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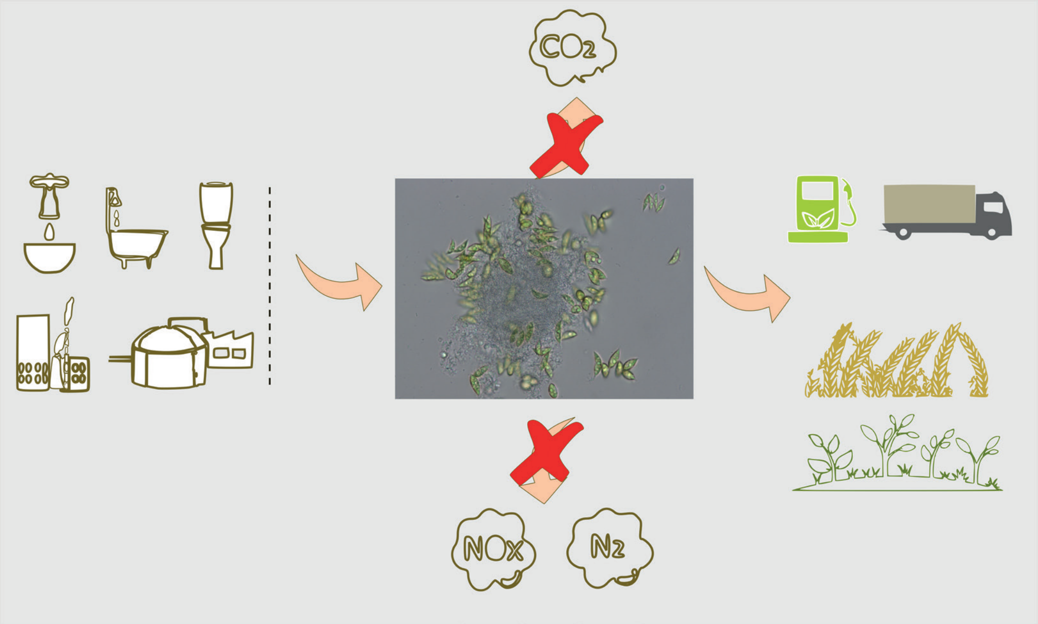
A passage to wastewater nutrient recovery units

Microalgal-Bacterial bioreactors

Anbarasan Anbalagan



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A PASSAGE TO WASTEWATER NUTRIENT RECOVERY UNITS
MICROALGAL-BACTERIAL BIOREACTORS

Anbarasan Anbalagan

Akademisk avhandling

som för avläggande av teknologie doktorsexamen i energi- och miljöteknik vid
Akademin för ekonomi, samhälle och teknik kommer att offentligen försvaras
tisdagen den 19 juni 2018, 09.00 i Delta, Mälardalens högskola, Västerås.

Fakultetsopponent: Professor Francisco Gabriel Acién Fernández, University of Almería

MÄLARDALEN UNIVERSITY
SWEDEN

Akademin för ekonomi, samhälle och teknik

Abstract

In recent years, the microalgal–bacterial process has been considered to be a very attractive engineering solution for wastewater treatment. However, it has not been widely studied in the context of conventional wastewater treatment design under Swedish conditions. The technology holds several advantages: as a CO_2 sink, ability to withstand cold conditions, ability to grow under low light, fast settling without chemical precipitation, and reducing the loss of valuable nutrients (CO_2, N_2, N_2O, PO_4). The process also provides the option to be operated either as mainstream (treatment of municipal wastewater) or side stream (treatment of centrate from anaerobic digesters) to reduce the nutrient load of the wastewater. Furthermore, the application is not only limited to wastewater treatment; the biomass can be used to synthesise platform chemicals or biofuels and can be followed by recovery of ammonium and phosphate for use in agriculture.

In the present study, the feasibility of applying the process in Swedish temperature and light conditions was investigated by implementing microalgae within the activated sludge process. In this context, the supporting operational and performance indicators (hydraulic retention time (HRT), sludge retention time (SRT) and nutrients removal) were evaluated to support naturally occurring consortia in photo-sequencing and continuous bioreactor configuration. Furthermore, CO_2 uptake and light spectrum-mediated nutrient removal were investigated to reduce the impact on climate and the technical challenges associated with this type of system.

The results identified effective retention times of 6 and 4 days (HRT = SRT) under limited lighting to reduce the electrical consumption. From the perspective of nitrogen removal, the process demands effective CO_2 input either in the mainstream or side stream treatment. The incorporation of a vertical absorption column demonstrated effective CO_2 mass transfer to support efficient nitrogen and phosphorus removal as a side stream treatment. However, the investigation of a continuous single-stage process as the mainstream showed a requirement for a lower SRT in comparison to semi-continuous operation due to faster settlability, regardless of inorganic carbon. Furthermore, the process showed an effective reduction of influent phosphorus and organic compounds (i.e. COD/TOC) load in the wastewater as a result of photosynthetic aeration. Most importantly, the operation was stable at the temperature equivalent of wastewater (12 and 13 ˚C), under different lighting (white, and red-blue wavelengths) and retention times (6 and 1.5 d HRT) with complete nitrification. Additionally, the biomass production was stable with faster settling properties without any physiochemical separation.

The outcomes of this thesis on microalgal–bacterial nutrient removal demonstrates that (1) photosynthesis-based aeration at existing wastewater conditions under photo-sequential and continuous photobioreactor setup, (2) flocs with rapid settling characteristics at all studied retention times, (3) the possibility of increasing carbon supplementation to achieve higher carbon to nitrogen balance in the photobioreactor, and (4) most importantly, nitrification-based microalgal biomass uptake occurred at all spectral distributions, lower photosynthetic active radiation and existing wastewater conditions.

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To my beloved mother, Saroja (Late)

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Västerås, Sweden, in May 2018
Anbarasan Anbalagan

Summary

We human beings are leading our lives seemingly as cool as a cucumber at the expense of clean freshwater and energy from fossil fuels. Our households generate wastewater rich in carbon, nitrogen and phosphorus (e.g. from lipids, proteins and carbohydrates, faeces, detergents). Wastewater treatment plants must therefore reduce the amount of nutrients in the wastewater to avoid water pollution. However, presently, crop nutrients are lost either as chemical sludge or as greenhouse and dinitrogen gases during the wastewater treatment.

Have you ever considered that the air that we breathe is a waste product of tiny naturally occurring organisms called microalgae, which live in lakes, rivers and oceans? Like plants, these creatures take up greenhouse gases and nutrients and release oxygen, increasing the oxygen content of their aquatic environment. A conventional wastewater treatment plant aerates wastewater mechanically so that bacteria can degrade the organic compounds in the water. The aim of this thesis is to consider the use of microalgae–bacteria symbiosis together in a wastewater treatment plant to reduce greenhouse gases and wastewater nutrients, to recycle and to recover crop nutrients from wastewater to the agricultural field.

Is it practical to utilise lab scale cultivation processes in the treatment facility conditions? Initially, I tried to cultivate the microalgae–bacteria combination in a wastewater tank at 2, 4 and 6 day intervals. Under these laboratory conditions, treatment times of 4 and 6 days were found to be effective for treating raw wastewater. However, the algal–bacterial nutrient removal process was effective only in the presence of added external phosphorus in treatment facility conditions.

Moreover, is it possible to utilise this process to remove CO_2 from industrial waste gases? A cultivation tank connected to a vertical tubular column with a waste gas supply was considered. The cultivated liquid was used to absorb the carbon dioxide in the column (similar to forcing CO_2 to dissolve in a soda stream) but by varying the liquid recirculation. As a result, there was almost complete nutrient removal at higher liquid recirculation

under laboratory conditions. Thus, waste gas rich in CO_2 can be utilised during the cultivation process.

Furthermore, is it possible to cultivate algae–bacteria in the low temperatures (~13˚C) and limited lighting conditions in Swedish wastewater plants? Longer (6 days) and shorter (1.5 days) treatment times showed that cultivation was stable in these conditions. However, the cultivated biomass showed higher aging of microalga–bacteria (time spent by microalgae–bacteria in the tank before removal) due to the rapid settling property. Thus, the sludge age influenced the removal of wastewater nutrients under lower lighting conditions.

Findings from this work suggest that identification of effective sludge age and treatment time can increase the nutrient removal capacity during wastewater treatment. In doing so, the treatment can be adjusted to increase nutrient removal and most importantly, the crop nutrients can be recovered alongside greenhouse gas capture and avoiding emission of greenhouse gases during wastewater treatment.

Swedish summary

Vi människor lever våra liv på bekostnad av rent sötvatten och energi från fossila bränslen. Hushållen generar avloppsvatten som är rikt på kol, kväve och fosfor (lipider, proteiner, kolhydrater, tvättmedel och rengöringsmedel).

Avloppsreningsverken behöver därför minska koncentrationen av näringsämnen i avloppsvattnet för att undvika vattenförorening. Växtnäringsämnena förloras emellertid antingen som kemiskt slam eller växthusgaser och kvävgas under avloppsreningen.

Har du någonsin funderat på att den luft som vi andas är en biprodukt från naturligt förekommande små varelser i sjöar, floder och hav, som kallas mikroalger? De tar upp växthusgaser med växtnäringsämnen och släpper ut syre, precis som växter gör. Det konventionella avloppsreningsverket luftar avloppsvattnet mekaniskt för att bryta ner organiska föreningar med hjälp av bakterier. I det sammanhanget eftersträvar jag att utnyttja en symbios mellan mikroalger och bakterier för att reducera växthusgaser och näringsämnen i avloppsvatten, för att återföra och ta till vara växtnäringsämnen från avloppsvatten på jordbruksmark.

Är det praktiskt möjligt att i fullskala använda samma tillväxtprocess som används i labbmiljö? Till en början undersökte jag att odla en blandning av mikoalger och bakterier i tankar med avloppsvatten med ett tidsintervall på 2, 4 och 6 dagar. Den mest effektiva behandlingen var att använda 4 eller 6 dagar vid odling i obehandlat avloppsvatten i laboratoriemiljö. Avskiljningen av näringsämnen var dock bara effektiv när extern fosfor fanns tillgängligt, utöver det fosfor som i normala fall finns i inkommande avloppsvatten till reningsverket.

Dessutom, är det möjlighet att använda den här processen för att kunna avskilja koldioxid från rökgas från industrier? En odlingstank med tillförsel av gas, utformad som en vertikal tubformad kolumn, studerades. Vätska från odlingen användes för att absorbera koldioxiden i kolumnen (liknande tillsats av ren koldioxid i en kolsyremaskin) men med variation av mängden vätska

som återcirkulerades. På så sätt kan avgaser rik på koldioxid nyttiggöras i odlingsprocessen.

Vidare, är det möjligt att odla en blandning av alger och bakterier vid den temperatur avloppsvattnet har i Sverige (~13˚C) och begränsad ljustillgång? Både längre (6 dagar) och kortare (1,5 dagar) behandlingstid visade att odlingen var stabil under dessa förhållanden. Dock åldrades den odlade biomassan mer, eller fick högre ”slamålder” (tiden som blandningen av mikroalger och bakterier stannar i tanken innan de avskiljs) på grund av snabb sedimentering i tanken. ”Slamåldern” påverkade alltså avskiljningen av näringsämnen från avloppsvattnet vid lägre ljustillgång.

Resultaten av denna studie indikerar att identifieringen av en effektiv slamålder kan öka kapaciteten för reduktionen av näringsämnen i tanken. Genom detta kan behandlingen anpassas för högre avskiljning av näringsämnen och, viktigast av allt, näringsämnen kan återföras samtidigt som växthusgaser fångas in, utan några utsläpp av växthusgaser under rening av avloppsvatten.

Tamil summary

மனிதர்களாகிய, நாம், வெளித்தோற்றத்தில் வெள்ளரிக்காய் போன்று குளுமையான வாழ்க்கையயை சுத்தமான தண்ணிர் மற்றும் புதைப்படிவ எரிபொருட்கள் (fossil fuels) மூலம் கிடைக்கும் சக்தியினால் வாழ்க்கயை அனுபவித்து வருகின்றோம். வீட்டில் இருந்து வெளியேற்றப்படும் கழிவு நீரில் (wastewater), கார்பன் (carbon), நைட்ரஜன் (nitrogen), மற்றும் பாஸ்பரஸ் (phosphorus) மிக அதிக அளவில் உள்ளது (எடுத்துக்காட்டாக, கொழுப்பு (lipids) , புரதம் (proteins) , கார்-போ-ஹைட்ரேட்ஸ (carbohydrates) போன்றவை மலம் மற்றும் டிட்டர்ஜென்டுக்கள் (detergents) மூலமாக பெறப்படுகின்றன. இதனால், நீர் மாசுப்படுவதை தவிர்க்க நீர் சுத்திகரிப்பு நிலையங்களில் (wastewater treatment plant) கழிவு நீரில் உள்ள செறிஊட்டப்பட்ட ஊட்டச்சத்துகளின் அளவைக் குறைக்க வேண்டும். ஆனால், கழிவு நீர் சுத்திகரிக்கப்படும் அதே வேலையில் தாவரங்களுக்கு தேவையான ஊட்டச்சத்துகள் கசடுகளாகவோ அல்லது வாய்வுவாகவோ இழக்கப்படுகின்றன.

நீங்கள் எப்போதாவது எண்ணியது உண்டா? நாம் சுவாசிக்கும் காற்றானது, ஏரி, ஆறு மற்றும் சமுத்திரத்தில் வாழும் நுண்ணுயிர்பாசியின் (microalgae) கழிவுகளாகும். இவை தாவரங்களைப் போல், பசுமையில்லா வாயுக்களை (green house gases) தாவர ஊட்டச்சத்துக்களுடன் சேர்த்து எடுத்துக்கொண்டு ஆக்சிஜன் வெளியிட்டு, நீர் சூழலில் ஆக்சிஜனின் (oxygen) அளவை உயர்த்துகின்றன. வழக்கமாக கழிவுநீர் சுத்தகரிப்பு நிலையங்களின் கரிமங்களை பாக்டீரியா சிதைப்பதர்காக இயந்திரத்தின் உதவியால் காற்றுபடும் படி வைப்பார்கள். இந்த ஆய்வறிக்கையின் நோக்கம் என்னவென்றால் நுண்ணுயிர்பாசி–பாக்டீரியாவின் (microalgae–bacteria) கூடி வாழ்வியலை (symbiosis) கழிவுநீர் சுத்தகரிப்பு வலையில் சேர்த்து கொண்டு பசுமையில்லா வாய்வுகள் மற்றும் கழிவுநீரில் உள்ள ஊட்டச்சத்துகளின் வெளியேற்றத்தை குறைத்து, கழிவுநீர் உள்ள தாவர ஊட்டச்சத்துகளை மீட்டு எடுத்து, அதை மறுசுழற்சி செய்து விவசாயத்துற்கு பயன்படுத்துவதாகும்.

ஆய்வுக்கூடத்தில் சாகுபடி செய்யும் முறை நடைமுறையில் சுத்திகரிப்பு நிலையங்களில் பயன்படுத்த முடியுமா? ஆரம்பத்தில் நான் நுண்ணுயிர்–பாசி பாக்டீரியத்தின் சேர்க்கையை கழிவுநீர் தொட்டியில் 2, 4 மற்றும் 6 நாட்கள் இடைவெளியில் சாகுபடி செய்ய முயற்ச்சித்தேன். இந்த ஆய்வுக்கூட சோதனையில் 4 மற்றும் 6 நாட்கள் சாகுபடி முறை கழிவுநீர் சுத்தகரிப்புக்கு

உகந்தாக இருந்தது. ஆனால்இ நுண்ணுயிர்பாசி–பாக்டீரியத்தின் ஊட்டச்சத்து பிரித்தெடுக்கும் செயல் முறையானது வெளியில் இருந்து பாஸ்பரஸ்ஸை சேர்த்த போதுதான் திறன்பட இருந்தது.

மேலும், இந்த செயல்முறையை பயன்படுத்தி தொழிற்சாலையில் இருந்து வெளியேற்றப்படும் கழிவு வாய்வுகளில் (waste gases) இருந்து கார்பன்-டை-ஆக்ஸைடை (carbon dioxide) நீக்குவதர்க்கும் பயன்படுத்த முடியுமா? ஒரு சாகுபடி தொட்டியை (tank) கழிவு வாயுக்கள் உள்ள செங்குத்தான குழாய் பாத்தியுடன் (vertical tubular column) இணைக்கப்பட்டது. சாகுபடி செய்யப்பட்ட திரவமானது (cultivated liquid) பாத்தியில் உள்ள கார்பன்-டை-ஆக்ஸைடை உறிஞ்சுவதற்கு பயன்படுத்தப்படுகின்றது (சோடா தயாரிக்க கார்பன்-டை-ஆக்ஸைடை உட்செலுத்துவது போல்) ஆனால் இங்கு திரவத்தின் மறு சுழற்ச்சியின் (liquid recirculation) மூலம் செய்யப்படுகிறது. இதன் விளைவாக கிட்டத்தட்ட ஊட்டச்சத்துகள் முழுவதும் திரவ மறுசுழற்சியை அதிகரிக்கப்படுவதன் மூலம் நீக்கப்பட்டது. இதனால் கார்பன்-டை-ஆக்ஸைடு அதிகம் உள்ள கழிவு வாயு நுண்ணுயிர்பாசி சாகுபடி செய்யும் முறைக்குப் பயன்படுத்தலாம்.

மேலும், இந்த நுண்ணுயிர்பாசி–பாக்டீரியா சாகுபடி முறையை ஸ்வீடனில் நிலவும் குறைந்த வெப்பநிலை (13˚C) மற்றும் வெளிச்சத்தில் இயங்கும் சுத்திகரிப்பு நிலையங்களில் பயன்படுத்த முடியுமா? நீண்ட (6 நாட்கள்) மற்றும் குறுகிய (1.5 நாட்கள்) செய்முறை நேரத்தில், சாகுபடியானது நிலைப்புத்தன்மை வாய்ந்ததாக இருந்தது. ஆனால் விரைவாக படியும் தன்மையுடைய கசடுகளினால் சாகுப்படி செய்யப்பட்ட உயிர் தொகுப்புப்பில் (biomass) வயது முதிர்ந்த நுண்ணுயிர்பாசி–பாக்டீரியாக்கள் காணப்பட்டன. எனவே வயதான கசடுகள் (aged sludge) ஊட்டச்சத்துக்கள் பிரித்தெடுப்பதை குறைந்த வெளிச்சத்தில் பாதிக்கின்றன.

இந்த ஆய்வு பரிந்துரைப்பது என்னவென்றால் கசடுகளின் வயது மற்றும் செய்முறை நேரத்தை திறன்பட அறிவதன் மூலம் கழிவுநீரில் இருந்து ஊட்டச்சத்துகளை பிரித்தெடுக்கும் திறன் முனணேற்றப்படும். இப்படி செய்வதன் மூலம் கழிவுநீர் சுத்திகரிப்பு செயல்முறையில் மாற்றம் செய்யப்பட்டு ஊட்டச்சத்துகள் பிரித்தெடுப்பது அதிகரிக்கப்படும். முக்கியமாக தாவர ஊட்டச்சத்துக்கள் மீட்டெடுக்கப்படும், பசுமையில்லா வாயுக்களை பிடித்து கொள்வதன் மூலம் அவற்றின் வெளியேற்றம் தடுக்கப்படும்.

List of papers

Publications included in the thesis

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Anbalagan** A, Schwede S, Lindberg CF, Nehrenheim E, 2016. Influence of hydraulic retention time on indigenous microalgae and activated sludge process. Water research 91, 277–284.

II. **Anbalagan A**, Schwede S, Lindberg CF, Nehrenheim E, 2017. Influence of iron precipitated condition and light intensity on microalgae activated sludge based wastewater remediation. Chemosphere 168, 1523–1530.

III. **Anbalagan A**, Cervantes AT, Posadas E, Rojo E, Lebrero R, González-Sánchez A, Nehrenheim E, Muñoz R, 2017. Continuous photosynthetic abatement of CO_2 and volatile organic compounds from exhaust gas coupled to wastewater treatment: Evaluation of tubular algal-bacterial photobioreactor. Journal of CO_2 Utilization 21, 353–359.

IV. **Anbalagan A**, Castro CJ, Schwede S, Lindberg CF, Nehrenheim E, Butler C, 2018. Influence of environmental stresses on microalgal-bacterial process during nitrogen removal**.** Manuscript.

V. **Anbalagan A**, Schwede S, Lindberg CF, Nehrenheim E, 2017. Continuous microalgae-activated sludge flocs for remediation of municipal wastewater under low temperature**.** 1st IWA Conference on Algal Technologies for Wastewater Treatment and Resource Recovery, UNESCO-IHE, Delft, Netherlands.

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My contribution to the papers

I. I designed, performed and evaluated the experimental work at Mälardalen University with the support of Mälarenergi AB. I wrote the manuscript in cooperation with the co-authors and acted as the corresponding author.

II. I designed, performed and evaluated the experimental work at Mälardalen University based on pilot scale operation at Mälarenergi AB. I wrote the manuscript in coordination with the co-authors and acted as the corresponding author.

III. I constructed, performed, and evaluated most of the experimental work. I wrote the manuscript in coordination with co-authors. Initial material design was obtained from Esther Posadas Olmos. Elena Rojo, a Masters student, performed the end stage of the experimental work under my supervision due to time constraints of my research stay. This work was carried out at the University of Valladolid and the cultivation and the centrate supplementation was considered based on the proposal from the International Water Association short term scientific mission submitted by Emma Nehrenheim, Raul Muñoz and me.

IV. I designed, constructed and performed the experimental work at the University of Massachusetts Amherst. In addition, Cynthia Castro performed part of the nutrient analysis due to equipment-related time constraints. I wrote the manuscript in coordination with co-authors and acted as the corresponding author (under internal revision).

V. I designed, constructed and performed the experimental work at Mälardalen University. I wrote the manuscript in cooperation with the co-authors and acted as the corresponding author.

All the co-authors read and approved original document of the published manuscript prior to submission.

Publications not included in the thesis

The author has also contributed to the following related publications, which are not included in the thesis:

I. **Anbalagan A**, Schwede S, Nehrenheim E, 2015. Influence of light emitting diodes on indigenous microalgae cultivation in municipal wastewater. Energy Procedia 75, 786−792, Abu Dhabi, United Arab Emirates.

II. Punzi M, **Anbalagan A**, Börner RA, Svensson BM, Jonstrup Mattiasson B, 2015. Degradation of a textile azo dye using biological treatment followed by photo−Fenton oxidation: evaluation of toxicity and microbial community structure. Chemical Engineering Journal 270, 290−299.

III. Punzi M, Nilsson F, **Anbalagan A**, Svensson BM, Jönsson K, Jonstrup M, Mattiasson B, 2015. Combined anaerobic−ozonation process for treatment of textile wastewater: evaluation of acute toxicity and mutagenicity removal. Journal of Hazardous Materials 292, 52−60.

IV. Schwede S, **Anbalagan A,** Krustok I, Lindberg CF, Nehrenheim E, 2016. Evaluation of the microalgae-based activated sludge (MAAS) process for municipal wastewater treatment on pilot scale. IWA World Water Congress, Australia.

V. **Anbalagan A**, Cervantes AT, Rojo E, Lebrero R, González-Sánchez A, Nehrenheim E, Muñoz R, 2016. Continuous CO_2 and volatile organic compounds (VOCs) removal in a tubular photo-bioreactor. International Conference on Applied Energy (ICAE) −2016, Beijing, China.

Contents

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Nomenclature

Abbreviations

ADP	Adenosine diphosphate
AS	Activated sludge
ATP	Adenosine triphosphate
APHA	American public health association
BPR	Biogas production rate
C	Carbon
CANON	Complete ammonium oxidation over nitrate
$CaCO_3$	Calcium carbonate
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
e^-	Electron
EPS	Extra polymeric substances
H^+	Hydrogen ion or proton
H_2O	Water
HCO_3^-	Bicarbonate ion
HRAP	High rate algal ponds
HRT	Hydraulic retention time
LED	Light emitting diodes
MAAS	Microalgae activated sludge process
MAB	Microalgae bacteria flocs
N	Nitrogen
N_2	Di-nitrogen
N_2O	Di-nitrogen oxide
NO_{2+3}^-	Sum of nitrite and nitrate
NH_4^+	Ammonium
NADP/NADPH	Nicotinamide adenine diphosphate (oxidised/reduced state)
NU	Nutrient

OB	Operational objectives
O_2	Oxygen
P	Phosphorus
PAR	Photosynthetically active radiation
PO_4-P	Phosphate-phosphorus
PSI	Photosystem I
PSII	Photosystem II
RC	Research challenges
RE	Removal efficiency
RNA	Ribonucleic acid
ROP-I	Rate of photosynthesis to the intensity
SRT	Sludge retention time
TOC	Total organic carbon
TN	Total nitrogen
TP	Total phosphorus
TS	Total solids
TSS	Total suspended solids
USA	United States of America
VS	Volatile solids
VSS	Volatile suspended solids
BPR	Biogas production rate

Symbols

NU_{excess}	$[mg\ L^{-1}]$	Nutrients in the excess sludge flow
BG.Y	$[mL\ g\ VS^{-1}]$	Accumulated biogas yield
NU_{in}	$[mg\ L^{-1}]$	Nutrients in the influent
NU_{out}	$[mg\ L^{-1}]$	Nutrients in the effluent
NU_{rec}	$[mg\ L^{-1}]$	Nutrients in the recirculated/remaining flow
Q_{excess}	$[L\ d^{-1}]$	Excess sludge flow
Q_{in}	$[L\ d^{-1}]$	Influent flow
Q_{out}	$[L\ d^{-1}]$	Effluent flow
Q_{rec}	$[L\ d^{-1}]$	Recirculation flow
T_C	[hrs]	Total cycle time
TSS_L	$[L\ d^{-1}]$	Suspended solids leaving the system
TSS_r	$[mg\ L^{-1}]$	Suspended solids in the reactor
V_L	$[L\ d^{-1}]$	Volume out
V_r	$[L\ d^{-1}]$	Volume of the reactor

1 Introduction

1.1 Background

A centralised wastewater treatment facility is an essential part of a society that harvests, treats and safely disposes of wastewater in natural water bodies. Therefore, all kinds of biological and physiochemical processes during municipal wastewater treatment are bound to sanitary measures for achieving environmental, social and economic sustainability. Based on national objectives set by the Swedish Environmental Protection Agency (SEPA), seven essential objectives can be identified in relation to wastewater treatment plants: 1. reduced climate impact, 2. clean air, 3. natural acidification only, 4. a rich diversity of plant and animal life, 5. a protective ozone layer, 6. flourishing lakes and streams, 7. a non-toxic environment (SEPA, 2012). In this context, it is always a consideration to utilise wastewater nutrients for agricultural purposes. This also reduces traditional nutrient run off from farmland to natural water courses and avoids escape of nutrients into the atmosphere as gases at the wastewater treatment site (Jeyanayagam et al., 2012). Modern wastewater treatment is expensive and highly energy consuming, mostly for external aeration, due to changes in lifestyle of city inhabitants. For instance, high water usage and protein-rich dietary requirements, immigration and housing, etc. Hence, there is always a search for alternatives, since the present technology is a century old and until now has operated with numerous process improvements to support biological processes (Jenkins and Wanner, 2014).

Municipal wastewater is a complex environment in which a wide variety of microorganisms thrives symbiotically as an entangled mass. Pollutants are present in the form of nitrogenous compounds (ammonium from animal and plants origin), phosphorus (phosphates from animal and plant origin), carbon (fats and lipids from animal and plants origin), volatile organic compounds, and other contaminants such as heavy metals and pharmaceuticals (Jenkins and Wanner, 2014; Tchobanoglous et al., 2014). A simplified overview of municipal wastewater treatment and its associated components at different steps is shown in Figure 1 (broken lines indicate water treatment and solid lines indicate onsite biogas generation).

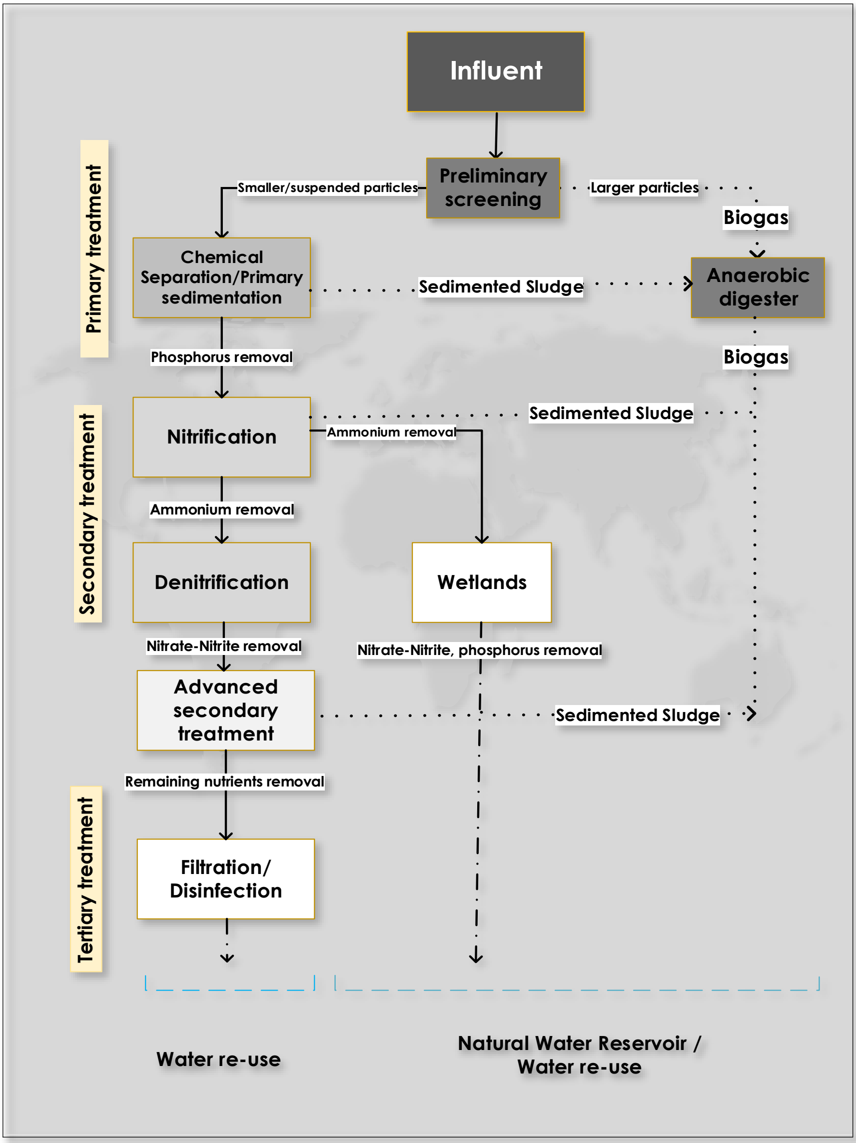

Figure 1. A simplified overview of municipal wastewater treatment and the associated process and how it may vary on a global scale (modified from Tchobanoglous et al. (2014) and Weismann et al. (2006)). The colour gradient reflects the turbidity of the wastewater (■-black to □-white). Solid lines (−) represent liquid treatment, dotted lines (•••) represent solid waste and dash-dotted lines (− •) represent discharged wastewater.

The activated sludge (AS) process is commonly used as a biological wastewater treatment (secondary treatment) in all parts of the world. The AS process is based on the removal of organic pollutants, as most of the carbon (C) is consumed by the bacteria through an external aeration step (i.e. oxygen, O_2). However, the process strictly requires additional chemical steps to reduce nitrogen (N) and phosphorus (P) content in the wastewater (primary, secondary and tertiary treatment) (Cuellar-Bermudez et al., 2017; Jenkins and Wanner, 2014). Further, in Sweden, the process is designed to meet Swedish regulations, which set high standards for N and P removal following the wastewater treatment to avoid eutrophication. The maximum allowed limit for nitrogen is up to 15 and 10 mg L^{-1} for personal equivalents (p.e.) of $\geq$10,000–100,000 and >100,000, whereas the phosphorus limits are 0.5 and <0.25 mg L^{-1} for the same p.e.s, respectively. However, there are variations in these standards among different counties within Sweden in pollution sensitive areas (EU, 1991; Mälarenergi AB, 2016; Naturvårdsverkets, 2016.; Uppsala Vatten och Avfall AB, 2015).

The activated sludge based wastewater treatment facility itself faces challenges related to the economy, stricter effluent requirements and sustainability. Recent developments such as complete autotrophic nitrogen over nitrite (CANON), aerobic granular sludge, membrane biofilm reactors and anaerobic ammonium oxidation (anammox)-based nitrogen removal have been envisioned as mainstream or side stream biological process in wastewater treatment plants (Wiesmann et al., 2006; Jenkins and Wanner, 2014) . Though these processes reduce external carbon usage and operate with less sludge, most of the N that is removed from the wastewater is emitted to the atmosphere as nitrogen gas (N_2) or nitrous oxide (N_2O) as a result of the denitrification step, and most of the C is emitted as carbon dioxide (CO_2) (Jenkins and Wanner, 2014). The impact of other issues such as membrane replacement, fouling, plastic carriers and polymers (after tertiary treatment to improve dewatering of sludge) on the environment remain unknown. Additionally, the operation must be modified according to the geographical location.

In recent years, the economics of municipal wastewater treatment plants have been given a greater importance over achieving the discharge limits of treated wastewater, while most of the nutrients are either lost as greenhouse gases due to aeration and anaerobic denitrification, or partly bound to chemical complexes (Campos et al., 2016). Overall, the amount of indirect CO_2 consumption has been too high in comparison to the recovery of valuable resources such as bio-methane (CH_4) and plant nutrients (N and P) during the operation of wastewater treatment plants (Acién et al., 2016; Campos et al., 2016; Cuellar-Bermudez et al., 2017) (Figure 1, solid line for biogas production during wastewater treatment). Conventional activated sludge (AS) and modified AS are targeted towards end-pipe solutions to achieve removal of nutrients at the expense of greenhouse gas emission and limited nutrient recovery from the treatment plant. Hence, the increase in emissions of greenhouse gases (CO_2, CH_4 and N_2O) from the treatment plants and increased usage of nitrogen in agriculture demand sustainable alternatives for wastewater treatment with recovery of nutrients in geographical locations with both low and high population densities (Daelman et al., 2012).

In this context, microalgae-based wastewater treatment refers to enrichment of freshwater microalgae in wastewater for removal of organic and inorganic pollutants in the presence of light. Microalgae are considered as a promising alternative for sequestration of wastewater nutrients like N and P and CO_2; they release O_2, which supports the microorganisms in wastewater that oxidise organic pollutants to CO_2, in addition to their own photo-degradation of pollutants (Cuellar-Bermudez et al., 2017). Moreover, abiotic (photo degradation and residence time of wastewater) and biotic degradation (photosynthesis and bacterial oxidation) can be achieved through microalgal–bacterial symbiosis. For these reasons, recent years have seen an increase in interest in microalgae cultivation for removal of emerging contaminants such as pharmaceuticals and aromatic hydrocarbons during wastewater treatment (Lebrero et al., 2016; Norvill et al., 2016).

In general, ‘photobioreactors’ refers to open or outdoor tanks that are employed to cultivate microalgae cultivation (Lundquist et al., 2010). High rate algal pond (HRAP)-type photobioreactor setups have been studied extensively for microalgal biomass cultivation in outdoor wastewater treatment (Muñoz and Guieysse, 2006). Engineering challenges in the use of microalgae for wastewater treatment, such as design and process observation, have been under consideration since at least 1957, when the use of land area for HRAP was considered (Lundquist et al., 2010). Another consideration is aimed at increasing the amount of biomass produced at the prevailing outdoor temperature (Lundquist et al., 2010). For instance, a conventional AS plant needs 1 ha to treat 30,000–50,000 p.e., whereas HRAP needs 30–50 ha (Acién et al., 2016). Therefore, the use of HRAP to achieve wastewater treatment is not feasible due to the high requirement for land and surface lighting, especially where lighting is not feasible during winter conditions. Further, closed photobioreactor concepts like tubular photobioreactors and algal biofilm reactors are advantageous for biomass production, but are generally not designed for primary wastewater treatment. Additionally, harvesting of microalgae has also been considered a major bottleneck for scaling up of the process (i.e. treatment capacity) (Lundquist et al., 2010).

However, the use of a flexible closed photobioreactor system (i.e. by combining outdoor and closed systems) concept in the activated sludge environment has the possibility to provide greenhouse gas mitigation and efficient nutrient recovery at the wastewater treatment site itself. This thesis covers strategies for understanding and enhancing the microalgae-based bacterial biomass production and nutrient removal process during municipal wastewater treatment. It is a continuation of my Licentiate dissertation (Anbalagan, 2016). Related works on molecular and metabolic aspects of microalgae–bacteria are described by Krustok (2016) and microalgal biomass conversion to bio-methane is discussed by Olsson (2018).

1.2 The significance of this study

The microalgal–bacterial process in photobioreactors is an emerging application in Nordic-like conditions. It ecological benefits have been widely reported in the literature. In this thesis, the applicability of operational variables (light, temperature, wastewater load, incorporation of CO_2) is studied for their effects on process performance variables such as pH, total oxygen concentration and removal of pollutants. The applicability of this process has been not been extensively addressed in the literature, and there are few long-term studies under conventional wastewater treatment conditions.

Therefore, this thesis collects work that elucidates the applicability of microalgal–bacterial cultivation by identifying effective operational variables in the activated sludge environment. Moreover, it includes additional reactor design considerations (treating wastewater and gas treatment), which cover broad as well as core knowledge. This study is put forward as an initial phase focusing on the concept of waste to resource recovery in the Västmanland region.

The primary operational objectives (OB) and the associated challenges are described in **papers I**–**V** as shown in Figure 2. The three primary objectives are as follows,

- To define effective treatment conditions at photobioreactor level (OB 1= OB 1.1 + OB 1.2).
- To upgrade photobioreactors as CO_2-utilising units (OB 2).
- To study Nordic/Nordic-like climatic conditions as an environmental stress factor (OB 3).

According to these objectives, four research challenges (**RC**) are identified to be addressed in this thesis:

RC 1. What are the initial conditions for the operation of the microalgal–bacterial process?

RC 2. What is the main limitation of photosynthetic nutrient removal in the local environment?

RC 3. Can nitrogen removal be achieved by efficient CO_2 addition?

RC 4. How do light spectrum and temperature influence the microalgae–bacterial photobioreactor at high sludge retention?

The most likely answers are derived from **papers**, I–V as shown in Figure 2.

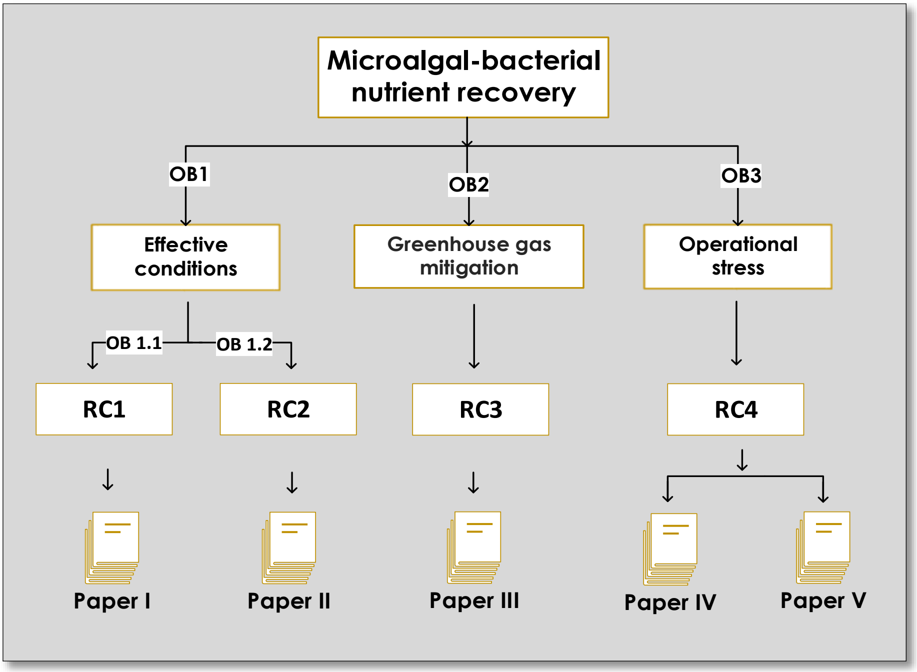

Figure 2. Doctoral thesis structure with appended papers (I-V). RC-Research challenges; OB-Objectives.

1.3 Thesis Outline

This thesis is divided into chapters based on the appended papers as follows.

Chapter 1 Introduction
This chapter covers the present situation, research objectives, challenges and structure of the entire thesis.

Chapter 2 Theoretical background
This chapter presents a detailed overview of the literature on algal–bacterial nutrient recovery, reviewing the previous and present situation, based on the respective objectives.

Chapter 3 Methods
This chapter provides an overview of the applied methodology and essential calculations used in this study.

Chapter 4 Results and discussions
This chapter presents and discusses the main findings.

Chapter 5 Conclusions
This chapter presents the significant conclusions and outlook from this work.

Chapter 6 Future directions
This chapter describes other possible outcomes from this study.

2 Theoretical background

This chapter covers the literature overview of present and past situation during wastewater treatment. Further, it elucidates the opportunities for using algal-bacterial nutrient recovery during wastewater treatment. Here, boxes are used to highlight the indicators of nutrient removal process from the perspective of photobioreactor operation.

2.1 Conventional wastewater treatment

Presently, the conventional biological wastewater treatment plant demands a large proportion of the energy used by the whole plant and varies according to the population density and geographical location (Bodík and Kubaská, 2013; Marcin and Mucha, 2015; Masłoń, 2017; Smith and Liu, 2017). Based on the literature, the electrical power consumption during the AS process alone is 0.2–0.8 kW h m^{-3} depending on the location, as shown in Figure 3. To provide a daily context, 1 kWh of power is required to run a toaster for 1 h (Swedish Energy Agency, 2015). In this regard, the average daily inflow received by Västerås wastewater treatment plant in Sweden varies from ~40,000 to 50,000 m^3 d^{-1} (including stormwater from rainfall and melting of snow) and the energy consumption is expected to increase in future due to population expansion (see Figure 4 for daily inflow).

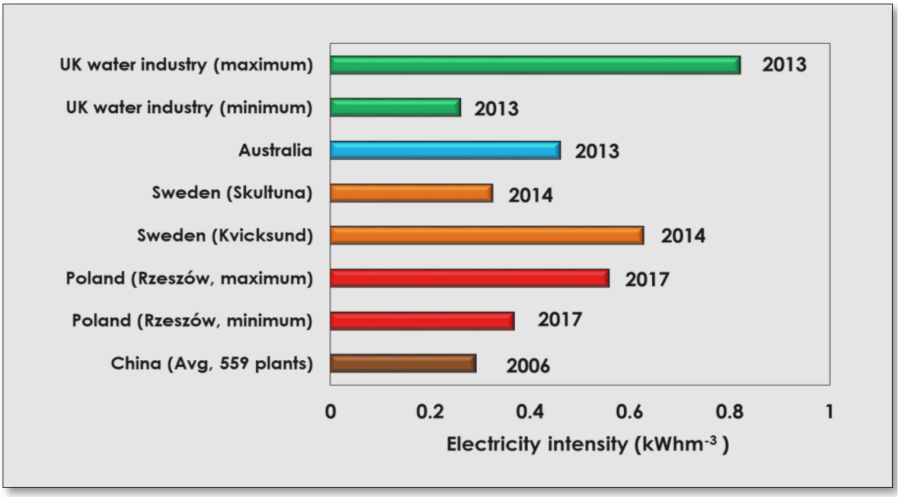

Figure 3. Electricity consumption of the biological process during wastewater treatment (years refer to reported years in the corresponding references).

As the conventional treatment is energy consuming owing to the requirement for aeration, it is a priority to recover chemical energy stored in wastewater based on an alternative biological treatment. In addition, this is likely to also avoid chemical consumption at different stages of the process. Moreover, Västerås wastewater treatment plant uses iron sulphate during the primary wastewater treatment since the AS process does not utilise P. Also, based on the preceding step, the addition of polymer (for dewatering of the sludge or to increase thickness of suspended solids) varies during the tertiary wastewater treatment (Marcin and Mucha, 2015) regardless of its environmental impact on the environment. In this context, it is essential to conserve all forms of energy and chemicals consumed in reducing chemical and biological sludge, as well as greenhouse gas emission at the treatment site. However, the plant in Västerås also utilises external carbon sources (ethylene glycol), which are also used accordingly in other treatment plants in Sweden during the nitrogen removal step (denitrification step) (e.g. UppsalaVatten AB avoids addition of external carbon sources).

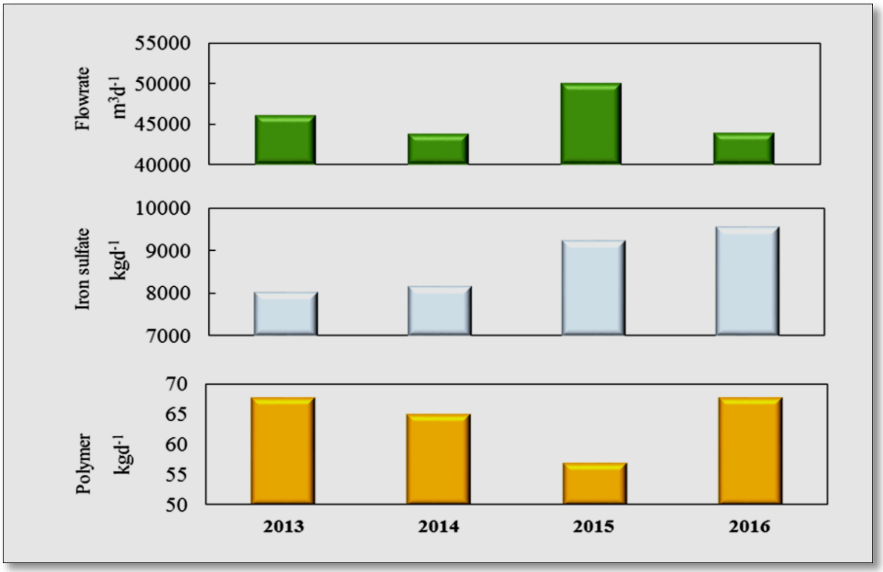

Figure 4. Daily inflow and chemical consumption at Kungsängen wastewater treatment plant, Västerås, Sweden (Mälarenergi AB, 2016, 2015, 2014, 2013). (Here, tonnes year^{-1} are converted to kilograms day^{-1})

2.2 Microalgae–bacteria in wastewater treatment

In recent years, microalgae cultivation has gained much attention for its ability to conserve wastewater nutrients in the form of biomass by utilising the nitrifying activity of conventional AS. Bioreactors are classified into open system (operated outdoor) and closed (operated indoors) configurations. Both types of system have their advantages and disadvantages, as shown in Table 1. However, combining the benefits of both systems is vital to overcoming operational and environmental challenges in different geographical locations. For instance, an open system is in direct contact with the environment, unlike a closed system. Hence, it is more dependent on weather, and CO_2 losses (~90%) are very high compared to a closed system (~75%) (Slade and Bauen, 2013). However, this could be avoided by combining advantages of both open and closed systems.

Table 1. Open versus closed microalgae cultivation, modified from (Grobbelaar, 2009).

Parameters	Open vs Close
• Contamination (insects, microbial, dust etc) • Weather dependence	Open >> Closed
• CO_2 losses • Process control	Open >> Closed
Productivity	Open ≤ Closed
• Harvesting/recovery of biomass • Light exposure	Open << Closed
Maintenance	Open << Closed

<< Lower; >> Higher; ≤ Lower than or equal to

In Nordic conditions (i.e. cold temperature and dark periods), a semi/flexible closed system has advantages over an entirely open system. In this context, microalgal symbiosis has been studied widely in various closed bioreactor configurations that could be advantageous for its application in municipal wastewater treatment, as shown in Table 2. Algal–bacterial symbiotic processes can be divided into two groups: algal symbiosis based on the solid phase of activated sludge (i.e. SAB, PAS, ALGAMMOX, OPG), and wastewater-derived bacterial symbiosis (MAAS). These can be operated either as a simple single-stage process or as a complex process (NA-A) by assimilating additional processes (Table 2). In a case such as photosynthestic granules, activated sludge alone acts as a seed for development of granules. These granules are similar to activated sludge granules with self-oxygen generation dominated by cyanobacteria (Milferstedt et al., 2017). However, other examples of such processes are adaptation of green microalgae towards nitrifying/heterotrophic bacterial communities.

Table 2. Recent trends in microalgae-bacterial cultivation.

Cultivation strategy	Microalgae	Bacteria	Highlights	References
Symbiotic alga-bacteria (SAB)	*Chlorella vulgaris* and organisms from oxidation ponds	Activated sludge	1. Partial and higher total nitrogen (TN) removal efficiency at higher lighting intensity (2000 PAR and 925 PAR*) and at influent chemical oxygen demand (COD) above 400 mg L^{-1}. 2. Efficient total organic carbon (TOC) removal 3. Longer settling time or higher sludge volume index. Faster settling at higher SRTs.	Medina and Neis, 2007; Gutzeit et al., 2005; Valigore et al., 2012
Photo-activated sludge (PAS)	*Scenedesmus quadricada, Anbaena variablis, Chlorella sp., Spirulina sp.,* canal water communities	Activated sludge, canal water communities	1. 67–85% ammonium oxidation (Lower N removal efficiency) 2. Air flotation-based settling 3. Low light intensity of 62 PAR*	Karya et al., 2013; van der Steen et al., 2015
Microalgae activated sludge (MAAS)	Lake water communities (non-filamentous and filamentous microalgae)	Nitrifying communities present in municipal wastewater	1. Efficient TN removal 2. COD removal 3. Partial P removal 4. Efficient gravity settling sludge volume index 5. Low light intensity (150 PAR*)	This study
ALGaeAMMonium Oxidation (ALGAMMOX)	*Chlorella sp.*	Anamox granules and nitrifying communities	1. Light intensity of 110 PAR. 2. Oxygen tolerant anammox community 3. Stability of granules under investigation.	Manser et al., 2016; Van de Vossenberg et., 2017
Algal granules (AG/OPG)	*Chlorella, cyanobacteria* of activated sludge origin	Activated sludge: nitrifiers, denitrifiers, methanogens and phosphate-accumulating bacteria	1. Nitrification/Denitrification 2. COD oxidation 3. Good settling 4. Low lighting intensity (150 PAR*)	Butler et al., 2016; Milferstedt et al., 2017; Stauch-White et al., 2017; Tiron et al., 2017; Abouhend et al., 2018

Cultivation strategy	Microalgae	Bacteria	Highlights	References
Novel anoxic-aerobic process (NA-A)	Green microalgae and cyanobacteria mixture	Activated sludge	1. CO_2 removal 2. Two stage process (anoxic-algal process) with lighting intensity of ~400 PAR. 3. Organic carbon and inorganic removal 4. Good settling	Alcántara et al., 2015; García et al., 2017

*PAR – Photosynthetic active radiation (see also Glossary)

2.3 Nutrient Removal

2.3.1 Bio nutrients from wastewater

Municipal wastewater is characterised by organic compounds of animal and plant origin. As an initial step, heterotrophic bacteria originating from wastewater convert most of the carbonaceous, nitrogenous and phosphate containing organic matter in the presence of oxygen into ammonium, phosphates and carbon dioxide as shown in equation 1 (See Box 1 for sensitive parameters based on wastewater nutrients). Later, the nitrogenous compounds are utilised by the nitrification process and P is recovered by chemical flocculation in the AS configuration of the wastewater treatment plant. An overview of different forms of N and P retrieved from municipal wastewater is shown in Figure 5. In general, most of the N (i.e. ~70–90% of ammonia and ~10–30% of organic N) and P obtained from the wastewater originate from degradation of proteins and amino acids of animal and plant origin. However, a small portion of inorganic N is introduced by industrial wastewater, and this varies according to geographical location. For instance, Westinghouse Electric Corporation contributes ~3–4 mg NH_4^+ L^{-1} d^{-1} of total nitrogen in the wastewater composition of Västerås wastewater treatment plant, Sweden (Mälarenergi AB, 2016, 2015, 2014, 2013).

$$Organic\ compounds\ + O_2 \\ \dashrightarrow Bacteria \dashrightarrow CO_2 + NH_4^+ + PO_4^{3-} + other\ endproducts + Energy \quad (1)$$

On the other hand, inorganic carbon or CO_2 is an essential nutrient source that originates from wastewater (equation 1) and is lost to the atmosphere due to the concentration gradient from the liquid (>~0.4 mg CO_2 L^{-1}, according to atmospheric gas/liquid equilibrium) to the gas phase due to intense nitrification in the conventional process (Posadas et al., 2016).

Further, CO_2 is an essential nutrient for algal photosynthesis. CO_2 exists in the wastewater as follows, based on acid dissociation (pK_a) of the wastewater,

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^-(\mathrm{p}K_a = 6.42, @15^{\circ}C) \leftrightarrow CO_3^{2^-} + H^+(\mathrm{p}K_a = 10.43, @15^{\circ}C) \qquad (2)$$

Other possible routes of CO_2 input can be implemented via soluble solid carbonates from flue gas (Na_2CO_3) or by absorption column using flue gas and flaring of biogas, and CO_2 from the atmosphere (Wang et al., 2008). CO_2 in flue gas is available at no cost and is readily available with up to 15 % CO_2, which can be easily incorporated during algal–bacterial cultivation. In this context, power plant gasifiers (such as the one at Mälarenergi AB) and biogas upgradation plants (Vafabmiljo AB and Gasum AB) in Västerås are a likely potential source for microalgal–bacterial biomass cultivation.

Box 1: Bio nutrient based parameters

Overall, from the perspective of photosynthetic bioreactor, the sensitive parameters of algal–bacterial symbiosis based on the strength of wastewater can be estimated as follows:

- Concentration of organic compounds (expressed as total suspended solids (TSS) and chemical oxygen demand (COD), or biological oxygen demand, (organic compounds ⇢ CO_2))
- Concentration of ammonium and phosphate
- pH and alkalinity of wastewater
- Effective CO_2 flow (if needed)

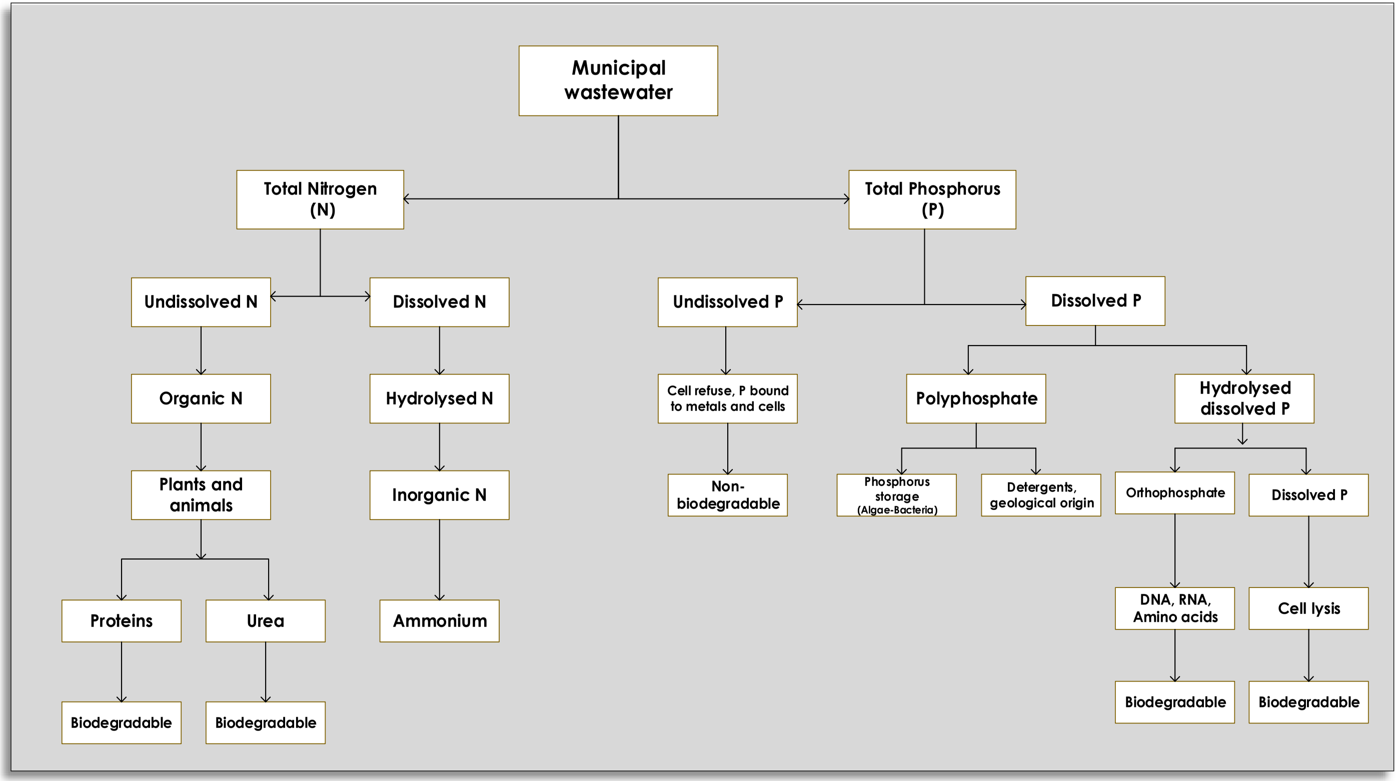

Figure 5. Speciation of nitrogen and phosphorus in wastewater (Tchobanoglous et al., 2014; Jenkins & Wanner, 2014).

2.3.2 Microalgal nutrient uptake

Based on oxygenic photosynthesis, algae are differentiated into eukaryotic (multicellular and differentiated into cellular organelles) and prokaryotic groups (unicellular and non-differentiated cellular organelles). In general, the shape of microalgae vary according to species, with sizes ranging from 0.2 to 200 µm (Pentecost, 1984; Reynolds, 2006). They are ubiquitous in freshwater and seawater. The most common structures found in wastewater are shown in Figure 6. In wastewater applications, eukaryotic microalgae are comprised of green (e.g. *Chlorella*), golden brown (e.g. *Diatoma*), and yellow-green algae (e.g. *Tribonema*); these are classified as eukaryotes (Reynolds, 2006).

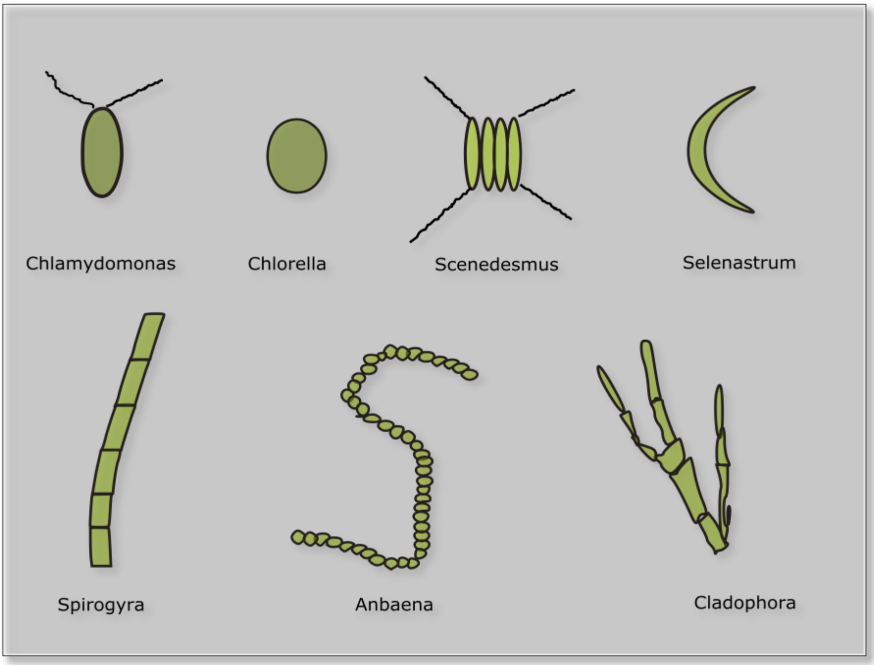

Figure 6. Examples of algae structures.

Cyanobacteria or blue-green algae are prokaryotes, and include unicellular (e.g. *Microcystis*) and filamentous (uniseriate, e.g. *Spirulina* and unbranched, e.g. *Anbaena*) organisms that come under the domain bacteria (Reynolds, 2006). Further, both groups, microalgae and cyanobacteria can be distinguished based on their light-harvesting photosynthetic pigments. For instance, the primary photosynthetic pigments chlorophylls *a*, *b* and *c* are part of the light-receiving antenna in the chloroplast of green and brown microalgae cells during photosynthesis. However, cyanobacteria possess proteins called phycobilin instead of chlorophyll *b* and *c* (Reynolds, 2006). Photosynthesis is the primary mode of nutrient assimilation among various microalgae. Photosynthesis involves light and dark reactions. The light reaction in eukaryotic microalgae are carried out in a specific cell organelle called the chloroplast. However, in the case of cyanobacteria, the chloroplast is absent and photosynthesis takes place in loosely dispersed thylakoid membranes in the cytoplasm. The photosynthetic mechanism is initiated by a photosystem containing a light-harvesting complex which is composed of 200–300 chlorophyll *a* molecules and up to 30% chlorophyll *b*; the rest of the phophotosystem is composed of carotenoids (carotenoids function as an antenna that receives light and transfers it to chlorophyll and are also considered as preotective lens for microalgae) (Borowitzka et al., 2016).

A simple photosynthesis pathway is shown in Figure 7. The sequence of events during the light reaction occurs in the photosystem architecture via three crucial components: photosystem II, photosystem I, the plastoquinone pool and the b_6/f cytochrome complex (in microalgae) or cytochrome *c* (in cyanobacteria). The functions of these components are as follows,

(i) Photosystem II: Light capture, ATP production ($ADP + H_3PO_4 + Energy \leftrightarrow ATP + H_2O$), hydrolysis of water ($2H_2O \rightarrow 4H^+ + 4e^- + O_2$) and excitation of chlorophyll at a wavelength of 680 nm.

(ii) Plastoquinone cycle and b_6/f cytochrome complex: acts as an electron transporter from photosystem II to photosystem I; this acts as a proton gradient for ATP generation.

(iii) Photosystem I: acts as an electron acceptor, and the chlorophyll unit is excited at an excitation wavelength of 700 nm. As a result, the excited electron from chlorophyll reduces NADP to NADPH.

Thus, the light reaction can be summarised according to equation (3),

$$2\ \mathrm{NADP} + 3\ \mathrm{ADP} + 3\ \mathrm{P} + 2\mathrm{H_2O} + 8\ \mathrm{e^-} \rightarrow\ 2\ \mathrm{NADPH} + 3\ \mathrm{ATP} + 3\ \mathrm{P} + 2\ \mathrm{H^+} + \mathrm{O_2} \qquad (3)$$

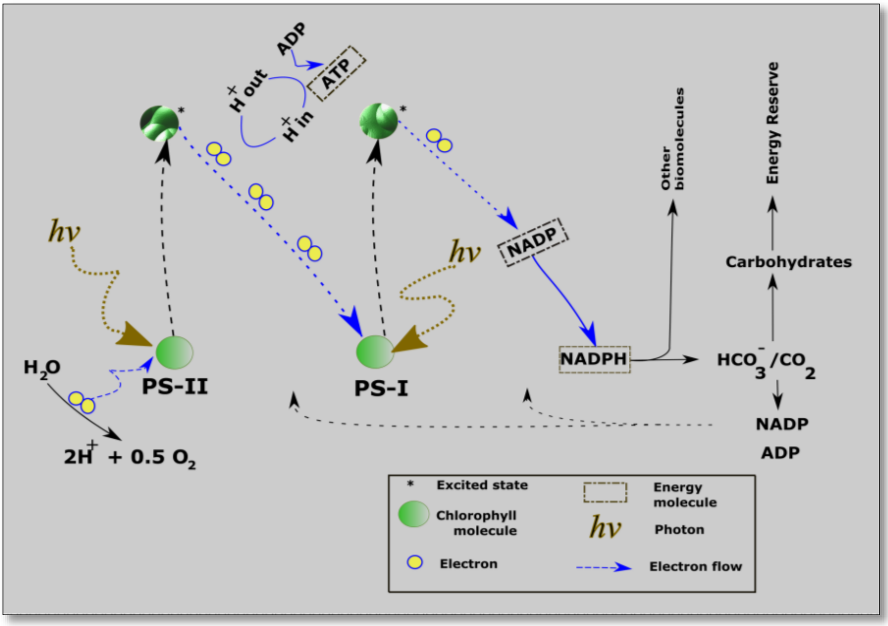

Figure 7. A simplified overview of Z scheme of photosynthesis through photosystems II and I (Reynolds, 2006).

The energy obtained in equation 3 of the light reaction, in the form of NADPH and ATP, is subsequently used for reduction of CO_2 to starch and glycogen or paramylon reserves and partly for deriving biomolecules as in equation 3. This reaction is also called the Calvin–Benson cycle, dark cycle or dark reaction. CO_2 is assimilated through active transport inside the cells, through diffusion and through active transport from the medium as HCO_3^- ($HCO_3^- \dashrightarrow CO_2$) or CO_2.

$$\begin{aligned} &CO_2 + 2\,NADPH + 3\,ATP + 2\,H^+ \\ &\qquad \rightarrow\ 0.167\,C_6H_{12}O_6 + H_2O + 2\,NADP + 3\,ADP + 3P \end{aligned} \tag{4}$$

The aggregate of the photosynthetic reaction can be summarised as follows (photosynthetic parameters can be obtained from Box 2),

$$H_2O + CO_2 + light\ energy\ \rightarrow 0.167\ C_6H_{12}O_6 + O_2 \tag{5}$$

Box 2: Photosynthetic parameters

From a wastewater treatment perspective, the following sensitive parameters of a photosynthetic reactor can be estimated as follows:

- Amount and intensity of incident light energy supplied and received during photobioreactor operation (photosynthetic active radiation).
- Oxygen production.
- The inorganic carbon demand of the algal cells based on the strength of wastewater (COD ⇢ CO_2).

Further, nutrient assimilation processes such as those for N (7.5% (w/w) of algal biomass) and P (1% (w/w) of algal biomass) take place in parallel with photosynthesis for production of biomolecules by utilising the energy retrieved from photosynthesis (as ATP and NADPH) (Cresswell et al., 1989). The importance of nutrient assimilation for the photobioreactor is summarised in Box 3.

Based on the availability of N (ammonium > nitrate > urea), it is mainly assimilated either as ammonium during active uptake or reduced from nitrate to ammonium through a passive uptake mechanism; additionally, urea is directly transported by the urea transporters (Borowitzka et al., 2016). Further, microalgal N transport across the membrane takes place through switching on and off of ammonium and nitrate transporters, which is driven by energy in the form of ATP. However, the presence of nitrite and ammonium in the medium affects the uptake of nitrate, as reported by Thacker and Syrett (1972). In this sense, nitrate is thermodynamically favourable to microalgae in natural aquatic bodies due to oxidation of ammonium by nitrifying bacteria (Li, 2013). A similar situation prevails in a wastewater environment due to the presence of nitrifying communities. Therefore, microalgae prefer feed-forward nutrient assimilation through a series of redox reactions to reduce nitrate to ammonia using enzymes, nitrate reductase and nitrite reductase, respectively (Figure 8a). Besides, this step is slow which demands high energy input and proton consumption (i.e. OH^- release; alkalinisation), and it occurs during ammonium-depleted conditions. This is a typical situation in HRAPs, which results in partial volatilisation of ammonium as ammonia (Gonçalves et al., 2016). In contrast to active ammonium uptake, feed-backward assimilation is widespread whenever microalgae encounter either accumulation of nitrate or high ammonium concentration in the system due to high density cultures in continuous photobioreactor cultivation (Scherholz and Curtis, 2013; Wang and Curtis, 2016). Additionally, this step is less energy demanding due to the direct ammonium transport into the cells and increases proton release in the system (i.e. acidification). Overall, each mole of nitrate and nitrite assimilation inside the cells releases 0.7 moles of OH^- ions during passive nutrient uptake; by contrast, ammonium uptake releases only protons,1.03 mole of H^+ per mole of ammonium, whereas uptake of urea has less effect on pH of the medium (Borowitzka et al., 2016). Therefore, it is vital to consider the N uptake mechanism for long term operation of the photobioreactor at an optimal pH (i.e. running a stable process without a proton imbalance) as reported by Wang and Curtis, (2016).

On the other hand, inorganic P plays a crucial role in the metabolism of microalgae, as shown in Figure 8b. In general, P removal takes place through both intracellular and extracellular mechanisms by transport of orthophosphate. Inside the cells, the P is assimilated into organic form through phosphorylation, which involves the generation of ATP (Figure 8 b). In addition, P plays a crucial role in synthesis of molecules that are central to biology such as DNA, RNA and protein. The uptake of P in microalgae is low, based on the microalgal biomass composition. However, the uptake increases whenever conditions are favourable. Excess P accumulates in cells as polyphosphates (acid insoluble phosphates or acid soluble phosphates) to perform various cellular functions as shown in Figure 8. Further, AS and microalgae contribute to Ca-mediated and CO_2-mediated $CaCO_3$-based P adsorption on the cell surface and secretion of extrapolymeric substances (EPS) during cell aggregation or biofilm formation (EPS is composed of 75–90% protein) which may favour removal of the P during symbiosis (Mañas et al., 2012; Sahoo et al., 2014; Santomauro et al., 2012).

Overall, the nutrient uptake during the photosynthesis process can be summarised according to equation 6,

$$\begin{aligned} &106\, CO_2 + 16\, HNO_3 + H_3PO_4 + 122\, H_2O \\ &\qquad \rightarrow \quad (CH_2O)_{106}\, (NH_3)_{16} H_3PO_4\, (algae) + 138\, O_2 \end{aligned} \tag{6}$$

Box 3: Important parameters

Based on the nutrient assimilation mechanism, the sensitive parameters for a photosynthetic reactor treating wastewater can be targeted as follows

- Ammonium levels (influent, reactor and effluent).
- Nitrate/nitrite levels (influent, reactor and effluent).
- Phosphate levels (influent, reactor and effluent).

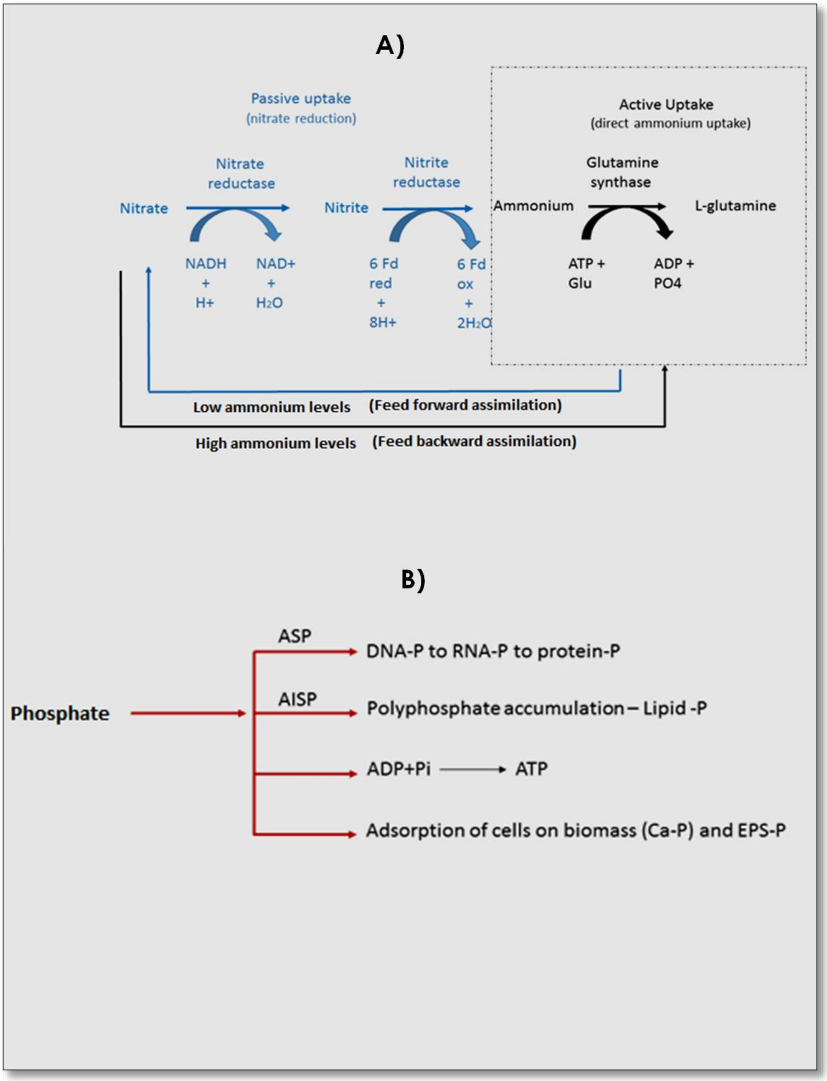

Figure 8. **Understanding of nitrogen and phosphorus removal by microalgae (Cai et al., 2013; Cuellar-Bermudez et al., 2016; Mañas et al., 2012; Scherholz and Curtis, 2013; Wang and Curtis, 2016). A) Nitrogen uptake and B) phosphorus uptake. Dash-dotted box, direct ammonium uptake; blue, ammonium assimilation; red, phosphate assimilation. [ASP-acid soluble polyphosphates; AISP-acid insoluble polyphosphates; ATP-adenosine triphosphate; ADP-adenosine diphosphate; ATP-adenosine triphosphate; ADP-adenosine diphosphate; Ca-calcium; EPS-extra polymeric substances; Fd-ferredoxin; Glu- glutamine; iP-inorganic phosphorus; RNA-ribonucleic acid; NADH-nicotinamide adenine diphosphate].**

2.3.3 Bacterial nutrient uptake

Nitrification is a two-step process, carried out by the gram-negative bacteria *Nitrosomonas* (coccus) and *Nitrobacter* (bacillus) in the presence of oxygen and CO_2 as carbon source at a pH range of 5.8–8.5 (Wiesmann et al., 2006). Similar to the active ammonium uptake by algae, bacterial nitrification results in acidification of the medium (i.e. release of H^+). *Nitrosomonas* and *Nitrobacter* oxidise ammonium to nitrite (nitritification), and nitrite to nitrate (nitratification), according to the catabolic and anabolic stoichiometry reactions in equations 7 and 8,

$$\begin{gathered} NH_4 + 1.98\,HCO_3 + 1.3\,O_2 \dashrightarrow \\ 0.0182\,C_5H_7O_2N\ (Bacteria) + 0.98\,NO_2 + 1.04\,H_2O + 1.89\,H_2CO_3 \end{gathered} \tag{7}$$

$$\begin{gathered} \mathrm{NO_2} + 0.02\,\mathrm{HCO_3} + 0.048\,\mathrm{O_2} + 0.005\,\mathrm{NH_4} + 0.005\,\mathrm{HCO_3} \dashrightarrow \\ 0.005\,C_5H_7O_2N\ (Bacteria) + NO_3 + 0.015\,H_2O \end{gathered} \tag{8}$$

Nitrifying bacteria can take up up to 12 mg O_2 L^{-1}, whereas higher concentrations of O_2 can lead to the toxic condition. Photosynthesis can result in high concentrations of total oxygen in the liquid medium of 130–300% of saturation. Therefore, it is important to consider such oversaturation in algal–bacterial symbiosis in the photobioreactor (Norvill, 2016). In brief, nitrification consumes alkalinity, as in the case of ammonium uptake by algae (Wiesmann et al., 2006; Jenkins and Wanner, 2014); for instance, 7 g of alkalinity is reduced for each gram of NH_4^+ that is oxidised during nitrification (based on equations 7 and 8, i.e. 2 g of alkalinity is consumed per mole of NH_4^+ that is oxidised). Furthermore, the nitrification process is considered to be complete when ammonium concentration levels in the effluent are below 1 mg L^{-1} (Hoa Binh T, 2013).

2.4 Importance of process variables in nutrient removal

Light, CO_2 availability and residence time are considered to be the three most important process variables that influence photosynthetic nutrient removal.

2.4.1 Light and its relation to algal–bacterial photobioreactors

Light is crucial for conversion of N and P by bacterial-mediated photosynthesis. In an ideal photobioreactor, the rate of photosynthesis (ROP) is directly related to the light intensity (I) according to the ROP–I curve. This relationship is divided into three zones: light limitation zone, maximum photosynthesis and saturation zone, and photoinhibition zone, analogous to the Michaelis–Menten equation (Béchet et al., 2013, See Box 4).

In general, microalgae can efficiently utilise visible wavelengths (350–750 nm) of electromagnetic radiation, light of these wavelengths are also referred as photosynthetically active radiation or PAR (Reynolds, 2006; Schulze et al., 2014). A PAR efficiency (allowing for electricity losses due to heat and generation of ultraviolet radiation) of ~40% has been measured for artificial lighting using fluorescent lamps in greenhouses (Darko et al., 2014). Light emitting diodes (LEDs) can provide higher PAR efficiencies of 80–100%, since LEDs can produce specific wavelengths (e.g. for activation of PSII at 680 nm and of PSI at 700 nm) or a wider spectrum with low ultraviolet and heat emission (Borowitzka et al., 2016; Darko et al., 2014). Table 3 shows the microalgal pigment requirement and the wavelength range of carotenoids, chlorophyll and phycobilins targeted by LED lamps.

In addition, photo-saturation of PAR around 150–400 µmol m^{-2} s^{-1} has been reported for artificial lighting in the literature (Ooms et al., 2016). Though it is species specific, most algal photosynthesis is inhibited at PAR levels higher than 200, equivalent to 10−17% of the summer (2000 µmol m^{-2} s^{-1}) and winter (1200 µmol m^{-2} s^{-1}) solar PAR radiation (Park et al., 2011). However, other factors like reflection, dissolved organic matter, mixing and algal–bacterial cell density or self-shading can significantly dilute the visible light spectrum resulting in reduced biomass productivity (Borowitzka et al., 2016).

Box 4: Importance of light utilisation in photobioreactors

Light utilisation in algal cells follows the ROP–I curve as follows (Béchet et al., 2013):

- Zone 1: light limitation and compensation zone, where the rate of respiration equals the rate of photosynthesis (i.e. CO_2 consumption = O_2 release).
- Zone II: level off zone, where light utilisation increases linearly and reaches a maximum photosynthesis rate or light saturation point.
- Zone III: photo-inhibition state, where excess light intensity results in damage to the photosystem. This causes a decrease in photosynthesis rate and microalgal productivity.

Light can also have adverse effects on nitrifying bacteria. For instance, *Nitrosomonas* is inhibited at a blue light wavelength of 410–415 nm (Alleman et al., 1987). The nitrifying activity may also be lower when PAR radiation exceeds 180 µmol m^{-2} s^{-1}; this can significantly affect microalgal photosynthesis (Vanzella et al., 1989; Vargas et al., 2016). In addition, Raven et al. (1992) and Thompson et al. (1989) report that limited lighting supports nitrate uptake by microalgae in preference to ammonium uptake.

Table 3. Light requirements of chlorophyll and carotenoid proteins. Adapted from (Schulze et al., 2014).

Pigments	**Cyanobacteria**	**Brown algae**	**Green algae**
Chl *a*	🟩	🟩	🟩
Chl *b*	🟥		🟩
Chl *c*		🟩	
β carotene	🟩		🟩
αcarotene	🟥		
Zeaxanthin	🟩	🟥	🟥
Phycobilins	🟩		
Violaxanthin			🟩
Neoxanthin			🟩
Loroxanthin			🟥
Diatoxanthin		🟥	
Diadinoxanthin		🟩	
Fucoxanthin		🟥	

Red cells represent low light intensity requirement at wavelengths of 560–610 nm and 480–550 nm; Green cells represent high light intensity requirement at wavelengths of 660–690 nm, 620–650 nm and 560–610 nm, 480–550 nm and 420–470 nm; white cells indicate the absence of pigment. Chl a- chlorophyll a; Chl b- chlorophyll b; Chl c- chlorophyll c.

2.4.2 Carbon addition

Wastewater has a low composition of inorganic C, which is dependent on the heterotrophic oxidation of bacteria. The requirement for C (which makes up 45–50 % (w/w) of algal biomass) for building algal biomass is higher than that for bacteria, as shown by equations 6, 7 and 8 (Slade and Bauen, 2013). This results in consumption of 1.83 kg CO_2 kg^{-1} algal biomass during photosynthetic growth. However, although nitrifiers and microalgae have higher and lower growth rates, respectively, both groups are dependent on inorganic carbon sources. The typical C:N ratio of wastewater (3–7) is low in comparison to the algal biomass (6–15) (Park et al., 2011). Therefore, it is vital to augment the cultivation medium with an external carbon source to support photosynthetic nutrient removal and biomass productivity. In this context, mass transfer of carbon dioxide plays a crucial role in microalgal cultivation. For instance, losses of CO_2 have been noticed in open ponds (HRAP); this can be avoided by using a closed system with a collection chamber and an absorption column (Posadas et al., 2016, 2015a, 2015b). An overview of external C addition in various closed photobioreactors is presented in Table 4.

Table 4. Closed photobioreactor studies using CO_2 addition.

Yield/ productivity	CO_2 source	Reactor type (volume)	Micro-organisms	Highlights	Reference
0.6–1.2 g L^{-1}	Sodium bicarbonate + carbonate addition (24 g L^{-1})	Tubular photobioreactor (100 L)	*Spirulina plantesis*	90% higher biomass yield than open pond using synthetic medium (comparative study).	Torzillo et al., 1986
Not reported	Pure CO_2 + air (100 mL min^{-1} + 2.5 L min^{-1})	Bubble column photobioreactor (1.5 L)	*Anbaena variablis*	Dual sparging increased the CO_2 absorption using synthetic medium. Low supply: 205 PAR.	Eriksen et al., 1998
1.2 g L^{-1} d^{-1}	Ambient air	Tubular photobioreactor (200 L)	*Phaeodactylum tricornutum*	High liquid velocity to reduce the oxygen accumulation in medium.	Acién Fernández et al., 2001
2. (15%)–2.1 g L^{-1} (2 %)	House exhaust gas containing 15 %, and 2 % CO_2	Hollow fiber membrane photobioreactor (0.5 L)	*Spirulina plantesis*	Synthetic wastewater containing nitrate. Removal (5 days HRT): 48% CO_2: (15%), 82–85% (2%); nitrogen removal: ~80%.	Kumar et al., 2010
Not reported	1% CO_2 and 22% O_2	Hollow fiber membrane photobioreactor (5 L)	*Chlorella vulgaris*	Higher CO_2 fixation rate with red and white light spectrum.	Fan et al., 2007
0.9 g L^{-1}	Flue gas 18 % CO_2 from coke oven + air 0.05 v/v min^{-1}	Air-lift photobioreactor- (100 L)	*Scenedesmus obliqus* WUST4	Actual flue gas, 67% removal of CO_2. Light supply: 12000–13000 lux	Li et al., 2011
51 g m^{-2} d^{-1}	0.2–5% CO_2	Twin panel sheet photobioreactor (0.72 m^2)	*Halochlorella rubescens*	Light supply of 1500 PAR. Basal medium.	Schultze et al., 2015
>3 mg L^{-1}	Synthetic biogas (24% CO_2)	Cylindrical photobioreactor (2.7 L)	*Mixed microalgal communites and activated sludge*	Anoxic reactor connected to a photobioractor (HRT of 2 d). Biogas containing CO_2, >80% CO_2 removal. Light supply: ~400 PAR.	García et al., 2016

2.4.3 Residence time or retention time

Hydraulic retention time (HRT) and solids or sludge retention time (SRT) refer to the average time that wastewater and microalgae−bacteria remain in the photobioreactor (Tchobanoglous et al., 2014). HRT and SRT are critical parameters in the nutrient uptake mechanism by microalgae and bacteria.

In a continuous open photobioreactor (HRAPs), HRT ranges of 6 days and up to 2 days have been considered as effective in the presence of external carbon source (Muñoz and Guieysse, 2006; Park and Craggs, 2007; Posadas, 2016). However, in the case of the closed photobioreactor, HRT ranges of between 3 to 0.5 days have been considered. For instance, Van Der Steen et al., (2015) studied HRT of 1 day resulting in removal of 33% of ammonium−N and 67% of nitrate-N in the treated wastewater under continuous lighting (250 PAR) with recirculation of sludge from the settler. Further, Abouhend et al. (2018) report HRT of 0.5−0.9 days with combined denitrification and nitrification of ammonium (28−70% of N removal) under discontinuous lighting (150 PAR) without recirculation.

However, SRT is given less importance during the photobioreactor operation. Gutzeit et al. (2005) report SRTs of 20−25 days under discontinuous lighting at a very high light intensity (2000 PAR) in relation to operational HRT of 2−3 d ays with synthetic and pretreated wastewater resulting in 68−87% of N removal. By contrast, Abouhend et al. (2018) observed fluctuating N removal under SRTs of 21 to 42 days due to sludge accumulation in the photobioreactor as discussed earlier. Further, Van Der Steen et al., (2015) and Karya et al.(2013) report ~67 to 85% of nitrification at SRTs of 30−15 days under lower lighting (66 PAR). Also, the same authors suggest SRT as a function of light transparency or absorption in the reactor due to slow growing nature of nitrifiers.

However, the continuous studies reporting effective SRT to HRT are very scarce in the literature under prevailing wastewater conditions in Sweden. Further, small scale studies (any volumes lower than 5L) limit the importance of SRT for photosynthetic nutrient removal; this remains to be considered during the photobioreactor operation.

2.5 Biogas production

Biogas production from microalgae sludge has been known since the 1950s (Tchobanoglous et al., 2014). Algal slurry is colloidal, and contains 60 to 67% N (in the form of ammonium) and has a minimum hydraulic retention time of less than 11 days with negligible gas production (i.e. absence of lag phase) during anaerobic digestion (Olsson et al., 2014; Oswald and Golueke, 1960; Passos et al., 2015). Oswald and Golueke (1960) suggest thermophilic digestion over mesophilic digestion with microalgal slurries; this may vary with the composition of the algal slurry. Microalgae are highly proteinaceous with complex carbohydrate structures; hence, several pre-treatments have been proposed (thermal, physiochemical and enzymatic hydrolysis) to increase their degradability (Passos et al., 2014; Ramos-Suárez et al., 2014). On the other hand, accumulation of inhibitory compounds or degradation products, volatile fatty acids and ammonium can also reduce the biogas production. However, the digestion of microalgae is species-specific (Olsson et al., 2014; Ramos-Suárez et al., 2014). The anaerobic wastewater generated following the digestion could be a potential source for microalgal biomass cultivation as a sidestream, reducing the load of the influent wastewater to the mainstream (Ledda et al., 2015)

3 Methods

This chapter provides an overview of applied methods and important calculations used in this study.

3.1 Sampling

Municipal wastewaters and natural waters of different origin were utilised for inoculum development for the photobioreactor operation as shown in Figure 9. In brief, four combinations of the mixed microalgal–bacterial community were developed as follows:

- Lake water communities (*Mälaren*, Sweden): pre-treated municipal wastewater (Kunsängen wastewater treatment plant, Västerås, Sweden).
- Lake water communities (*Mälaren*, Sweden): raw municipal wastewater (Kunsängen wastewater treatment plant, Västerås, Sweden).
- Seawater (Pacific Ocean, Cantabria Sea), sediments (Cantabria Sea) and pure colony (*Nanochloropsis* sp and *Tetraselmis* sp): 15% centrate (Valladolid wastewater treatment plant, Spain).
- Pure strains (*Chlorella vulgaris*, *Scenedesmus quadricada* and *Oscillatoria* sp.): pre-treated municipal wastewater (Amherst wastewater treatment plant, USA).

Further, as shown in Figure 9, municipal wastewater was collected at a different location based on the experimental objectives as follows:

- Raw wastewater was collected between Summer and Autumn 2014 and between Autumn and Winter 2016–2017 from Västerås wastewater treatment plant (Sweden) – **Paper I** and **V**.

- Pre-treated wastewater (after iron sulphate coagulation step) was collected from the pre-sedimentation basin during Spring–Summer 2015 at Västerås wastewater treatment plant (Sweden) – **Paper II**.
- Anaerobic effluent or centrate was collected during Autumn–Winter 2015–2016 from Valladolid wastewater treatment plant (Spain) following the centrifugal separation in the anaerobic digester – **Paper III**.
- Pre-treated wastewater (without any coagulation) was collected during Winter 2017 from Amherst wastewater treatment plant, Amherst (USA). Synthetic wastewater was freshly fed to the reactors – **Paper IV**.

At each experimental stage, the wastewater was freshly collected and stored in a cold room (or) fridge (4–6 ^{0}C) for 2–4 days before experimental investigation if needed.

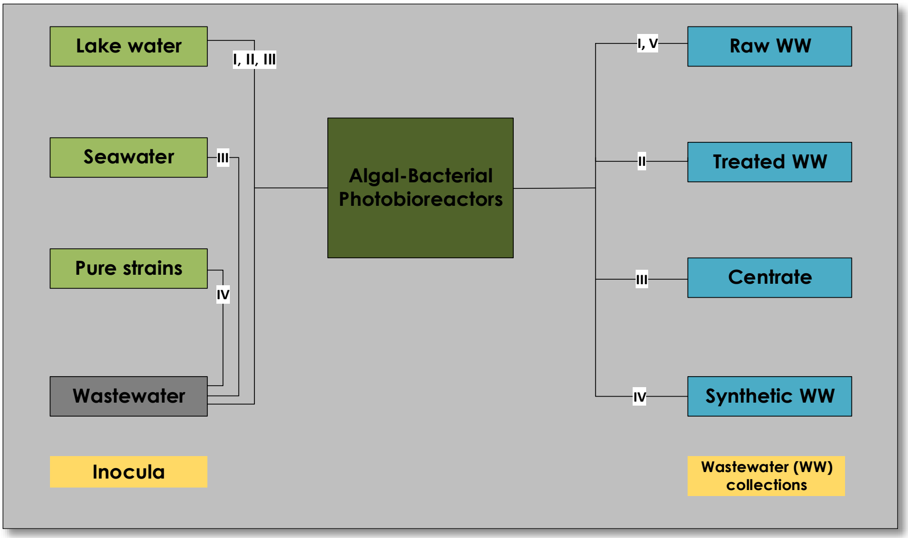

Figure 9. A simplified overview of inoculum preparation and wastewater collection. Roman numbers refer to the appended papers (I–V). Light green (microalgal communities), dark green (photobioreactor), grey (wastewater bacterial communities) and blue (wastewater sources).

3.2 Photobioreactors

The photobioreactor configurations of different experimental are shown in Figure 10. An overview of photobioreactor operational conditions in the different experiments is shown in Table 5. All the reactors were constructed and the trial runs were performed before experimentation in the laboratory environment. The photobioreactor was scaled up from 200 mL to 110 L working volume as follows,

- **Paper I**: Duplicate semi-continuous photobioreactors with 22 L working volume were used with external carbon dioxide addition on demand based on pH control.
- **Paper II**: Initially, a fed-batch photobioreactor volume of 250 mL was used to define the effective PO_4-P additions in the iron sulphate-treated wastewater due to growth inhibition at pilot scale tests (1 m^3 at Mälarenergi AB, Västerås). Secondly, a batch photobioreactor volume of 1.2 L with temperature control was utilised for a total oxygen response study at different light intensities. Finally, a semi-continuous photobioreactor volume of 22 L was used to perform nutrient removal studies with external carbon addition by setting light intensity defined from the batch study.
    - Two different PO_4-P additions, 6 and 3 mg P L^{-1} were tested.
- **Paper III**: Initially, duplicate batch photobioreactors were investigated for CO_2 and volatile organic pollutant removal from the exhaust gas using centrate by selection of effective natural communities using the same strategy as in **paper I**. Later, a continuous 110 L photobioreactor that was connected to an absorption column was studied for continuous nutrient, CO_2 and volatile organic compounds removal.
    - Four stages were tested: I (acclimatisation); II (15% centrate and recirculation liquid to gas ratio of 1); III (15% centrate and recirculation liquid to gas ratio of 15) and IV (30% centrate and recirculation liquid to gas ratio of 15).
- **Paper IV**: 5 L photobioreactors were used to study environmental stress factors: limited organic and inorganic carbon supply (250 mg L^{-1}), cold temperature, light spectrum and uncontrolled pH. The experiment was carried out in duplicate with LEDs of different colours (white, red or blue).

  - Two stages were studied: stage I (without external carbon addition and sludge wastage) and stage II (with external carbon addition and sludge wastage).

- **Paper V**: A continuous photobioreactor of 75 L connected to a sedimentation cone (4.5 L) was operated under activated sludge configuration to study the effect of cold temperature and limited lighting conditions on nutrient removal.
  - Seven stages were tested: I (seeding), II (acclimatisation to continuous wastewater supply and higher sludge recirculation rate), III (99 % CO_2 addition (9 mL d^{-1}), IV (alkalinity addition), V (reduced sludge recirculation) and VI (reduced sludge recirculation without CO_2 gas flow).

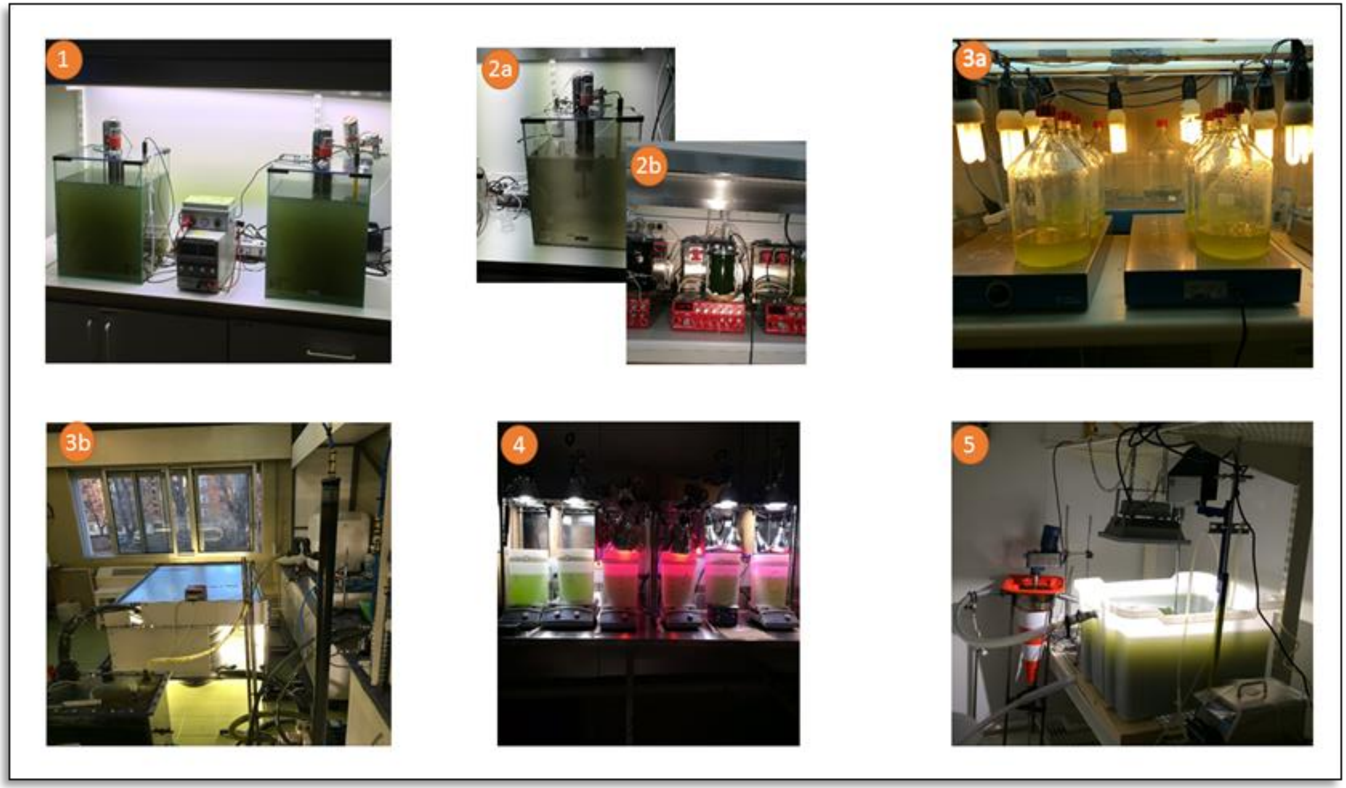

Figure 10. Overview of algal–bacterial photobioreactor configurations during initial cultivation stages. 1, paper I; 2a and b, paper II; 3a and b, paper III; 4, paper IV; and 5, paper V.

Table 5. Operation of various photobioreactors used in this study

Operation	Papers				
	I	II	III	IV	V
HRT (days)	2, 4, 6	4	14, 7	1.5	6.3 ± 0.5
SRT (days)	2, 4, 6	7	14,7	10–55	13-50
Lighting (hrs, light:dark)	24:0	24:0	12:12	24:0	24:0
pH	Controlled	Controlled	Not controlled	Not controlled	Controlled
Temp. (˚C)	22	22	22 (<20 atnight)	12	13
Mixing (rpm* or m s^{-1}**)	100*	100*	0.25**	~350*	99*
CO_2 (%)* / HCO_3^- **(mg C L^{-1}) addition	99*	99*	23*	75**	99* & 180**
Lighting (PAR in µmol $m^{-2}s^{-1}$)	Fluorescent lamp (150)	Fluorescent lamp (150)	Cool white LED lamp (1500)	Cool white, red or blue LED lamps (110)	Cool white LED lamp (250)
Vertical Absorption Column	-	-	Yes	-	-
Days of operation	44	20	137	50	151

3.3 Biogas kinetics

Biogas potential tests were performed using different algal–bacterial biomass with and without thermal pre-treatment as described in the **paper I** at biomass to inoculum ratio of 2:1; this is shown in Figure 11. The inoculum from anaerobic digester of Västerås wastewater treatment plant (Sweden) was acclimatised to 37 ˚C before experiments.

Figure 11. Biogas potential tests.

3.4 Chemical Analysis

3.4.1 Liquid analysis

All the nutrients (total N, total P, total organic carbon (TOC), ammonium, nitrate, nitrite) and wastewater characteristics (COD, TSS, VSS) were analysed using APHA standard methods (American Public Health Association, 1998). Additionally, nutrients were also analysed using ready-made test kits (Dr Lange Test kits) and analytical instruments (ion chromatography and flow injection analyser) according to the APHA standard methods as elucidated in **papers I–V**. In all investigations, analytical instruments were calibrated (R^2=0.99) with freshly prepared standards and reagents during each run.

3.4.2 Gas and light analysis

The biogas pressure was measured using a digital pressure gauge as described in **paper I**. Gases/volatile gases were collected using a 100 µL gas-tight syringe and analysed by gas chromatography using standard calibration of pure gases as described in **paper III**. Light intensity was measured using a PAR meter (**paper I–III**, **V**) or a luminous flux (LUX) meter (**paper IV**).

3.4.3 Pigments analysis

Chlorophyll *a* content was determined using the method of Bellinger and Sigee, (2010) in **papers I**, **II** and **V**. Total chlorophyll (chlorophyll *a*, *b* and *c*) was determined using APHA standard methods in **paper IV** (Clesceri et al., 1998). Pearson correlation was performed to identify the relationship among microbial growth parameters (total oxygen concentration to chlorophyll *a* (**paper I**), biomass to chlorophyll *a* (**paper II**) and also to identify the relationship between dissolved and undissolved nutrients using *Microsoft Excel*.

3.5 Overview of calculations

The HRT was calculated as shown in equation 9, and the SRTs were calculated according to equation 10 (**paper II** and **IV**) and 11 (**paper V**) based on the reactor configuration. The accumulated biogas yield was calculated as presented in Anbalagan (2016). Based on the reactor configurations, the removal efficiencies (REs) were calculated as shown in equations 12 (**paper I** and **II**), 13 (**paper III**) and 14 (**paper IV** and **V**). In addition, nutrient accumulation effect has been considered from recirculation in the inlet in equation 14 (**paper IV**).

$$\text{HRT (d)}: \ \frac{\mathrm{V}}{Q_{in}} \tag{9}$$

$$\text{SRT (d)}: \ \frac{TSS_r * V_r * T_c}{TSS_L * V_L * 24} \tag{10}$$

$$\text{SRT (d)}: \frac{V_r * TSS_r}{(Q_{out} * TSS_{out}) + (Q_{excess} + TSS_{excess})} \tag{11}$$

$$RE\ (\%): \frac{(NU_{in} - NU_{out})}{NU_{in}} * 100 \tag{12}$$

$$RE\ (\%)\text{:}\ \frac{(Q_{in}*NU_{in)}-(Q_{out}*NU_{out})}{Q_{in}*N_{out}}*100 \tag{13}$$

$$RE\ (\%)\ \text{:}\ \frac{((Q_{in}*NU_{in})+(Q_{rec_{in}}*NU_{rec_{in}}))-((Q_{out}*NU_{ou)}+Q_{excess}-NU_{excess})}{((Q_{in}*NU_{in})+(Q_{rec_{in}}*NU_{rec_{in}}))}*100 \tag{14}$$

$$BPR\text{:}\ \frac{BG.Y_1}{BG.Y_{nthday}}*100,\frac{BG.Y_2}{BG.Y_{nthday}}*100\ldots\frac{BG.Y_n}{BG.Y_{nthday}}*100 \tag{15}$$

4 Results and discussion

In this chapter, the relevant results from different papers are presented and evaluated based on the experimental investigation.

4.1 Wastewater characterisation

Four different types of wastewater have been utilised in this study as discussed in the previous chapter. Overall, the wastewaters collected at different times exhibited heterogenic profiles throughout the experimental period as shown in Table 6.

Table 6. Characteristics of different wastewaters during nutrient removal studies in this work

Papers	**Season (Year)**	**Location**	**Type of wastewater (WW)**	**COD, mg L^{-1}**	**TOC, mg L^{-1}**	**TN (or sum of NH_4+NO_2+$_3$), mg L^{-1}**	**TP (or PO_4-P), mg L^{-1}**
I	Autumn, Winter (2014)	Sweden	Raw WW	342±73	164±29	30±7	4.70±0.70
II	Spring, Summer (2015)	Sweden	Pre-treated WW	238±70	-NM-	33±4	7±0.20 (6), 4.50±0.30 (3)
III	Autumn, Winter (2015–2016)	Spain	Centrate	-NM-	-NM- (56)	112±18, 200±68 (700)	4.40±1.80, 7.50±1.30 (36.16)

Papers	**Season (Year)**	**Location**	**Type of wastewater (WW)**	**COD, mg L^{-1}**	**TOC, mg L^{-1}**	**TN (or sum of NH_4+NO_{2+3}), mg L^{-1}**	**TP (or PO_4-P), mg L^{-1}**
IV	Spring, Summer (2017)	USA	Synthetic WW	-	74±9	42±3	6±0.80
V	Autumn, Winter (2016–2017)	Sweden	Raw WW (primary storage tank)	213±92	-NM-	52±8	5.80±1

(**Papers I**, **II**, **IV**, **V** – average composition of influents during the whole experimental investigation; **Paper III** – average composition of 15 and 30% centrate throughout the experiment). The values in the parentheses for **papers II** and **III** are two different external PO_4-P additions and composition of 100 % centrate. NM, not measured. Acetate was used as carbon source in **Paper IV**).

4.2 Microalgal–bacterial biomass

4.2.1 Evaluation of chlorophyll, suspended solids and total oxygen levels.

The influence of process conditions on the chlorophyll *a* content in the biomass has a direct influence on the total oxygen concentration. Therefore, the contribution of chlorophyll *a* in the cultivated TSS has been evaluated in this study. Figure 12 (A–D, primary Y-axis) presents how chlorophyll *a* as a proportion of TSS varies among different **papers** (**I**, **II**, **IV** and **V**) in response to operational variables and wastewater characteristics. Chlorophyll *a* varied relative to TSS with HRT (**paper I**), P addition (**paper II**), operational SRT (i.e. >8 mg g^{-1}, chla:TSS; **papers I** and **V**) and spectral distribution (**paper IV**, stage II). In general, the trend showed a decrease in chlorophyll levels relative to TSS at 2 days HRT due to washout of biomass in comparison to 6 and 4 days HRT in **paper I**. In **paper II**, the levels varied slightly due to different levels of phosphorus at 4 days HRT. However, both cases recorded rapidly settleable flocs during the operation (i.e. based on sludge volume index (**papers I**–**II**).

Noticeably, in **paper IV**, the inclusion of the red-blue spectrum with white LEDs resulted in a significantly higher chlorophyll *a* to TSS ratio than with the white light spectrum, or with red-blue LEDs alone at higher SRTs (>28 d) and lower HRT (1.5 d) (Figure 12 C, Stage II). Finally, the continuous operation showed varying chlorophyll *a* to TSS levels due to bio-flocculation and higher SRT (**paper V**) . For instance, with reduced recirculation of sludge from the settler, chl *a*:TSS was higher than in other conditions due to the reduction of sludge in the main tank (Figure 12 D, **paper V**).

Furthermore, chlorophyll *a* was directly linked to photosynthetic total oxygen in the liquid as shown in Figure 12 A–D (secondary Y−axis). It was noted that the total oxygen levels in the cultivation liquid varied in different groups (**paper I–V**) according to the trend in chlorophyll *a*:TSS. For instance, HRT had a greater influence on total oxygen generation in the liquid during raw wastewater treatment due to washout of biomass (**paper I,** see also biomass trend in next section), whereas the decrease in phosphorus addition (6 to 3 mg L^{-1}) influenced the total oxygen concentration during the treatment of iron flocculated wastewater (**paper II**) as a result of the decrease in chlorophyll *a*:TSS (see also oxygen evolution rate based on light intensity in **paper II**). Further, the lower HRT (1.5 days, **paper IV**) and higher HRT (6 days, **paper V**) conditions showed higher total oxygen concentration in the cultivation liquid at 12–13 ˚C. Therefore, the optimal chlorophyll *a*:TSS ratio is essential for supporting total oxygen behaviour in the reactor; this is also linked to organic compounds removal, as discussed in the next section.

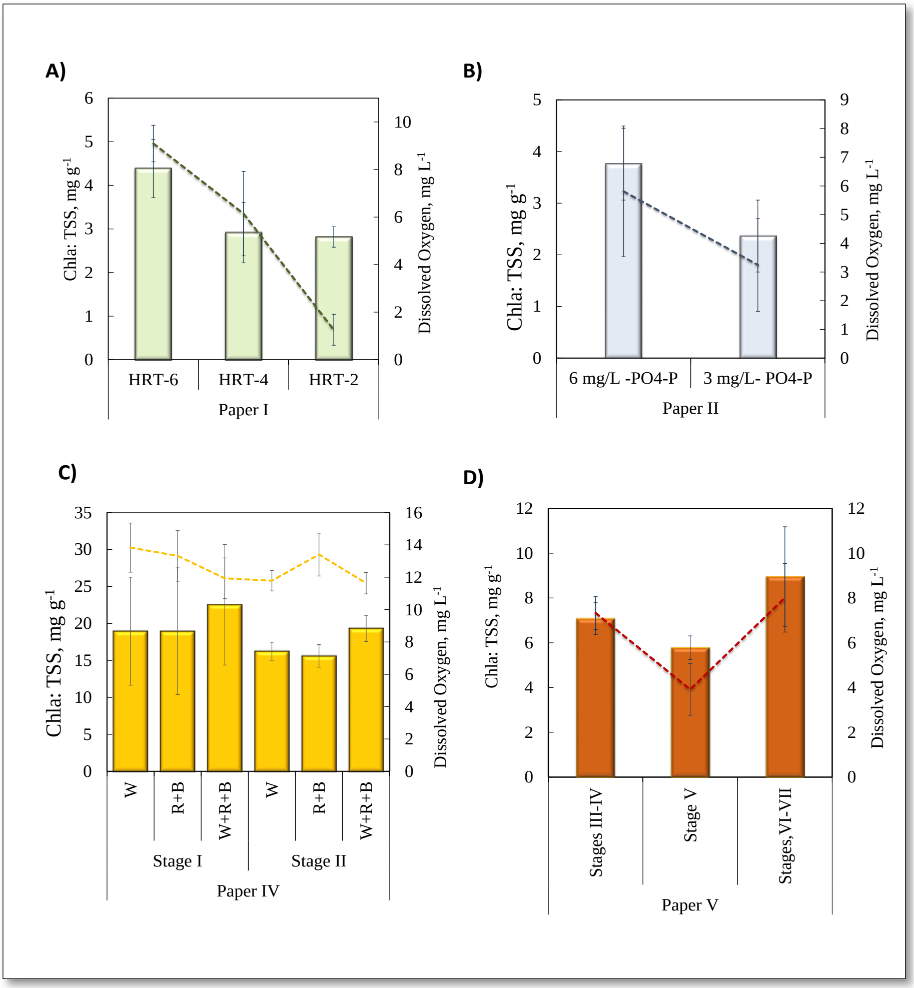

Figure 12. Overview of chlorophyll *a* (chl. *a*) to algal–bacterial suspended solids or total suspended solids (TSS) and total oxygen is represented as dissolved oxygen concentration profile in various studies. Paper I and IV represent average of duplicates reactors. 🟩, 🟦,🟨,🟥 -chla: TSS and --, --, --, -- -dissolved oxygen concentration

In addition, chlorophyll *a* to TSS levels in the effluent are a direct measure of efficient settling characteristics of microalgal–bacterial biomass. For instance, **paper V** showed a chlorophyll *a* to TSS ratio of below 0.01 mg chl. *a* g^{-1} TSS during 152 days of continuous operation (not shown). Gutzeit et al., 2005 and Medina and Neis (2007) report chlorophyll *a* to TSS trends of up to 2.5 mg chl. *a* g^{-1} TSS during the sedimentation process at HRTs of 4 and 2 days, respectively. Thus, the chl. *a* to TSS trend can be considered to ensure the strength of settling characteristics in the sludge. Also, the settling characteristics of algal–bacterial floc could be considered at all operational HRT and SRTs for biopolymer application or for improving settling properties of activated sludge, as noted by Wágner et al. (2016).

4.2.2 Biomass and organic compounds removal

Table 7 shows the biomass profile of different studies and its relevance to organic compounds removal determined as removal efficiencies of TOC, COD and acetate in wastewater. Here, the volumetric biomass productivity and organic compounds removal were used as a performance indicator for sludge production and wastewater treatment in **papers I**, **II**, **IV** and **V**.

As can be seen in **paper I**, stable COD and TOC removal efficiencies were obtained with decreasing biomass concentrations in the chemostat condition (HRT = SRT) in response to the influent (see Table 6). However, the COD removal efficiency exhibited a slight decrease in **paper II** with higher biomass concentrations of between 6 and 3 mg L^{-1} of P addition in comparison to **paper I** (Table 7) due to lower COD levels in the influent (Table 6). Overall, the residual COD and TOC values in both **papers (I** and **II)** are likely to be due to excretion of organic compounds like EPS, which has been linked to floc formation and faster settling properties of the sludge (Cuellar-Bermudez et al., 2017). In addition to this, algal aeration (see total levels) had favourable effects on the removal of COD and TOC removal, as shown in the previous section.

In **paper IV**, TOC removal was stable in all lighting conditions (Table 7). It is likely favoured by higher biomass concentrations at all lighting that demand higher TOC in the medium to enable bacterial oxidation based nitrogen uptake by microalgae. Further, this was supported by complete acetate removal (~99 %) in all lighting conditions regardless of biomass development without external inorganic carbon addition, and with external inorganic carbon addition (Table 7). Notably, Kim et al., (2013) obtained higher daily biomass productivity using white light than with different ratios of red and blue light with *Scenedesmus* sp.

However, the biomass concentration increased or decreased at different stages depending on sludge accumulation (in main tank and in the settler) in various stages in **paper V** (Table 7). This had a significant impact on SRT (>15 d) of the continuous photobioreactor operation. On the other hand, the COD removal remained stable regardless of varying recirculation and retention of sludge in the main tank.

In general, regardless of the biomass concentration in the photobioreactor, organic compounds removal was stable. Biomass concentration had a positive influence on bio-flocculation properties (i.e. based on SVI and effluent biomass concentration), which resulted in higher SRT and biomass accumulation during different operational periods. In addition, Silva et al., (2016) suggest lower recirculation of sludge or higher sludge removal to avoid low biomass productivity in a flat panel photobioreactor. Therefore, it is essential to operate the photobioreactor with optimal sludge recirculation, which is less likely to hinder biomass production and nutrient removal in the long run. Further, additional measures like maintaining SRT below 10 days in closed membrane photobioreactors may delay instable photobioreactor operation and deposition of sludge in deep reactor setups (Honda et al., 2012). Therefore, long-term operating conditions of a continuous sludge cultivation system remain to be determined; lower sludge internal recirculation or no recirculation may be needed to maintain nutrient removal, as presented in the next section.

Table 7. Biomass and organic compounds removal.

Papers	COD (%)	TOC (%)	TSS (mg L^{-1})
I	77 ± 10 **(HRT-6d)** 80 ± 03 **(HRT-4d)** 76 ± 09 **(HRT-2d)**	81 ± 04 72 ± 3 72 ± 5	242 ± 17 134 ± 13 98 ± 18
II	63 ± 10 **(6 mg PO_4-P L^{-1})** 78 ± 01 **(4 mg PO_4-P L^{-1})**	-	377 ± 02 408 ± 03
IVa	-	91 ± 03 (W) 92 ± 03 (R+B) 93 ± 03 (W+R+B)	936±353 711±239 623±233
IVb	-	93 ± 06 90 ± 07 89 ± 07	1343 ± 71 1073 ± 39 1025 ± 79
V	81 ± 09 **(Stage III-IV)** 85 ± 05 **(Stage V)** 81 ± 08 **(Stage VI-VII)**	-	545 ± 139 660 ± 47 498 ± 69

W-white LED, R+B-red and blue LED

4.2.3 Biomass and inorganic carbon removal

Figure 13 shows a specific case (**paper III**) in which volumetric biomass productivity (primary Y-axis) and inorganic carbon removal (secondary Y-axis) were used as a performance indicator in an enclosed photobioreactor (in

= out) to maximise the algal biomass based on N removal (see also next section for N and P removal).

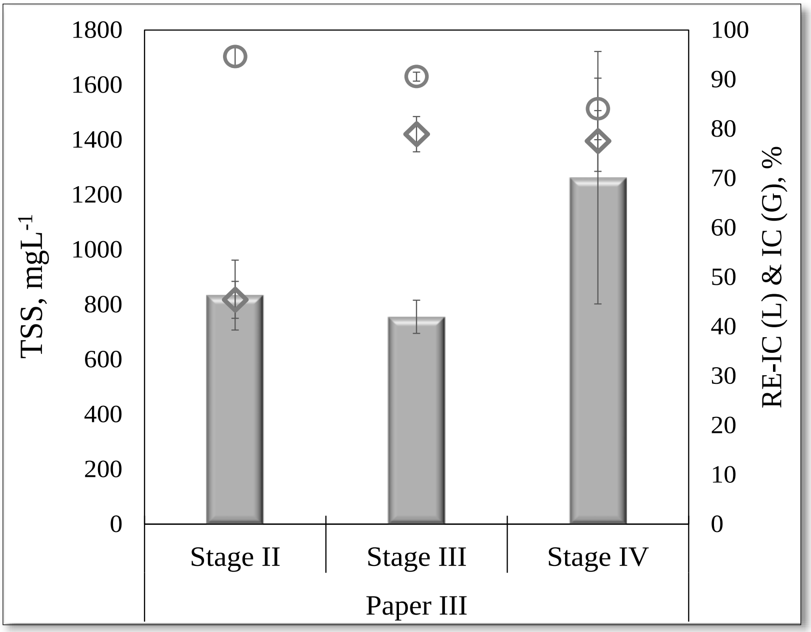

Figure 13. Total suspended solids (TSS, ■) and inorganic carbon removal from the liquid (○) and gas phase (◊) during wastewater treatment.

Stable biomass production was seen at different stages of the experimental investigation in the presence of external CO_2 using 15 % centrate as shown in stage II and III (Figure 13). The removal of CO_2 was higher in the liquid (94%) than the gas phase (45%) in stage II due to the lower liquid to gas recirculatio ratio (L/G = 1) maintained in the vertical absorption column. Conversely, th removal increased in liquid (90%) and gas phase (79%), with an increase i L/G ratio of 15 in stage III due to higher mass transfer in the liquid as a resul of photosynthetic growth. In both stages (II and III), the biomas concentrations obtained were in the range of 834 ± 127 an 755 ± 60 mgL^{-1} TSS, though a slight variation was observed with centrate collection. Subsequently, the increment in centrate concentration from 15% t 30% in stage IV resulted in a slight decrease in CO_2 removal efficiency of the liquid phase (84%) relative to the gas phase (77%). However, the biomas concentration (1262 mgL^{-1} TSS) exhibited wide fluctuations in comparison to other stages due to higher nutrient concentration in the centrate due t seasonal variation in stage IV.

As presented in **paper III**, the application of effective inorganic carbon supplementation through a vertical absorption column can be implemented to achieve continuous biomass production coupled with efficient CO_2 removal during wastewater treatment, to support the mass transfer of CO_2 in the cultivation medium. As a result, total oxygen concentration in the medium above saturation level (i.e. more than 10 mg L^{-1}) at L/G ratios of 1 and 15 were observed during this study. Additionally, the pH was slightly alkaline (~pH 8) at L/G of 1 and neutral at L/G of 15. However, the total oxygen levels were lower (~3–6 mg L^{-1}) at a higher centrate concentration (30%) in neutral pH conditions (See also Table 6).

4.3 Bionutrient removal

Here residual N and P concentration in the effluent (primary Y-axis) and removal efficiencies (secondary Y-axis) were used to predict the performance of the process in different studies, as shown in Figure 14 (A–F) and 15 (G–J). In general, N and P removal was stable in all stages with complete nitrification in different studies.

4.3.1 Nitrogen removal

In **papers I** and **II**, proof of concept studies supported stable nitrogen removal efficiencies in raw and pre-treated wastewater at different HRTs (Figure 14, A and C). Additionally, using a HRT of 4 (64 ± 16%N removal) and 6 days (81 ± 4%) had a more favourable effect on nitrogen removals than a HRT of 2 (39 ± 18%) days of HRT in **paper I**. The residual concentration increased with increase in HRT (6, 4 and 2 d) as follows: 6 ± 2, 9 ± 4 and 16 ± 4 mg N L^{-1}, respectively (Figure 14 A). Hence, 4 days HRT and 6.75 days SRT were used in **paper II** to evaluate the nitrogen removal based on the previous study with the addition of 6 and 3 mg P L^{-1} in iron-flocculated wastewater. However, higher removal efficiencies (87–93%) were obtained with lower residual nitrogen concentration (around 2–10 mg L^{-1}) (Figure 14 C). In both cases, the pH of the system was maintained at 7.5–7.8; in this condition, the ammonium volatilisation had less impact on the nitrogen removal.

Further, in **paper III**, CO_2 supply based on vertical tubular columns at L/G ratios of 1 and 15 increased availability of inorganic carbon in the medium (15% centrate, stage II and III). As a result, the removal efficiency of nitrogen increased in stages II and III at 15% centrate load. The removal efficiency then decreased with the increase in centrate concentration from 15 to 30% of centrate (stage IV). The removal efficiencies were recorded at 92% in stages II and III with nitrogen levels of below 10 mg L^{-1} (nitrate levels of below

2 mg L^{-1}) suggesting complete nitrification at neutral pH conditions (~7). The removal efficiency of N was 43–84% with residual nitrogen levels of 50–140 mg L^{-1} in the effluent (Figure 15 F). By contrast, Ledda et al., (2015) report similar results in an enclosed tubular photobioreactor in outdoor lighting without an absorption column and experimentally confirmed that P limitation at a higher centrate concentration (30% centrate) is likely to be possible.

Different light spectra were considered to determine the influence of N uptake efficiency during the microalgal–bacterial process using a mixed community composed of pure colonies as described in **paper IV** (Figure 15 G). In the first stage, a stable removal efficiency was observed with residual TN concentrations in the range of 24–21 mg N L^{-1} using combined white and red−blue light (Figure 15 G). The higher residual concentration observed in the reactors was most likely due to remaining TN left with every semi-continuous cycle; this can be compared to recirculation of a continuous system (as seen in paper V). In the second stage, the addition of bicarbonate had less effect on the N removal efficiency at higher SRT due to slower nitrification at elevated pH. The residual concentration observed in white light increased slightly, however, the N levels in the blue-red light mixture remained in a similar range. Conversely, in both stages, the removal efficiency was slightly unstable with mixed lighting (~48–57%) in comparison to other lighting groups as indicated by residual N concentrations, 28–24 mg N L^{-1} (Figure 15 G). The nitrite-nitrate levels in the reactor were recorded below 2 mg L^{-1}. Thus, the uncontrolled pH (pH>8) influenced the nitrification-based N uptake at a retention time of 1.5 d (see also feed-backward nitrogen uptake as described in Figure 8). This was confirmed by the residual ammonium concentration, as discussed in **paper IV**.

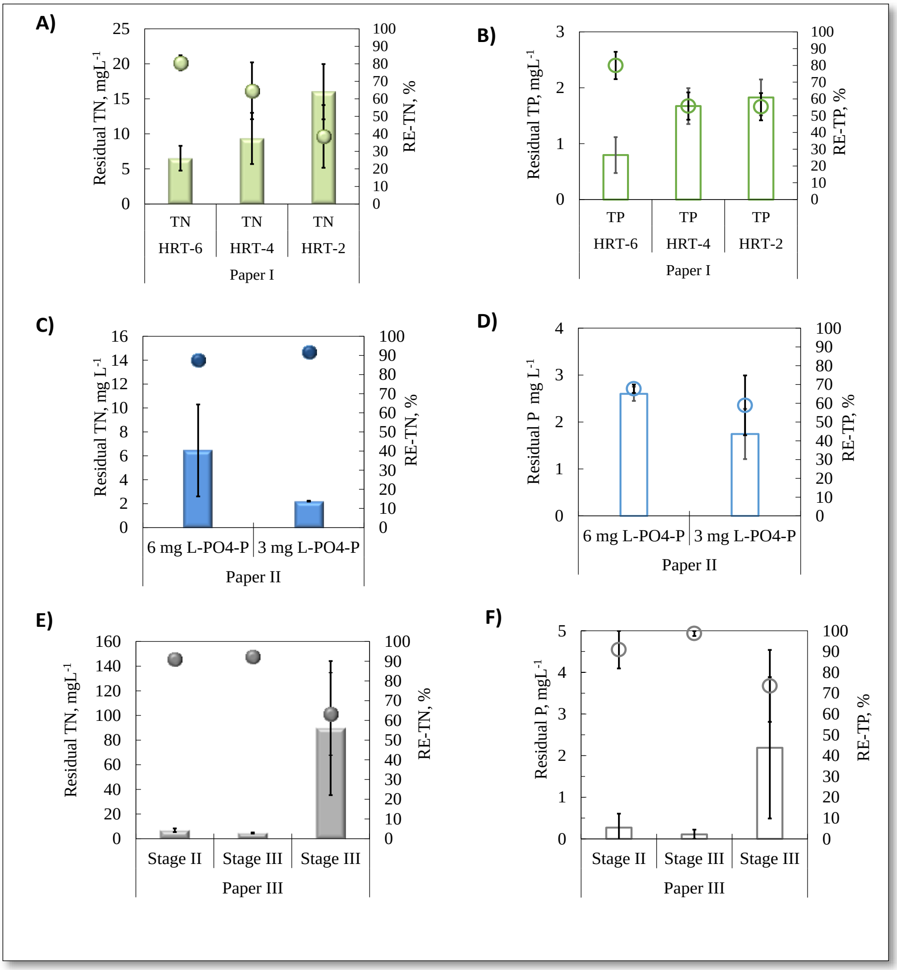

Figure 14. Effluent nutrients and removal efficiencies from Papers I–III. (■, ■,■ – represents residual total nitrogen; □, □, □ – represents residual total phosphorus concentration; ●, ●, ● – represents TN removal efficiency; ○, ○, ○ – TP removal efficiency)

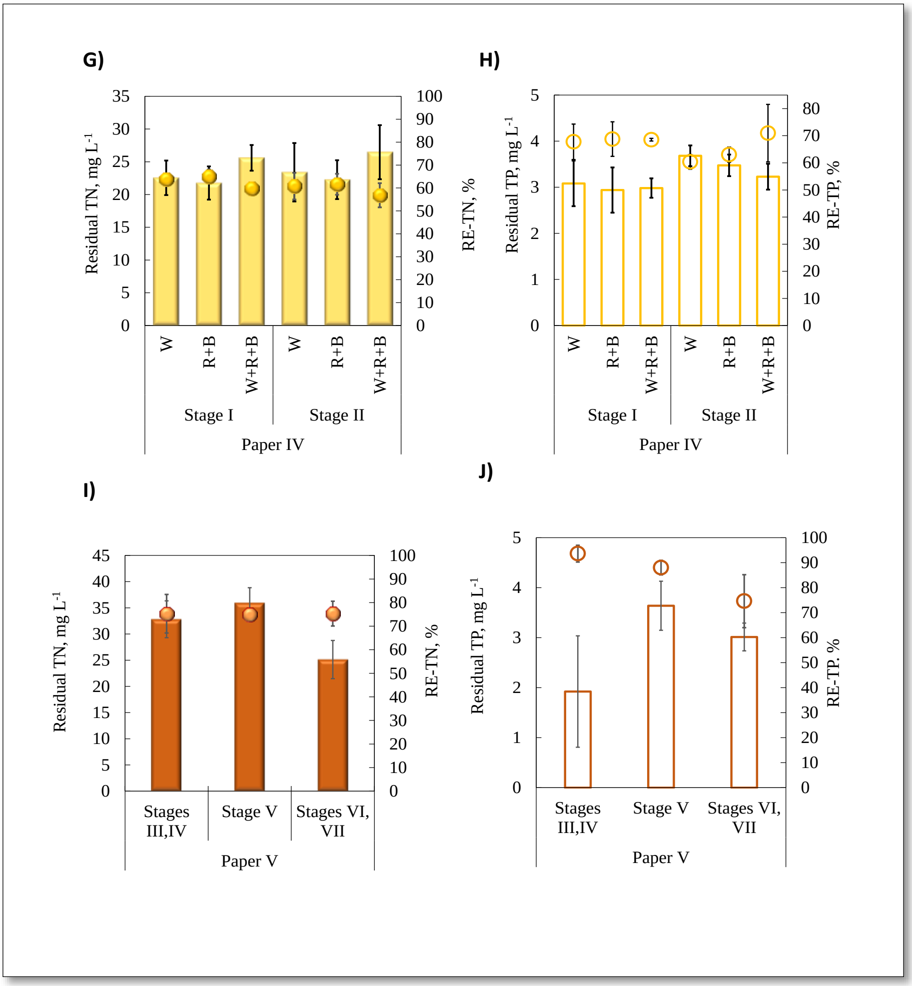

Figure 15. Effluent nutrients and removal efficiencies from papers IV-V. (■, ■ – represents residual total nitrogen; □, □ – represents residual total phosphorus concentration; ●, ● – represents TN removal efficiency; ○, ○ – TP removal efficiency).

In the final investigation, in **paper V**, the algal–bacterial process under activated sludge configuration was considered (Figure 15 I), to verify the effect of sludge recirculation on nitrogen removal. At higher recirculation rate, N accumulation increased (>30 mg N L^{-1}), regardless of an increased alkalinity or external CO_2 addition (Figure 15 I). A two-fold decrease in sludge recirculation also had no effect on the nitrate accumulation levels, with only a slight reduction due to the reduction of N load (26 ± 2 mg N L^{-1}). This was confirmed with similar removal efficiency of different stages (i.e. ~75%

removal efficiency at stages, III & IV, V, and VI & VII). Further, the system needed a longer adaptation time to reach stable conditions due to faster settling characteristics. However, a complete ammonium removal was observed, indicating complete nitrification at 6 days HRT with a significant decrease in pH (pH<7) in comparison to the photo-sequencing reactor (**paper IV**). In addition, the process behaviour in **papers IV** and **V** have been hypothesised to occur as follows: recirculated nitrite-nitrate acts as a signal for nitrate-based growth of microalgae and slower nitrification due to competition for inorganic carbon source. As suggested by García et al., (2017), incorporation of the anoxic zone before the microalgal process or absorption column can circumvent the reduction of nitrite-nitrate accumulation. Zambrano and Nehrenheim (2017) also suggest that there is an accumulation of nitrate during mathematical simulation of the algal–bacterial model at higher SRT and dilution of light intensity.

4.3.2 Phosphorus removal

In all studies, microalgal based P removal was the leading cause of P removal as obtained in Figure 14 (B, D, F) and 15 (H, J).

In **paper, I** (Figure 14 B), the P removal efficiency increased with the increase in HRT, more than 64% at 6 days and more than 50% at 4 and 2 days, with the natural variation of influent P (see also Table 6). The resulting final concentrations of P were 0.70 ± 0.50, 1.7 ± 0.20 and 1.8 ± 0.20 mg P L^{-1}, respectively (Figure 14 B). However, the residual concentration of P was high in the presence of high or low P after addition of iron sulphate for flocculation at 4 days HRT in **paper II**. At high P levels in wastewater, uptake of excess P resulted in residual P of ~2.6 mg P L^{-1} in the effluent and a slight decrease in residual P with a two-fold decrease in P concentration as described in **paper II (**Figure 14**)**. In both cases (**paper I** and **II**), the removal of P obtained was in the similar range.

Further, almost complete removal of P was observed at a diluted centrate concentration of 15 % at 7 days HRT, as presented in **paper III**. This result can be positively related to higher C and N removal as observed in previous sections. By contrast, the system exhibited unstable operation due to higher centrate load (30% centrate), which resulted in lower P efficency (~55%) with residual P above 3 mg P L^{-1} (Figure 15 F).

On the other hand, the lighting had less impact on P removal efficiency at 1.5 d HRT either in the absence (stage I) or presence of inorganic carbon (stage II) as shown in Figure 15 H. The residual P was in the range of 2.5–3 mg P L^{-1} in stage I and 3.2–4 mg P L^{-1}in stage II. Higher observed P levels are likely to vary due to P remaining in the reactor at each cycle and higher SRT (>28 d) regardless of daily sludge wastage. Altogether, the P removal efficiency was in the range of 55–70 % in both stages. Also, the pH of the system was higher than 8 which had less effect on precipitation of P at colder conditions.

TP levels recorded in **paper IV** were similar to those in **paper V** except in stages III and IV (1–3 mg P L^{-1}, ~93% removal) due to the influent concentration of the wastewater. Residual P in stages V–VII were above 3 mg L^{-1} with removal efficiencies of ~88% in stage V and 60–80 % in stages VI and VII. However, the higher removal efficiencies achieved in this study was due to higher recirculation load in the single stage activated sludge configuration.

4.4 Biogas kinetics

Biogas kinetics or biogas production rate of algal–bacterial biomass obtained from different HRTs is shown in Figure 16. Most of the accumulated biogas synthesised was obtained between 9 and 11 days (>80% of accumulated biogas) in the case of thermally treated sludge. By contrast, untreated biomass showed delayed gas production, recording similar levels (>80% of accumulated biogas) after 13 days. Though thermal pre-treatment resulted in faster kinetics, the biomass at 6, 4 and 2 days HRT did not show any significant improvement in the biogas yield. The accumulated gas yields following 25 days of digestion at 6 and 4 days were 268 ± 2 and 258 ± 11 mL gVS^{-1}, and the corresponding yields after the treatment were 263 ± 10 and 266 ± 16 mL gVS^{-1}, respectively. However, the obtained biomass concentration had a significant effect on the biogas yield regardless of pre-treatment. For instance, 2 days HRT showed higher biogas yield in comparison to 6 and 4 days HRT. The biogas yields at 2 days HRT were 349 ± 10 (untreated) and 308 ± 19 (treated) mL gVS^{-1}. The reason for lower gas production following the thermal pre-treatment could be lower retention of algae in the sludge at an SRT of 2 days or degradation of volatile substances during hygenisation of the sludge. In this context, Ge et al., 2013 found higher gas yields in an aerobic activated sludge process by maintaining low SRTs. Overall, other potential pre-treatments remain to be determined in the long run. Furthermore, there may be inhibitory effects from higher ammonium content of the algal sludge, and growth inhibition may be reduced by the co-digestion concept as suggested by Olsson et al. (2014).

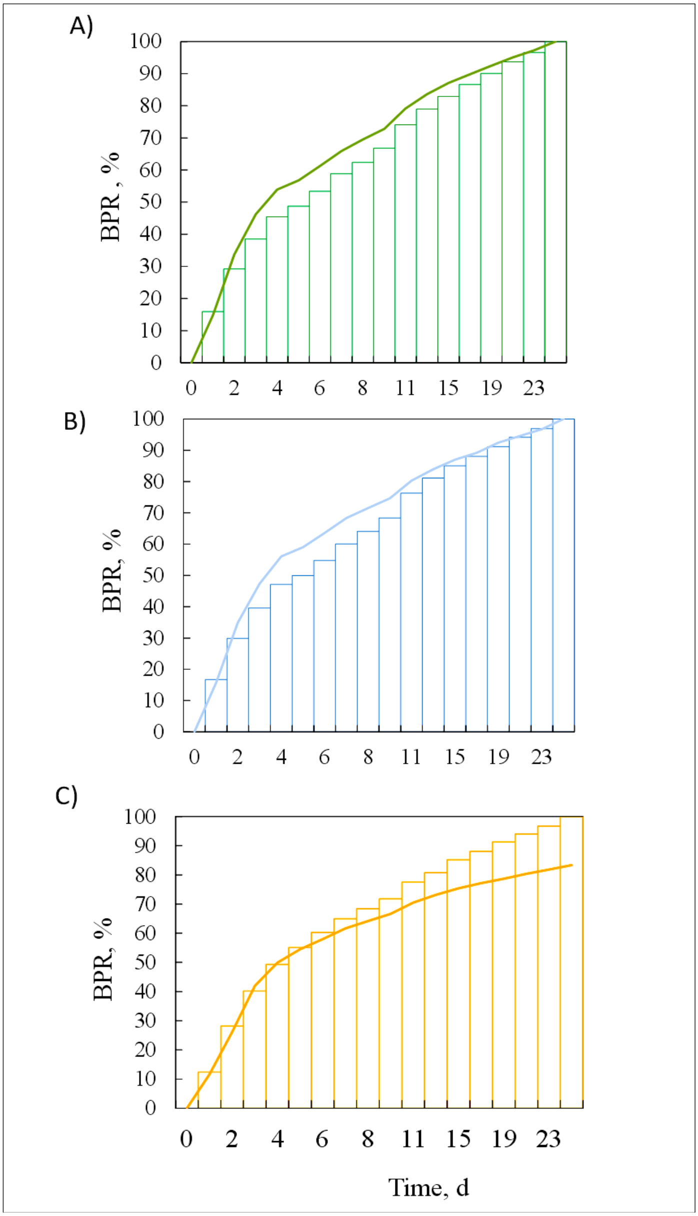

Figure 16. Biogas production rate (BPR in %) at various biomass during anaerobic monodigestion in paper I. □, □, □ – 6, 4 and 2 days HRT (untreated) and —, —, — – 6, 4, 2 days HRT (thermal pre-treatment).

5 Conclusions

The experimental investigations presented in this thesis cover only the prevailing conditions in the wastewater environment and the feasibility of a microalgal–bacterial process utilising native microbial communities and microbial models in the prevailing environment. In conclusion, the following answers have been obtained regarding the following four challenges indicated earlier,

RC 1. What are the initial conditions for the operation of the microalgal–bacterial process?

Paper I: When considering the electricity consumption during the algal cultivation, 6 and 4 days are shown to be effective at low light intensity over 2 days HRT in Swedish summer conditions.
In addition, thermal pre-treatment (or hygenisation of sludge at 120 ˚C) of the collected biomass from the HRT study demonstrated faster biogas kinetics with sludge at effective HRT (6 and 4 days) than with untreated sludge under mesophilic condition. However, the final biogas yields were similar regardless of pre-treatments, suggesting consideration of other treatment option to improve the accessibility of the microalgal cells for enhanced biogas production.

RC 2. What is the main limitation of photosynthetic nutrient removal in the local environment?

Paper II: In local conditions, the iron flocculation step limits algal–bacterial photosynthesis and N removal in wastewater. Thus, the external addition of P to the pre-treated wastewater favoured the removal of nitrogen. As defined in **RC 1**, 4 days of HRT could be an effective condition for N removal. However, in both cases, SRT is maintained below 7 days. Therefore, this study and the previous study suggest that biomass concentration has a direct impact on N and P removal due to the low light intensity in the photobioreactor in summer conditions.

Altogether (**paper I** and **II**), the COD or TOC removal suggests effective organic compound removal using algal–bacterial oxygenation without external aeration. Additionally, the faster settling of sludge favours avoiding expensive downstream processes for easier separation of solid and wastewater.

RC 3. Can nitrogen removal be achieved by efficient CO_2 addition?

Paper III: Efficient supply of CO_2 promotes a high removal efficiency of N and P in the centrate in an enclosed bioreactor coupled to an absorption column. Therefore, this type of system is recommended to improve the C to N ratio of the photobioreactor and utilise CO_2 free of cost from regional resources in the vicinity of the wastewater treatment site by utilising native microalgal communities. In regional conditions, 300 to 330 $m^3$of centrate is produced every day from the anaerobic digester at Uppsalavatten and Västerås wastewater treatment plant (Marcin and Mucha, 2015). Hence, this could be a vital nutrient source for microalgal cultivation and carbon removal at the treatment site itself and partly reduce the influent load of the wastewater treatment.

RC 4. How light spectrum and temperature can influence the microalgae-bacterial photobioreactor at high sludge retention?

Papers IV and **V**: The influence of combined red and blue light and white light had similar effects on nutrient removal efficiencies in the study in **paper IV**. In addition, this study suggests higher biomass in the system can be maintained with good settling properties at HRT of below 2 days. However, the depletion of organic carbon and higher sludge wastage during the process remains to be considered during this process. This can be avoided by supplementing external CO_2 into the process to support the N uptake as seen in the case of **RC 3** and to avoid unfavourable pH during the treatment.

As shown in **paper V**, the continuous process (6 days of HRT) suggests faster settling properties, supporting higher SRT which can bring increased deterioration of biomass in the system and turbidity, which is subject to further investigation on the optimal design of a connected settler. In addition, higher liquid to sludge ratio in the recirculated sludge adds more complexity to the understanding of the nutrient removal mechanism by increasing nitrogen load or accumulation of nitrogen into the cultivation tank. Altogether, temperature equivalent to that of wastewater (12–13˚C) in **paper IV** and **V** did not show any impact on biomass cultivation in either case.

In sum, regardless of whether the experiment is performed under semi-continuous or continuous conditions, the single-stage algal–bacterial process is able to achieve stable oxygenation in cold wastewater conditions; this is an important finding that sheds light on algal technologies in the Nordic conditions for biorefinery and biofuel development. However, lighting plays a crucial role, and can increase the energy consumption during the process; further, this requirement can be reduced by reducing the load on the photosynthetic process by incorporating a separate bacterial process or a two-stage process with the addition of a CO_2 absorption column by alteration of the photobioreactor design.

6 Future directions

The thesis has shown how microalgae could be implemented as mainstream or side stream during wastewater treatment with the possibility of converting carbon dioxide to useful biomass and oxygen. However, other related possibilities remain to be considered as follows:

- The unstable SRTs (i.e. due to faster gravity settling) experienced are an important operational parameter in this study, and influence the process performance. Therefore, more research should be conducted to overcome the nitrogen accumulation due to SRT and to understand the nutrient mechanisms by avoiding cone-shaped settler designs or otherwise improving designs for capturing and recirculating biomass. The applicability of a process with lower sludge recirculation remains to be determined in future photobioreactor designs (a simplified overview of event-based process improvements addressed in the whole study is shown in supplementary Figure S in the Annexes.).
- Faster settling of biomass suggests that other reactor designs such as a contact stabilisation reactor or reactor in series could be more favourable for avoiding sludge-related nitrogen accumulation (i.e. deterioration) in the cultivation tank. On the other hand, this research demonstrates good settling ability of the algal–bacterial sludge. In local conditions, polymer is used to thicken the sludge. The synergy of algal sludge on different types of sludge remains to be determined during sedimentation of conventional or modified activated sludge processes.
- Current research also shows the applicability of concentrated PAR radiation such as blue and red wavelengths for the system, which favour part of the filtered light energy for electricity generation from sunlight. This could reduce electricity generation and utilisation during the process in the peak summer conditions. However, the

scope of electricity generation is somewhat remote from the present objectives.

- In terms of nutrient removal, identifying the activity of enzymes involved in the conversion of ammonium, nitrite and nitrate (nitrification and algal assimilation) during the different operational shifts may elucidate the removal mechanisms. In addition, the factors that cause or affect nutrient conversion rates could be more easily identified, which will be valuable for improving the microalgal nutrient removal in bioreactors. Further, increased hydraulic retention time can favour degradation of emerging contaminants such as pharmaceuticals and volatile organic compounds present in the wastewater (Norvill, 2016).

Overall, there is also a great need for studies to estimate the industrialisation potential for the use of microalgae as the main medium for wastewater treatment plants.

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Glossary

Anoxic: A respiration process taking place in the absence of molecular oxygen by bacteria or archea. In nature, anoxic bacteria prefer nitrate instead of oxygen to carry out respiration.

Aerobic granular sludge: Granules formed from activated sludge in a bubble column with high settling properties. The granule is composed of four zones: nitrifiers (oxidising ammonia bacteria), denitrifiers (oxidising nitrate bacteria), phosphate accumulating bacteria and glycogen accumulating bacteria.

Anaerobic ammonium oxidation (anammox): The conversion of ammonium to nitrite and then to di-nitrogen under anaerobic conditions is referred to as the anammox process; it is performed by anamox bacteria (*Candidatus Brocadia anamoxidans*).

Activated sludge: A complex entangled portion of wastewater, which is composed of bacteria, archaea and protozoa as dominant microbes along with other organisms. This portion is responsible for the conversion of organic compounds or COD to CO_2 and oxidation of ammonium and nitrate during activated sludge process of wastewater treatment.

Adenosine tri phosphate (ATP): ATP is referred to as the "energy currency of life". ATP is a nucleoside tri phosphate that transports chemical energy required for the functioning of the cells.

Biogas: The gas mixture (methane, carbon dioxide, hydrogen sulphide and other trace gases) obtained during the breakdown of organic compounds in the absence of air or oxygen in a closed container. For instances, cow rumen contains methanogens which can potentially release biogas that causes global warming.

Biogas/bio-methane potential tests: A closed bottle assay used to determine the accumulated biogas or bio-methane per gram of volatile solids at a certain period during the anaerobic digestion. This is a batch operation.

Batch operation: A closed operation; the wastewater/sludge is only added at the beginning and not during the wastewater treatment/anaerobic digestion.

Completely autotrophic nitrogen removal over nitrate (CANON): The autotrophic conversion of ammonium to dinitrogen under oxygen limitation by ammonium oxidising bacteria and nitrite oxidising bacteria.

Fed-batch operation: The fed batch operation is a semi continuous operation where the wastewater is fed into the bioreactor/vessel for nutrient supplementation intermittently and to maintain the retention time.

Nicotinamide adenine di phosphate (NADP/H): NADP is a reducing agent for most of the biosynthetic reactions, e.g. during Calvin’s cycle (i.e. assimilation of carbohydrate). NADP refers to oxidised state (released during Calvin’s cycle), and NADPH refers to the reduced state (generated during photosynthesis) the forms during the the light and dark reactions or oxidation-reduction reactions.

Chemical oxygen demand (COD): The oxygen required to degrade (oxidise) the pollutants entirely in wastewater is measured during this step. The COD procedure is performed to indirectly identify the suspended and dissolved substances (both organic and inorganic form) present in wastewater. It is always expressed in mg L^{-1} of oxygen.

Greenhouse gases: Gases that absorb, accumulate or trap heat energy in the earth’s atmosphere instead of allowing it to radiate out to space (the greenhouse gas effect works on a similar principle to the greehouses that we use for growing plants). This results in heating of earths atmosphere by increasing global temperature.

High rate algal ponds (HRAPs): Shallow, open raceway ponds that have been used for municipal and agricultural wastewater treatment using microalgae (the depth varies from 0.1–0.3 metres).

Hydraulic retention time/residence time: The average length of time that wastewater remains in a storage unit or a treatment unit.

Light emitting diodes (LEDs): Semiconductor devices that emit monochromatic light when an electric current passes through them. Commercially available LEDs have wavelengths between red (650 nm) and blue-violet (480–400 nm).

Methane (CH_4): A greenhouse gas generated from oil and gas plants, animal digestion and wetlands. It has a greenhouse gas potency 28 times that of carbon dioxide.

Dinitrogen monooxide (N_2O): Also called nitrous oxide or laughing gas.

Generated from agriculture, fossil fuel combustion and wastewater management. It is also used as a propellant and anesthetic, and as oxidisers in rocker motors and internal combustion engines.

Personal equivalent (p.e.): One personal equivalent is defined as the load of organic compounds that are biodegradable. Therefore, 1 p.e. is equivalent to biochemical oxygen demand (BOD_5) of 60 g of oxygen per day.

Polymers: Used to coagulate the suspended biomass in the treated wastewater. Polymers are organic molecules made up of either positively (cationic) or negatively (anionic) charged chains of monomers.

Pigments: Proteins or substances that absorb light in the visible range of the electromagnetic spectrum.

Photosynthetic active radiation (PAR): For photosynthesis, plants (including algae) require light of wavelength between 350 and 750 nm from the sun or artificial light. The radiation in this range of wavelengths is called photosynthetic active radiation. It is expressed in photosynthetic photon flux density (PPFD) as µmol m^{-2} s^{-1} or µEinstein m^{-2} s^{-1} (i.e. the micromoles of photon incident on a surface of 1 m^2 per second).

pKa: An index used to express the strength of acids. It is defined as the negative logarithm of the acid ionisation constant. The larger the value of pKa, the weaker the acid.

Sludge: Thick, soft, wet mud or a similar viscous mixture of liquid and solid components, especially the product of an industrial or refining process (*definition from the Oxford English Dictionary*).

Sludge retention time (SRT): The average time that sludge resides in the system.

Sludge volume index (SVI): The sludge volume index is used to evaluate the settling properties of sludge, if the sludge volume index is above 150 mL g^{-1} it is considered as weak sludge.

Total organic carbon (TOC): The total organic carbon refer to amount of organic carbon present in the organic compounds in the water. It is widely used to estimate wastewater quality.

Wastewater: Water that has been released from households and business activities (*definition from the Oxford English Dictionary*).

Annex

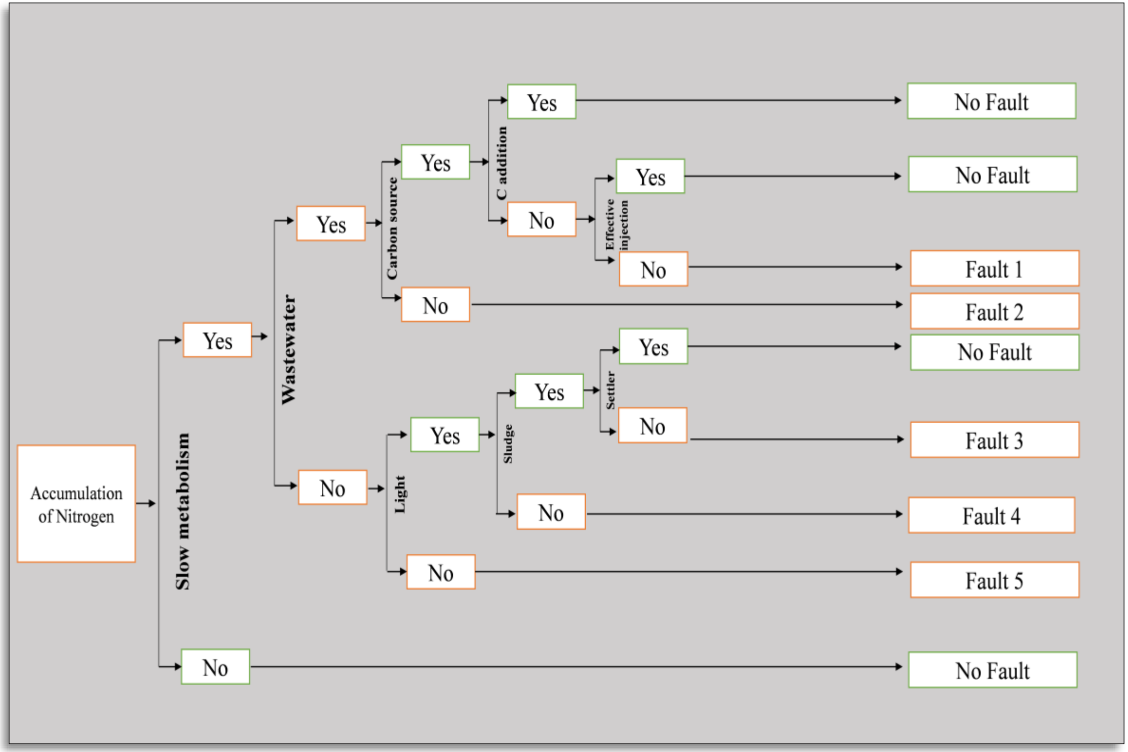

Figure S1. Event-based fault location for a photobioreactor based on one specific issue. Here nitrogen accumulation as a result of metobolism can be avoided by the right actions, based on operational events by asking ‘yes’ or ‘no’ questions. Faults (orange boxes) represent a slow process and no fault (green boxes) represents a recovered process or no action needed. Faults: 1 (conneceted to quality of wastewater or wastewater storage), 2 (unbalanced wastewater, e.g. iron flocculation step), 3 (clogging or sludge bulking), 4 (higher sludge age in the tank) and 5 (higher light requirement).