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NUTRIENT RECOVERY FROM SOURCE-SEPARATED HUMAN URINE BY MICRO-ALGAE IN CONTINUOUSLY FED RACE-WAY POND

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ABSTRACT

SONJA SAARNIO: Nutrient Recovery from Source-Separated Human Urine by Microalgae in Continuously Fed Raceway Pond
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Nitrogen and phosphorus are essential nutrients for all organisms and recovery and reuse of these resource are reasonable (Yuan et al. 2012). Nutrient recovery reduces the need for fertilizer production, which has significant environmental impacts. (Cordell et al. 2009, Childers et al. 2011) Municipal wastewaters are one important nutrient source and a major part of the nutrients in wastewaters originate from urine. Therefore, it is effective to treat urine separately after source-separation. (Chang et al. 2013, Tuantet et al. 2014a, Zeeman et al. 2008) There are laboratory-scale batch studies of nutrient recovery by microalgae (Martinez et al. 2000, Samori et al. 2013, Zhu et al. 2013, Lv et al. 2018) and a few pilot-scale studies (Adamsson, 2000; Posadas et al., 2015) that have successfully demonstrated nitrogen and phosphorus recovery, also in Nordic climate (Chatterjee et al. 2019).

The aim of this study was to cultivate microalgae *Scenedesmus acuminatus* in 15 times diluted source-separated human urine in continuously fed outdoor raceway pond in Nordic climate and evaluate biomass production and nutrient recovery. The study was conducted in Tampere, Finland from the end of June to the beginning of October. The inoculum cultivated in laboratory and in 400 L raceway pond was inoculated to 2000 L raceway pond with 15 times diluted urine. Highest biomass production (OD 1.4 and TSS 1.1 g/L) was achieved after 32 days of cultivation. Efficiency of microalgal growth started to decrease around day 50 when the temperature decreased below 25 °C (pH < 8.5). Around 30 % of the nitrogen in the influent was present as ammonium. On average 26 % (2.7 – 51 %) of total nitrogen entered was captured by microalgal cells and 46 % (0 – 100 %) of total nitrogen entered was volatilized. On average 36 % (0 – 100 %) of the phosphorus entered was captured by microalgae or precipitated. Based on the results it could be concluded that microalgae can be used to recover nutrients from diluted source-separated human urine in open raceway ponds in Nordic climate conditions without addition of trace elements, artificial light or CO_2.

TIIVISTELMÄ

SONJA SAARNIO: Ravinteiden talteenotto erilliskerätystä ihmisvirtsasta mikrolevien avulla jatkuvasyöttöisessä avoaltaassa
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Typpi ja fosfori ovat välttämättömiä ravinteita kaikille organismeille ja rajallisen saatavuuden vuoksi niiden kierrätys on tärkeää (Yuan et al. 2012). Ravinteiden kierrätys vähentää tarvetta lannoitteiden valmistukselle, millä on merkittävät ympäristövaikutukset. (Cordell et al. 2009, Childers et al. 2011). Jätevedet ovat tärkeä ravinteiden lähde ja suurin osa jätevesien ravinteista on peräisin virtsasta. Siksi sen käsittely erilliskerättynä on tehokasta ja kannattavaa. (Zeeman et al. 2008, Chang et al. 2013, Tuantet et al. 2014a,) Ravinteiden talteenottoa mikrolevien avulla on osoitettu toimivaksi laboratoriomittakaavan tutkimuksilla (Martinez et al. 2000, Samori et al. 2013, Zhu et al. 2013, Lv et al. 2018) sekä muutamissa pilot-mittakaavan kokeissa (Adamsson, 2000; Posadas et al., 2015), myös pohjoismaisissa olosuhteissa (Chatterjee et al. 2019).

Tämän tutkimuksen tarkoituksena oli kasvattaa mikrolevälajia Scenedesmus acuminatus 15 kertaa laimennetussa erilliskerätyssä ihmisvirtsassa jatkuvasyöttöisessä altaassa pohjoismaisissa olosuhteissa ja tutkia biomassan tuottoa ja ravinteiden talteenottoa. Tutkimus toteutettiin Tampereella, Suomessa kesäkuun lopusta lokakuun alkuun. Laboratoriossa ja 400 litran altaassa kasvatettu inokulaatti lisättiin 2000 litran altaaseen 15 kertaa laimennetun virtsan kanssa. Suurin biomassa tuotto (OD 1.4 ja TSS 1.1 g/L) saavutettiin 32:n kasvatuspäivän jälkeen. Mikrolevien kasvun tehokkuus alkoi heikentyä noin 50 päivän jälkeen, lämpötilan laskettua alle 25 °C (pH < 8.5). Noin 30 % syötteessä esiintyvästä typestä oli ammonium-muodossa. Keskimäärin 26 % (2.7 – 51 %) syötetystä kokonaistypestä saatiin otettua talteen mikroleväsoluihin ja 46 % (0 – 100 %) syötetystä kokonaistypestä haihtui. Keskimäärin 36 % (0 – 100 %) syötetystä fosforista kerääntyi mikroleväsoluihin tai saostui. Tulosten perusteella voidaan todeta, että mikroleviä voidaan hyödyntää ravinteiden talteenotossa erilliskerätystä virtsasta avoaltaissa pohjoismaisissa ilmasto-olosuhteissa ilman lisättyjä hivenaineita, keinotekoista valonlähdettä tai hiilidioksidia.

PREFACE

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LIST OF SYMBOLS AND ABBREVIATIONS

EBPR	enhanced biological phosphorus removal
MFC	microbial fuel cell
PHA	poly-β-hydroxyalkanoates
RwP	raceway pond
WWTP	wastewater treatment plant
COD	chemical oxygen demand
N_{tot}	total nitrogen
OD_{660}	optical density with wavelength 660 nm
P_{tot}	total phosphorus
T	temperature
TSS	total suspended solids
VSS	volatile suspended solids

1. INTRODUCTION

Nitrogen and phosphorus are essential nutrients for all organisms and recovery and reuse of these resources are reasonable (Yuan et al. 2012). Nutrient recovery reduces the need for fertilizer production, which has significant environmental impacts. Phosphorus for the fertilizers is produced by mining but the reservoirs are limited and depleting. Nitrogen is produced mainly by energy intensive Haber-Bosh process. (Cordell et al. 2009, Childers et al. 2011) Increasing population, the increasing need for fertilizers and environmental issues are driving improvements to recovery technologies of phosphorus and nitrogen.

Municipal wastewaters are one important nutrient source. Yearly 2.5 – 4.0 kg of nitrogen and 0.22 – 0.37 kg of phosphorus are produced per person (Karak & Bhattacharyya 2011). Major part of the nutrients in wastewaters originate from urine. Therefore, it would be reasonable to treat urine separately after source-separation. (Zeeman et al. 2008, Chang et al. 2013, Tuantet et al. 2014a)

Microalgae has drawn attention in recent years and several studies have reported the efficiency of microalgae to recover nutrients (Cuellar-Bermudez et al. 2017). There are laboratory-scale batch studies of nutrient recovery by microalgae (Martinez et al. 2000, Samori et al. 2013, Zhu et al. 2013, Lv et al. 2018) and a few pilot-scale studies (Adamsson, 2000; Posadas et al., 2015), that have successfully demonstrated nitrogen and phosphorus recovery, also in Nordic climate (Chatterjee et al. 2019). Microalgae have many advantages in the removal of nitrogen and phosphorus, such as (1) availability of sufficient solar energy as energy source, (2) simultaneous fixation of CO_2, (3) lack of extra organic carbon requirement and (4) economic potential of harvested algal biomass (e.g. feedstock, fertilizers, biofuels) (Aslan and Kapdan 2006, Xin et al. 2010, Patel et al. 2017). However, economic efficiency of nutrient recovery by microalgae depends for example on the cultivation conditions (e.g. location and climate) and technology used in cultivation, harvesting and treatment of biomass.

The aim of this study was to cultivate microalgae *Scenedesmus acuminatus* in 15 times diluted human urine in continuously fed outdoor raceway pond in Nordic climate and to evaluate biomass production and nutrient recovery. The study was conducted in Tampere, Finland from the end of June to the beginning of September.

2. NUTRIENT RECOVERY

Phosphorus (P) and nitrogen (N) are two of the main nutrients and they are essential for all organisms (Madigan et al. 2017). However, the phosphorous resources are limited, fertilizer production has significant environmental impacts and the need for fertilizers has increased as a result of increased population (Yuan et al. 2012). Phosphorus for the fertilizers is produced by mining but the reservoirs are limited and depleting. Nitrogen is produced mainly by the energy intensive Haber-Bosh process. (Cordell et al. 2009) Nutrient recovery decreases the need for fertilizer production, which has significant environmental impacts.

2.1 Phosphorus

Phosphorus is essential for all organisms (Ashley et al. 2011, Yuan et al. 2012, Ruttenberg 2013). It exists in nature primarily as organic and inorganic phosphates (Madigan et al. 2017). However, phosphorus is one of the least biologically available nutrients, because the forms in which it exists in the biosphere are often unavailable for plants and they can only absorb the soluble inorganic form of phosphorus dissolved in soil solution (Asley et al. 2011). In abiotic nature, phosphorus exists for example as phosphate-containing minerals in rock and dissolved phosphates in freshwaters and marine waters. Biotic phosphorus reservoirs include mainly nucleic acids DNA and RNA, bones and phospholipids of living organisms and ATP (adenosine-5′-triphosphate), which is the primary carrier of chemical energy in cells (Childers et al. 2011, Asley et al. 2011, Madigan et al. 2017). Phosphorus cycles through living organisms, water and soils, and Earth's crust (Madigan et al. 2017). Thus, phosphorus is a limiting nutrient to growth and production in wide range of marine, freshwater, and terrestrial ecosystems. Phosphorus is released to these ecosystems through the weathering of rocks. In addition, there is no known alternative nutrient for phosphorus. (Childers et al. 2011, Madigan et al. 2017). Figure 1 presents the natural phosphorus cycle and human effect on it.

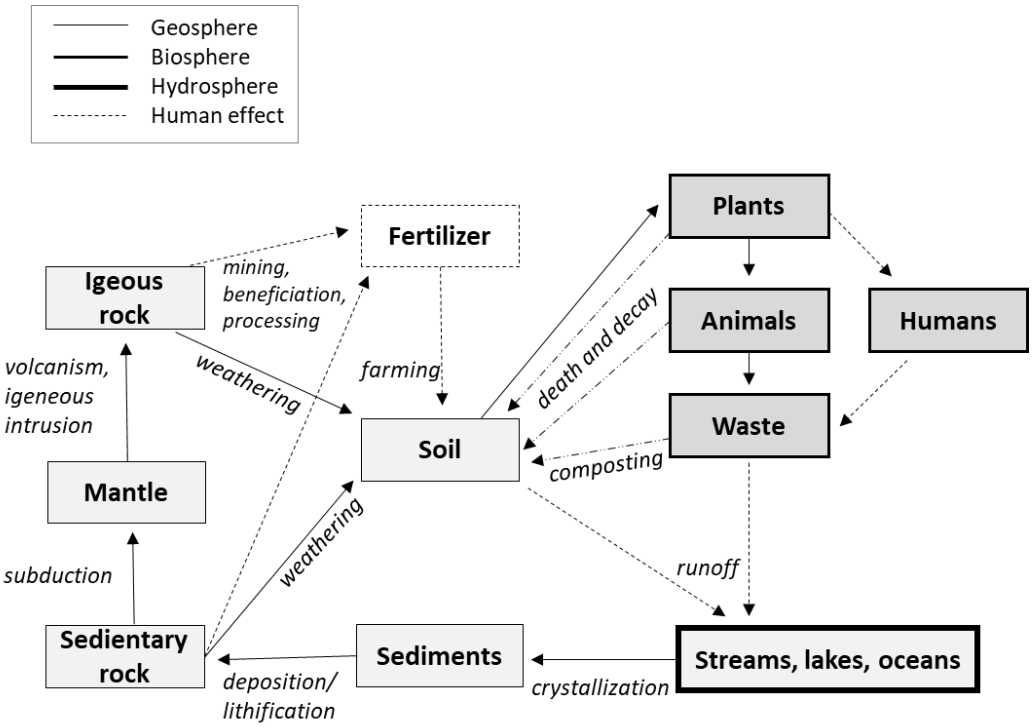


Figure 1. *Natural phosphorus cycle and human effect (InTeGrate 2018, modified)*

Phosphorus is mainly used in fertilizers. Phosphorus is produced by mining from sources from which 85 % are controlled by five countries (China, US, Morocco, Russia and Jordan). (Asley et al. 2011) However, the phosphorus reservoirs are limited and depleting (Cordell et al. 2009). Based on several studies (Childers et al. 2011, Asley et al. 2011), the depletion of mined phosphorus sources is concerning because the global production of phosphorus is widely thought to hit a peak this century (Cordell et al. 2009, Walan et al. 2014). Global phosphorus reserves are limited and while timelines for peak phosphorus are contentious, it is clear that the phosphorus that remains is of lower grade and more difficult to access than ever before (Cordell et al. 2011, Yuan et al. 2012). It has been predicted that the diminishing global phosphorus reserves might lead to not only environmental but also social problems in the future. During human history the Sanitation Revolutions and the Green revolution have had the greatest impact for the global phosphorus cycle. Investing in renewable phosphorus sources through local phosphorus recovery from wastes, such as human excreta, manure, food and organic waste, can simultaneously reduce dependence on a finite resource, reduce water pollution and increase communities' phosphorus security. (Asley et al. 2011, Childers et al. 2011)

2.2 Nitrogen

As phosphorus, nitrogen is an essential element for life. In nature, the main sinks of nitrogen are primary rocks, sedimentary rocks, the deep-sea sediment, the atmosphere, and the soil-water pool (Mortenson 2018). Nitrogen exists in organisms, for example in pep-

tides, proteins, enzymes, chlorophylls, energy transfer molecules (ADP, ATP), and genetic materials (RNA, DNA). (Barsanti & Gualtieri, P. 2014) Through biological nitrogen fixation, biomass burning and lightning, nitrogen gas (N_2) is converted to reactive nitrogen (N_r),

The main reason for organisms to utilize nitrogen compounds are to use it as a nutrient source, energy source or as terminal electron acceptor, when the appearance of oxygen is limited (Mortenson 2018). Nitrogen exists in several oxidation states, but mainly as nitrogen gas (N_2). However, most of the micro-organisms cannot use nitrogen gas as a nitrogen source by nitrogen fixation. In nature nitrogen exists also as combined with other elements, for example as ammonia (NH_3) or nitrate (NO_3^-). Nitrogen volatilizes from soils to the atmosphere primarily as N_2 and N_2O formed in denitrification and from alkaline soils as NH_3. When pH is neutral, NH_3 exists as ammonium (NH_4^+) which is released by aerobic decomposition. However, it is quickly recycled and converted to amino acids by plants and micro-organisms. (Madigan et al. 2017) The nitrogen cycle is a complicated group of processes and new microbial nitrogen processes are still being discovered (Stein & Klotz 2016). Processes of nutrient cycle are visualized in figure 2.

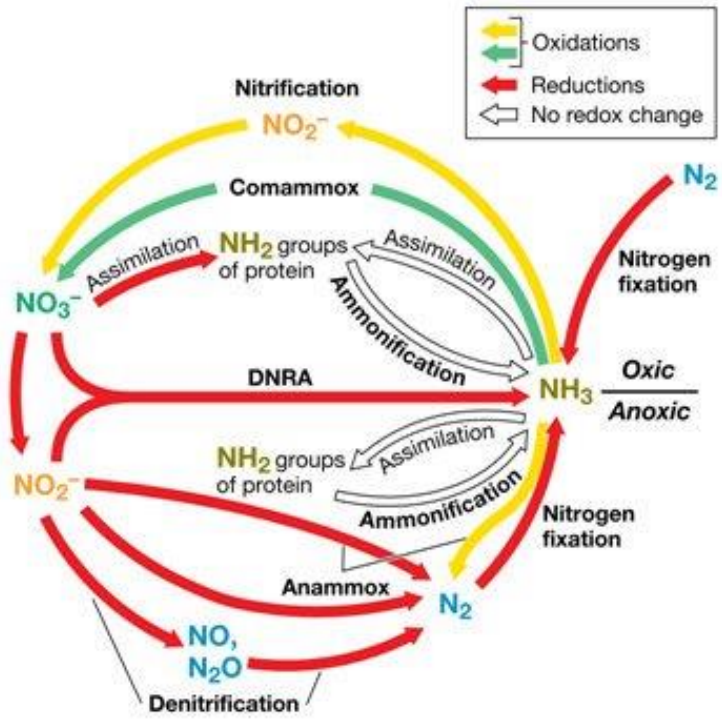


Figure 2. *Nitrogen cycle (Madigan et al. 2017)*

The key processes in nutrient cycle are nitrification, denitrification, N_2 fixation, ammonification and Anammox (Stein & Klotz 2016, Madigan et al. 2017):

Nitrification	$NH_4^+ \rightarrow NO_2^-$	
	$NO_2^- \rightarrow NO_3^-$	(1)
Denitrification	$NO_3^- \rightarrow N_2$	(2)
N_2 fixation	$N_2 + 8\,H \rightarrow NH_3 + H_2$	(3)
Ammonification	$organic - N \rightarrow NH_4^+$	(4)
Anammox	$NO_2^- + NH_3 \rightarrow 2\,N_2$.	(5)

Humans have a great impact on the global nitrogen cycle. The main effectors are the reduction of N_2 to NH_4^+ by industrial processes, agriculture that increase crop yields, and burning of fossil fuels (Capone 2001). Anthropogenic sources double the natural nitrogen fixation. The use of nitrogen fertilizers increased around 800 % from 1960 to 2000. In addition, over 60 % of nitrogen used as a fertilizer is washed out of the root zone or volatilized in the atmosphere, which increases the need for fertilizer production and therefore the environmental impacts of the agriculture. (Canfield et al. 2010) Agricultural systems also release N_2O formed in denitrification, which is a greenhouse gas and has a great warming potential in the atmosphere (Ravishankara et al. 2009, Stein & Klotz 2016). In addition, NO_3^- pollution has contributed to increasing dead zones in coastal regions (Stein & Klotz 2016).

Nitrogen fertilizers are mainly produced by the industrial energy intensive Haber-Bosch process (Cordell et al. 2009). The process requires high temperatures and pressures of 500 °C and 20 MPa and consumes over 1 % of the world's energy sources (Smith 2002, Kandemir et al. 2013).

2.3 Nutrient content of urine

An adult person produces 1 – 1.5 L of urine per day (Jönsson et al. 2004, Karak et al. 2011, Chang et al. 2013) Yearly that means 2.5 – 4.0 kg of nitrogen and 0.22 – 0.37 kg of phosphorus produced per person (Karak & Bhattacharyya 2011). Urine consist of substances with low molecular weight (Jönsson et al. 2004). Nitrogen exists in urine mainly as urea (75 – 90%) and the remaining nitrogen is present mainly as ammonium and creatinine (Jönsson et al. 2004, Lahr et al. 2016). Table 1 presents the reference characteristics of fresh undiluted human urine.

Table 1. *Characteristics of fresh undiluted human urine*

Parmeter	Reference range of human urine	References
pH	4.5 – 8.2	Jönsson et al. 2004, Heinonen-Tanski & Wijk-Sijbesma 2005, Maurer et al. 2006
NH_4^+-N (mg/L)	2500 – 8100	Heinonen-Tanski & Wijk-Sijbesma 2005, Maurer et al. 2006, Chang et al. 2013
N_{tot} (mg/L)	8000 – 10,000	Heinonen-Tanski & Wijk-Sijbesma 2005, Maurer et al. 2006, Chang et al. 2013
P_{tot} (mg/L)	700 – 2000	Heinonen-Tanski & Wijk-Sijbesma 2005, Maurer et al. 2006, Chang et al. 2013
Urea (mg/L)	5000 – 9000	Heinonen-Tanski & Wijk-Sijbesma 2005, Maurer et al. 2006, Chang et al. 2013
COD (mg/L)	8000 – 10,000	Heinonen-Tanski & Wijk-Sijbesma 2005, Maurer et al. 2006, Chang et al. 2013

Urine is degraded by bacterial urease enzymes to ammonium and carbon dioxide quickly according to equation 6 (Jönsson et al. 2004):

$$CO(NH_2)_2 + 3\,H_2O \xrightarrow{Urease} 2\,NH_4^+ + OH^- + HCO_3^- \,. \quad (6)$$

Urine can also be converted to fertilizer products by storing it in a sealed container several months, when urea is hydrolysed into ammonia due to increasing pH during storage (Lahr et al. 2016):

$$CO(NH_2)_2 + 2\,H_2O \xrightarrow{Urease} NH_4^+ + NH_3 + HCO_3^- \,. \quad (7)$$

As a result of storing, the pH of the urine rises approximately from 6 to 9 destroying potential pathogens. (Jönsson et al. 2004, Lahr et al. 2016)

Nitrogen as ammonium is directly available for plants. Some of the plants prefer nitrate as a fertilizer, but ammonium is converted by microbes to nitrate in soil (Jönsson et al. 2004) Phosphorus exists in urine almost entirely (95 – 100 %) as inorganic form as phosphate. Phosphate ions are directly available for plants. Therefore, nitrogen and phosphorus are as plant-available forms in urine as in chemical fertilizers. (Jönsson et al. 2004) Urine is a good source of nutrients because it contains all trace elements that are needed for micronutrient growth (Jönsson et al. 2004).

2.4 Source-separation of urine

The major part of the nutrients in municipal wastewaters originate from urine (Chang et al. 2013). Only 1 % of the volume of wastewaters is urine, but it contains 40 – 50 % of phosphorus load and 70 – 80 % of nitrogen load in domestic wastewaters (Larsen & Gujer 1996, Jönsson et al. 2004, Zeeman et al. 2008; Zeeman and Kujawa-Roeleveld 2011,

Chang et al., 2013, Lahr et al. 2016). That makes treatment of highly diluted municipal wastewaters costly and inefficient for nutrient and energy recovery (Tuantet et al. 2014a). Source separation of urine makes the treatment and recovery more efficient (Zeeman et al. 2008). (Larsen & Gujer 1996, Tervahauta et al. 2013)

Source separation of urine can be done by urine-diverting water or dry toilets (UDT), urinals or vacuum toilets (Otterpohl et al. 2003, Kvarnström et al. 2006). In UDTs urine and faeces are collected into two separate sections and stored in separate tanks. UDTs can be utilized in urban areas but also with developed wastewater management and pipelines. (Kvarnström et al. 2006) In NoMix-projects (Lienert & Larsen 2010) urine source separation was tested in seven Northern and Central European countries with 2700 respondents. Users were asked about the idea generally, design, hygiene, smell, and seating comfort of NoMix-toilets. The users were also asked about the use of urine-fertilizers in food production. The new NoMix-technology was widely accepted in general (80 %), but also problems were found. The most judged aspects were odours, hygiene and seating comfort. NoMix-toilets were judged more critically when the toilets were used at users' home compared to when the toiles were used at institutions. (Lienert & Larsen 2010) Source separation has also been tested in Hiedanranta area in Tampere, Finland, in which this study was conducted.

2.5 Nutrient recovery from urine

Phosphorus and nitrogen can be recovered from urine by several methods, such as struvite precipitation, stripping, chemical precipitation and microbial fuel cells (Cieślik and Konieczka, 2017, Xu et al. 2017). Struvite precipitation can be applied to phosphorus and nitrogen recovery from urine. By adding magnesium, phosphorus and ammonium are precipitated as struvite ($NH_4MgPO_4 \cdot 6H_2O$), which can be separated from urine (Jaffer et al. 2002, Kapdasli et al. 2006, Ronteltap et al. 2010, Xu et al. 2017). The advantage in struvite precipitation is that both phosphorus and nitrogen can be recovered at the same time as a product that can be used as a fertilizer as such (Maurer et al. 2006).

Phosphorus can be precipitated also by chemical precipitation, for example as calcium phosphate (Davis 2010), or recovered by enhanced biological phosphorus removal (EBPR) (Bao et al. 2007, Yuan et al. 2012). EBPR processes are based on the ability of polyphosphate accumulating organisms (PAOs) to uptake excess orthophosphate and store it as polyphosphate. With PAOs phosphorus content of 15 – 20 % of the cell dry weight can be reached. PAOs can uptake phosphorus under aerobic conditions as polyphosphate, glycogen and poly-β-hydroxyalkanoates (PHAs). (Yuan et al. 2012) Under anaerobic conditions PAOs gain energy through the hydrolysis of polyphosphate and uptake short-chain volatile fatty acids (VFAs), which is unique ability for microorganisms under anaerobic conditions. Under aerobic conditions PAOs utilize oxidation of stored PHAs as the energy source for biomass growth, glycogen replenishment, P uptake and polyphosphate storage. (Oehmen et al. 2007, Zhou et al. 2010) Process design of EBPR

can be provided in two ways. In a continuous system the bioreactor can be divided into anaerobic, anoxic and aerobic zones. Batch reactor system can be divided temporally by anaerobic, anoxic and aerobic periods. Feeding is provided to the anaerobic zone or period. (Tchobanoglous et al. 2003)

Nitrogen can be recovered by ammonia stripping (Xu et al. 2017), ion exchange or hydrolysis (Kapdasli et al. 2006). In ammonia stripping, ammonia is stripped under vacuum and absorbed under pressure, resulting in e.g. a 10 % ammonia solution, which is further processed (Maurer et al. 2006). Ammonia can also be stripped from source-separated human urine without vacuum in high temperature (> 50 °C) and pH (pH > 9), e.g. with air stripping (Liu et al. 2015). The ammonia stripped out can be recovered by sulfuric acid absorption (Liu et al. 2015). Ammonia stripping is the most common technology for ammonium recovery (Carey et al. 2016) and ammonium removal efficiency of 99 % has reported at 35 °C and pH around 11 (Xu et al. 2017). The disadvantage in air stripping is its high energy consumption, although it is cost-efficient (Carey et al. 2016, Xu et al. 2017). In ion exchange zeolite is added to a urine solution, and the N-loaded product is collected. The process can be combined with struvite precipitation by adding MgO. (Maurer et al. 2006) High ammonium recovery efficiency by ion exchange has been reported, but it is not widely studied (Tarpeh et al. 2017). Recently there have been increasing amount of studies about nutrient recovery from urine by microalgae. In enzymatic hydrolysis urea is converted to ammonia and carbamate, which decomposes to carbonic acid and ammonia ($NH_2(CO)NH_2 + 2H_2O \rightarrow NH_3 + NH_4^+ + HCO_3^-$) (Udert et al. 2003, Kapdasli et al. 2006).

Both nutrients can also be removed by microbial fuel cells (MFC) (Kuntke et al. 2012), which have been studied in laboratory scale. There are many applications of MFCs (Hamelers et al. 2010). In MFCs, bacteria catalyze the oxidation of organic substrate and produce electrons at the anode, which are used to reduce an electron acceptor at the cathode (Figure 3). The anode and cathode are usually separated by an ion exchange membrane. Produced electrons are transported from the anode to the cathode, which induces a charge transport across the membrane to neutralize the charge. In case of nitrogen recovery, ammonium-ion transport occurs across the membrane through the anode to the cathode. The high pH in the cathode results in to a formation of volatile NH_3, which can be recovered. (Hamelers et al. 2010, Kuntke et al. 2012)

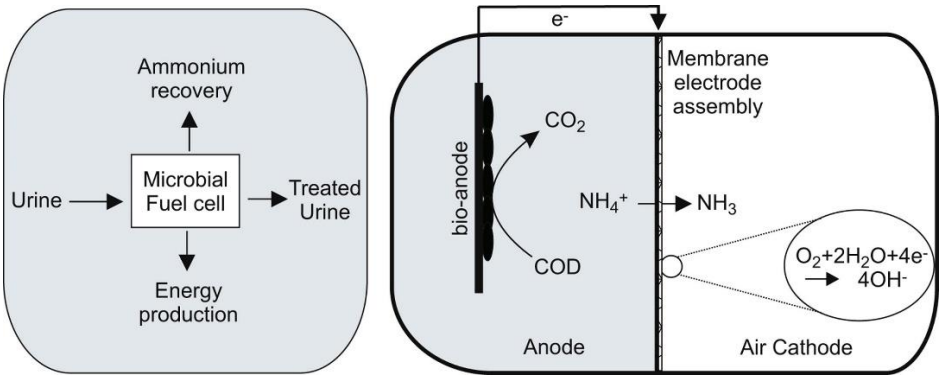


Figure 3. *Ammonium recovery from urine by microbial fuel cell. (Kuntke et al. 2012)*

3. MICROALGAE AND NUTRIENT RECOVERY

3.1 Microalgae growth and metabolism

There is a large variety of microalgae species which differ from each other in cellular organizations (unicellular or colonial) (Barsanti & Gualtieri 2014). However, the basic metabolic and growth characteristics are similar.

3.1.1 Microalgae metabolism and nutrient uptake

Microalgae convert inorganic nitrogen to its organic form by assimilation (Figure 4). Assimilation occurs in several steps. First, translocation of the inorganic nitrogen occurs across the plasma membrane. Then, the oxidized nitrogen is reduced through nitrite to ammonium. Finally, the ammonium is incorporated into amino acids. (Cai et al. 2013) In the presence of ammonium, microalgae prefer ammonium over nitrate. Because less energy is required for assimilation when ammonium is used, effective microalgae growth is achieved in ammonium rich wastewaters (Barsanti & Gualtieri 2006).

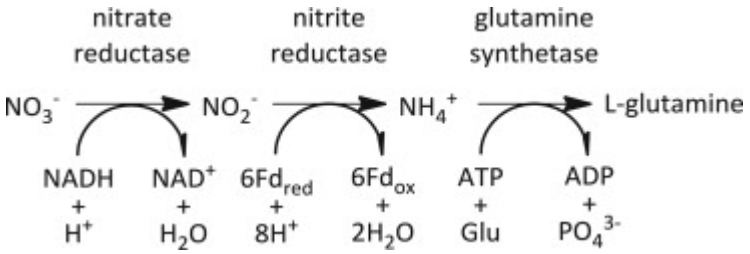


Figure 4. *Simplified schematic of the assimilation of inorganic nitrogen (Cai et al. 2013)*

Also, phosphorus is a key factor in metabolism of microalgae. Inorganic phosphate is essential for microalgae cell growth and metabolism (Cai et al. 2013). Phosphorus as $H_2PO_4^-$ or HPO_4^{2-} is incorporated into organic compounds by the process of phosphorylation including the generation of ATP from adenosine diphosphate (ADP) (Martinez et al. 1999). The oxidation of respiratory substrates, the electron transport system of the mitochondria or light by photosynthesis can serve as energy source (Cai et al. 2013).

Microalgae remove nitrogen and phosphorus from water mainly by uptake into algal cells, but nitrogen is removed also by ammonia stripping and phosphorus by precipitation through increased pH (Hoffman 1998, Bich et al. 1999, Aslan & Kapdan 2006, Garcia et

al. 2006, Xin 2010). The efficiency of the nutrient uptake by microalgal cells depend on the growth conditions (such as light intensity, pH and temperature), the nutrient (mainly nitrogen and phosphorus) concentrations and the N/P ratio of the medium (Kapdan and Aslan 2008, Xin et al. 2010, Beuckels et al. 2015 and Whitton et al. 2016, Bryant and Appah 2017). The concentrations of nitrogen and phosphorous have a direct impact on microalgal growth kinetics which affects nutrient removal mechanisms and capacities (Xin et al. 2010, Yao et al. 2015). The optimal N/P ratio for nutrient uptake is species-dependent (Xin et al. 2010).

3.1.2 Environmental conditions and growth of microalgae

The efficiency of microalgal growth and biomass production depends on the growth conditions, such as light intensity, temperature, pH, nutrients and CO_2 availability. Typically, microalgae grow autotrophically, i.e. they use sunlight as the energy source and inorganic carbon (CO_2) along with inorganic nutrients (N, P, etc.) to form biochemical energy through photosynthesis (Huang et al. 2010). Parmar et al. (2011), Wahidin et al. (2013) and Iasimone et al. (2018) have reported that the light intensity and photoperiod (light and dark) cycles are the prime factors that determine the growth rate of microalgae cultivation. Light is the energy source of microalgae for synthesizing cell protoplasm, but additional light does not increase the biomass production above the light saturation limit (Cheirsilp & Torpee 2012, Wahidin et al. 2013). Higher culture depth and high concentrations of microalgal cells increase the light requirements (Wahidin et al. 2013). Light is also important because it is the only factor that cannot be easily controlled, compared to e. g. pH and CO_2 and nutrient availability (Bechet et al. 2013).

Microalgal cultivation has been successfully reported at temperatures in the range of 5 – 40 °C and the influence of cultivation temperature has been found to be species-dependent (Chen et al. 2008, Converti et al. 2009, Xin et al. 2011, Cassidy 2011, Singh & Singh 2015). Optimal pH and tolerance for pH changes are also species-dependent. For example, *Scenedesmus* species can grow in a wide range of pH (4 – 11) (Ren et al. 2013). Most favourable pH for microalgae growth has been reported to be 7 – 9.

CO_2 is essential for microalgal photosynthesis. Growth and aeration with certain CO_2 concentration can improve the biomass production and nutrient uptake of microalgae cells. The optimal CO_2 concentration is medium and strain dependent. However, surplus CO_2 can decrease the biomass growth and nutrient removal if the pH of the medium is decreased due to CO_2 sparging. (Yao et al. 2015)

The largest amounts of elements used by microalgae are non-mineral elements, that include carbon, hydrogen and oxygen, which are taken up mainly as CO_2, and water (H_2O) (Jönsson et al. 2004). Along with essential macronutrients (N and P), several trace elements (K, Fe, Mg, S, Ca, Zn, Cu, Mn) are required for microalgae growth (Jönsson et al. 2004). Even though the requirements are species-dependent, it is possible to evaluate the

essential micronutrients by microalgal composition analysis (Jaatinen et al. 2016). Ammonium is toxic to microalgal cells in high concentrations (Konig et al. 1987). However, some species e.g. *Chlorella Sorokiniana* can have high tolerance for it (up to 1,400 mg NH_4-$^+$-N /L) (Tuantet et al. 2014a, Collos and Harrison, 2014).

Algae also form mutually beneficial partnerships with other organisms, which enable both to survive in conditions that they could not endure alone. They live with fungi to form lichens, or inside the cells of reef-building corals, in both cases providing oxygen and complex nutrients to their partner and in return receiving protection and simple nutrients. (Barsanti & Gualtieri 2014) In many studies nutrient recovery from wastewaters has often been done with single microalgae species or monocultures. However, in outdoor conditions or scaled-up systems other microalgae species and bacteria (especially heterotrophs) are introduced into the cultures. The interactions between microalgae and bacteria can increase the efficiency of microalgal growth. (Ramanan et al. 2016)

3.2 Cultivation of microalgae

3.2.1 Cultivation technologies

Microalgae can be cultivated in open, closed or hybrid systems (Table 2) (Cai et al. 2013, Cuellar-Bermudez et al., 2017). All these systems are used on land and utilize suspended microalgae cultures in an aquatic environment (Cai et al. 2013). The selection of reactor type depends for example on the economic resources and the characteristics of used microalgae species, such as light and CO_2 requirements and sensitivity for contaminants (Parmar et al. 2011, Cai et al. 2013). To stabilize microalgal growth, the alterations in cultivation conditions should be minimized by monitoring the conditions and mixing the biomass (Patel et al. 2017). The culture can be fed as batch, semi-continuously or continuously (Abe et al. 2008). Hygienization of the feed is often required before feeding (Cai et al. 2013).

Typical open system for microalgae cultivation is a raceway pond. Currently, open ponds are the most often used cultivation systems because they are easy to scale up. (Parmar et al. 2011, Cai et al. 2013) Because the photosynthesis by microalgal cell requires sufficient sunlight, the ponds are low, around 0.3 m. The algae culture is mixed and circulated around the raceway track by paddlewheels. There are also applications of the cultivation ponds, where they have been built near power plants or wastewater treatment plants (WWTP) to utilize carbon dioxide from flue gas, wastewater or saltwater, respectively, in the microalgae cultivation. The main disadvantage in open systems are water loss by evaporation and contamination. (Cai et al. 2013)

Typical closed systems are flat plate reactors, tubular photobioreactors, and bag systems. Light availability and gas exchange can be optimized in flat plates and photobioreactors. They can also be moved according to the orientation of the sun. Bag systems consist of

plastic bags (around ø 0.5 m) and aeration systems. Advantages of closed systems are that water evaporation and contamination can be avoided and photosynthesis efficiency increased. However, the costs of the closed systems are usually higher compared to open systems. In bag systems one disadvantage is the lack of mixing. (Cai et al. 2013) In addition, one biotechnical challenge in photobioreactors is the physics of light distribution and its utilization, because too high light intensity may result in photo-inhibition or overheating (Parmar et al. 2011).

Hybrid systems consist of two cultivation stages; the inoculum is cultivated in closed photobioreactor and used in the second cultivation stage in open ponds. Therefore, cultivation of the inoculum can be monitored better and contaminations avoided. However, large-scale applications can be conducted with lower costs of second stage. (Cai et al. 2013)

Also, immobilization technologies have been designed for microalgae cultivation, mainly to make the harvesting of the culture easier. Currently, there are six types of immobilization methods (covalent coupling, affinity immobilization, adsorption, confinement in liquid–liquid emulsion, capture behind semipermeable membrane, and entrapment) and five types of bioreactors (fluidized-bed, packed-bed, parallel-plate, air-lift, and hollow-fiber). However, the studies and applications of the immobilized systems are still at laboratory scale. (Cai et al. 2013)

Table 2. *Summary of cultivation systems of microalgae*

Cultivation process	**Scale**	**References**
Open systems		
Raceway ponds	Large scale (most commonly used)	Parmar et al. 2011, Cai et al. 2013
Closed systems		Parmar et al. 2011,
Flat plate reactor	Large scale	Cai et al. 2013,
Tubular photobioreactor	Large scale	Slegers et al. 2011,
Bag system	Lab scale	Slegers et al. 2013,
Hybrid systems	Large scale	Cai et al. 2013
Immobilization technologies	Lab scale	Cai et al. 2013

3.2.2 The overall process for nutrient recovery with microalgae

Different microalgae species have been studied widely for nutrient recovery (Abe et al. 2008). The most widely studied microalgae species for nitrogen and phosphorus removal are *Scenedesmus, Chlorella and Spirulina* (Patel et al., 2017). Microalgae have successfully been cultivated in human urine without dilution (Tuantet et al. 2014a, Tuantet et al. 2014b) and in different dilutions (Adamsson 2000, Jaatinen et al. 2015, Tao et al. 2017,

Chatterjee et al. 2019). However, based on these studies optimal nutrient recovery was reached by diluting the urine used as a feed. Economically it is effective to use low dilution, so the optimal dilution must be found in all cases.

To utilize cultivated microalgae, harvesting of the culture is needed. Current algae harvesting methods include chemical, mechanical, electrical, and biological techniques, such as centrifugation, foam fractionation, filtration, flocculation and gravity sedimentation. Challenges in different harvesting methods are small cell size, highly diluted culture, the large consumption of chemicals and energy and rupture of the microalgal cells. (Cai et al 2013, Chen et al. 2013) In figure 5, the overall scheme for nutrient recovery with microalgae is visualized.

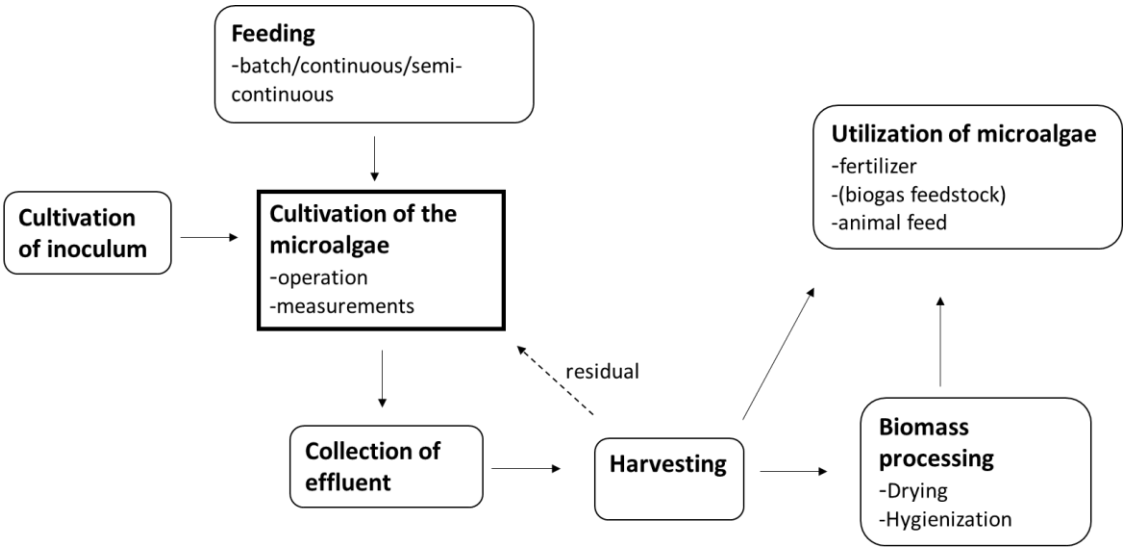


Figure 5. *Flowchart of microalgae utilization in nutrient recovery*

3.3 Utilization of microalgae as nutrient source

Microalgae is well known as a biofertilizer in agriculture (Renuka et al. 2016). Microalgae contains large amounts of carbohydrates, lipids, proteins, pigments, vitamins, trace elements and plant growth-promoting substances (Spolaore et al. 2006, Coppens et al. 2016) There are several studies of use of microalgae biomass as a biofertilizer for crops and other plants (Uysal et al. 2015, Coppens et al. 2016, Renuka et al. 2016, Renuka et al. 2018) and also wastewater-grown microalgae have been used as fertilizer. Microalgae have been shown to improve soil nutrient availability, plant growth, fruit quality, nutritional characteristics, grain yield and production of plant hormones (Renuka et al. 2016, Coppens et al. 2016, Renuka et al. 2018). Microalgal biofertilizer have also been shown to reduce erosion of soil by regulating the water flow into soils and improving soil fertility and stabilize pesticides (Yan et al. 2013, Renuka et al. 2016). However, microalgae accumulate toxic heavy metals and therefore the utilization of microalgae cultivated in

wastewater is questionable, although trace amounts of metals can be used as micronutrients (Renuka et al. 2016).

Different types of microbial biofertilizers are available, such as carrier based, liquid formulations or pellets (Renuka et al. 2016), or dried biomass can be pulverized (Coppens et al. 2016). As a carrier in the biofertilizers can be used for example vermiculite or compost. (Renuka et al. 2016) After harvesting of microalgal biomass it is often dried. (Renuka et al. 2016, Coppens et al. 2016) The biomass can also be pasteurized (Coppens et al. 2016).

Based on economical assessments microalgal fertilizers are not competitive commercial horticulture fertilizers. Microalgal based fertilizers could be used in integrated plant nutrition systems instead, which combines inorganic, organic and biofertilizers to optimize the use of fertilizers and minimize environmental impacts. (Coppens et al. 2016) In addition, the economic efficiency can be affected with the selection of cultivation process and the cost of production of chemical fertilizers is increasing (Coppens et al. 2016, Bhalamurugan et al. 2018) and in life cycle analysis, microalgae have been regarded as cost-effective and eco-friendly fertilizers (Mahapatra et al. 2018).

3.4 Advantages of nutrient recovery from urine with microalgae

Microalgae have many advantages in the removal of nitrogen and phosphorus, including the following (Aslan and Kapdan 2006, Xin et al. 2010, Patel et al. 2017): (1) availability of sufficient solar energy, (2) simultaneous fixation of CO_2, (3) lack of extra organic carbon requirement (as compared to biological nitrification-denitrification), (4) discharge of oxygenated effluents into water bodies, (5) decreased water pollution and greenhouse gas emissions (6) avoidance of sludge handling problems, and (7) economic potential of harvested algal biomass (for e.g. feedstock, fertilizers, biofuels, and so on). To maximize benefits and minimize environmental impacts of the outdoor cultivation, improvements in engineering practices are required (Bechet et al. 2013).

The produced microalgal biomass can be utilized in several ways. Microalgae are widely used to produce different products, such as dietary supplements for animal feed, polyunsaturated fatty acids, anti-oxidants, coloring substances, fertilizers and soil conditioners, and a variety of specialty products such as bioflocculants, biodegradable polymers, cosmetics, pharmaceuticals, polysaccharides and stable isotopes for research purposes. In addition, microalgae can be used for example to produce bio-oil or as a feedstock in biogas production in wastewater treatment. (Spolaore et al. 2006, Parmar et al. 2011, Bhalamurugan et al. 2018)

4. MATERIALS AND METHODS

In this study the microalgae *Scenedesmus acuminatus* was first cultivated in laboratory, and then in batch raceway pond (400 L) in a greenhouse. Subsequently, the microalgae biomass was used as inoculum in bigger raceway pond (2000 L) operated as a continuous system. The microalgae culture was cultivated with 15 times diluted source-separated urine. The study was conducted in Hiedanranta area in Tampere, Finland. The city of Tampere is planning to build a residential area of 20 000 inhabitants in Hiedanranta. In addition, circular economy will be optimised in the area. Source separation has already been used in smaller scale and has been planned to be used in the residential area of Hiedanranta.

4.1 Source-separated urine

Source-separated urine (from now on called urine) used in this study was collected from source separating toilet system in cultural center in Hiedanranta, Tampere. The urine was stored for approximately 6 months in 1000 L plastic tanks in closed containers at ambient temperature 5 – 20 °C. During storing the pH of the urine rises destroying potential pathogens (Jönsson et al. 2004).

Characteristics of the urine were determined in the beginning of the study. The urine sample was analyzed for pH, optical density (OD_{660}), total nitrogen (N_{tot}), total phosphorus (P_{tot}), chemical oxygen demand (COD), NH_4^+, phosphate (PO_4^{3-}), total suspended solids (TSS) and volatile suspended solids (VSS).

4.2 Experimental setup

Two raceway ponds with a working volume of 400 L and 2000 L were used in this study (Figure 6). Both ponds were located in a greenhouse. Both ponds had a paddle wheel with four paddles. Urine and influent tanks (1000 L) as well as effluent tank were located in containers next to the raceway ponds (Figure 6).

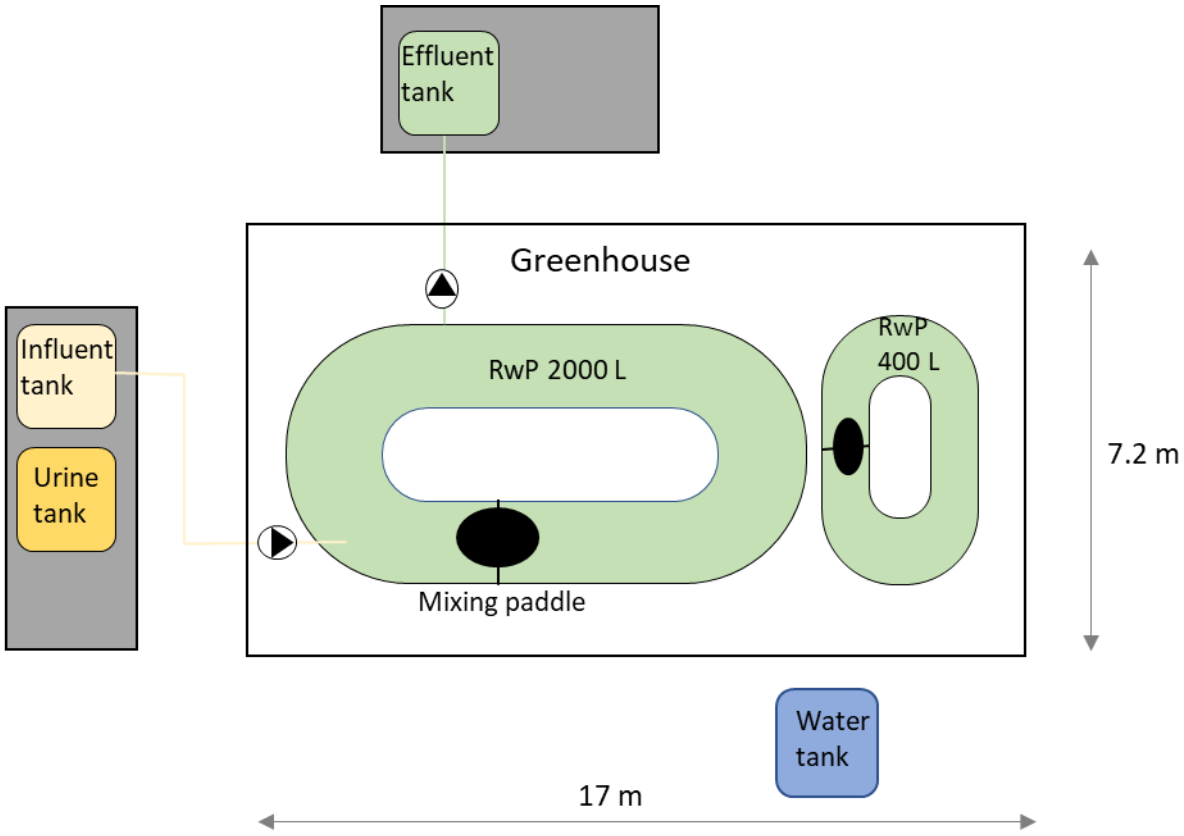


Figure 6. *Illustration of the experimental setup; the raceway pond was located in the greenhouse and the influent and effluent tanks next to the greenhouse.*

Urine was diluted with tap water (1:15) into 1000 L plastic influent tank, which was filled when the volume was low (< 200 L). Urine was pumped to the pond from the bottom of the tank without mixing. Characteristics of the influent were followed by taking samples and analysing pH, OD_{660}, N_{tot}, P_{tot}, COD, ammonium, phosphate, TSS and VSS of the dilution. Samples were taken three times a week 9 – 12 a.m. from the influent tube and stored at (4± 1) °C before analyses.

The feed was pumped from the tank with submersible pump and conducted to the big pond with plastic tubes (Masterflex Tygon Fuel and Lubricant, P/S 24). The effluent flow was pumped to an effluent tank in a container.

4.3 Cultivation of *Scenedesmus acuminatus*

The microalgae *Scenedesmus acuminatus* was first cultivated in laboratory, and then in batch raceway pond (400 L) in a greenhouse. Subsequently, the microalgae biomass was used as inoculum in bigger raceway pond (2000 L) operated as a continuous system. The microalgae biomass was cultivated with 15 times diluted source-separated urine.

4.3.1 Cultivation of inoculum

Freshwater microalgae *Scenedesmus acuminatus* was obtained from the Culture Collection of Algae (SAG) at the University of Göttingen, Germany. The microalgae was first cultured in laboratory in 1 L glass photobioreactors (PBR) (Figure 7) in N8 media (Praveenkumar et al. 2014) as batch with the composition of 0.5055 g/L KNO_3, 0.7400 g/L KH_2PO_4, 0.2598 g/L Na_2HPO_4, 0.0500 g/L $MgSO_4 \times 7\ H_2O$, 0.0175 g/L $CaCl_2$, 0.0115 g/L FeNaEDTA × 3 H_2O, $ZnSO_4 \times 7\ H_2O$, 0.0130 g/L $MnCl_2 \times 4\ H_2O$, 0.0183 g/L $CuSO_4 \times 5\ H_2O$ and 0.0070 g/L $Al_2(SO_4)_3 \times 18\ H_2O$. Exceptionally $CaCl_2$ was used instead of $CaCl_2 \times 2\ H_2O$, which is not as soluble as its hydrous form. The media was filtered with 0.45 µm Millipore filter and pH was adjusted to 7.7 (Xu et al. 2015) by adding NaOH. 80 mL of washed stock culture was inoculated in 700 mL of N8 medium in four PBRs. Initial optical density with wavelength 660 (OD_{660}) was 0.246.

Prepared PBRs were set under continuous illumination of the fluorescent lamp (Osram L 18W/965, Biolux Germany) with the intensity of 40µmol photons $m^{-2}\ s^{-1}$. Air and Carbon dioxide (CO_2) were added to the bottom of the PBRs and dispersed with glass distribution tubes (diameter 22mm, Duran Group Germany). The gas flow was controlled by the flow meter (Dwyer Instruments Inc., USA) at the constant flowrate of 0.2 L/min and 20 mL/min, respectively. Distilled water was added to replace the water loss caused by evaporation in the beginning of the cultivation to keep the volume constant. The growth of the culture was followed by measuring OD_{660} and pH five times a week. Condition of the microalgae was also checked with microscope. When enough biomass was grown (OD_{660} 0.9), centrifuged culture was removed in N8 medium to get 5L of inoculum.

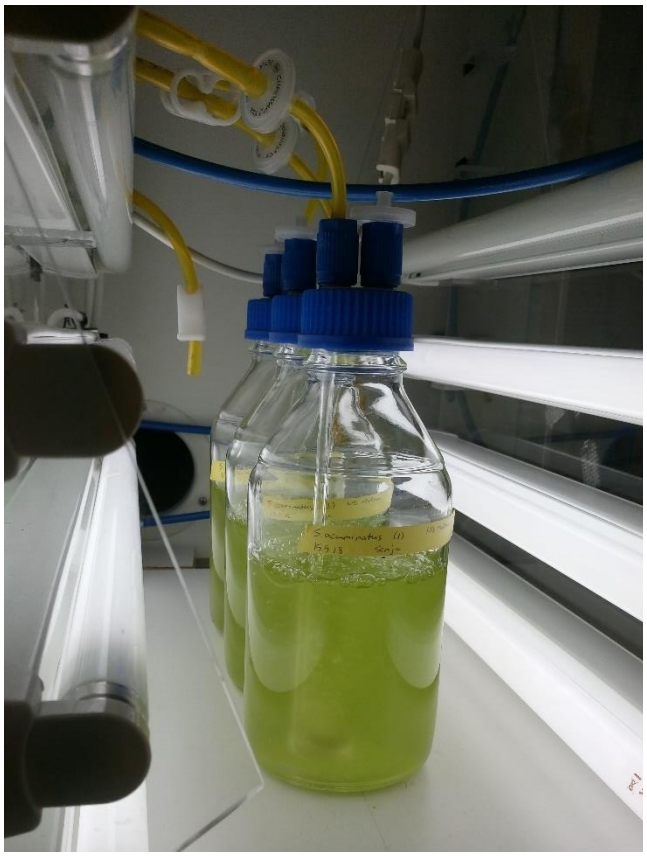


Figure 7. *Cultivation of S. acuminatus in N8 medium in photobioreactors (day 1)*

Five litres of the prepared culture (OD_{660} = 0.9) was inoculated into raceway pond (400 L) with 395 L of 15 times diluted urine to reach 400 L. The pond contents were continuously mixed with paddle wheel with a constant velocity of 10 rpm. Water was added to replace evaporated water on the 5th and 9th day of the experiment. In addition, on the 16th day 45 L of diluted urine (5:40) was added because of low nutrient levels in the culture. The pond was operated as a batch system for 20 days to get enough biomass (OD_{660} = 0.9) to inoculate the culture to the big pond.

The growth was followed by measuring temperature of the culture and light intensity on site and taking samples to measure pH and OD_{660} three times a week. In addition, N_{tot}, P_{tot}, ammonium, phosphate, COD, VSS, TSS were measured in laboratory once a week.

4.3.2 Cultivation in raceway pond and operation

About 350 L of the biomass (OD_{660} = 0.9) cultivated in the 400 L pond was used as inoculum in the 2000 L pond (from now on called raceway pond) (Figure 8) with 15 times diluted urine to reach 2000 L. The pond was first run as a batch system for 13 days. Then continuous feeding was started with the diluted urine with a flow rate of 130 L/d. Constant influent feeding without effluent pumping was continued until the volume of the culture reached the desired volume of 2000 L. High temperature in the greenhouse (20 – 50 °C in sampling time) increased water evaporation and the requirement of additional feeding.

Figure 8. *The raceway pond on day zero*

Effluent pumping was started on day 39, and it was continued with a constant flow rate of 55 L/d. The liquid volume of the pond was kept constant controlling the effluent flow rate and evaluating evaporation of water from the pond. Trace elements were added on day 77 and 88 using modified N8 medium without macronutrients containing N and P (KNO_3, KH_2PO_4 and Na_2HPO_4). The final concentration of the modified N8 medium was 1/10 of the concentration of N8 medium used in laboratory cultivation.

The pond content was continuously mixed with paddle wheel with a constant velocity of 15 rpm. Sample from the influent, pond and effluent were taken mainly three times a week before midday (9 – 12 a.m.). Samples were taken from the upper part of the biomass and the microalgal biomass that had settled on the pond's shore was brushed off before sampling. The influent sample was taken from the end of the tube.

The temperature of the water and the light intensity were measured on site. The samples were analyzed pH, OD_{660}, ammonium, TSS and VSS on the sampling day and stored at (4 ± 1) °C until analysing N_{tot}, P_{tot}, COD and phosphate. Samples analyzed for COD were preserved with 0.01 mL of H_2SO_4 per 10 mL of sample. Table 3 presents the summary of the operation of the pond.

Table 3. *Summary of the operation of the pond*

Week	Day	Operation
0	0	Inoculation
2	11	Continuous feeding started
6	39	Effluent feeding started
10	71	Modified N8 medium added
12	88	Modified N8 medium added

4.4 Analytical methods and calculations

4.4.1 Analytical methods

Temperature of the culture and light intensity (Apogee, MQ-200 Quantum sensor and meter, λ 410 – 655 nm) were measured on site. Temperature was measured on the top of the biomass. Light intensity was measured every time at the same spot on the floor in the middle of the greenhouse.

pH was measured by pH electrode and optical density (OD_{660}) was determined at 660 nm using UV-VIS spectrophotometer (Shimadzu UV-1700 PharmaSpec). TSS and VSS were measured according to standard methods (SFS-EN 872 2015, SFS 3008 1990). About 50 – 100 mL of the sample was filtered depending on OD through glass fibre filter of pore size 1.6 µm (Whatman GF/A). COD was measured for filtered and nonfiltered samples according to standard methods (SFS 5504). Before the analyses, samples were filtered with 0.45 µm PTFE filters (CHROMAFIL Xtra PET 45125, Machinery Nagel, Germany).

Ammonium, N_{tot}, P_{tot} and phosphate were analyzed with HACH Lange kits (LCK 303, LCK 350, LCK 349, LCK 338) according to the protocol by the manufacturer (HACH 2019). N_{tot} and P_{tot} were measured from filtered and nonfiltered samples. Ammonium and phosphate were measured from filtered samples. Filtration was done with 0.45 µm PTFE filters (CHROMAFIL Xtra PET 45125, Machinery Nagel, Germany).

The culture was visualised by bright-field microscope (Axio-Imager, Carl Zeiss, Germany) and photographed using an AxioCam HRc CCD camera equipped with AxioVision software (Carl Zeiss, Germany).

4.4.2 Calculations

The nutrient recovery was based on the weekly mass of nutrients present in the influent, pond and effluent, which were calculated based on the measured nutrient concentrations

and the respective liquid volumes; influent volume, estimated volume of the culture in the pond and volume of the effluent.

The mass of nutrients in the pond at a certain time point was calculated by summing the influent mass of nutrients to the nutrient mass in the pond of previous week and subtracting the mass of nutrients in the effluent. The difference in calculated mass and the measured mass of N_{tot} in the pond was assumed to be the mass of nitrogen volatilized as NH_3. The mass of nitrogen captured in microalgae cells (nitrogen recovery rate) was calculated by subtracting the mass of nitrogen in liquid phase in the pond from the mass of N_{tot} in the pond. Therefore, the nitrogen volatilized as NH_3 was not counted to the recovery rate. The mass of phosphorus captured in microalgae cells was calculated by subtracting the mass of phosphate of the mass of P_{tot} in the pond.

The nutrient recovery of nitrogen and phosphorus as percentage was calculated by dividing the values of nutrients captured in the cells with the entering total nutrient mass:

$$Nutrient\ recovery\ (\%) = \frac{Nutrient\ in\ the\ pond - Nutrient\ in\ liquid\ phase}{Nutrient\ entering}. \quad (8)$$

The weekly mass flow calculations were based from day 4 onwards and nutrient recovery calculations from week 4 onwards.

5. RESULTS

The raceway pond was operated for 106 days from the middle of June to the beginning of October. Results from the pond were followed after starting the pond. The characteristics of diluted urine used as a feed was followed during the cultivation.

5.1 Urine and influent characteristics

The urine used in this study was analysed in the beginning of the study (Table 4) and the influent was followed three times a week from the beginning of the experiment.

Table 4. *Characteristics of the source-separated urine used in this study*

pH	OD_{660}	N_{tot} mg/L	P_{tot} mg/L	COD mg/L
9,08	0,184	3590	196	6340

In table 5 is presented the pH, OD, COD and nutrient concentrations of the influent during the study. These results are also visualized in figure 9. OD_{660} of the influent varied between 0.033 and 0.098 except for three peaks on days 39, 53, 62 (0.56, 0.26 and 0.16 respectively). pH dropped from the initial pH 9 to the lowest value of 8.55 during first 34 days, increased to 9.0 until day 55 and varied between 9.0 and 8.7 the rest of the run. COD varied between 180 and 530 mg/L except for the days 39 – 43 when the COD decreased under 80 mg/L.

Table 5. *pH, OD_{660}, COD and nutrient concentrations of the influent and nutrient loading rates (mean value and range) during days 18 – 92*

	Influent					Influent filtered			
	pH	OD_{660} µm/m²s	COD (mg/L)	N_{tot} (mg/L)	P_{tot} (mg/L)	NH_4^+ (mg/L)	N_{tot} (mg/L)	P_{tot} (mg/L)	PO_4^{3-} (mg/L)
Mean	8.8	0.103	339	855	15,6	177	598	13,1	12,7
Range	[8.6-9.0]	[0.033-0.26]	[0.0-529]	[196-1050]	[11.8-17.5]	[122-220]	[458-1140]	[11-16.1]	[10.7-15.9]
n	26	26	28	14	14	30	11	11	11
	Loading rate (g/m³ d)			N_{tot}	P_{tot}				
Mean				34	0.87				
Range				[17-65]	[0.34-1.2]				

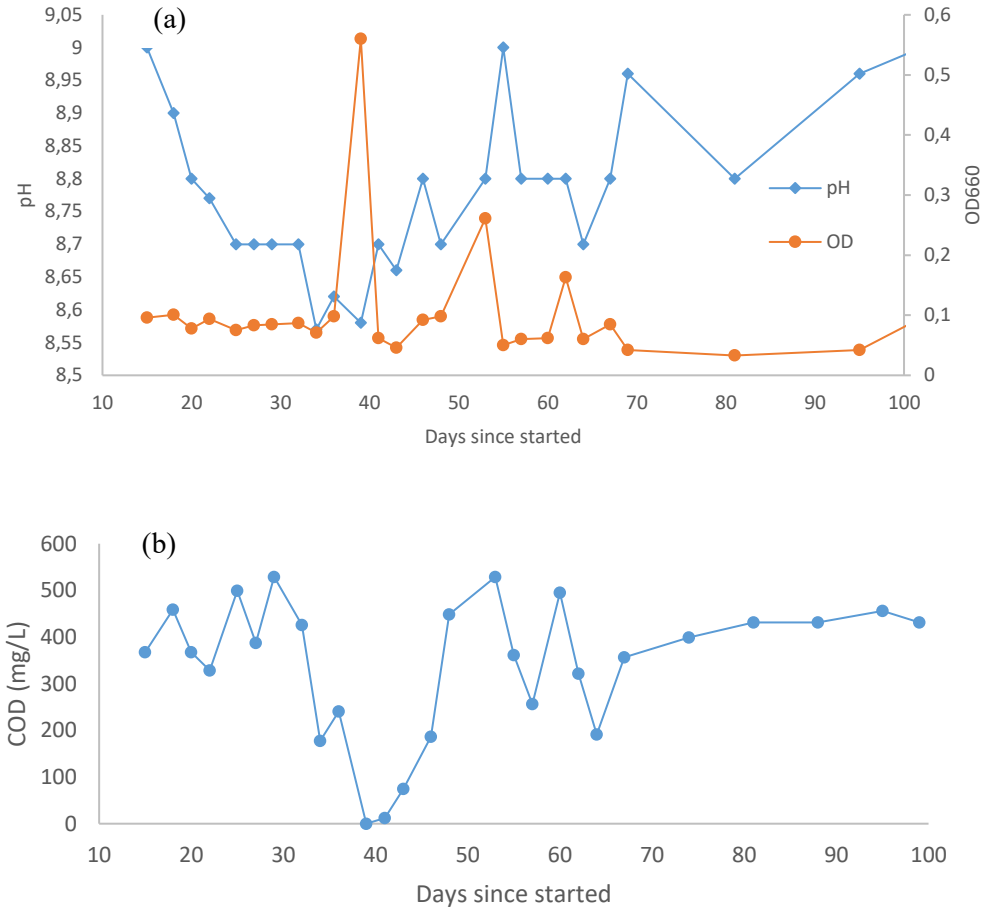


Figure 9. *pH, OD_{660} and COD of the influent during the run*

The concentration of ammonium in the influent decreased during the run and varied between 380 and 120 mg/L. On average 30 % of nitrogen in influent was present as ammonium. N_{tot} concentration increased in the beginning of the run and started to decrease after 30 days from 1050 to 200 mg/L. From the concentration of the ammonium in influent it can be seen that it occurs the days when the influent tank was filled being highest on average day after filling. Nutrient calculations do not include the results from day 39 because the values of N_{tot} and P_{tot} varied significantly from other concentrations (influent N_{tot} 246 mg/L, P_{tot} 365 mg/L; influent filtered N_{tot} 219 mg/L, P_{tot} 18,3 mg/L). Influent sample on day 39 was rigid and difficult to filtrate. In the influent concentrations of phosphate and P_{tot} there was variety during the run. The concentrations in the influent varied between 11 and 17 mg/L.

5.2 Growth of *Scenedesmus acuminatus* and the operation of raceway pond.

5.2.1 Cultivation of inoculum

The microalgae *S. acuminatus* was grown 14 days in laboratory in photobioreactors and then days in 400 L raceway pond (Figure 10). Then 350 L of the culture was used as inoculum in the raceway pond. OD of the inoculum was 0.84 and pH 10.2. TSS of the inoculum was 0.5 g/L and VSS 0.4 g/L. The microalgae culture was grown as batch with the 15 times diluted urine for 20 days before starting the continuous feeding.

Growth of the S. *acuminatus* cells were examined before starting the cultivation of the inoculum and the culture in the raceway pond.

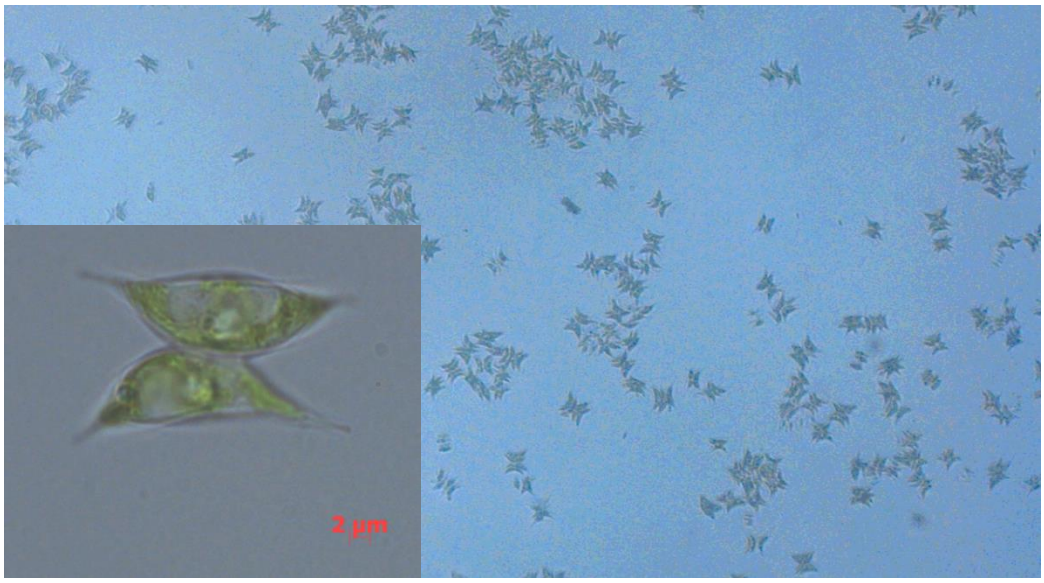


Figure 10. *Laboratory culture of S. acuminatus used as inoculum for the raceway pond*

5.2.2 Cultivation in raceway pond and operation

Cultivation was followed altogether 106 days after starting the raceway pond. Continuous feeding was started on 13th day. Cultivation was discontinued on 106th day after significant decrease in biomass production. Addition of trace elements to support weakened growth of the biomass did not affect OD_{660}, COD, TSS or VSS or the biomass. The pond was operated with HRT of approximately 23 d.

The continuous feeding of the influent was started on day 13. However, the feeding was interrupted a couple of times during the cultivation. Therefore, the feeding was adjusted to compensate the feeding during the power outages or pump breaks. The effluent flow rate varied between 50 – 120 L/d.

Light intensity variation during the cultivation is presented in figure 11. Light intensity was measured in the middle of the greenhouse floor. Part of the greenhouse was covered in shadow of the container next to it during sampling hours, but the measurement point was not under that shadow. In addition, occasional cloudiness made the measured values not unambiguous. In addition, light intensity varied during day and night.

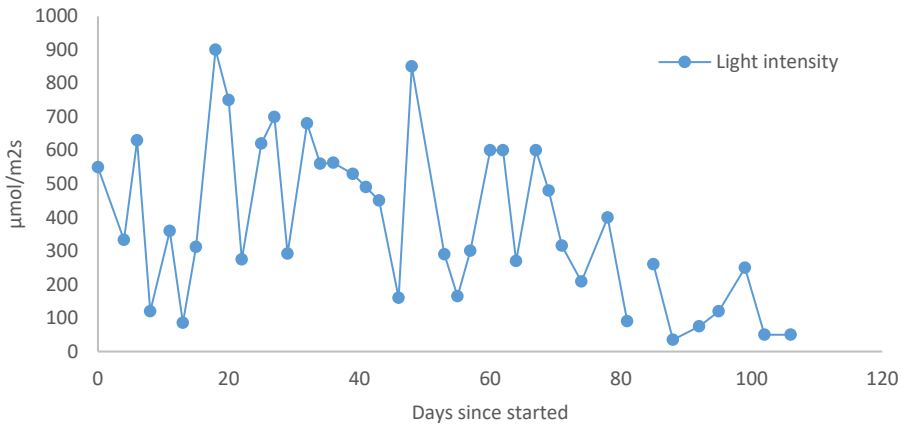


Figure 11. *Light intensity in the greenhouse*

In the figure 12 the values of T, pH, OD, COD, TSS and VSS of the culture during the cultivation are presented. Temperature varied between 17 – 29 °C and after 43 days the temperature started to decrease until it was below 10°C in the end of the run. In addition, the ambient temperature varied during day and night (2.2 – 30 °C) (Ilmatieteen laitos 2018). The highest pH reached was 9.7 around day 11. After the peak value the pH stayed between 8 and 9, until it dropped significantly to 6.

COD started to increase approximately at the same time that the continuous feeding was started. In the beginning of the run, the biomass content (OD and COD) increased until day 25 and started decreasing after 50 days, that is, after the temperature started to decrease. The OD of the culture did reach a highest value of 1.4 and decreased under 0.5 during last cultivation days.

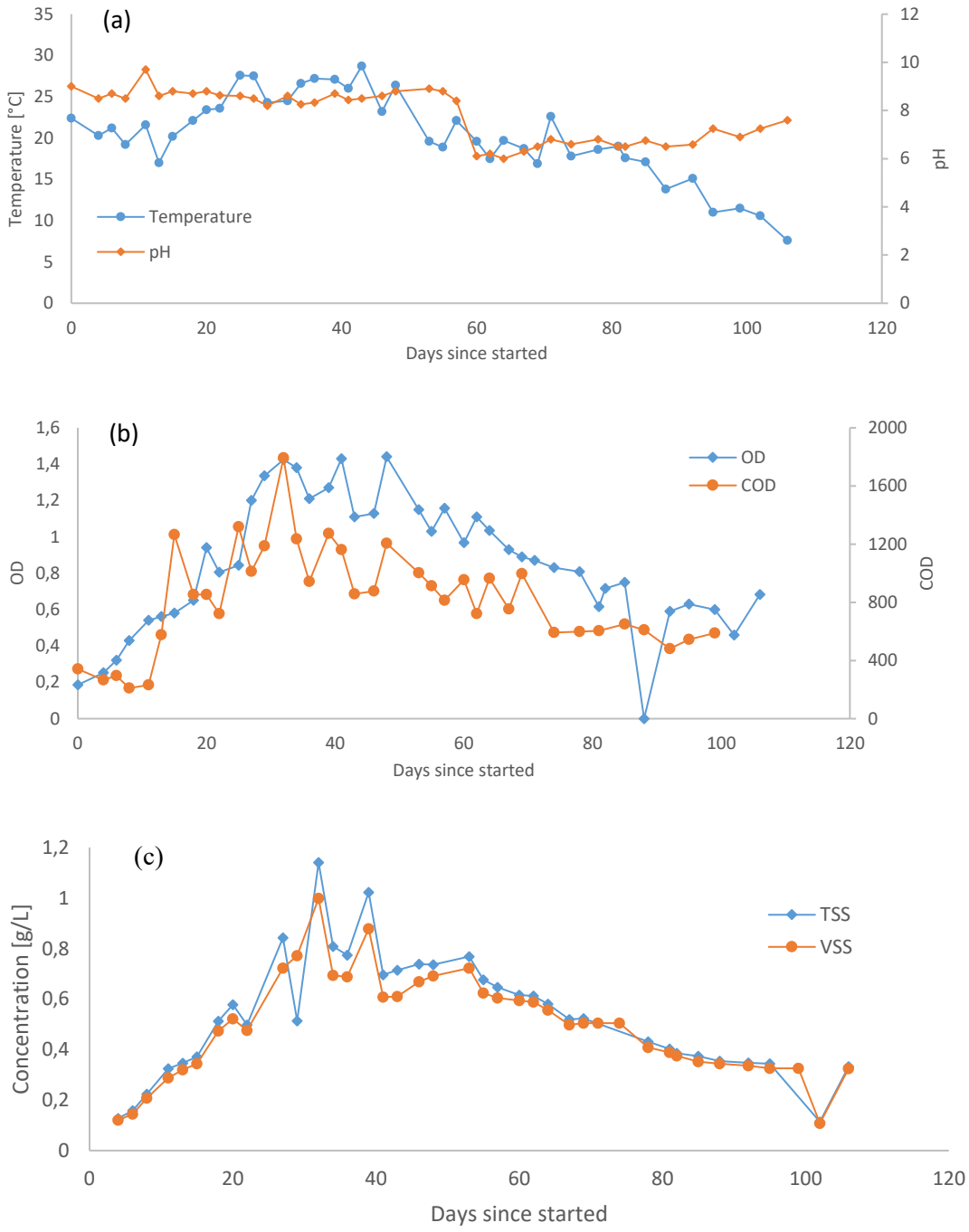


Figure 12. *pH, temperature, OD, COD, TSS and VSS of the microalgae biomass during the run*

In addition to *S. acuminatus* in the culture also some other organisms (other algae species or fungi) were grown and *S. acuminatus* cells flocculated to the mycelium in the culture. Some of the cells were slightly expanded.

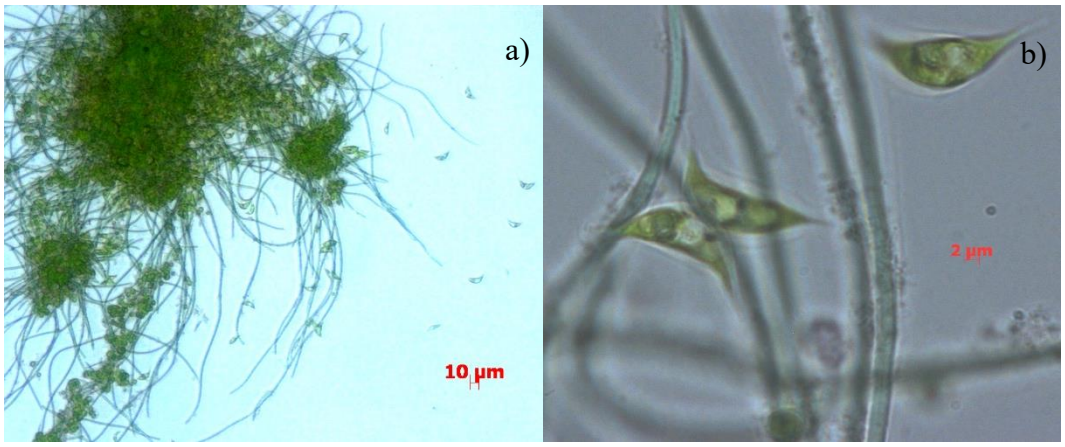


Figure 13. *Biomass culture on day (a) 53 and (b) 41. In figure (a) The S. acuminatus cells (b) are surrounded with the mycelium.*

5.3 Nutrients and recovery

The measured influent N_{tot} concentration varied from 200 to 1050 mg/L, while ammonium ranged from 120 to 220 mg/L. Concentration of ammonium in the pond varied from 0 to 100 mg/L increasing after day 13 when the continuous feeding started (Figure 14 and Table 5). N_{tot} concentration increased until day 81 from 80 to 630 mg/L.

Phosphate and P_{tot} concentrations varied during the cultivation. Concentration of phosphate started to increase from 6 mg/L after starting the continuous feeding. After the start of the feeding the concentration of phosphate varied between 4 and 14 mg/L. The concentration of P_{tot} increased from 11 to 22 mg/L during the first 40 days and after that the concentration of Ptot started to decrease.

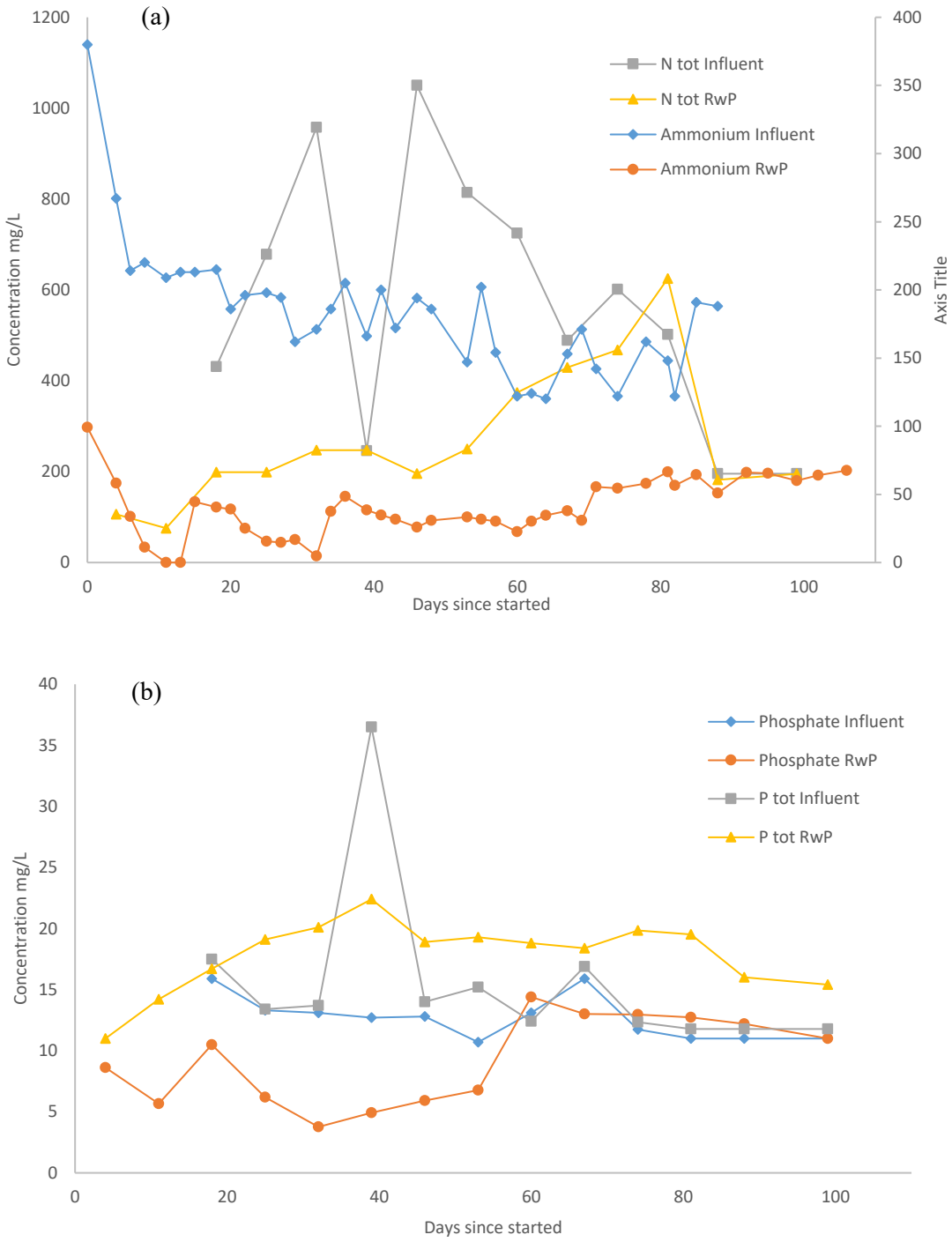


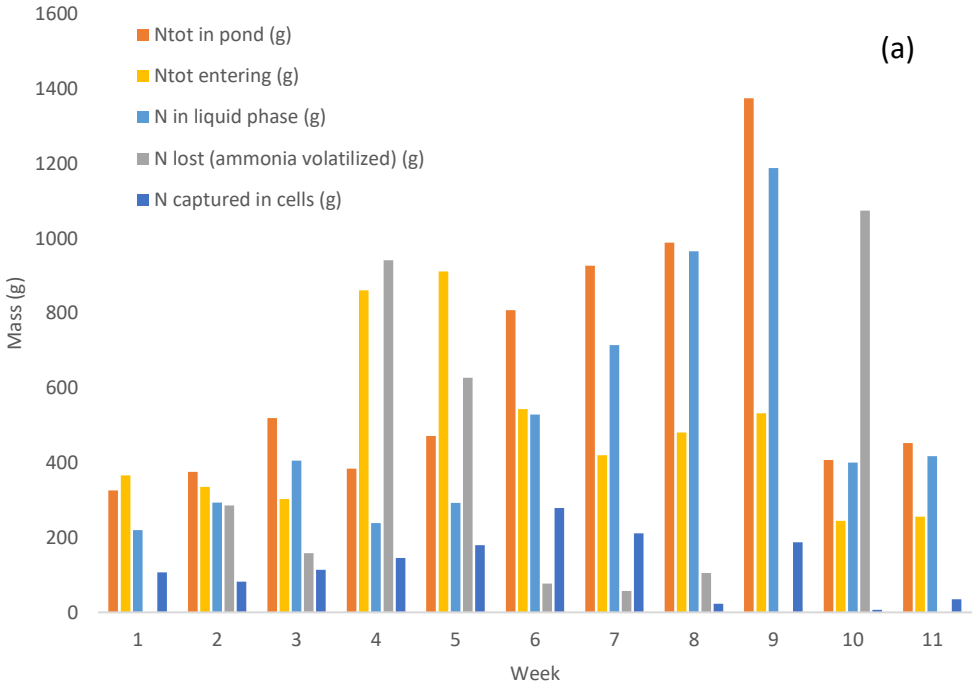
Figure 14. *Nitrogen (a) and phosphorous (b) concentrations in the influent and the raceway pond during the run*

Nutrient recovery was determined from the day 15 onwards, the week after the continuous feeding had started (Figure 15). The phosphate used by microalgal cells and precipitated on the bottom of the pond is included in precipitated and captured phosphate in Figure 15b.

Total nitrogen entering the pond started to increase after week three and started to decrease after week six. The maximum ammonium volatilization occurred around week four

when over 85 % of entered nitrogen was volatilized. During eight weeks 46 % (range 0 – 100 %) of the ammonium was volatilized on average. More ammonium was volatilized, when the temperature and pH was high (> 25 °C, > 8.5, respectively). The total nitrogen amount and nitrogen in the liquid phase started to increase after week four. Another volatilization peak was during week 10. After week four the temperature and biomass growth also started to decrease. On average 26 % (2.7 – 51 %) of total nitrogen entering the pond was captured by algal cells during the cultivation with maximum weekly average of 51 %. On average 57 % (12 – 100 %) of total nitrogen entering the pond was left in liquid phase.

The weekly mass of total phosphorus entering the pond varied between 5 and 16 g and it was in the form of phosphate. The weekly mass of phosphorus in the pond varied between 30 and 42 g per week. In the pond the mass of total phosphorus (phosphate) started to increase after five weeks. So, the greater part of the phosphorus precipitated in the pond or was used by microalgal cells. On average 36 % (0 – 100 %) of the phosphorus entered was captured by microalgae or precipitated.



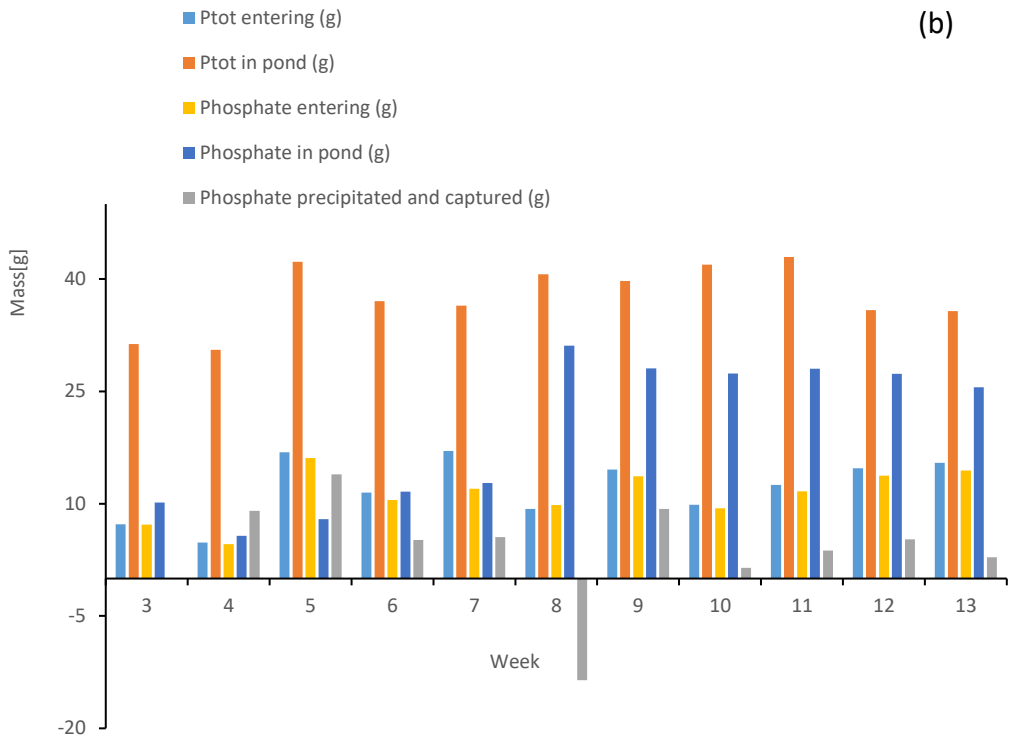


Figure 15. *Mass of nitrogen (a) and phosphorus (b) in algae biomass in different forms and fractions during the cultivation.*

The weekly recovery rate of nitrogen varied between 1 – 50 % being in most weeks from 17 to 50 % (Figure 16). The maximum weekly average of nitrogen captured was 51 % (Figure 16). Maximum weekly average recovery rate for phosphorus was 188 % and minimum 0 %, while in the most weeks phosphorous recovery ranged from 16 to 100 %. On weeks 3 and 8 the recovery of phosphorus was negative (- 150 %). On week 3 there were also peaks in N and P concentrations in the influent. Less phosphorus was recovered when temperature and biomass production started to decrease. In the same time nitrogen recovery slightly increased, until it decreased towards the end of the cultivation.

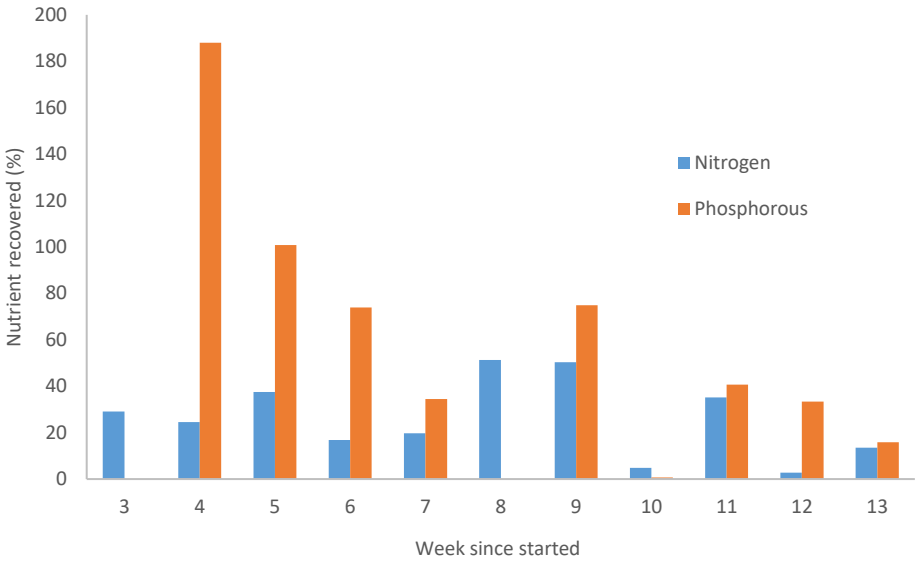


Figure 16. *Recovery rate of nitrogen and phosphorus*

The ratio of nitrogen and phosphorus in influent and effluent is presented in figure 17. N/P ratio in the influent increased from the beginning of the cultivation from 25 up to 75 and started to decrease after 46th day to 17 with the exception on day 39 when the N/P ratio was 7. On that day the nutrient concentrations in the influent differed significantly from those of the other days. N/P ratio in the effluent increased in the beginning from 6 to 52 and varied between 42 and 17 after day 32. Nitrogen recovery was higher when the N/P ratio was lower in effluent (figure 16 and 17). An effect to the phosphorus recovery was the opposite; phosphorus recovery was directly proportional to the N/P ratio in the effluent.

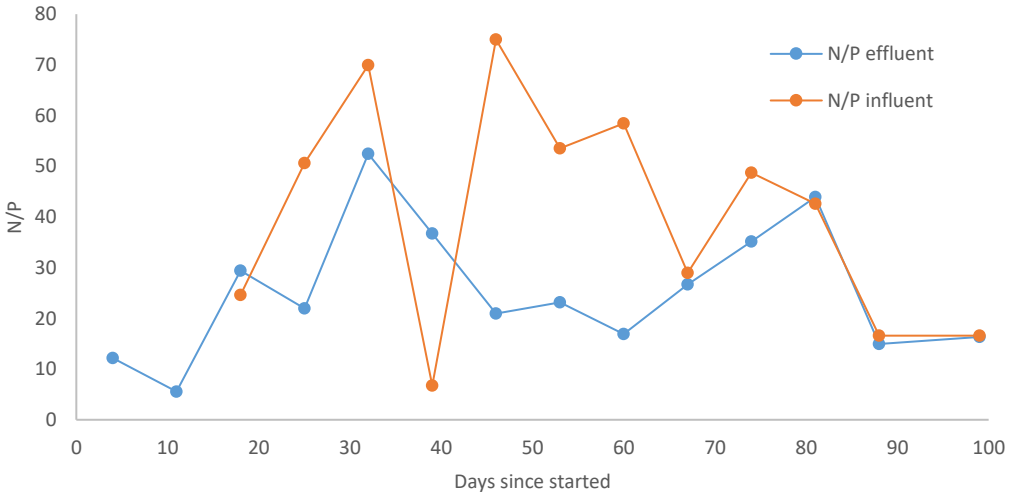


Figure 17. *N/P ratio in influent and effluent (liquid phase) during cultivation*

6. DISCUSSION

6.1 Characteristics of urine and influent

The source-separated urine used in this study was stored for half a year before use. In Granatier (2018) the characteristics of stored urine were similar to this study (N_{tot} 3600 mg/L, P_{tot} 200 mg/L and COD 6300 mg/L). In other studies found in literature (Jönsson et al. 2004, Heinonen-Tanski & Wijk-Sijbesma 2005, Maurer et al. 2006) fresh urine was used in which the nutrient concentrations and COD were higher (N_{tot} 8000 – 10,000 mg/L, P_{tot} 700 – 2000 mg/L and COD 8000 – 10,000 mg/L) than in this study. The differences in the characteristics of the urine were most likely caused by the storing of the urine in this study. During the storing of the urine the pH had increased, and probably phosphorus and solids had precipitated and some of the nitrogen had volatilized.

There was some variation in the characteristics of the influent during the study. The mass of nutrients in the influent was most diluted after filling the tank and most concentrated when the liquid content was low (< 200 L). The influent was pumped from the bottom of the tank and there was no mixing in the tank, which may have resulted in precipitation on the bottom of the influent tank. Therefore, precipitation had probably occurred also already in the urine tank. Mixing in both urine and influent tank would keep the influent quality constant. On day 39 the influent was very rigid, and samples were taken when the liquid volume was at its lowest, so the sample was from the bottom of the container. That could explain the higher concentrations of the nutrients compared to the other days. Filling of the influent tank did not seem to have affected the concentrations of phosphate and P_{tot} in the influent as apparently as to ammonium and N_{tot} concentrations.

6.2 Cultivation of *Scenedesmus acuminatus*

In this study microalgae was cultivated in raceway pond with source-separated urine and was for the first time fed continuously. Several studies have reported the efficiency of microalgae to recover nutrients (Cuellar-Bermudez et al. 2017), but just a few in pilot-scale (Adamsson, 2000; Posadas et al., 2015; Chatterjee et al. 2019) and all these studies have used batch or semi-continuous systems. The results of this study indicate that cultivation of microalgae in open ponds continuously fed by diluted source-separated urine and nutrient recovery is possible also in Nordic climate (temperature 2.2 – 30 °C) and light (light intensity 35 – 900 $\mu mol(ms^2)^{-1}$ forenoon) conditions. The pond was operated for 100 days from July to October in 2018 and day length varied from 7 to 20 hours. The pond was operated with HRT of approximately 23 d. The average nutrient loading rates were 0.034 kgN/m^3d (17 – 65 kgN/m^3d) and 0.87 $\cdot 10^{-3}$ kgP/m^3d ($0.34 \cdot 10^{-3}$ – $1.2 \cdot 10^{-3}$ kgP/m^3d).

There was variation in growth parameters and nutrients during the study and several factors might have affected them, such as temperature, light intensity, nutrient concentrations, trace elements and CO_2. Temperature, pH and growth parameters of microalgae (OD, COD_{tot}, TSS, VSS) were followed during the cultivation. OD of the culture reached the highest value of 1.4 during the cultivation. That was lower compared to previous studies (OD > 7) where *S. acuminates* has been cultivated in laboratory and in open ponds (Tao et al. 2017, Granatier 2018). That could be because of mycelium, which caused flocculation of the culture. Mycelium could have affected also the growth of the microalgae and thereby the nutrient recovery; microalgae cells could have a mutual partnership with other organisms (Barsanti & Gualtieri 2014). pH of the culture dropped below optimal value of 8 for *S. acuminatus* (Ren et al. 2013) after temperature decreased below 20°C. In addition, OD of the culture dropped below 1.2 and VSS below 0.7. However, *Scenedesmus* species can grow in a large pH scale (4 – 11) with the same microalgal biomass production compared to in optimal pH and has strong tolerance for pH changes (Ren et al. 2013). From the microscopic picture it was seen that the OD of the culture did not increase like in the laboratory conditions likely because of flocculation. The mycelium was also seen in the culture samples of flocculating biomass. Ammonium concentration likely did not affect the biomass production significantly even though ammonia is toxic to microalgae cells in high concentrations (Konig et al. 1987). The microalgal class in which *S. acuminatus* is included can have a high tolerance for ammonia (up to 1,400 mg NH_4^+-N /L) (Tuantet et al. 2014a, Collos and Harrison 2014).

Temperature and light intensity could have been important parameters that affected the microalgal growth. During the study the measured growth parameters started to decrease when the temperature decreased below 20°C. Decreased temperature, shortened day light and cloudy weather during this study could have affected the growth kinetics and weakened the biological functions of the microalgae and therefore decreased the biomass production and nutrient recovery. Parmar et al. (2011), Wahidin et al. (2013) and Iasimone et al. (2018) have reported that the light intensity and photoperiod (light and dark) cycles are the prime factors that determine the growth rate of microalgae cultivation. The biomass production efficiency is an important factor, because nutrient removal efficiency is directly related to algae growth (Hussain et al. 2017). Granatier (2017) also concluded in a similar study that the temperature and light had significant effects on microalgal growth in cultivation of *S. acuminatus* in raceway ponds. Xin et al. (2011) studied in batch cultures the growth of microalgae *Scenedesmus* sp. LX1 under different cultivation temperatures and they concluded that the microalgae could grow well between temperatures 10 and 30 °C. However, based on studies of growth of different microalgae species (Chen et al. 2008, Converti et al. 2009, Xin et al. 2011, Cassidy 2011) the influence of cultivation temperature is species-dependent. In Granatier (2018) *S. acuminatus* was cultivated in 15 and 20 times diluted urine as a batch and semi-continuous system and the growth efficiency was decreased and stayed constant when ambient temperature decreased under 20

°C. Christov et al. (2001) found that when cultivating *Scenedesmus* sp. at lower temperatures the chlorophyll and protein levels were reduced, while levels of carotenoids, saccharides, and lipid were increased, when microalgae was grown between 15 and 36 °C. The optimal temperature for *Scenedesmus* sp. is 20 – 40 °C when grown in photobioreactors (Sánchez et al. 2008). Cassidy (2011) determined the optimal growth temperature for *Scenedesmus* sp. to be 30 °C, when it was grown in M-8 medium and urea.

In this study CO_2 was not added during the run, so CO_2 could have been a limiting factor for microalgae growth. CO_2 is essential for microalgae growth and CO_2 aeration with certain concentration can improve the efficiency of biomass growth and nutrient uptake of microalgae (Yao et al. 2015, Hussain et al. 2017). In this study trace elements were added two times at the end of the cultivation to improve the growth of microalgae, but they did not influence the growth. Scarcity of trace elements could be improbable, because urine contains all the micronutrients required for growth of microalgae (Jönsson et al. 2004). Jaatinen et al. (2016) found that dilution of urine and thus decreased trace element concentrations did not affect overall microalgal biomass growth. However, weakened microalgae growth in diluted urine without additional trace elements has been reported (Tuantet et al. 2014a, Tuantet et al. 2014b).

Further development of microalgal technologies for cultivation has various options. Using more battle wheels in the pond would decrease the flocculation and precipitation of microalgae. Cultivation could be conducted in closed system, such as photobioreactor, instead of open raceway pond. In photobioreactors cultivation conditions can be controlled effectively and less area would be needed in vertical cultivation (Nwoba et al. 2016). In addition, the effect of cultivation conditions on microalgae growth could be studied by controlling for example CO_2 or temperature.

6.3 Nutrient recovery

Phosphorus was mainly recovered by microalgae cells (weekly average 36 %). However, part of the phosphorus can be present as a precipitate on the algae cells or on the bottom of the pond. In high pH (< 8.5) phosphorus precipitates for example as struvite (Chang et al. 2013), so part of the phosphorus present in urine had likely precipitated already in urine tank during storage. There are several factors that might have affected phosphorus recovery rate during the run. In the beginning of the cultivation, in higher temperatures and pHs, phosphorus recovery was higher than 100 %. That might be the result of phosphate precipitation, not only uptake by microalgal cells. Since the ammonium is volatilized in high pH, hardly any struvite is formed at higher pHs (Hao et al. 2008). However, other compounds (Ca_3PO_4, $CaHPO_4$, Mg_3PO_4, etc.) can be formed at the existence of Ca and Mg at alkaline pHs, when $H_2PO_4^-$ transforms into HPO_4^{2-} and PO_4^{3-} (Hao et al. 2008, Chang et al. 2013). On weeks 3 and 8 the recovery of phosphorus was negative, which can be due to solubilisation of precipitates containing phosphorus due to changing pH

(Chang et al. 2013) or phosphorus and nitrogen release from dying algal cells (Jaatinen et al. 2016). The same assumption of phosphorus solubilisation and release was done in Chatterjee et al. (2019) in the case of decrease of phosphorus uptake.

On average 26 % (2.7 – 51 %) of total nitrogen entered was captured by microalgal cells and 46 % (0 – 100 %) of total nitrogen entered was volatilized. The nutrient recovery rate did not include the nitrogen volatilized as ammonium. Therefore, evaluating water treatment efficiency (including ammonia volatilization) of the run on average 72 % of the total nitrogen was removed. Thus, only nitrogen recovered by microalgal cells can be utilized, if the ammonia is not collected in the run. Ammonium is volatilized as ammonia in high pH (> 8.5) (Madigan et al. 2017) and more ammonia is volatilized in higher temperatures (Lei et al. 2017), which could be seen as a reduction in volatilization since temperature and pH started to decrease around week 7. In warm weather ammonia can be volatilized from the algae ponds even at lower pHs (< 9) (Garcia et al. 2000). Nitrogen reduction might have also happened during storage, because ammonium can precipitate with phosphate as struvite (Chang et al. 2013). Around 30 % of the nitrogen in the influent was present as ammonium, which is available for microalgae (Barsanti & Gualtieri 2006).

Only a few pilot scale studies have been conducted on nutrient recovery by microalgae (Table 6). In Adamsson (2002) *S. acuminatus* was grown in 50 times diluted urine in 130 L tanks as semi-continuous operation in greenhouse. The highest microalgal yield resulted was 287 mg of dry weight/L in 12 weeks. During the cultivation the average recovery rate of total nitrogen was 67.1%, and for phosphate-P 36.2%. Nitrogen removal rate was much higher compared to this study (on average 26 %). However, nutrient concentrations were also lower in this study (N < 40 mg/L, P < 10 mg/L and on average N 270 mg/L and P 18 mg/L). The study was conducted between May and September and the temperature of biomass varied between 10 and 20 °C. The biomass was harvested three times a week (10 L) and replaced with diluted urine. Chatterjee et al. (2019) conducted a comparable study to this study as a batch and semi-continuous operation with similar experimental design from the middle of July to the middle of October. VSS of the *S. acuminatus* culture varied between 0.2 and 0.5 g/L when fed with 15 times diluted urine and nutrients were recovered effectively. Compared to this study total nitrogen and phosphorus was removed more effectively (38 % of phosphorus, 52 % of TN) when fed with 20 times diluted urine. With 15 times dilution the recovery rates were 13 and 22 %, respectively. The culture was harvested two times per week with semi-continuous feeding. However, the ambient temperature decreased below 15 °C during the operation with 15 times dilution. In addition, pH dropped below 9, since it had fluctuated between 8.5 and 11 and OD of the culture decreased below 1. Posadas (2015) conducted a study where *Scenedesmus* sp. was cultivated in outdoor raceway ponds (700, 800, 850 L) in Spain to study biomass production and nutrient recovery from secondary domestic wastewater. Without pH control and CO_2 addition removal in total nitrogen of 97 ± 0%, 98 ± 2% and

97 ± 0% and in total phosphate of 62 ± 1, 61 ± 1 and 56 ± 1% were achieved with maximum TSS of 397 ± 8, 321 ± 30 and 350 ± 27 mg/L, respectively. In addition, in this study the biomass production decreased towards the end of the cultivation, which was not the case in other studies in same scale. The daily ambient temperature varied approximately between 8 and 18 °C (December) and pH around 8 – 9 during the cultivation.

Table 6. *Summary of the results of nutrient recovery from urine by microalgae in pilot-scale studies*

Process type and scale	Feed	Microalgae specie	Maximum biomass yield	Average nutrient recovery	Reference
Continuous raceway pond 2000 L	1:15 diluted urine	*S. acuminatus*	TSS 1.1 g/L	N_{tot} 26 % P_{tot} 36 %	This study
Batch Tank 130 L	2 % diluted urine	S. *acuminatus*	287 mg of dry weight/L	N_{tot} 67.1 % P_{tot} 36.2 %	Adamsson 2002
Semi-continuous raceway ponds 700, 800, 650 L	domestic wastewater	*Scenedesmus sp.*	TSS 397 ± 8, 321 ± 30, 350 ± 27 mg/L	N_{tot} 97 ± 0%, 98 ± 2%, 97 ± 0% P_{tot} 62 ± 1%, 61 ± 1%, 56 ± 1%	Posadas 2015
Semi-continuous and batch raceway ponds 2000 L	1:15 and 1:20 diluted urine	*S. acuminatus*	VSS 0.2 – 0.5 g/L	N_{tot} 52 % P_{tot} 38 %	Chatterjee et al. 2019

In this study N/P ratio varied in the effluent between 6 and 52 and both nutrients were removed more efficiently with higher influent N/P ratio values (N/P > 40). Xin et al. (2010) studied the effect of different nitrogen and phosphorus concentrations on microalgae growth and nutrient uptake with *Scenedesmus* sp. LX1. The initial nutrient concentrations were much lower in Xin et al. (2001) (N_{tot} 2.5 – 25 mg/L, P_{tot} 0.1 – 2.0 mg/L) compared to this study (on average 270 and 18 mg/L, respectively) and therefore the results are not comparable. In addition, the cultivation was conducted under controlled conditions in artificial climate chamber. However, the N/P ratio was also analysed and almost 100% phosphorus was removed at any N/P ratios in the conditions of their research, but the nitrogen removal was obviously influenced by the N/P ratios (Xin et al. 2010). The optimal N/P ratio for nutrient recovery was found to be between 5 and 8. Xin et al. (2010) also concluded that *Scenedesmus* sp. LX1 has the ability to over-uptake nitrogen or phosphorus, when the N/P ratio in growth medium does not match the normal elementary composition of microalgal cells, but only at the optimum N/P ratio nitrogen and phosphorus could both be efficiently removed. Kapdan and Aslan (2008) found the optimum N/P

ratio of 8 for *Chlorella vulgaris* and Rhee (1978) reported that Scenedesmus sp. required an N/P ratio of approximately 13.5 to grow without limitations. So, the optimum N/P ratio depends on the microalgae species and growth conditions (Xin et al. 2010). Beuckels et al. (2015), Whitton et al. (2016) and Bryant and Appah (2017) also found that more phosphorus than nitrogen is removed from diluted human urine by *Scenedesmus obtusiusculus* and *Scenedesmus obliquus* and that it is inversely proportional with N/P ratio of the biomass and light intensity.

7. CONCLUSIONS

Based on this study, it can be concluded that microalgae can be used to recover nutrients from diluted source-separated human urine in open raceway ponds in Nordic climate (temperature 2.2 – 30 °C) and light conditions without addition of trace elements, artificial light or CO_2. In this study the microalgae *Scenedesmus acuminatus* was cultivated in 15 times diluted urine in continuously fed outdoor raceway pond. The pond was operated for 100 days from July to October in 2018 and day length varied from 7 to 20 hours. The pond was operated with HRT of approximately 23 d. The average nutrient loading rates were 0.034 kgN/m^{3}d (17 – 65 kgN/m^{3}d) and 0.87 $\cdot 10^{-3}$ kgP/m^{3}d ($0.34 \cdot 10^{-3}$ – $1.2 \cdot 10^{-3}$ kgP/m^{3}d).

Highest biomass production (OD 1.4 and TSS 1.1 g/L) was achieved after 32 days of cultivation. Efficiency of microalgal growth started to decrease around day 50 when the temperature decreased below 25 °C (pH < 8.5). The mycelium was also seen in the culture samples of flocculating biomass.

Around 30 % of the nitrogen in the influent was present as ammonium. On average 26 % (2.7 – 51 %) of total nitrogen entered was captured by microalgal cells and 46 % (0 – 100 %) of total nitrogen entered was volatilized. Nutrient concentrations and changes in temperature and pH probably mostly affected to the nitrogen recovery efficiency.

On average 36 % (0 – 100 %) of the phosphorus entered was captured by microalgae or precipitated. Part of the phosphorus could be present as a precipitate on the algae cells or on the bottom of the pond. Changes in phosphorus recovery were probably result of cultivation conditions (e.g. temperature, light intensity, CO_2), nutrient concentrations, solubilisation of precipitates containing phosphorus and the release of phosphorus and nitrogen from dying algal cells. N/P ratio varied in the effluent between 6 and 52 and both nutrients were removed more efficiently with higher influent N/P ratio values (N/P > 40).

Further studies are required, and process optimization is needed to enable more efficient nutrient recovery. Biomass production and nutrient recovery could be improved by improving experimental design of the cultivation in raceway pond. Further development of microalgal technologies for cultivation has various options. Using more battle wheels in the pond would decrease the flocculation and precipitation of microalgae. Cultivation could be conducted in closed system, such as photobioreactor, instead of open raceway pond. In addition, the effect of cultivation conditions on microalgae growth could be studied by controlling for example CO_2.

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