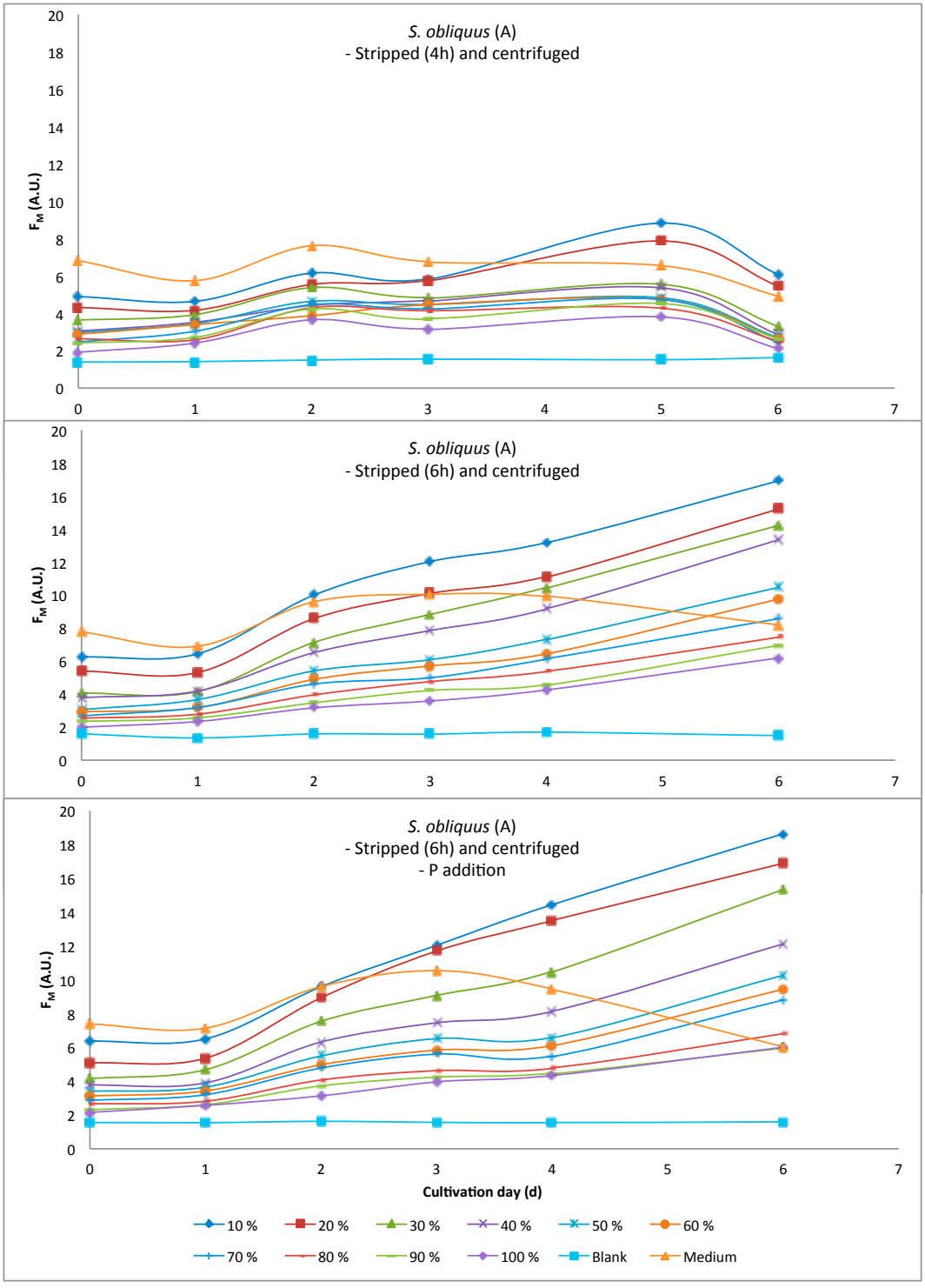


Figure 44. The longer stripping time increased the intensities on each dilution and also on 100 % reject water medium of S. *obliquus*. The addition of external P had an ambivalent influence to the growth of *S. obliquus*.

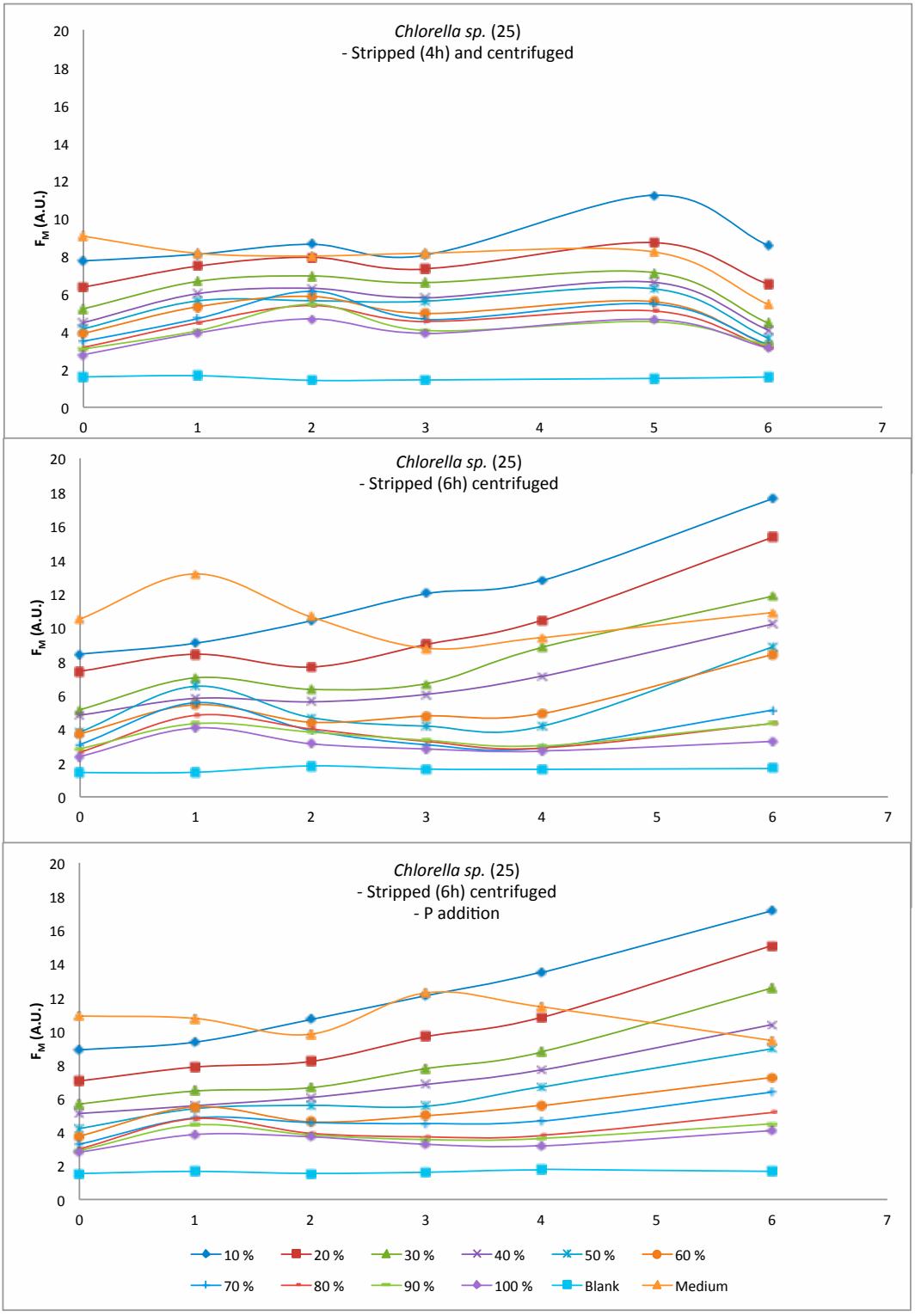


Figure 45. The longer stripping time increased F_M intensities in culture mediums of *Chlorella sp.* However, above reject water dilutions of 70 %, the differencies were negligibly between the stripping times. The addition of P had a negligible effect to the F_M measurements.

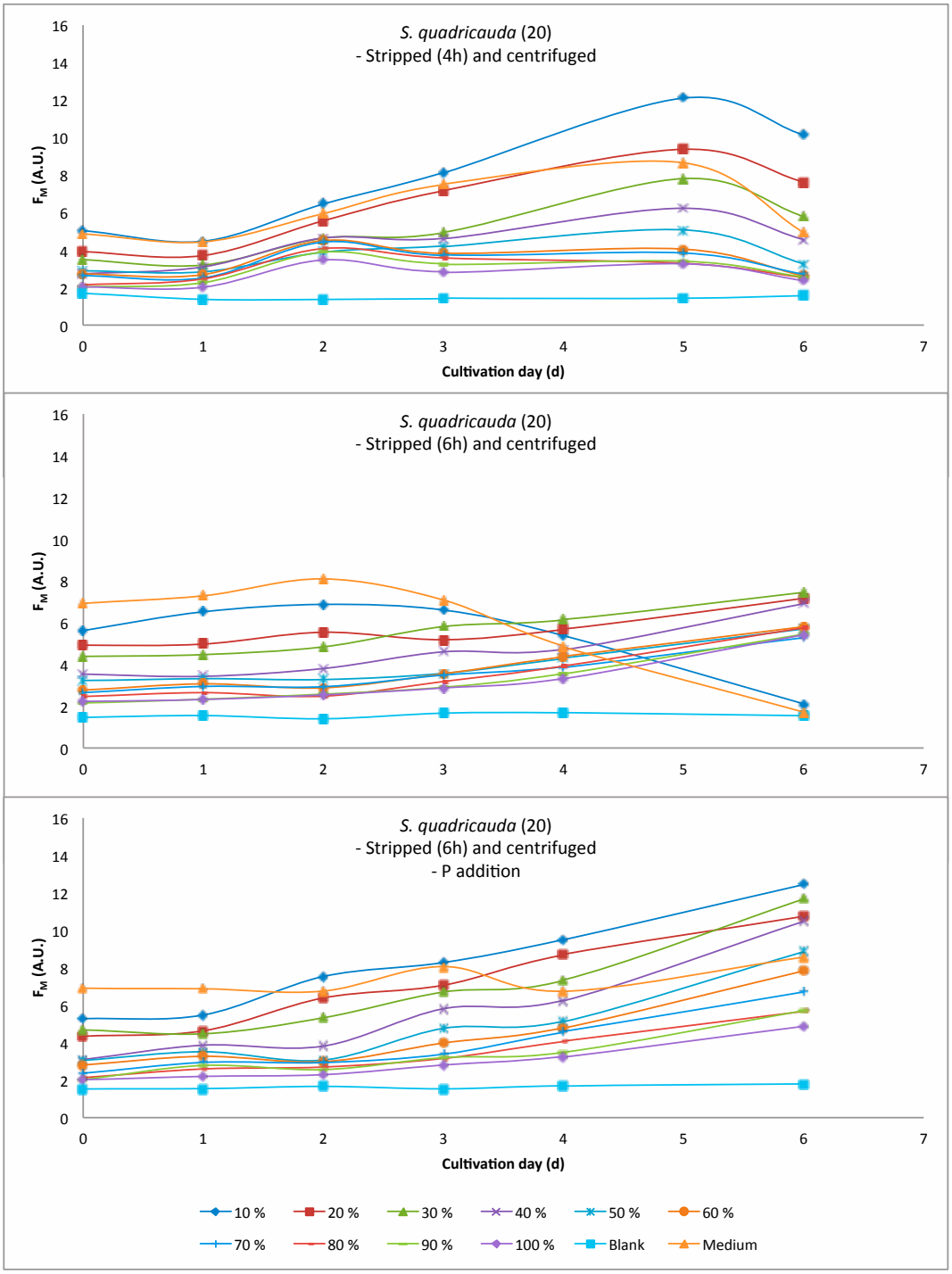


Figure 46. *S. quadricauda* showed increased F_M intensities on stripped (6 h) dilutions above 40 % compared to mediums that were stripped for four hours. P addition seemed to affect positively to the growth. *S. quadricauda:* the F_M values were higher when P was added in dilutions below 50 % compared to the medium without the P addition.

Figure 56 demonstrates the biomass production of *S. obliquus* and *Chlorella sp.* in the unfiltered and centrifuged reject water samples that were stripped for four hours. Based on the figure, the biomass production was effective in both samples. The bottoms are brownish in terms of unfiltered sample due to higher content of solid particles. The biomass production differenced probably only slightly in the unfiltered and centrifuged sample.

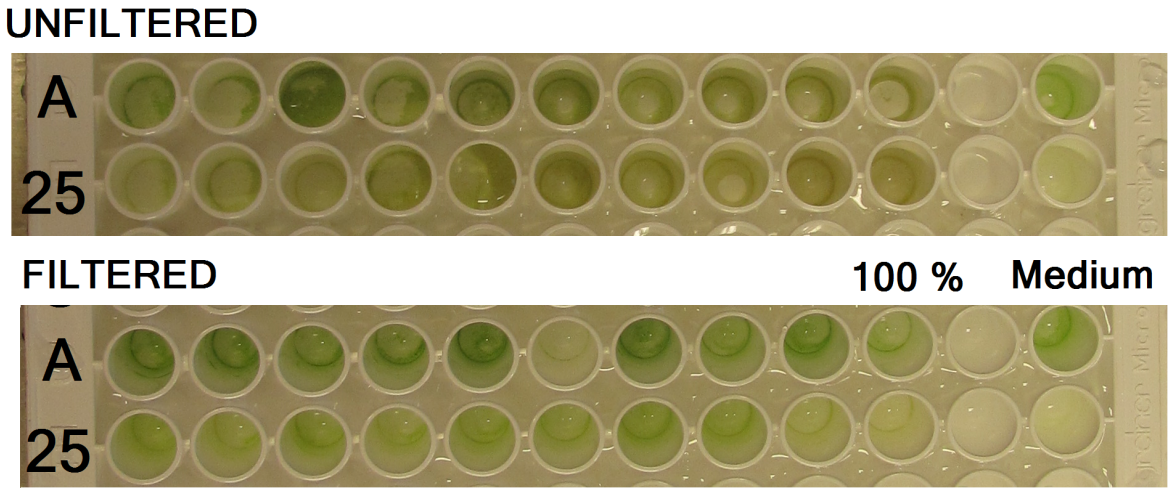


Figure 47. Rinsed unfiltered and centrifuged microplates after six days cultivation period in the stripped RW (4 h). Photographed by Sara Merin.

6.6. Erlenmeyer flask cultivation (Viikinmäki)

The specie *S. obliquus* was one of the greatest biomass producers in high concentrations of reject water based on previous microplate tests and therefore it was selected for the cultivation test in Erlenmeyer flasks. In this experiment reject water was stripped for fifteen hours and it was unfiltered. At the end of the experiment after 10 cultivation days, three singular mediums diluted to the concentration of 50 % were extremely green including *S. obliquus* biomass (Figure 57). On the contrary, the algal growth in flasks filled with 100 % reject water without P addition (Samples 1–3) was negligible. The measured F_M/F_V ratio curves of each Erlenmeyer medium are presented in the Figure 58. *S. obliquus* grew efficiently in the 50 % reject water without any notable lagging time. Also, the exponential growth phases were easy to observe and therefore the growth rates were able to calculate (Figure 59). The 100% reject water mediums with P addition (Samples 4–6) showed some cellular growth but the biomass formation remained weak after the experiment since there was no green color. Also, since the light availability decreases with

increasing water pond depth it can be feasible that *S. obliquus* was inhibited resulted from the lack of light. Also, a presense of bacteria or other e.g. heavy metals and drug residuals can be the sources of the inhibiton. *S. obliquus* showed good growth in the stripped 100 % reject water in the previous microplate tests, which indicates that *S. obliquus* is perhaps more tolerance high ammonium nitrogen concentrations than to the low light availability or high particle content.

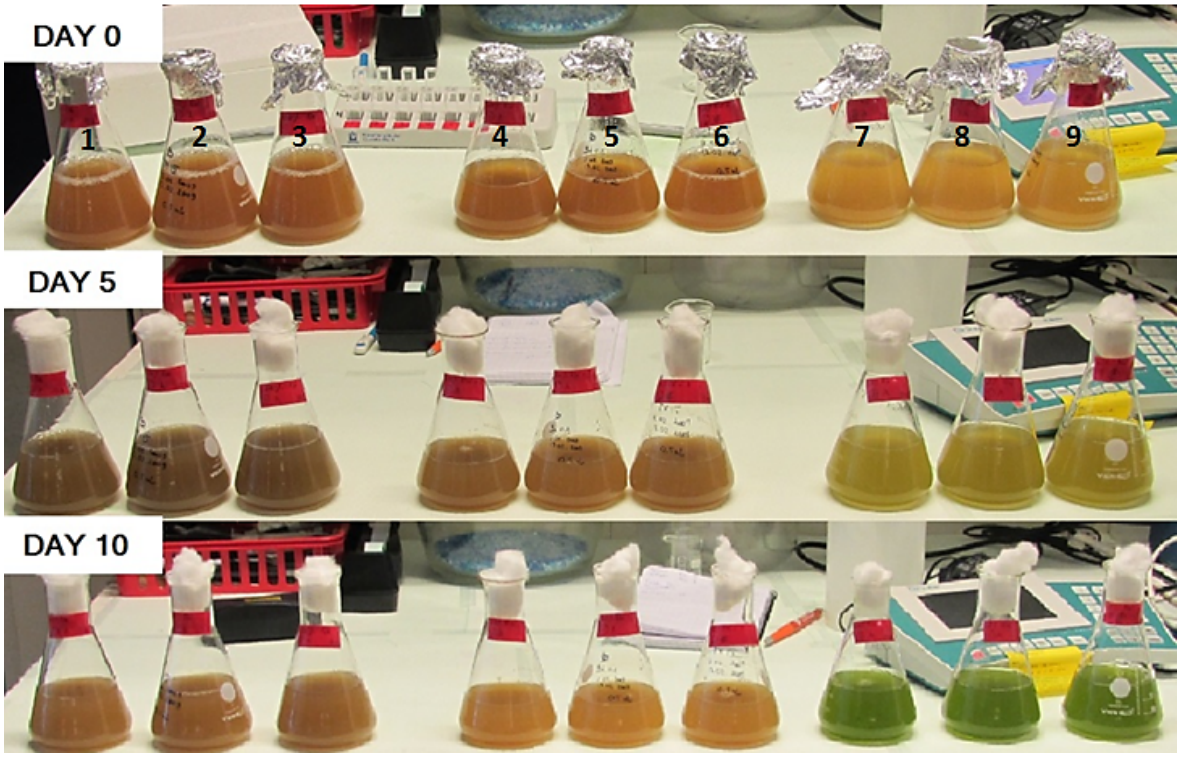


Figure 48. *S. obliquus* grew rapidly in 50 % RW whereas the growth was weak in 100 % RWs. The three 100 % RW mediums (Samples 1-3) on the left, the three 100 % RW mediums with P addition (Samples 4–6) are in the middle and the three 50 % RW (Samples 7–9) on the right. All the mediums were ammonia stripped for fifteen hours. There was a slight color difference between 100% RW and 100% RW with the P addition. The occasion for this is perhaps microalgal or bacterial growth or effect of chemical reactions followed by addition of $NaH_2PO_4 \cdot H_2O$ solution as external P. Photographded by Sara Merin.

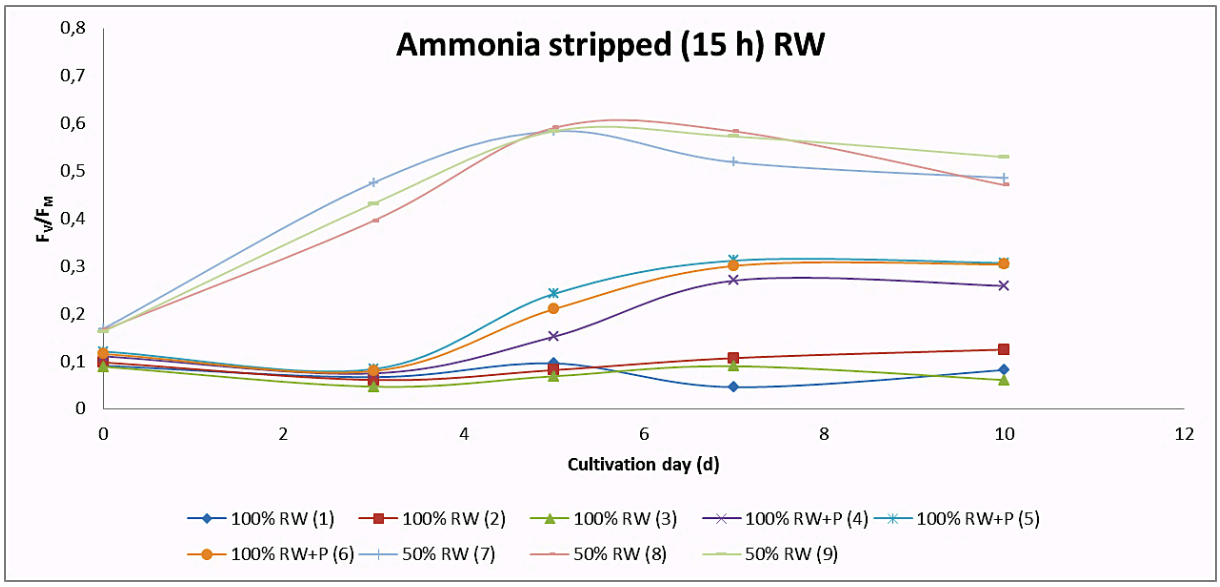


Figure 49. F_V/F_M ratios of *S. obliquus* culture mediums in function of cultivation days in stripped RW.

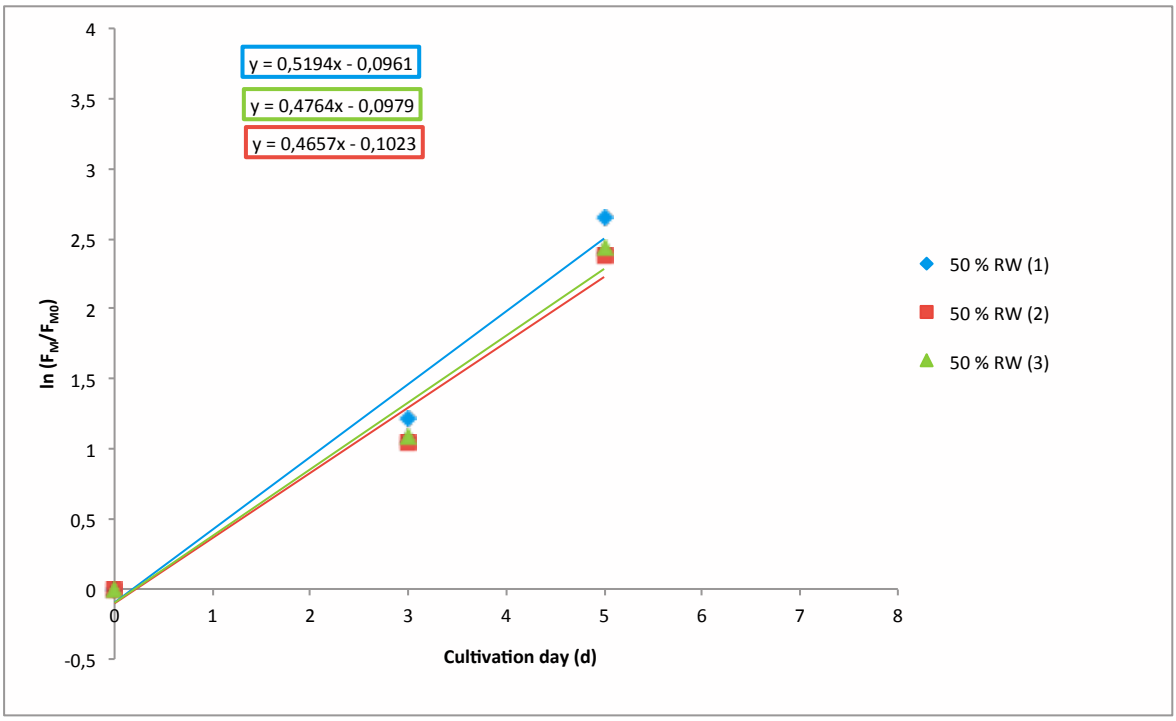


Figure 50. $\ln(F_M/F_{M0})$ plots of *S. obliquus* in 50 % reject water. The slopes of the lines are the growth rate.

6.7. Nutrient uptake efficiencies

As high as 99 % and 89,7 % NH_4-N and PO_4-N uptake rates were achieved after 27 days cultivation of *S. obliquus*, respectively. However, the final concentration of the medium was compared with a newly stripped sample (15 h) in the ammonia stripping experiment.

In addition, nutrient analyses from the undiluted culture mediums resulted to disconnected results in terms of NH_4-N and PO_4-P concentration since they were remarkably higher (62,67 mg/l and 43,55 mg/l (NH_4-N); 7,12 mg/l and 7,62 mg/l (PO_4-P)) in the mediums of Algae I and II (Samples 1 and 2), respectively, compared to the nutrient concentrations of 9,50 mg/l (NH_4-N) and 1,61 mg/l (PO_4-P) in the newly stripped sample (15 h) (Table 18). Therefore the nutrient composition of the mediums and stripped samples must be viewed and comparised critically.

Although ammonia stripping operational time was same for the nutrient removal samples (Samples 1–3) and ammonia stripping efficiency (Sample 9), the origin for the significantly lower NH_4-N concentration in the Sample 9 is probably from a smaller stripping volume. The volume of the stripped reject water for culture mediums (Samples 1–3) was around 800 ml whereas it was 200 ml (Sample 9) for nutrient analyses. Also, the solid particles in the reject water may disturb used analysis methods although the samples were centrifuged. More trustable nutrient uptake results can be achieved by analyzing mediums in the beginning of the cultivation period. This experiment, unfortunately, failed this opportunity. Furthermore, a nutrient analysis from mediums with a shorter cultivation period should have considered since *S. obliquus* in the mediums of 50 % reject water (Samples 1–3) grew rapidly already from the first day (Chapter 6.2.6.).

Table 18. Nutrient analysis results. The methods are presented in the Table 16.

	NO_3+NO_2	**PO_4**	**NH_4**	**TOTN**	**TOTP**	**N:P ratio**
Sample	*mg/l*	*mg/l*	*mg/l*	*mg/l*	*mg/l*	-
1. Algae I (100 % RW)	0,19	7,12	62,67	104,46	9,28	19
2. Algae II (100 % RW with P addition	0,01	7,62	43,55	85,01	9,50	13
3. Algae III (50 % RW)	0,01	0,08	0,03	23,51	1,45	1
4. 100% RW (before stripping)	0,05	1,33	744,74	879,64	10,51	1238
5. **2h** stripped	0,03	1,21	203,48	421,61	7,87	373
6. **4h** stripped	0,02	1,28	47,48	352,98	11,01	82
7. **6h** stripped	0,02	1,45	69,18	271,74	11,39	106
8. **8h** stripped	0,02	1,57	72,58	219,91	12,03	102
9. **15h** stripped	0,14	1,61	9,50	304,65	18,18	13

Still and all, the nutrient analysis confirmed that *S. oblicuus* reduced effectively nutrients from the diluted reject water (50 %). The analyzed NH_4-N and PO_4-P concentrations from the culture mediums of Algae I (62,67 mg/l and 7,12) and Algae II (43,55 mg/l and 7,62 mg/l) were higher compared to the medium of Algae III (0,03 mg/l and 0,08 mg/l). The culture medium of Algae I, II and III was originated from the same stripped sample. Thus, the reduction of NH_4-N by *S. obliquus* is verifiable in the diluted 50 % sample.

6.8. Flocculation by addition of cationic and anionic polymers

Tested polymers from Kemira showed a small or negligible precipitation effect on diluted (25 %) reject water from Envor Group Oy whereas a part of them performed a great precipitation effect in the undiluted reject water from the Viikinmäki WWTP. Figure 60 shows the polymer addition results in the Viikinmäki reject water. The polymers C-491, C-492, C-494 and A-150 performed the greatest settling effect of the precipitated matter; and the color of the reject water is notable lighter compared to the initial condition. A polymer A-150 was the only anionic polymer that precipitated solid matter. Its anionic charge was the highest of the tested anionic polymers. The precipitation effect of A-150 operated, however, slowly whereas cationic polymers started to precipitate solid matter immediately after the addition into the reject water and mixing. The greatest operated cationic polymers C-491 and C-492 possess a low cationic charge. C-491 probably operated greater than C-492 based on the reject water color in the Figure 60. The cationic charge increases with a larger number of the commercial polymer name. Thus, the precipitation effect weakened when the tested polymer had a greater cationic charge.

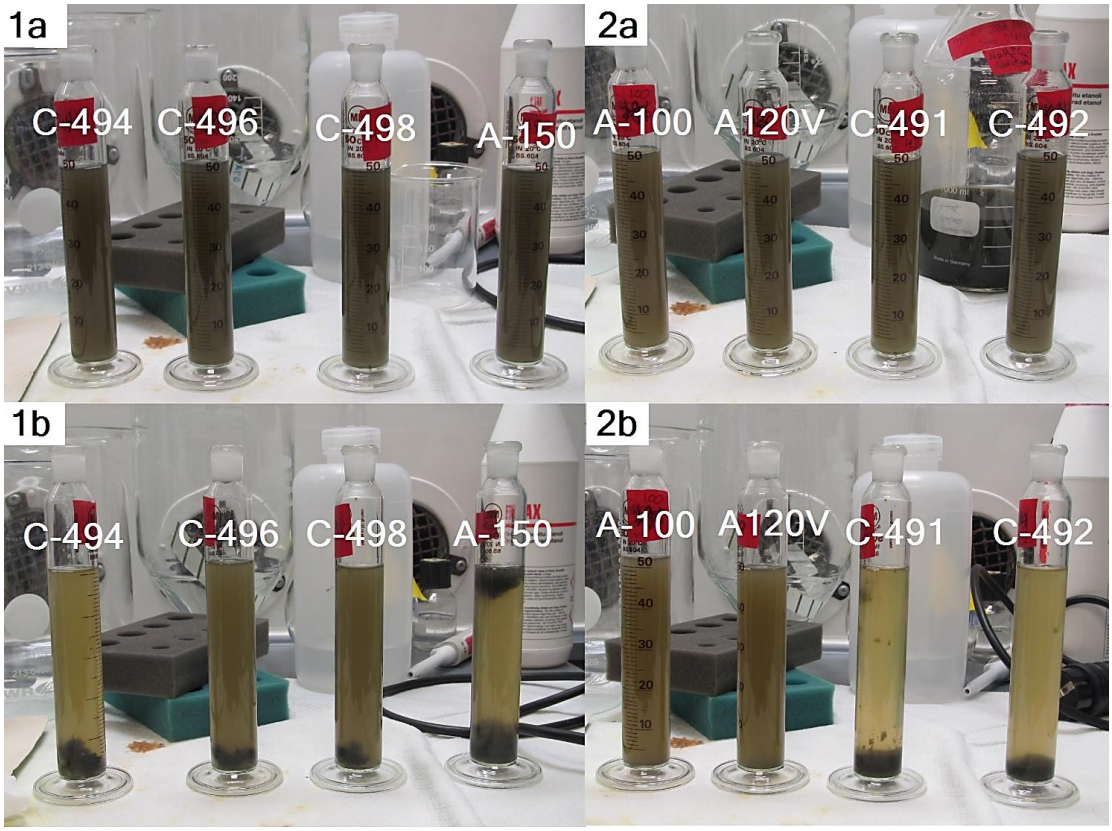


Figure 60. Polymer flocculation results in the Viikinmäki RW. Pictures 1a and 2a shows samples before the addition of the flocculant and 1b and 2b shows the results after the addition and mixing. The samples were settled for an hour after the addition. Photographed by Sara Merin.

6.9. Flocculation by addition of ferric sulfate (PIX-105)

The samples reacted and foamed strongly after the PIX-105 addition into the 100 % reject water samples (Figure 61) from Envor Group Oy. The foaming was more intensive after increasing PIX-105 dosage. The precipitated solid matter settled gradually on the bottom of the flasks after stirring was intercepted. The PIX-105 dosage of 33 g/l (undiluted PIX-105 in grams per liter reject water) resulted to the brightest reject water, which was noted after pouring the samples into test tubes (Figure 62). The largest dosage resulted to deep orange watercolor, which is probably due to iron content followed by the overdose of PIX-105. The volume of the precipitated matter was large, which indicates that the concentration of the total solids is high in the raw reject water.

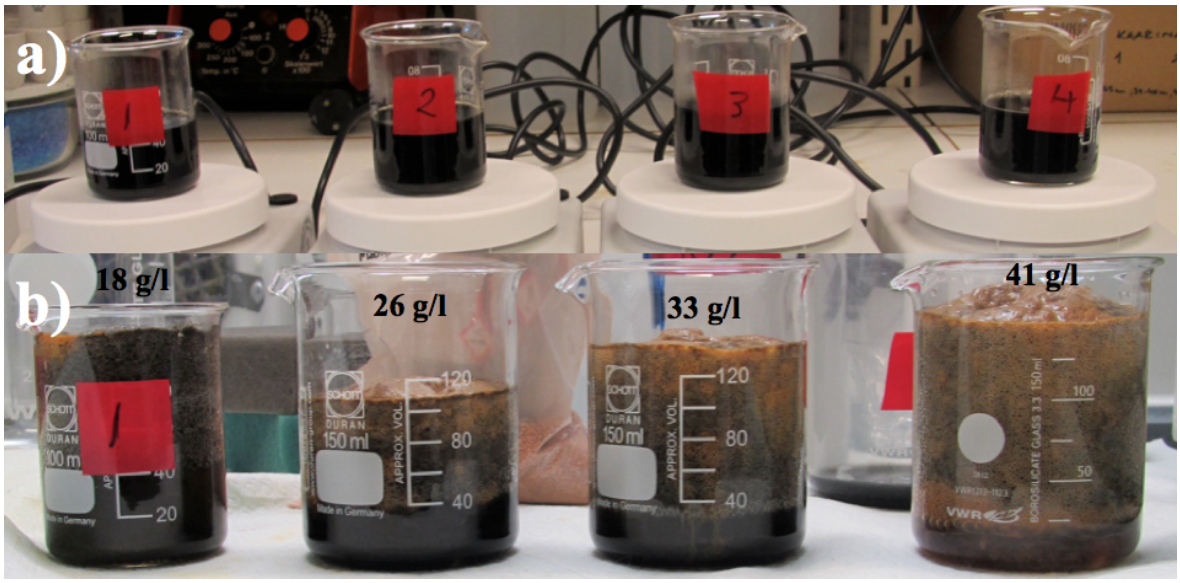


Figure 61. a) 100 % RW samples from Envor Group Oy before the addition of PIX-105 b) The samples after the PIX-105 addition of various dosages (Appendix 6). The samples were settled for an hour. The sample 1 foamed more than expected. Thus, samples 2,3 and 4 were sifted into larger containers before the PIX-105 addition. The dosage unit is undiluted PIX-105 in grams per liter. Photographed by Sara Merin.

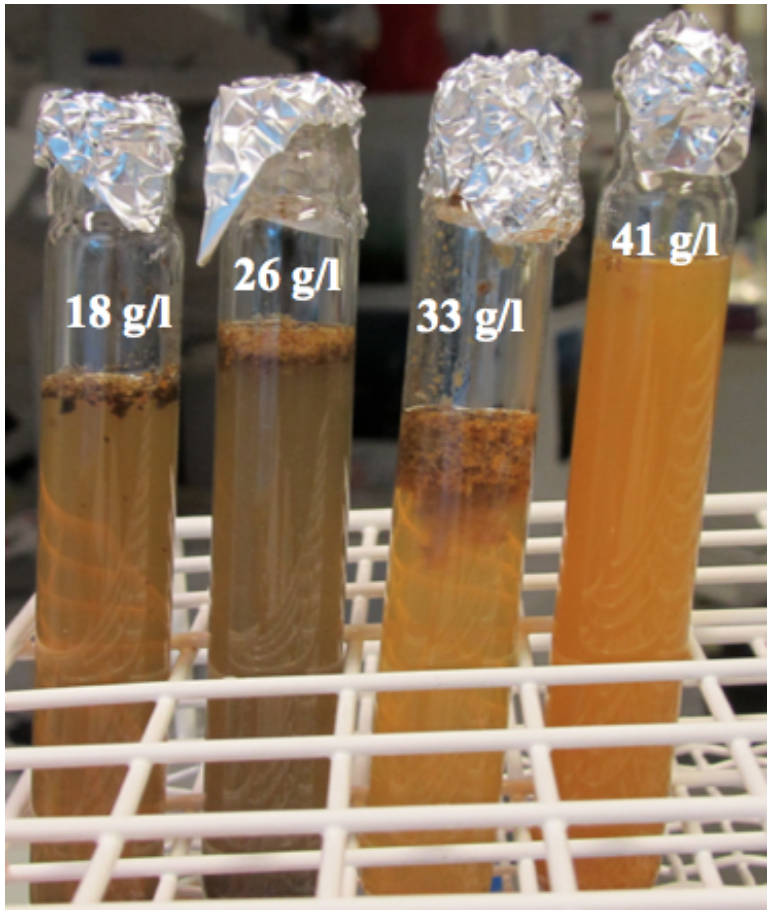


Figure 62. 100 % RW from Envor Group Oy after the PIX-105 addition. These treated RWs were poured from the decanter glasses that are in Figure 57. Photographed by Sara Merin.

PIX-105 addition into reject water from the Viikinmäki WWTP also resulted to precipitation of solid particles (Figure 63). The required dosage was approx. quarter of the beneficial PIX-105 dosage of the reject water from Envor Group Oy, which probably indicates about lower solid and phosphorus content. Moreover, the foaming was notable weaker in the Viikinmäki samples. In the end of the flocculation experiment, a combination of PIX-105 and anionic polymer C-491 was tested into the reject water from Viikinmäki. C-491 was added before PIX-105. It was noted that the reject water remained more orange compared to the sample in which only PIX-105 was added. This probably indicates about a smaller need for a PIX-105 addition after a polymer addition since the polymer may participate part of the solid particles first.

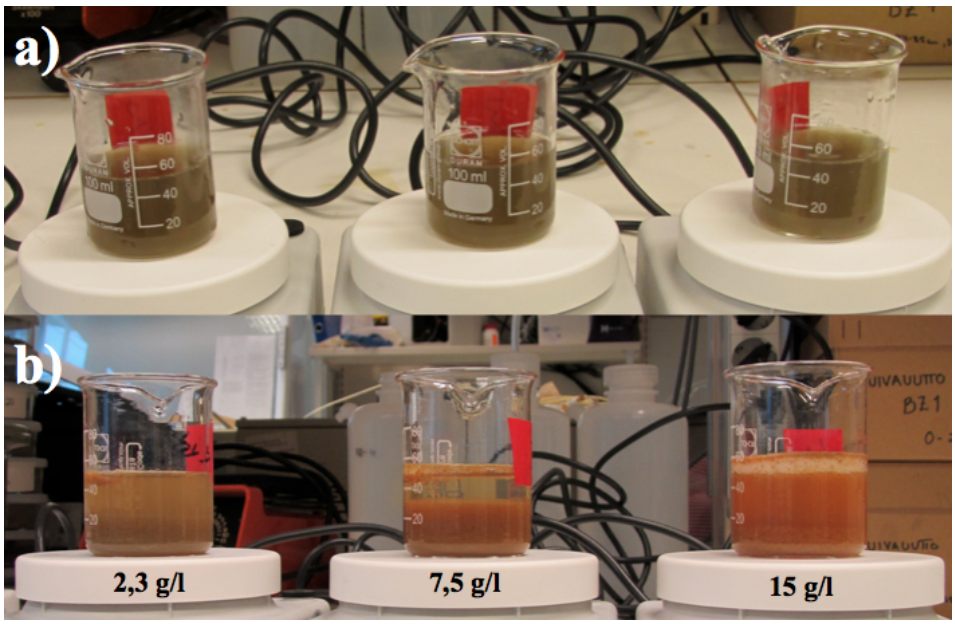


Figure 63. a) Viikinmäki RW samples before the PIX-105 addition. b) PIX-105 addition resulted precipitation. The dosage of 7,5 g/l resulted to the clearest layers of the purified water and precipitated matter. The dosage of 15 g/l resulted to a surplus ferric sulfate since the watercolor changed to deep orange probably due to excessively high iron concentration. The tested dosages are represented in Appendix 6. Photographed by Sara Merin.

6.10. Final discussion about the microalgae cultivation in Envor Group Oy reject water

Successful cultivation experiments were observed in the 12,5 % dilutions in which five microalgae strains: 1) *Golenkinia brevispicula Hegewald et Schnepf*, 2) *S. quadricauda*, 3) *Pediastrum simplex Meyen*, 4) *Monoraphidium contortum (Thuret)* and 5) *Sorastrum*

spinulosum Nägeli showed efficient growth. The lagging time, however, varied depended on the specie. The shortest lagging time (2–3 days) was in the culture of *Golenkinia brevispicula Hegewald et Schnepf*. The cultures of *Golenkinia brevispicula Hegewald et Schnepf*, *Pediastrum simplex Meyen* and the combination of the five species showed effective growth in the 25 % reject water. This indicates their capability to adapt light limited conditions since the dilution was remarkable dark. *Golenkinia brevispicula Hegewald et Schnepf* was probably the most adatable for the 25 % dilution according to the F_M intensities. The tested combination culture achieved approx. the same maximal F_M intensity in the last day of the cultivation period as the culture of *Golenkinia brevispicula Hegewald et Schnepf* in the 25 % dilution. The lagging time was, however, five days longer for the multiculture. Hence, any positive benefits resulted by the multicultural medium is difficult to conclude. Also, the dominating cultures in the combination were unknown. In terms of plate tests, 1) *Monoraphidium contortum (Thuret),* 2) *S. obliquus,* 3) *Selenastrum capricornutum Printz* 4) *Chlorella sp.* and 5) *Euglena gracialis* were the most adabtable for the medium. However, all the cultures probably collapsed in the dilutions that included reject water more than 30 %.

The dark color in reject water from Envor Group Oy probably inhibited the growth of the microalgae. The color is probably resulted from a high total solid content that leads to decreased availability of essential light in the water. The separation of the solid particles was difficult to decrease by a centrifuge and it did not improve the color or the reject water enough to create a more beneficial culture medium for microalgae. However, the solid particles in the reject water probably affected to the fluorescence intensities measured by fluorometers, thus the algal growth results may be uncertain.

6.11. Final discussion about the microalgae cultivation in Viikinmäki reject water

The N/P ratio was extremely high (1776) in the raw reject water from the Viikinmäki WWTP, which was probably not preferable for the most microalgae. However, species *S. obliquus, Chlorella sp., Monoraphidium contortum* and *S. quadricauda* showed growth in the centrifuged but strongly diluted reject water mediums, which indicates their capability to live under phosphorus weak conditions. There were ambivalent results about the influence of the decreased N/P ratio by a P addition. The decreased N/P ratio had mostly a

small positive or negligible influence to the microalgal growth. Based on the nutrient analysis, N/P ratio was decreased to a more balanced level after reject water was ammonia stripped followed by the ammonium nitrogen reduction and evaporation. This may explain the negligible effect of the P addition in the stripped mediums since then they included an excessively amount of phosphorus.

The experiments proved potentiality of nutrient uptake by the specie *S. obliquus*. *S. obliquus* showed to remove P and N compounds efficiently from 50 % reject water from the Viikinmäki WWTP. The reject water medium included negligible amount of NH_4-N (0,08 mg/l) and PO_4-P (0,03 mg/l) after 27 days cultivation period.

The oversupply of ammonium nitrogen (NH_4-N) was founded to constitute the main inhibiting factor for certain microalgae species. Ammonia stripping resulted to a more beneficial culture medium for these species. The stripping operation time of six hours resulted to higher F_M intensities on strong reject water concentrations compared to the operation time of four hours. In addition to operation time, ammonia stripping with continous magnet stirring was noticed to improve the removal of ammonia resulting to a more adaptable reject water medium. This is probably due to that stirring releases gaseous ammonia near to the air-water interface where it can eject from the liquid phase. After the stripping, the color of the reject water was lighter and more reddish. In terms of the red color, a possible origin for this is a high iron content. However, the origin for the color change remained unsolved.

Ammonia stripping caused precipitation. One reason for that may be the phosphorus precipitation since orthophosphate exists mostly in a solid form under high pH conditions. Secondly, the reject water probably contained calcium (Ca^{2+}) that can precipitate with orthophosphate forming tertiary calcium phosphate ($Ca_3(PO_4)_2$). $Ca_3(PO_4)_2$ can precipitate also magnesium (Mg^{2+}). Other possible precipitation compounds that formed during the ammonia stripping are struvite, limestone and ferric phosphate (Lehtovaara 2016). A presence of iron can improve the flocculation effect without binding itself to phosphorus (Karttunen p. 143). Therefore, the flocculation during ammonia stripping can be originated from the iron concentration in the reject water since a certain co-digestion plant reported about a high (33 mg/l) iron concentration. The iron concentration in the reject water from Viikinmäki was, however, unknown.

6.12. Final discussion about the flocculation

Kemira polymers showed a weak precipitation effect of solid matter in the Envor Group Oy reject water whereas they performed greatly in the reject water from Viikinmäki WWTP. Especially cationic polymers C-491 and C-492 with a low cationic charge performed both efficient precipitation and settling. Anionic polymer A-150 with a high anionic charge also precipitated solids effectively but the settling operated significantly slower compared to the cationic polymers. However, this indicates that the reject water includes both negatively and positively charged particles since cationic polymers neutralize negatively charged particles whereas anionic polymers neutralize positively charged particles (Tchobanoglous pp. 485–486).

PIX-105 (ferric sulfate) showed great a precipitation effect of solid matter in both reject water samples. PIX-105 can be added for improving light conditions in reject water. A dosage of 33 g of PIX-105 per liter reject water was observed to result to the brightest water in terms of reject water from Envor Group Oy. Reject water from Viikinmäki WWTP required less PIX-105 for precipitation of solids: a dosage of 7,5 g/l was observed to result to the most effective precipitation effect. A lower dosage indicates about the lower concentration of solids and P since PIX-105 precipitates especially phosphorus compounds (Kemira 2016). Also, the volume of the precipitation was smaller compared to the PIX-105 treated reject water from Envor Group Oy. In terms of microalgae cultivation, the precipitation of P may affect negatively to the growth of algae. An addition of cationic polymer C-491 and ferric sulphate PIX-105 can probably be a solution for reducing consumption of PIX-105 for purification of Viikinmäki reject water since the polymer probably precipitates part of solid particles first.

7 CONCLUSIONS

The quality of the considered reject waters from the Envor Group Oy co-digestion plant and Viikinmäki WWTP differenced significantly. The different type of the feedstocks, digestate dewatering technology, ammonia stripping and effects followed by the municipal WW treatments constitute factors that affect to the reject water composition. Feedstocks with a richer content of various compounds e.g. trace metals and vitamins result to more efficient and complete degradation by anaerobic bacteria under anaerobic conditions. The

water content of the feedstock material has an influence at least to the biogas production but its effect to the reject water quality is unclear.

Compared to the reject water from Envor Group Oy, reject water from the Viikinmäki WWTP was observed to be more beneficial as a culture medium for microalgae probably due to its lighter color and lower solid particle content. The lighter color of the reject water is probably resulted from more diverse content of the digested municipal wastewater sludge compared to biowaste and agricultural waste that Envor Group Oy principally utilizes as feedstock material. However, the phosphorus concentration was much lower in the Viikinmäki reject water, which was probably due to the use of ferrous sulfate during the municipal wastewater treatment process.

The four most adaptable species for reject water medium were probably 1) *S. obliquus* 2) *Chlorella sp.* and 3) *Monoraphidium contortum* 4) *S. quadricauda* since they grew both in reject water from Envor Group Oy and Viikinmäki WWTP. *S. obliquus* may be able to produce the most efficiently biomass based on the fluorescence measurements and the strongest visible green color in the stripped reject water mediums. A tolerance towards high ammonium nitrogen concentration, high pH, low light conditions and presence of bacteria is strongly specie depended, which was also observed during the experiments because only certain species adapted stripped Viikinmäki reject water as a culture medium. Also, e.g. *Golenkinia brevispicula Hegewald et Schnepf* and *Pediastrum simplex Meyen* survive under light limited conditions based on cultivation tests in 25 % reject water from Envor Group Oy. Nevertheless, these same species showed poor growth in reject water from the Viikinmäki WWTP, which may indicate about poor tolerance towards ammonium nitrogen concentration or e.g. bacteria. Therefore, the microalgae specie must be selected carefully for a cultivation installation at a biogas plant.

The effective growth of 1) *Golenkinia brevispicula Hegewald et Schnepf*, 2) *S. quadricauda*, 3) *Pediastrum simplex Meyen*, 4) *Monoraphidium contortum (Thuret)* and 5) *Sorastrum spinulosum Nägeli* was observed in the dilutions of 12,5 % reject water from Envor Group Oy co-digestion plant. Summarily, the reject water was unsuitable for algal cultivation in strong concentrations probably due to its extremely dark color. The solid particles that probably caused the dark color and the inhibition can be removed by flocculation and filtration. Ferric sulfate (PIX-105) was observed to constitute a suitable

additive for forming precipitation that can be removed lastly by settling or filtering. The dosage of 7,5 g of PIX-105 per a liter of reject water resulted to the most effect precipitation effect. However, the effects of ferric sulfate addition into reject water must be investigated in terms of microalgae cultivation. The addition of cationic and anionic polymers resulted to a negligible precipitation effect in the reject water.

Ammonia stripped and diluted reject water from Viikinmäki WWTP can be suitable for effective biomass production of microalgae. The ammonia stripping leaded to decreased ammonium nitrogen (NH_4-N) content and balanced N/P ratio, which created a beneficial culture medium for certain species. In the 50 % dilution of reject water, the growth was rapid and *S. obliquus* removed effectively nitrogen and phosphorus compounds. Addition of polymer precipitated solid particles effectively without centrifugation from the reject water. The cationic polymers C-491, C-492 and C-494 by Kemira with a low cationic charge resulted to the greatest flocculation effect. Also, the addition of the anionic polymer A-150 with a high anionic charge resulted to effective flocculation but the operation time of the settling was longer compared to the cationic polymers. Addition of ferricsulfate (PIX-105) was also a functional chemical for precipitation of solid particles from the reject water. The dosage of 33 g of PIX-105 per a liter of reject water resulted to the most effect precipitation effect. A polymer or PIX-105 addition is perhaps more attractive for improving reject water for culture medium compared to energy-intensive centrifugation. However, the purchasion of chemicals causes also costs.

The maintenance of the culture medium in reject water may encounter difficulties since numerous factors have an influence to the growth of microalgae. However, since many microalgae species have a capability to adapt various environments, they can also be adaptable for possible changes. Mixotrophic and heterotrophic cultures may be more advantageous for cultivation under light limited conditions. Also, diverse cultures can be more stable and resilient towards contamination. The advantages of diverse cultures should be examined extensively.

A reject water culture medium should be monitored and maintenanced for ensuring favorable conditions for microalgae since e.g. pH value, a precense of certain bacteria, lack of light and an excess of ammonium nitrogen can have a fateful influence for a culture comfort. However, pH level has no radical variation between co-digestion plants, which

was observed based on the inverviews of co-digestion plants in Finland. A co-digestion plant that utilizes mostly same feedstock material between digestion batches may prevent radical changes in the quality of generated reject water. Also, the functionality of digestate dewatering can be improved by optimization and monitoring with persistent measurements of the solid content, which is founded to lead both greater reject water quality and lower energy consumption.

Based on personal interviews of workers at co-digestion plants, the attitudes towards cultivation of microalgae were positive. Hence, the potential of microalgae cultivation installation can be more achievable. A pilot system of microalgae cultivation installation should be examined since there are numerous unsolved factors effecting to the system. For example, essential separate treatments for reject water before leading it to the cultivation pond, biomass harvesting technologies, light availability, effects to the composition of new reject water after co-digestion with algal biomass, biogas yield maximation and contamination risks are all components which will have a part in the overall functionality of the system. Also, microalgal growth in a larger volume of reject water may performs totally differently compared to laboratory-scale experiments.

Lastly, a comprehensive investigation about energy and greenhouse gas balance is required for the clearing profitability of the installation especially for achieving an energy positive and carbon negative culture installation. Moreover, the system needs to be economically feasible and profitable, thus an estimation of cost is required. In the best scenario, microalgae cultivation system will create an effective cycle of materials and energy, which reduces impurities from reject water and increases biogas yield.

REFERENCES

Aaltonen H. 2013. Flokkulantin käyttö sakeuttimella (Usage of Flocculant In Thickener). Thesis. Satakunta University of Applied Sciences. 47 p. Available: https://www.theseus.fi/handle/10024/53570 [Referenced 2.9.2016].

Act on Fertiliser Products 539/2006.

Aittomäki E., Eerikäinen T., Leisola M., Ojamo H., Suominen I. & von Weymarn N. 2002. Bioprosessitekniikka. 445 p. ISBN: 9789510269954.

Abdel-Raouf N., Al-Homaidan A.A. & Ibraheem I.B.M. 2012. Microalgae and wastewater treatment. Saudi Journal of Biological Sciences, 19. pp. 257–275. DOI: 10.1016/j.sjbs.2012.04.005.

Barsanti L. & Gualtieri P. 2014. Algae Anatomy, Biochemistry, and Biotechnology. 2nd Edition. 345 p. ISBN: 978-1-4398-6732-7.

Becker E.W. 1994. Microalgae: Biotechnology and microbiology. 295 p. ISBN: 0-521-35020-4.

Beneragama C.K. & Goto K. 2010. Chlorophyll a: b Ratio Increases Under Low-light in 'Shade-tolerant' Euglena gracilis. Tropical Agricultural Research, 22 (1). pp. 12–25.

BioKymppi Oy. 2016. Information label. [pdf-file]. Available: http://www.bio10.fi/binary/file/-/id/1/fid/114 [Updated 11.4.2016] [Referenced 25.9.2016].

Biotehdas Oy. 2016. Information label. VamBio. [pdf-file]. Available: http://www.biotehdas.fi/media/uploads/pdf/tuoteseloste-vambion-perus-2016-002.pdf [Updated 11.4.2016] [Referenced 25.9.2016].

Biovakka. 2016. Vehmaan biokaasulaitos. Laitoksemme. [online]. Available: http://www.biovakka.fi/vehmaan-biokaasulaitos [Referenced 25.9.2016].

Bitton G. 2005. Wastewater Microbiology. McDonald 3rd Edition. 746 p. ISBN: 0-471-65071-4.

Boisvert S., Joly D & Carpentier R. 2006. Quantitative analysis of the experimental O-J-I-P chlorophyll fluorescence induction kinetics. FEBS Journal, 273 (20). pp. 4770–4777. DOI: 10.1111/j.1742-4658.2006.05475.x.

Bowker M.A., Reed S.C., Belnap J. & Phillips S.L. 2002. Temporal Variation in Community Composition, Pigmentation, and Fv/Fm of Desert Cyanobacterial Soil Crusts. Microbial Ecology, 43. pp. 13–25. DOI: 10.1007/s00248-001-1013-9.

Cai T., Park S. & Li Y. 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renewable and Sustainable Energy Reviews, 19. pp. 360–369. DOI: http://dx.doi.org/10.1016/j.rser.2012.11.030.

Campbell N.A. & Reece J.B. 2008. Biology. 8th edition. 1393 p. ISBN 978-0-321-53616-7.

Campos J., Moura D., Costa A., Yokoyama L., da Fonseca Araujo F., Cammarota M. & Cardillo L. 2013. Evaluation of pH, alkalinity and temperature during air stripping process for ammonia removal from landfill leachate. Journal of Environmental Science and Health, Part A, 48. pp. 1105–1113. ISSN: 1093-4529. DOI: 10.1080/10934529.2013.774658.

Chen C., Yeh K., Aisyah R., Lee D. & Chang J. 2010. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. Bioresource Technology, 102. pp. 71–81. DOI: 10.1016/j.biortech.2010.06.159.

Chinnasamy S., Ramakrishnan B., Bhatnagar A. & Das K. 2009. Biomass Production Potential of a Wastewater Alga Chlorella vulgaris ARC 1 under Elevated Levels of CO2 and Temperature. International Journal of Molecular Sciences, 10 (2). pp. 518-532. DOI:10.3390/ijms10020518.

Cho S., Luong T.T. Lee D., Oh Y., & Lee T. 2011. Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. Bioresource Technology, 102. pp. 8639–8645. DOI: 10.1016/j.biortech.2011.03.037.

Christensen T. 2011. Solid Waste Technology & Management. Volume 2. 1052 p. ISBN: 978-1-4051-7517-3.

Christensson M. Ekström S., Andersson C. Le Vaillant E. & Lemaire R. 2013. Experience from start-ups of thefirst ANITA Mox Plants. Water Science & Technology, 67 (12). pp. 2677–2684. DOI: 10.2166/wst.2013.156.

Constantine T. 2006. North American Experience With Centrate Treatment Technologies For Ammonia and Nitrogen Removal. Proceedings of the Water Environment Federation, WEFTEC 2006: Session 61 through Session, 70. pp. 5271–5281. DOI: 10.2175/193864706783763291.

Coombs J., Hall D.O. Long S.P. & Scurlock J.M.O. 1985. Techniques in Bioproductivity and Photosynthesis. 2nd Edition. 298 p. ISBN: 0-08-031999-8.

Courtesy of Ron George. 2013. Disinfection Chart. [online]. Legionella prevention. Plumb-Tech Design & Consulting Services LLC. Available: http://www.legionellaprevention.org/disinfect.html [Referenced 23.8.2016].

Correll D.L. 1999. Phosphorus: A Rate Limiting Nutrient in Surface Waters. Poultry Science, 78 (5). pp. 674-682. DOI: 10.1093/ps/78.5.674.

de Clippeleir H., Yan X., Verstraete W. & Vlaeminck S. 2011. OLAND is feasible to treat sewage-like nitrogen concentrations at low hydraulic residence times. Applied Microbiology and Biotechnology, 90. pp. 1537–1545. DOI: 10.1007/s00253-011-3222-6.

Demirbas A. & Demirbas M. 2011. Importance of algae oil as a source of biodiesel. Energy Conversion and Management, 52. pp. 163–170. DOI: 10.1016/j.enconman.2010.06.055.

Demirbas A. & Demirbas M. 2010. Algae Energy, Algae as a New Source of Biodiesel. 198 p. ISSN 1865-3529. ISBN 978-1-84996-04. DOI: 10.1007/978-1-84996-050-2.

Directive 2014/94/EU of the European Parliament and of the Council of 22 October 2014 on the deployment of alternative fuels infrastructure.

Directive 2014/94/EU of the European Parliament and of the Council of 22 October 2014 on the deployment of alternative fuels infrastructure.

Ek M., Bergström R., Bjurhem J.-E., Björlenius B. & Hellström D. 2006. Concentration of nutrients from urine and reject water from anaerobically digested sludge. Water Science and Technology, 54 (11–12). pp. 437–444. DOI: 10.2166/wst.2006.924.

Equipements Scientifiques SA 2008. Aquapen-C AP-C 100. [pdf-file]. ES ISO 9001: 2008. Available: http://www.es-france.com/pdf/AquaPen.pdf [Referenced 29.8.2016].

Envor Group. Biokaasulaitos. [Online]. Available: http://envor.fi/teknologia/biokaasu/biokaasulaitos [Referenced 25.9.2016].

Ficara E., Uslenghi A., Basilico D. & Mezzanotte V. 2014. Growth of microalgal biomass on supernatant from biosolid dewatering. Water Science & Technology, 69 (4). pp. 896–902. DOI: 10.2166/wst.2013.805.

Finnish Food Safety Authority Evira. 2016. Regulation for fertilizer products. [Online]. Available: http://www.ravitsemusneuvottelukunta.fi/files/attachments/fi/kasvit/lannoitevalmisteet/raportit/tyyppinimiluettelo_konsolidoitu_31_3_2016.pdf [Referenced 26.9.2016].

Finnish Government Decree on Landfills 331/2013.

Fred T. 2013. Mädätys HSY:n jätevedenpuhdistamoilla. [powerpoint presentation]. Digestion structure and equipment technology seminar. HSY 2016. [Referenced 29.8.2016]. Available: http://www.vesiyhdistys.fi/pdf/Tommi_Fred_HSY.pdf.

Fux C. & Siegrist H. 2004. Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: environmental and economical considerations. Water Science and Technology, 50 (10). pp. 19–26.

Gasum Biotehdas Oy. 2016. Informative label. [online] Available: http://www.biotehdas.fi/media/uploads/pdf/tuoteseloste-vambion-perus-2016-003.pdf. [Referenced 11.8.2016].

Gonzáles C., Marciniak J. Villaverde S., García-Encina P. & Muñoz R. 2008. Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. Applied Microbiology and Biotechnology, 80 (5). pp. 891–898.

González-López C.V., Acién Fernández F.G., Fernández-Sevilla J.M., Sánchez Fernández J.F. & Molina Grima E. 2012. Development of a Process for Efficient Use of CO2 from Flue Gases in the Production of Photosynthetic Micro-organisms. Biotechnology and Bioengineering, 109 (7). pp. 1637–1650. DOI: 10.1002/bit.24446.

Google Earth. 2009. [map program and service].

Grasshoff, K., Kremling, K. and Ehrhardt, M., 1999. Methods of Seawater Analysis; Third, Completely Revised and Extended Edition. Wiley-VCH.

Gustavsson D. 2010. Biological sludge liquor treatment at municipal wastewater treatment plants – a review. Vatten 66. pp. 179–192. Sweden.

Hannon M., Tran M., Gimpel J.A., Rasala B.A. & Matfield S. 2010. Biofuels from algae: Challenges and potential. Biofuels. 1(5). pp. 763–784. DOI: 10.4155/bfs.10.44.

Heinonen T. 2014. Biokaasulaitoksen rejektiveden kierrätys prosessivetenä (Reject water recycling as a process water in a biogas plant). Thesis. 34 p. HAMK University of Applied Sciences. Finland.

Hellinga C., Schellen A.A.J.C., Mulder J.W., van Loosdrecht M.C.M. & Heijnen J.J. 1998. The SHARON Process: An Innovative Method for Nitrogen Removal from Ammonium-rich Waste Water. Water Science and Technology, 37 (9). pp. 135–142.

Helsinki Region Environmental Services (HSY). 2016. Authority. Jätevedenpuhdistus pääkaupunkiseudulla 2015 - Viikinmäen ja Suomenojan puhdistamot. 69 p. ISBN: 978-952-6604-95-4. ISNN: 1798-6095 (pdf).

Herrmann C., Kalitaa N., Walla D., Xiaa A. & Murphy J. 2016. Optimised biogas production from microalgae through co-digestion with carbon-rich co-substrates. Bioresource Technology, 214. pp. 328–337.

Hienonen L. 2009. Rejektiveden biologisen erilliskäsittelyn koeajot Viikinmäessä. (Separate biological treatment of reject water on trial at the Viikinmäki WWTP). Master's Thesis. 118 p. Helsinki University of Technology.

Ho S., Huang S., Chen C., Hasunama T., Kondo A. & Chang J. 2012. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. Biosource Technology. 135. pp. 191–198. DOI: http://dx.doi.org/10.1016/j.biortech.2012.10.015.

Huttunen M. & Kuittinen V. 2016. Suomen biokaasulaitosrekisteri n:o 19, Tiedot vuodelta 2015. Reports and Studies in Forestry and Natural Sciences. Publications of the University of Eastern Finland. 42 p. ISBN: 978-952-61-2161-1 (pdf). ISSN: 1798-5692.

Inthorn D., Sidtitoon N., Silapanuntakul S. Incharoensakdi A. 2002. Sorption of mercury, cadmium and lead by microalgae. Science Asia, 28. pp. 253–261.

Jeppo Biogas Ab. 2016. Information label. [pdf-file]. [Updated 23.3.2016] Available: http://www.jeppobiogas.fi/assets/analys/TUOTESELOSTE-2016.pdf [Referenced 25.9.2016].

Jeyanayagam S. 2005. True Confessions of the Biological Nutrient Removal Process. Florida Water Resources Journal. January 2005. pp. 37–46. Available:

http://www.deq.state.va.us/Portals/0/DEQ/Water/Wastewater/TrueConfessions_000.pdf [Referenced 21.9.2016].

Jones C. & Mayfield S. 2012. Algae biofuels: versatility for the future of bioenergy. Current Opinion in Biotechnology. 23. pp. 346–351. DOI 10.1016/j.copbio.2011.10.013.

Joutsan Ekokaasu Oy. 2016. Biokaasulaitos. [online]. Available: http://www.joutsanekokaasu.fi/4 [Referenced 25.9.2016].

Juhe H. Plant manager. 2016. Private phone interview. Envor Group Oy. [18.5.2016].

Juura J. Production engineer. 2016. Private email. [22.6.2016],

Tekniikka ja Talous 27. 2.9.2016. Anturi siellä, mittari täällä, liete tuolla. [Professional yield journal]. pp. 16–17.

Karttunen E. 2004. RIL 124-2 Vesihuolto II. 684 p. ISBN: 951-758-438-5. ISSN: 0356-9403.

Kemira. 2016. Ferric sulfate. Application & Product Finder. Industries & Applications. [online]. Available: http://www.kemira.com/en/industries-applications/pages/ferric-sulfate.aspx [Referenced 29.9.2016].

van Kempen. R, Mulder J.W., Uijterlinde C.A. & Loosdrecht M.C.M. 2001. Overview: full scale experience of the SHARON® process for treatment of rejection water of digested sludge dewatering. Water Science and Technology, 44 (1). pp. 145–152.

Kieniewicz A. 2006. A Reverse Osmosis (RO) Plant for Sewage Treatment and Nutrient Recovery - The Influence of Pre-treatment Methods. Master's thesis. 57 p. Royal Institute of Technology. Architecture and Built Environment. ISSN 1651-064X.

Koroleff, F. 1983: Determination of ammonia. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (eds.), Methods of Sea Water Analysis, Verlag Chemie, Weinheim (2. ed.) pp. 150–157.

Koroleff, F. 1983. Determination of nutrients. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (eds.), Methods of Sea Water Analysis, Verlag Chemie, Weinheim (2. ed.). pp: 36–138, 164–169.

Kouvolan Vesi Oy. 2016. Biolaitos. [online]. Available: http://www.kouvolanvesi.fi/biolaitos/ [Referenced 25.9.2016].

Kuenen J. 2008. *Anammox bacteria*: from discovery to application. Nature Reviews Microbiology, 6. pp. 320-326. DOI: 10.1038/nrmicro1857.

Kuglarz M., Grűbel K. & Bohdziewicz J. 2015. Chemical precipitation and ammonia air stripping as effective pre-treatment methods before membrane filtration of co-digestion effluents. Desalination and Water Treatment, 55. pp. 1672–1682. DOI: 10.1080/19443994.2014.942560.

Kuokkanen Anna. 2016. Development engineer. Helsingin seudun ympäristöpalvelut (HSY). Helsinki Region Environmental. Private interview. [29.6.2016].

Kuosma Kristiina. Process engineer. Stormossen biogas plant. Private phone interview. [6.7.2016].

Kymäläinen M. & Pakarinen O. 2015. Biokaasuteknologia: raaka-aineet, prosessointi ja lopputuotteiden hyödyntäminen. 197 p. ISBN: 978-951-784-770-4. ISSN: 1795-4231.

Lakaniemi A. 2012. Microalgal Cultivation and Utilization in Sustainable Energy Production. Doctoral Thesis. Tampere University of Technology. 80 p. ISBN: 978-952-15-2792-0.

Lakaniemi A., Hulatt C., David T., Tuovinen O. & Puhakka J. 2011. Biogenic hydrogen and methane production from Chlorella vulgaris and Dunaliella tertiolecta biomass. Biotechnology for Biofuels, 4:34. DOI: 10.1186/1754-6834-4-34.

Lakeuden Etappi. 2014. Information label. RANU-maanparannusrae. [pdf-file]. Available: http://www.etappi.com/files/8114/2684/2210/TUOTESELOSTE_30_10_2013_versio_1_26_2_2015_ranu.pdf [Referenced 25.9.2016].

Lakowicz J. 2006. Principles of Fluorescence Spectroscopy. 3rd edition. 698 p. ISBN-10: 0-387-31278-1. ISBN-13: 978-0387-31278-1.

Larkum AWD. 2010. Limitations and prospects of natural photosynthesis for bioenergy production. Current Opinion in Biotechnology, 21. pp. 271–276.

Latvala M. 2009. Paras käytettävissä oleva tekniikka (BAT) Biokaasun tuotanto suomalaisessa toimintaympäristössä. Suomen Ympäristö, 24. 114 p. ISBN: 978-952-11-3497-5. ISSN: 1238-7312.

Lazár D. 2006. The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. Functional Plant Biology, 33. pp. 9–30. DOI: 10.1071/FP05095.

Lee A., Lewis D. & Ashman P. 2012. Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. Biomass and Bioenergy, 46. pp. 89–101. DOI: 10.1016/j.biombioe.2012.06.034.

Lehto P. 2010. Biokaasulaitoksen rejektivesien vaikutus jätevedenpuhdistamon toimintaan ja rejektiveden erilliskäsittelyn tarpeellisuus (The impact of reject water from a biogas plant in wastewater treatment plant and the necessity of pre-treatment). Master's thesis. 62 p. Lappeenranta University of Technology.

Lehtomäki A., Paavola T., Luostarinen S., Rintala J. 2007. Biokaasusta Energiaa Maatalouteen – Raaka-aineet, Teknologiat ja Lopputuotteet. Research reports in biological and environmental sciences, 85. University of Jyväskylä. ISBN 978-951-39-3076-9. ISSN 1795-6900.

Lehtovuori J. 2016. Area Sales Manager/Municipal & Industrial. Kemira. [Private phone interview 15.8.2016].

Lei X., Sugiura N., Feng C. & Maekawa T. 2006. Pretreatment of anaerobic digestion effluent with ammonia stripping and biogas purification. Journal of Hazardous Materials, 145 (3). pp. 391–397.

Levin D., Pitt L. & Love M. 2004. Biohydrogen Production: Prospects and Limitations to Practical Application. International Journal of Hydrogen Energy. 29. pp. 173–187. DOI: 10.1016/S0360-3199(03)00094-6.

Li K., Liu S. & Liu X. 2014. An overview of algae bioethanol production. International Journal of Energy Research, 38. pp. 965-977. DOI: 10.1002/er.3164.

Li Y, Chen Y., Chen P., Min M., Zhou W., Martinez B., Zhu J. & Ruan R. 2011. Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresource Technology, 102. pp. 5138–5144. DOI: 10.1016/j.biortech.2011.01.091.

Li, Y., Horsman, M., Wu, N., Lan, C. Q. & Dubois-Calero, N. 2008. Biofuels from microalgae. Biotechnology progress, 24. pp. 815–820. DOI: 10.1021/bp070371k.

Lindell P. 2012. Demon-prosessin käynnistämisen kriittiset parametrit ja käytännön toteutus (Critical parameters of Demon-process start-up and practical implementation). Master's Thesis. 74 p. Tampere University of Technology.

Liu S. 2007. Food and Agricultural Wastewater Utilization and Treatment. 277 p. ISBN: 978-0-8138-1423-0.

Manninen K., Huttunen S., Seppälä J., Laitinen J. & Spilling K. 2015. Resource recycling with algal cultivation: environmental and social perspectives. Journal of Cleaner Production, 134. pp. 494–505. DOI:10.1016/j.jclepro.2015.10.097.

Mata T., Martins A. & Caetano N. 2009. Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews, 14 (1). pp. 217–232. DOI: 10.1016/j.rser.2009.07.020.

Markou G. & Georgakakis D. 2010. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. Applied Energy, 88. pp. 3389–3401. DOI: 10.1016/j.apenergy.2010.12.042.

Masłoń A. & Tomaszek J.A. 2007. Deammonification process of sludge digester liquors in biofilm systems. Environment Protection Engineering, 33 (2). pp. 175–182.

Matintalo S. Regional Manager. 2016. Biokaasulaitos ja jätteiden käsittely Hallavaaran jätekeskuksessa. [Powerpoint presentation]. Loimi-Hämeen jätehuolto Oy. Available: http://www.lhj.fi/UserFiles/lhj/File/Aineistopankki/Seminaariaineistot/2016/9_Matintalo_Omistajapaiva%202016%20Sanna%20Matintalo.pdf [Referenced 11.8.2016].

Marttinen, S., Paavola, T., Ervasti, S., Salo, T., Kapuinen, P., Rintala, J., Vikman, M., Kapanen, A., Torniainen, M., Maunuksela, L,, Suominen, K., Sahlström, L., Herranen, M. 2013. Biokaasulaitosten lopputuotteet lannoitevalmisteena (Fertiliser products from biogas plants). MTT Agrifood Research Finland Report, 82. 70 p. ISBN: 978-952-487-431-1. ISSN 1798-6419.

McDonald M. 2003. Photobiology of Higher Plants. 368 p. ISBN: 978-0-470-85523-2.

Min M., Wang L., Li Y., Mohr M., Hu B., Zhou W., Chen P. & Ruan R. 2011. Cultivating Chlorella sp. in a Pilot-Scale Photobioreactor Using Centrate Wastewater for Microalgae Biomass Production and Wastewater Nutrient Removal. Applied Biochemistry and Biotechnology, 165. pp. 123–137. DOI 10.1007/s12010-011-9238-7.

Ministry of Agriculture and Forestry Decree 24/11

Mostafa M., Gharieb M.M. & Abou-El-Souod G.W. 2009. Biodegradation of dyes by some green algae and cyanobacteria. International Biodeterioration & Biodegradation, 63. pp. 699–704. DOI: 10.1016/j.ibiod.2009.04.010.

Mulder J.W., Duin J.O.J., Goverde J., Poiesz W.G., van Veldhuizen H.M., van Kempen R. & 2006. Roeleveld P. Full-Scale Experience with the Sharon Process through the Eyes of

the Operators. Proceedings of the Water Environment Federation. WEFTEC 2006: Session 61 through Session 70. pp. 5256–5270. DOI: 10.2175/193864706783763444.

Muñoz R. & Guieysse B. 2006. Algal–bacterial processes for the treatment of hazardous contaminants: A review. Water Research, 40. pp. 2799–2815. DOI: 10.1016/j.watres.2006.06.011.

Murchie E.H. & Lawson T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. Journal of Experimental Botany, 64. pp. 3983–3998 . DOI: 10.1093/jxb/ert208.

Neves V., Sales E. & Perelo L. 2016. Influence of lipidextraction methods as pre-treatment of microalgal biomass for biogas production. Renewable and Sustainable Energy Reviews, 59. pp. 160–165. DOI: 10.1016/j.rser.2015.12.303.

Nguyen, M.T., Choi, S.P., Lee, J., Lee, J.H., Sim, S.J., 2009. Hydrothermal acid pretreatment of Chlamydomonas reinhardtii biomass for ethanol production. Journal of Microbiology and Biotechnology, 19 (2). pp. 161–166.

Normington D. Globar product manager. 2012. Kemira Water Treatment Seminar. [Powerpoint presentation]. Available: http://www.hidrosis-ws.com/images/pdfler/3-En-Er%20Kemiraseminars-Polymerr-3.pdf [Referenced 29.8.2016].

Ogbonna J., Yoshizawa H. & Tanaka H. 2000. Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. Journal of Applied Phycology, 12 (3). pp. 277–284. DOI: 10.1023/A:1008188311681.

Oswald W.T. & Gotaas H.B. 1957. Photosynthesis in sewage treatment. Transactions of the American Society of Civil Engineers 122. pp. 73–97.

Othman M.Z., Uludag-Demirer S. & Demirer G.N. 2009. Enhanced Nutrients Removal in Conventional Anaerobic Digestion Processes. World Academy of Science, Engineering and Technology, 58. pp. 1206–1212.

Paajanen Irene. Quality Manager. Envor Group Oy. Private phone call. [28.9.2016].

Parades D., Kuschk P., Mbwette T.S.A., Stange F., Müller R.A. & Köser H. 2007. New Aspects of Microbial Nitrogen Transformations in the Context of Wastewater Treatment – A Review. Engineering in Life Sciences 7 (1). pp. 13–25. DOI: 10.1002/elsc.200620170.

Park J., Jin H., Lim B., Park K. & Lee K. 2010. Ammonia removal from anaerobic digestion effluent of livestock waste using green alga Scenedesmus sp. Bioresource Technology, 101. pp. 8649–8657.

Park K., Kweon J., Chantrasakdakul P., Lee K. & Young Cha H. 2013. Anaerobic digestion of microalgal biomass with ultrasonic disintegration. International Biodeterioration & Biodegradation, 85. pp. 598–602.

Park S. & Li Y. 2012. Evaluation of methane production and macronutrient degradation in the anaerobic co-digestion of algae biomass residue and lipid waste. Bioresource Technology, 11. pp. 42–48. DOI: 10.1016/j.biortech.2012.01.160.

Partanen E. 2010. Mädätysjäännöksen tuotteistamismahdollisuudet Kymenlaaksossa (Possibilities of productization of digestate in region Kymenlaakso). Master's thesis. 140 p. Lappeenranta University of Technology.

Pauli S.-L. & Kaitala S. 1995. Optimal growth conditions for Acinetobacter isolates from activated sludge treating forest-industry wastewaters. Applied Microbiology and Biotechnology, 43. pp. 746–754. DOI: 10.1007/BF00164783.

Pauli S.-L. & Kaitala S. 1996. Phosphate Uptake Kinetics by Acinetobacter Isolates. Biotechnology and Bioengineering, 53 (2). pp. 304–309. DOI: 10.1002/(SICI)1097-0290(19970205)53:3<304::AID-BIT9>3.0.CO;2-M.

Pereda I., Irusta R., Motalvo S. & del Valle J.L. 2006. Solid mining residues from Ni extraction applied as nutrients supplier to anaerobic process: optimal dose approach through Taguchi's methodology. Water Science & Technology, 54 (9). pp. 209–219. DOI: 10.2166/wst.2006.871.

Piiparinen J. 2016. Researcher. Finnish Environmental Institute. Private conversation. May 2016.

Pitman. 1999. Management of Biological Nutrient Removal Plant Sludges – Change or Paradigms?. Water Research, 33 (5). pp. 1141–1146.

Polprasert C. 2007. Organic Waste Recycling. 3rd edition. 540 p. ISBN: 184339121X. ISBN13: 9781843391210.

Price N., Harrison G., Hering J., Hudson R., Pascale N., Brian P. & François M. 1989. Preparation and Chemistry of the Artificial Algal Culture Medium Aquil. Biological Oceanography, 6 (5–6). pp. 443¬–461.

Priyadarshani I. & Rath B. 2012. Commercial and industrial applications of micro algae – A review. Journal of Algal Biomass Utilization, 3 (4). pp. 89–100. ISSN: 2229-6905.

Päijät-Hämeen jätehuolto. 2016. Biokaasu- ja kompostointilaitos. Kujalan jätekeskus. [online]. Available: http://www.phj.fi/kujalan-jatekeskus/muut-alueella-toimijat/kompostilaitos [Referenced 25.9.2016].

Renault F., Sancey B., Badot P.-M. & Crini G. 2009. Chitosan for coagulation/flocculation processes – An eco-friendly approach. European Polymer Journal, 45. pp. 1337–1348. DOI: 10.1016/j.eurpolymj.2008.12.027.

Richmond A. & Hu Q. 2013. Handbook of Microalgae Culture, Applied Phycology and Biotechnology. 2nd edition. 705 p. ISBN 978-0-470-67389-8.

Richmond J. 2004. Algal Nutrition. Handbook of Microalgal Culture: Biotechnology and Applied Phycology. 588 p. ISBN 0–632–05953–2.

Rosén B. & Huijbregsen C. 2003. The ScanDeNi® process could turn an existing under-performing activated sludge plant into an asset. Water Science & Technology, 47 (11). pp. 31–36.

Ruissalo M.K. 2006. Reject water treatment at sewage treatment plant. Master's Thesis. 96 p. University of Jyväskylä. Department of Biological and Environmental Science.

Ruiz-Marin A., Mendoza-Espinosa L. & Stephenson T. 2009. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresource Technology, 101. 58–64 pp. DOI: 10.1016/j.biortech.2009.02.076.

Rusten B. & Sahu A. 2011. Microalgae growth for nutrient recovery from sludge liquor and production of renewable bioenergy. Water & Science Technology, 64,6. pp. 1195–1201. DOI: 10.2166/wst.2011.722.

Sahu A., Siljudalen J., Trydal T., Rusten B. 2013. Utilisation of wastewater nutrients for microalgae growth for anaerobic co-digestion. Journal of Environmental Management, 122. pp. 113–120. DOI: 10.1016/j.jenvman.2013.02.038.

Saidou H, Korchef A., Ben Moussa S. & Ben Amor M. 2009. Struvite precipitation by the dissolved CO2 degasification technique: impact of the airflow rate and pH. Chemosphere 74. pp. 338–343. DOI: 10.1016/j.chemosphere.2008.09.081.

Sakarika M. & Kornaros M. 2016. Effect of pH on growth and lipid accumulation kinetics of the microalga Chlorella vulgaris grown heterotrophically under sulfur limitation. Bioresource Technology, 219. pp. 694–701. DOI: 10.1016/j.biortech.2016.08.033.

Sambusiti C., Bellucci M., Zabaniotou A., Beneduce L. & Monlau F. 2015. Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review. Renewable and Sustainable Energy Reviews, 44. pp. 20–36. DOI: 10.1016/j.rser.2014.12.013.

Sanitas. 2013. Reject water Treatment Models –Literature Review. Sustainable and Integrated Urban Water System Management. [research report] Deliverable Nr: 2.9. Available: http://www.sanitas-itn.eu/wp-content/uploads/2013/10/Deliverable_2.9_Reject-Water-Treatment-Model.pdf [Referenced 29.9.2016].

Sayre R. 2010. Microalgae: The Potential for Carbon Capture. Bioscience, 60 (9). pp. 722–727. DOI: 10.1525/bio.2010.60.9.9.

Schenk P., Thomas-Hall S., Stephens E., Marx U., Mussgnug J., Posten C., Kruse O & Hankamer B. 2008. Second Generation Biofuels: High Efficiency Microalgae for Biodiesel Production. Bioresource, 1. pp. 20–43. DOI: 10.1007/s12155-008-9008-8.

Schultz O., Glunz S.W. & Willeke G.P. 2004. Multicrystalline Silicon Solar Cells Exceeded 20 % Efficiency. Progress in Photovoltaics: Research and Applications, 12. pp. 553–558. DOI: 10.1002/pip.583.

Schumacher G., Blume T. & Sekoulov I. 2003. Bacteria reduction and nutrient removal in small wastewater treatment plants by an algal biofilm. Water Science and Technology, 47 (11). pp. 195–202.

SFS 3032. Veden ammoniumtypen määritys (The determination of ammonium nitrogen concentration). 6 p.

Shelef D. & Sukenik A. 1988. Flocculation of Microalgae with Cationic Polymers – Effects of Medium Salinity. Biomass, 17. pp. 65–76.

Sialve, B., Bernet, N. & Bernard, O. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology, 27. pp. 409–416.

Singer P. & Lawler D. 1982. Treatbility Analysis of Digester Supernatant and Other Return Flows to Improve Wastewater Treatment Plant Efficiency. [technical report]. Water Resources Research Institute of the University of North Carolina. Available: http://repository.lib.ncsu.edu/dr/handle/1840.4/1718 [Referenced 13.6.2016].

Singh G. & Thomas P. 2012. Nutrient removal from membrane bioreactor permeate using microalgae and in a microalgae membrane photoreactor. Bioresource Technology, 117. pp. 80–85. DOI: 10.1016/j.biortech.2012.03.125.

Singh J. & Gu S. 2010. Commercialization potential of microalgae for biofuels production. Renewable and Sustainable Energy Reviews 14. pp. 2596–2610. DOI: 10.1016/j.rser.2010.06.014.

Sliekers O. 2003. Ammonium oxidation at the oxic/anoxic interface. 144 p. DUP science. Delft University Press. The Netherlands. ISBN: 90-407-2459-8.

So P. & Dong C. 2002. Fluorescence Spectrophotometry. Introductory article. Encyclopedia of Life Sciences. Macmillan Publishers Ltd, Nature Publishing Group. Available: http://web.mit.edu/solab/Documents/Assets/So-Fluorescence%20spectrophotometry.pdf. [Referenced 24.9.2016].

Sonck M. 2012. Cultivation of Microalgae for the Fixation of Carbon Dioxide from Power Plant Flue Gas. Master's Spolaore Thesis. Aalto University. Espoo.

Spilling K. Senior Research Scientist. Finnish Environmental Institute. Private conversation. July 2016.

Spilling K., Seppälä J. & Tamminen T. 2011. Inducing autoflocculation in the diatom Phaeodactylum tricornutum through CO2 regulation. Journal of Applied Phycology, 23 (6). pp. 959–966. DOI: 10.1007/s10811-010-9616-5.

Spolaore P., Joannis-Cassan C., Duran E. & Isambert A. 2012. Commercial applications of Microalgae. Journal of Bioscience and Bioengineering, 101. pp 87-96.

Strasser R. & Govindjee. 1992. The Fo and the O-J-I-P Fluorescence Rise in Higher Plants and Algae. Regulation of Chloroplast Biogenesis, 226. pp. 423–426. ISBN: 978-1-4613-6485-6. DOI: 10.1007/978-1-4615-3366-5_60.

Strasser RJ, Tsimilli-Micheal M. & Srivastava A. 2004. Analysis of the chlorophyll a fluorescence transient. In: Papageogiou GC and Govindjee (Eds) Advances in Photosynthesis and Respiration, 19. Springer, Berlin, Germany. pp. 321-362. ISBN: 978-1-4020-3217-2. ISNN: 1572-0233. DOI: 10.1007/978-1-4020-3218-9_12.

Stockenreiter M., Haupt F., Seppälä J., Tamminen T. & Spilling K. 2016. Nutrient uptake and lipid yield in diverse microalgal communities grown in wastewater. Algal Research, 15. pp. 77–82. DOI: 10.1016/j.algal.2016.02.013.

Strous M., Heijnen J. J., Kuenen, J. G., Jetten, M. S. M. 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Applied Microbiology and Biotechnology, 50 (5). pp. 589–596. DOI: 10.1007/s002530051340.

Strous M., Kuenen J. G. and Jetten M. S. M. 1999. Key physiology of anaerobic ammonium oxidation. Applied and Environmental Microbiology, 65 (7). pp 3248–3250.

Sun, Y. & Cheng, J.Y. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology. 83 (1), pp. 1–11.

Tam N.F.Y. & Wong Y.S. 1996. Effect of Ammonia concentrations on growth of Chlorella Vulgaris and Nitrogen Removal from Media. Bioresource Technology, 57. pp. 45–50.

Tchobanoglous G., Burton F. & Stensel H. 2003. Wastewater engineering: treatment and reuse. 4th edition. 1736 p. ISBN: 0-07-041878-0.

Tchobanoglous G., Theisen H. & Vigil S. 1993. Integrated Solid Waste Management, Engineering Principles and Management Issues. ISBN: 0-07-063237-5.

Virtanen J. & Aaltonen E.-K. 2013. Kokkolan biokaasulaitoksen ja jätevedenpuhdistamon yhteenvetoraportti vuodelta 2013. [pdf-report] Pohjanmaan vesi ja ympäristö ry. Available: http://www.vesiensuojelu.fi/pohjanmaa/wp-content/uploads/2012/07/BKL-JVP_vuosiyhteenvetoraportti_2013.pdf [Referenced 4.10.2016].

Vesitalous 1/2011. Biokaasulaitokset. Ympäristöviestintä YVT Oy. [Professional field journal]. 59 p. Available: http://vesitalous.mobie.fi/wp-content/uploads/2011/10/vt_12011.pdf [Referenced 26.9.2016].

Vänskä Janna. 2016. Quality and Environment Engineer. Private phone interview. Bio Kymppi Oy. [9.6.2016].

Wäger F. Delhaye T. & Fuchs W. 2010. The use of ceramic microfiltration and ultrafiltration membranes for particle removal from anaerobic digester effluents. Separation and Purification Technology, 73. pp. 271–278. DOI: 10.1016/j.seppur.2010.04.013.

Wäger-Baumann F. 2011. Physical and Biological Methods for the Treatment of the Liquid Fraction of Anaerobic Digester Effluent. Dissertation. Dissertation. University of Natural Resources and Applied Life Sciences. Vienna. [Referenced 19.5.2016]. Available: https://zidapps.boku.ac.at/abstracts/oe_list.php?paID=3&paSID=8522&paSF=-1&paCF=0&paLIST=0&language_id=DE [Referenced 29.9.2016].

Wäger-Baumann F. & Fuchs W. 2011. Process Variant for the Treatment of Anaerobic Digester Effluent with a Membrane Bioreactor. Environmental Engineering Science, 28 (9). pp. 611–627. DOI: 10.1089/ees.2010.0153.

Wang L., Li Y., Chen P., Min M., Chen Y., Zhu J. & Ruan R.R. 2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresource Technology, 101. pp. 2623–2628. DOI: 10.1016/j.biortech.2009.10.062.

Wang L., Min M., Li Y., Chen P., Chen Y., Liu Y., Wang Y. & Ruan R. 2009. Cultivation of Green Algae Chlorella sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. Applied Biochemistry and Biotechnology, 164 (4). pp. 1174-1186. DOI 10.1007/s12010-009-8866-7.

Ward A.J., Lewis D.M. & Green F.B. 2014. Anaerobic digestion of algae biomass: A review. Algal Research, 5. pp. 204–214. DOI: 10.1016/j.algal.2014.02.001.

Weber T. & Deutsch C. 2010. Ocean nutrient ratios governed by plankton biogeography. Nature, 467 (7315). pp. 550–554. DOI:10.1038/nature09403.

Lachat Instruments. 1997. QuikChem method 31-107-04-1-A. Determination of nitrate in brackish or seawater by flow injection analysis.

Wett B., Rostek R., Rauch W. & Ingerle K. 1998. pH-controlled reject-water-treatment. Water Science Technology, 37 (12). pp. 165–172.

Williams. 2012. Methane Production from Anaerobic Co-digestion of Chlorella Vulgaris and Wastewater sludge. Master's thesis. p. 91. San Diego State University. Department of Civil, Environmental and Construction Engineering. Available: http://scholarworks.calstate.edu/bitstream/handle/10211.10/3554/Williams_Carissa.pdf?sequence=1 [Referenced 7.9.2016].

Xiong J., Kurade M., Kurade M., Kim J., Roh H., Jeon B. 2016. Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga Chlamydomonas mexicana. Journal of Hazardours Materials. DOI: 10.1016/j.jhazmat.2016.04.073.

Yen H. & Brune D. 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresource Technology, 98. pp. 130–134. DOI: 10.1016/j.biortech.2005.11.010.

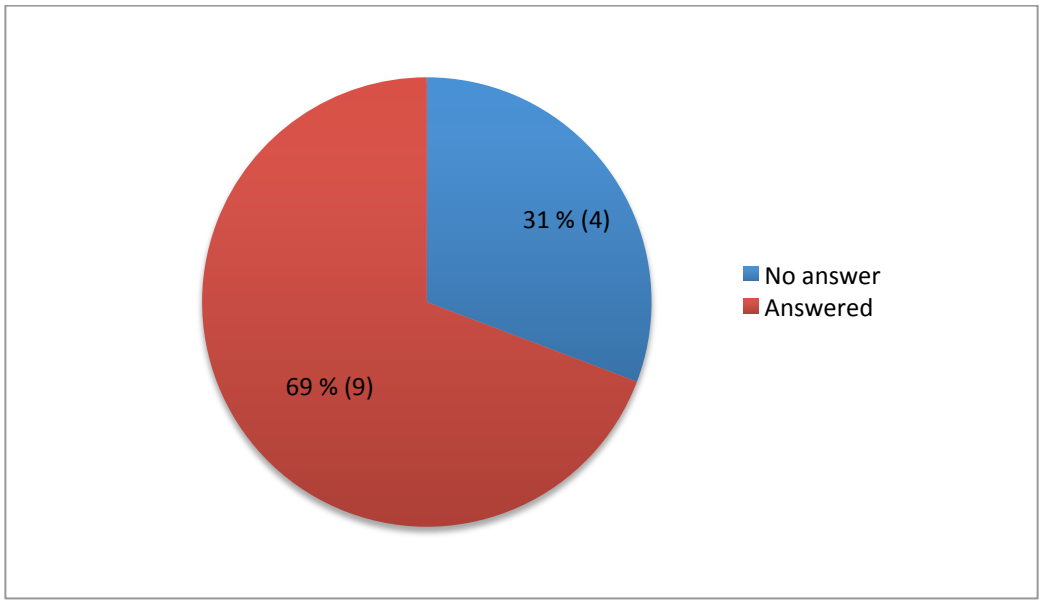
Yuan X., Wang M., Park C., Sahu A. & Ergas S. 2012. Microalgae Growth Using High-Strenght Wastewater Followed by Anaerobic Co-Digestion. Water Environment Research, 84 (5). pp. 396–404. DOI: 10.2175/106143011X13233670703242.

Zwietering M.H., Jongerburger I., Romboust F.M. & van't Riet K. 1990. Modeling of the Bacterial Growth Curve. Applied and Environmental Microbiology, 56 (6). pp. 1875–1881.

Appendix 1. Interview results

Interviewed persons and answer rate

Contact person	The biogas plant	Questionnaire sent	Answer date	Answerer
Joonas Juura	Gasum Biotehdas Oy	15.6.2016	21.6.2016	Joonas Juura
Ossi Lehtonen	Biovakka Suomi Oy, Turku	15.6.2016	No answer	
Jani Haapanala	Biovakka Suomi Oy, Vehmaa	15.6.2016	1.7.2016	Jani Haapanala
Heikki Juhe	Envor Biotech Oy	15.6.2016	No answer	
Janne Käld	Jeppo Biogas Ab	15.6.2016	17.6.2016	Kurt Stenvall
Petri Parhiala	Joutsan Ekokaasu Oy	15.6.2016	No answer	
Sari Pilli	Kouvolan Vesi Oy	15.6.2016	21.6.2016	Virpi Jääskeläinen
Niko Wassholm	LABIO Oy	15.6.2016	22.6.2016	Niko Wassholm
Veli-Matti Nyysti	Laihian kunta	15.6.2016	22.6.2016	Veli-Matti Nyysti
Antero Bäcklund	Lakeuden Etappi	15.6.2016	17.6.2016	Antero Bäcklund
Stig Kjellman	Oy Pohjanmaan Biokaasu	15.6.2016	No answer	
Karin Salmi	Loimi-Hämeen jätehuolto (before Satakierto Oy)	15.6.2016	17.6.2016	Juha Paturi
Johan Saarela	Stormossen	15.6.2016	30.6.2016	Johan Saarela



Answer rate for the reject water questionnaire sent by a letter

(continues)

	Process type		Reject water treatments			Utilization	Reference
Co-digestion plant	**Thermo-philic**	**Meso-philic**	**Separate treat-ment**	**No systematic treatment**	**Leaded to WWTP**	**RW utilization as a fertilizer**	
BioKymppi Oy		X		X		Yes	Vänskä 2016
Biotehdas Oy		X		X	X	No	Juura 2016
Biovakka Suomi Oy, Vehmaa		X	X		X	Yes + utilization in the industry	Haapanala 2016
Envor Biotech Oy		X	X		X when not utilized as fertilizer	Yes at the field cultivation period	Juhe 2016
Jeppo Biogas Ab,		X		X		Digestate utilized as a wholeness	Stenvall 2016
Kouvolan Vesi Oy	X			X	X	No	Jääskeläinen 2016
LABIO Oy		X (DRY)		No reject water			Wassholm 2016
Laihian kunta		X		X	X	No	Veli-Matti Nyysti 2016
Lakeuden Etappi		X	X		X	No	Bäcklund 2016
Loimi-Hämeen jätehuolto (Before Satakierto Oy)	X			X	X	No	Paturi 2016
Stormossen	X		Separate process water treatment plant		X	Yes, digested mixture of RW sludge+ other sludges are utilized as fertilizers after dewatering (Appendix 2) Separated liquid phase is recycled back to the treatment process	Saarela 2016, Kuosma 2016

(continues)

Reject water cleaning results at the co-digestion plants

Stormossen Ab (Process water treatment plant)

Characterize	Raw reject water	After treatment	Reduction
Total N	844 mg/l	321 mg/l	62 %
Total P	64 mg/l	10 mg/l	84 %
COD	4916 mg/l	1464 mg/l	70 %
Alkalinity	54 mg/l	7 mg/l	87 %

Biovakka Oy, Vehmaa (Volatilization treatment)

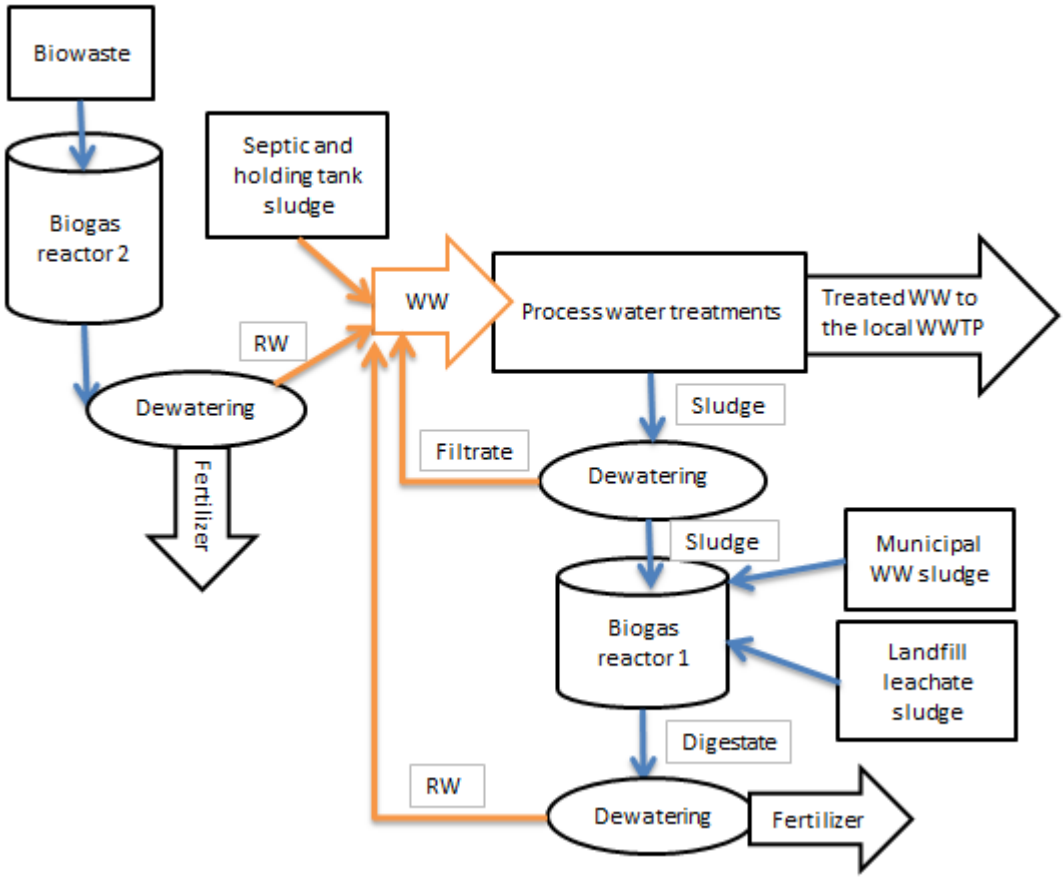
Characterize	Raw reject water	After treatment	Reduction
Total N	6000 mg/l	3–15 mg/l	99,75–99,95 %
Total P	800 mg/l	0,01 mg/l	99,99 %
COD	-	15–80 mg/l	-
pH	8	6–8	-

Lakeuden Etappi (DEMON process)

Characterize	Raw reject water	After treatment	Reduction
Total N	640 kg/d	12 kg/d	98 %
Total P	9,2 kg/d	2,3 kg/d	75 %
COD	5900 mg/l	1200 mg/l	80 %
BOD	367 kg/d	43 kg/d	88 %
pH	8,3	-	-

Appendix 2. Reject water streams at the Stormossen Ab biogas plant

Based on interview with Kuosma (2016).



Appendix 3. The characterizes of reject water from Viikinmäki WWTP

The measurements of reject water from Viikinmäki WWTP in June 2016. The measurements of 29th of June are measured from the reject water sample that was tested for algal cultivation in this study.

Date 2016	BOD_7ATU mg/l	SS mg/l	P_{tot} mg/l	PO_4-P mg/l	N_{tot} mg/l	NH_4-N (in)_Lab mg/l	pH	alkalinity mmol/l	COD_sol mg/l	BOD_7 mg/l	N/P ratio
3.6.		987,5							1500	130	-
7.6	644,34	975	13,118	0,883	957,843	820,2	7,53	73,16			2053,346
9.6.	596,17	820									-
13.6.	580,78	816,7	12,404	2,041	903,616	738,577	8,02	66,29	1700	170	799,9371
21.6.	603,29	1750	18,977	0,559	917,622	749,953	7,99	65,4	1300	160	2965,687
23.6.	606,06	1000							1500	130	
29.6.		980	11,461	0,846	1003,56	679,578	8,04	57,41	1500		1775,71

Appendix 4. MWC (Modified WC Medium) culture medium content

Stock	Mass per liter
$CaCl_2$ * 2 H_2O	36,8 g
$MgSO_4$*7 H_2O	37,00 g
$NaHCO_3$	12,60 g
K_2HPO_4*3 H_2O	11,40 g
$NaNO_3$	85,00 g
Na_2O_3Si*5 H_2O	21,20 g
Combined trace elements	
$EDTANa_2$	4,36 g
$FeCl_3$*6 H_2O	3,15 g
$ZnSO_4$*7 H_2O	0,01 g
$CoCl_2$*6 H_2O	0,022 g
$MnCl_2$*4 H_2O	0,18 g
Na_2MoO_4*2 H_2O	0,006 g
H_3BO_3	1,00 g
Vitamin mix	
Thiamine HCl	0,1 g
Biotin	0,0005 g
Cyanocobalamin	0,0005 g

Reference:

Guillard R.R.L. & Lorenzen C.J. 1972. Yellow-green algae with chloro- phyllide c. J Phycol 8, pp. 10–14.

Appendix 5. Modified acid medium (MAM) culture medium content

Stock	**Mass per liter**
$(NH_4)_2SO_4$	50 g
$CaCl_2$*2 H_2O	1 g
$MgSO_4$*7 H_2O	50 g
KH_2PO_4	30 g
NaCl	3,0
Na_2EDTA*2 H_2O	20
$FeSO_4$*7 H_2O + H_2SO_4	4,98 g/l + 1ml
Trace metals	
H_3BO_3	2,86 g
$MnCl_2$*4 H_2O	1,81 g
$ZnSO_4$*7 H_2O	0,222 g
$NaMoO_4$.2 H_2O	0,390 g
$CuSO_4$*5 H_2O	0,079 g
$Co(NO_3)$2*6 H_2O	0,0494 g
Vitamin mix 100 ml	
Biotin	0,1 mg / ml (solution)
B12	5 mg/5 ml (solution)
Thiamine HCl	20 mg / 100 ml

Reference:

Olaveson, Mary M. & Pamela M. Stokes. 1989. Responses of the acidophilic alga Euglena mutabilis (Euglenophyceae) to carbon enrichment at pH 3. Journal of Phycology. 25. pp. 529-539.

Appendix 6. PIX-105 dosages

PIX-105 dosages that were tested on reject water.

Sample	PIX-105 (25 %)		PIX-105 (100 %)		
	Dosage $ml/50ml_{RW}$	**Dosage** ml/l_{RW}	**Dosage** ml/l_{RW}	**Dosage** g/l_{RW}	**Dosage** $g/m^3{}_{RW}$
Envor 50 % (1)	0,3	6	1,5	2,25	2250
Envor 50 % (2)	0,6	12	3	4,5	4500
Envor 50 % (3)	0,9	18	4,5	6,75	6750
Envor 50 % (4)	1,2	24	6	9	9000
Envor 100 % (1)	2,4	48	12	18	18000
Envor 100 % (2)	3,4	68	17	25,5	25500
Envor 100 % (3)	4,4	88	22	33	33000
Envor 100 % (4)	5,4	108	27	40,5	40500
Viikinmäki 100 % (1)	0,3	6	1,5	2,25	2250
Vikinmäki 100 % (2)	1	20	5	7,5	7500
Viikinmäki 100 % (3)	2	40	10	15	15000

The dosages with mass per liter reject water unit were estimated utilizing the density of PIX-105 (1,5 g/cm^3).