INDIGENOUS MICROALGAE-ACTIVATED SLUDGE CULTIVATION SYSTEM FOR WASTEWATER TREATMENT

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To my mother
Abstract

“Everything is everywhere, but the environment selects the best duo”; natural selection holds the key to the relationship between the micro-algal communities in Lake Mälaren and the activated sludge organisms in the municipal wastewater of Västerås. Interest in microalgae-based activated sludge wastewater treatment has been increasing in recent decades as a way to meet the demand for sustainable wastewater treatment. Bacteria assisted microalgae cultivation is vital for reducing the emission of greenhouse gases (GHG) in the long term and for the prevention of excess biological and chemical sludge in conventional wastewater treatment sites.

This licentiate thesis focuses on process parameters such as light intensity, retention time and nutrient composition and the influence of micro-algal activated sludge (MAAS) on waste water treatment efficiency. The laboratory investigations will later be used to design a pilot scale approach.

Results from a semi-continuous MAAS process using raw wastewater showed combined removal of nitrogen (N) and phosphorus (P) at 2, 4 and 6 days of residence time. The removal of P was stable and a maximum removal efficiency from untreated wastewater of 50–65 % was achieved. The average N removal efficiencies at 6, 4 and 2 days of residence time were 82, 65 and 39 % respectively. In general, the removal of nutrients increased as expected with increased residence time. In addition, the MAAS sampled at different residence times showed a high biogas potential during batch tests. Most importantly, no lag phase was observed during the anaerobic mono-digestion.

Addition of P to the iron flocculated wastewater (treated wastewater) increased the N removal efficiency to 88 and 92 % at 4 days of residence time. Overall, the consumption of iron will be reduced in the future by using the MAAS process. The flocculation step may also be shifted to after the MAAS process. This will retain the P that increases N removal in the MAAS process and thus also helping to reduce chemical sludge.

Photosynthetic active radiation studies showed that the required light intensity increases with the concentration of biomass in a PBR operated at 14°C (water temperature). The MAAS floc is key for efficient utilisation of light and oxygen synthesis. However, the evaluation of the process needs to be verified by comparing continuous and discontinuous (i.e. intermittent lighting hours) artificial lighting conditions. This will enable the safe discharge of wastewater with minimal power consumption.
Additionally, rapid settleability of the MAAS was observed in all the semi-continuous studies. Therefore, expensive harvesting techniques can be avoided with the help of MAAS.

The experiments described here only cover laboratory operation, and the results have to be verified in pilot scale operation in prevailing Swedish weather conditions. The primary focus will be to achieve the discharge limits set by Mälarenergi AB (Nitrogen: $<15 \text{ mg L}^{-1}$; Phosphorous: $<0.5 \text{ mg L}^{-1}$) with minimal residence time.
Municipal wastewater is mainly composed of water containing anthropogenic wastes that are rich in nutrients such as carbon, nitrogen and phosphorous. The cost of biological treatment of wastewater is increasing globally due to the population growth in urban areas. In general, the activated sludge (AS) process is a biological nutrient removal process used in wastewater treatment plants (WWTPs). The AS is composed of different microorganisms among which bacteria play a crucial role in wastewater treatment (WWT). During the process, air is bubbled through the medium to supply oxygen, and methanol is added to improve removal of nitrogen, which is released as a gas. Phosphorous is removed using chemical precipitation. The current process requires electrical energy, precipitation chemicals and handling of excess sludge, and it emits carbon dioxide \((\text{CO}_2)\) as a by-product. This process has been used in WWTPs since 1914, although numerous modifications have been implemented to meet the stringent regulations in the European Union and other locations.

Microalgae are microorganisms that, like plants, also perform photosynthesis. They are green and reproduce quickly using available nutrients (nitrogen and phosphorous) and \(\text{CO}_2\) from their environment in the presence of light. As a result of photosynthesis, oxygen is released as waste gas. The oxygen synthesised during this process can be utilised by the AS bacteria, resulting in the microalgae activated sludge (MAAS) process. The main advantage of this process is the combined removal of nutrients from the aqueous phase.

The goal of this research is to implement indigenous microalgae cultivation in the activated sludge process to consume \(\text{CO}_2\) and recover nutrients from wastewater. This study was performed to improve the understanding of processes such as light utilisation, nutrient removal and recovery of biomass from wastewater in closed photo-bioreactors. Photo-bioreactors are vessels where cultivation is carried out in the presence of light. In this study, initial experiments investigate the influence of the light spectrum on photosynthetic growth in micro-algal cultivation. This is followed by a look at operational challenges of the microalgae cultivation during the AS process. Experiments are then performed on the process in the photo-bioreactors with different wastewater treatment times. The results show that 2–6 days of treatment can be used to reduce nutrients in wastewater if the process is optimised further. Nutrient ratio is also analysed for the availability for micro-algal growth. The recovered MAAS after the wastewater treatment subjected to anaerobic mono digestion showed biogas yields of about 60–80% within 5 to 9 days.
The experimental verification of chemically precipitated wastewater shows that phosphorus was limiting for micro-algal growth. Additionally, the optimal oxygen supply as a function of light response was verified for photo-bioreactors. The outcome of this study shows that identification of the right conditions can reduce the treatment time. In so doing, stable nutrient removal and reduction of precipitation chemicals can be achieved, as well as improved recovery of valuable nutrients like phosphorous and nitrogen.
Sammanfattning

Avloppsvatten innehåller allt det vi spolar ner i avloppet, och i det finns höga halter av viktiga näringsämnen så som fosfor, kväve och kol. Kostnaden för att rena avloppsvatten ökar i världen på grund av ökad urbanisering och ökade krav på reningen.

Reningsprocessen domineras av aktivslamprocessen, i vilken mikroorganismer (främst bakterier) renar vattnet från kol och kväve medan det mesta av fosforn fälls ut med kemikalier. Processen är mycket energikrävande på grund av den stora mängden luft som behöver pumpas in för att förse bakterierna med syre, och även sammantaget är det en energi- och resurskrävande process som därtöver kräver tillskott av mer kol, oftast som ren alkohol, för att kväve-reningen ska fungera.

I den här avhandlingen har ett alternativ till konventionell, bakteriell avloppsvattenrenning studerats; mikroalgbaserad aktivslamprocess (MAAS). Bakgrunden är att gröna mikroalger, i likhet med växter, utnyttjar en fotosyntes i vilken algerna snabbt kan föröka sig om det finns näringsämnen, ljus och koldioxid. Eftersom avloppsvattnet innehåller allt nödvändiga näringsämnen kan det räcka med att tillsätta koldioxid under rätt ljusförhållanden för att en livskraftig algkultur ska växa till. Algerna producerar även syre i processen som i sin tur kan användas av bakterierna i MAAS- processen i en intressant symbios. Mest intressant är mikroalgbaserad vattenrenning ur ett resursutvinningsperspektiv, eftersom näringsämnena assimileras i algerna och därmed förblir i slammets och kan sedan utvinnas som en gödselprodukt.

Målet med MAAS-forskningen har varit att använda koldioxid, näringsämnen och en algkultur från svenska förhållanden, i detta fallet Mälaren, för att bygga upp en effektiv kultur för vattenrenning. Samtidigt vill vi i forskningen optimeras processen utifrån hur mycket ljus som behöver tillföras, hur snabbt mikroorganismerna kan rena vattnet och vilka andra ämnen som kan störa processen.

I den här avhandlingen har olika ljuskällor undersöks, med särskilt fokus på effektiva LED-lampor. Därefter har målet varit att optimerar processen så att uppehållstiden, dvs den tid det tar för en viss volym vatten att renas, skulle kunna sänkas från 6 dagar (vilket anses vara internationell standard) till 4 eller till och med 2 dagars uppehållstid. Slutligen studerades effekterna av fällningskemikalier i mikroalgkulturen med slutsatsen att mikroalger blir begränsade av fällningskemikalier, men att mikroalgemäntäremot har goda förutsättningar att rena vattnet från betydande mängder fosfor och därmed minska mängderna av fällningskemikalier som måste tillsättas.
Preface

It would have been like searching for water in the desert if I hadn’t had their supervision: Senior Lecturer Emma Nehrenheim for considering me for this position, all encouragements, and moral support; Adjunct Professor Carl-Frederik Lindberg for his calm supervision, support and inspiration to think about everything in a practical way; Post-Doctoral Researcher Sebastian Schwede for his continuous encouragement and dedication to research, even if he ordered me to make a space shuttle in the evening he would ask me about it the next morning, “Hej Anbu, how is it going with our space shuttle?”… until it was completed.

It would have been like searching water in the desert if I hadn’t had their friendship: all my colleagues at the department and especially my office mates and neighbouring office mates, for fruitful suggestions with my licentiate. Most importantly, all the smiles and the sound discussions.

Special thanks to

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- Thomas Wahl for moral support, showing me “Swedish-ness” and for enabling me to stay closer to the school.
- Saywan Jamal and Joakim Jansson for all their technical support.

Last but not least special thanks to my family and friends outside Västerås for endless support in critical situations.
List of papers

This licentiate thesis is composed of the following papers which are represented by their roman numerals and are included at the end of the thesis:


List of papers not included


My contributions to the papers

The research presented in this thesis was supervised by Emma Nehrenheim (EN), Sebastian Schwede (SS) and Carl-Fredrik Lindberg (CFL), except for paper I which was supervised by EN and SS.

**Paper I**: I performed the experiment and wrote the manuscript in coordination with the supervisors. Lights were obtained from Stig Nohlert.

**Paper II**: I performed the planning and experimental set up of MAAS process in coordination with EN and CFL. BMPs were planned and performed together with SS. I wrote the manuscript in coordination with the supervisors.

**Paper III**: I performed the planning and experimental work, and wrote the manuscript in coordination with the supervisors.
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## Nomenclature

### Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{665}$</td>
<td>-</td>
<td>Absorbance of chl. $a$ at 665 nm</td>
</tr>
<tr>
<td>$A_{750}$</td>
<td>-</td>
<td>Absorbance due to turbidity at 750 nm</td>
</tr>
<tr>
<td>$C_i$</td>
<td>[mg L$^{-1}$]</td>
<td>Initial concentration of nutrients or COD in the incoming wastewater</td>
</tr>
<tr>
<td>$C_f$</td>
<td>[mg L$^{-1}$]</td>
<td>Final concentration of nutrients or COD in the outgoing wastewater</td>
</tr>
<tr>
<td>$E_w$</td>
<td>[mg]</td>
<td>Empty weight of the crucible</td>
</tr>
<tr>
<td>$f$</td>
<td>[cm mg L$^{-1}$]</td>
<td>Extinction coefficient of acetone, (87.67 \text{ L g}^{-1} \text{ cm}^{-1}) (\times) (1000 \text{ mg g}^{-1})</td>
</tr>
<tr>
<td>$FS_{550}$</td>
<td>[mg]</td>
<td>Fixed solids remaining in crucibles after constant incineration at 550°C for 2 hours</td>
</tr>
<tr>
<td>$H$</td>
<td>[m]</td>
<td>Settled MAAS height</td>
</tr>
<tr>
<td>$p$</td>
<td>[m bar]</td>
<td>Atmospheric pressure in the room</td>
</tr>
<tr>
<td>$p_a$</td>
<td>[m bar]</td>
<td>Ambient pressure</td>
</tr>
<tr>
<td>$p_m$</td>
<td>[m bar]</td>
<td>Measured pressure</td>
</tr>
<tr>
<td>$p_o$</td>
<td>[1013 m bar]</td>
<td>Normalised pressure</td>
</tr>
<tr>
<td>$p_w$</td>
<td>[m bar]</td>
<td>Vapour pressure of the water as a function of temperature</td>
</tr>
<tr>
<td>$SV$</td>
<td>[mL L$^{-1}$]</td>
<td>Settled sludge volume</td>
</tr>
<tr>
<td>$t$</td>
<td>[hr$^{-1}$] or [d$^{-1}$]</td>
<td>Time</td>
</tr>
<tr>
<td>$T_a$</td>
<td>[$^\circ$C]</td>
<td>Ambient temperature</td>
</tr>
<tr>
<td>$T_o$</td>
<td>[0$^\circ$C]</td>
<td>Normalised temperature</td>
</tr>
<tr>
<td>$TS$</td>
<td>[mg g$^{-1}$]</td>
<td>Total solids</td>
</tr>
<tr>
<td>$TS_{105}$</td>
<td>[mg]</td>
<td>Mass of total solids in crucibles after constant drying at 105°C for 24 hours</td>
</tr>
<tr>
<td>$TSS$</td>
<td>[g L$^{-1}$]</td>
<td>Total suspended solids / Dry weight</td>
</tr>
<tr>
<td>$V$</td>
<td>[mL]</td>
<td>Calculated gas volume</td>
</tr>
<tr>
<td>$VS$</td>
<td>[mg g$^{-1}$]</td>
<td>Volatile solids</td>
</tr>
<tr>
<td>$V_h$</td>
<td>[mL]</td>
<td>Volume of headspace in the bottles</td>
</tr>
<tr>
<td>$V_o$</td>
<td>[mL g Vs$^{-1}$ d$^{-1}$]</td>
<td>Normalised gas production</td>
</tr>
<tr>
<td>$V_s$</td>
<td>[m hr$^{-1}$]</td>
<td>Settling velocity of MAAS in the cones</td>
</tr>
<tr>
<td>$V_{sl}$</td>
<td>[mL]</td>
<td>Volume of sample used</td>
</tr>
<tr>
<td>$V_{sol}$</td>
<td>[mL]</td>
<td>Volume of acetone used</td>
</tr>
</tbody>
</table>
$W_w$ [g] Wet weight
$Y_{BG}$ [mL g VS$^{-1}$] Yield of accumulated biogas

**Abbreviations**

- **BPR**: Biogas production rate
- **COD**: Chemical oxygen demand
- **Chl. $a$**: Chlorophyll $a$
- **DO**: Dissolved oxygen
- **GHG**: Greenhouse gas emission
- **HRAPs**: High rate algal ponds
- **LED**: Light emitting diodes
- **MAAS**: Micro-algae activated sludge
- **MAB**: Microalgae bacteria
- **PAR**: Photosynthetic active radiation
- **PBRs**: Photo-bioreactors
- **PWM**: Pulse width modulation
- **$r_d$**: Residual concentration
- **SBR**: Sequential batch reactor
- **SRT**: Sludge retention time
- **SVI**: Sludge volume index
- **TOC**: Total organic carbon
- **TP**: Total phosphorous/Phosphorous
- **TN**: Total nitrogen/Nitrogen
- **TW**: Treated wastewater
- **UW**: Untreated wastewater
- **WWTP**: Wastewater treatment plan
1 Introduction

1.1 Background

Eutrophication is water pollution which is partly caused by discharge of effluent rich in major plant nutrients such as nitrogen and phosphorous. The effluent from conventional biological treatment enters lakes or coastal waters directly due to urbanisation. In Sweden, eutrophication is now a well-known problem to water resources planners due to the expansion of the population (from 7.5 million in 1960 to 9.85 million in 2015) (Statistics Sweden, 2016). These circumstances attracted a great deal of attention to upgrading the conventional process in the 1960s to maintain water quality (SEPA, 2012). Biological processes are efficient at removing carbon and nitrogen only, at the expense of energy. Developments in chemical engineering led to the introduction of chemical precipitation and a nitrogen removal process (i.e. addition of carbonaceous nutrient, methanol) (Teixeira et al., 2010). This has simplified the biological treatment of wastewater in several cities in Sweden. However, the long-term consequences of these processes have been given less importance in considerations of the sustainability of water treatment.

For instance, iron sulphate is commonly used as a chemical precipitant for concentrating solids and phosphorous in wastewater. However, commercial iron sulphate also contains impurities such as arsenic, zinc, nickel and lead, which are therefore indirectly added to the wastewater treatment process (QC Corporation, 2016). Heavy metals thus accumulate in the sludge as a result of the chemical precipitation. In addition, the accumulation of precipitated solids and sulphates may cause corrosion of concrete and sewer pipes in subsequent stages of the process. These consequences are less important in conventional wastewater treatment (Firer et al., 2007; Haaning Nielsen et al., 2005).

The present wastewater treatment plants (WWTPs) that utilise the activated sludge process use more electrical energy for aeration in comparison to other steps, such as clarifiers, sludge pump, heating and sludge dewatering. Aeration accounts for up to 75% of the energy consumption in WWTPs (Amand et al., 2013). In future, stringent effluent discharge limits in the European Union will increase the energy cost due to the aeration, chemical steps and handling of sludge. Therefore, the removal of nitrogen and mitigation of greenhouse gas emissions (GHG) during the treatment will remain a big challenge in future (Park and Dolan, 2015). On the other hand, the cost of the WWTPs can be reduced if the energy and chemical consumption can be mitigated.
In recent years, photo-bioreactors (PBRs), an alternative solution containing microalgae and activated sludge bacteria, have been considered for removal of nutrients in different wastewaters (Posadas et al., 2013; Tiron et al., 2015; Weinberger et al., 2012). To date, the cultivation of microalgae in open PBRs (raceway ponds) and closed PBRs have emerged as attractive candidates for wastewater treatment (Alcántara et al., 2015; Muñoz and Guieysse, 2006). In both the open and closed reactors, the installation and harvesting steps are the main bottlenecks for wastewater treatment (Suh and Lee, 2003).

It is important to acknowledge the potential of ecological processes like naturally growing microalgae and bacteria for eco-technological applications. From this perspective, microalgae cultivation in wastewater containing bacteria is not new in Sweden. The cultivation of algae to treat wastewater at low temperature has been reported in Stockholm and Luleå (Grönlund et al., 2004; Karin Larsdotter, 2006). In addition, the cultivation has been evaluated in combination with flue gas in Umeå (Axelsson, 2012; Broberg et al., 2011; Processum, 2016). All the available investigations used open PBRs where the pH was not well controlled (Broberg et al., 2011; Grönlund, 2004; Karin Larsdotter, 2006). The previous studies were more proofs of concept than studies to evaluate ideal conditions. Therefore, they are not sufficient for designing a PBR, especially for treating municipal wastewater at WWTPs. Moreover, the open PBR design requires large land areas and light coverage which are not practically feasible for current WWTPs. Therefore, the present study addresses strategies for improving wastewater treatment in closed PBRs and the scalability of sludge harvesting. This type of PBR construction in activated sludge units is expected to be useful for minimising the risk of greenhouse gas (GHG) emissions, recovering plant nutrients and sludge reduction in the near future.

1.2 Objectives and delimitation

The overall objective of the present research is to experimentally establish favourable conditions for microalgae in the activated sludge units. The objectives are defined based on previous knowledge on microbial analysis from PBRs (Krustok, 2016). Combining these results may provide a possible solution for continuous nutrient removal and biomass production in the prevailing Swedish wastewater treatment conditions (i.e. availability of light, characteristics of wastewater and temperature).

This licentiate thesis aims to explore the application of indigenous microalgae originating from a freshwater lake and activated sludge bacteria originating from wastewater. Establishing optimal micro-algal cultivation in the PBR is a straightforward approach to support the naturally occurring activated sludge process for the removal of carbon. By doing so, the microalgae activated
sludge/microalgae-bacterial (MAAS/MAB) process can be implemented with the same infrastructure as the conventional activated sludge process.

The application of MAAS is an emerging technology and has been reported in the literature during the last few decades. However, there is no full scale industrial application so far in the activated sludge environment. The criteria for the kind of reactor designs used in this study are shown in Figure 1.1, which is based on local conditions and also from the literature.

1.3 Research Challenges

From the PBR design criteria and in order to meet the demand for sustainable wastewater treatment practice, the following research challenges are put forward from a WWTP perspective.

1. What kind of lighting is suitable for microalgae cultivation? Does the specific wavelength of the LEDs influence the cultivation period? How does intensity of light affect the photosynthesis process in PBRs? (Papers I and III)

2. What are the optimal conditions for the MAAS in untreated wastewater? (Paper II)
3. What is the effect of iron flocculated wastewater during the MAAS cultivation? (Paper III)

4. What is the biogas potential of fresh MAAS obtained at various times during the treatment period? (Paper II)

5. What are the settling properties of MAAS? (Paper II and III)

Other applications: How can we simultaneously produce biomass and upgrade exhaust gas in a continuous closed PBR using reject water as a nutrient supplement? (Ongoing work)

1.4 Thesis outline

This thesis is structured around the appended papers with the following chapters:

Chapter 1 Background
This chapter covers the background, research objectives, challenges and the outline of the thesis.

Chapter 2 Theoretical Background
This chapter presents a detailed literature review of background knowledge of previous work based on the research objectives.

Chapter 3 Methodology
This chapter provides an overview of the experimental methodology and important calculations used in this study.

Chapter 4 Results and discussion
This chapter presents the main results and discussion.

Chapter 5 Conclusions
This chapter describes the major conclusions and the outlook from this study.

Chapter 6 Future work
This chapter elucidates the ongoing work and planned work for the coming years.
2 Theoretical Background

2.1 Activated sludge in wastewater treatment (WT)

Wastewater treatment in most towns or cities relies on the practice of traditional activated sludge process as biological treatment to remove nitrogen from wastewater. Figure 2.1 shows an overview of the activated sludge based wastewater treatment process, which celebrated its 100th anniversary in 2014. Incoming municipal wastewater (high strength wastewater) has to undergo a primary treatment which includes mechanical, physical and chemical treatments. This step is referred to as the primary step. After this step, the wastewater is referred to as a medium or low strength wastewater. This step removes most of the solid particles, which contribute up to 40% of the chemical oxygen demand (COD) (Beal et al., 2012). The solids or deposited waste obtained after this step is called the primary sludge. Subsequently, the wastewater containing carbohydrates, proteins, lipids and fats undergoes biological treatment. This step is called the secondary step. The nutrients are consumed in growth of bacterial and other microbial cells. The bacterial process also plays a major role in the removal of ammonium (NH₄) in the presence of oxygen (air). In short, activated sludge bacteria oxidise nitrogen in the form of NH₄ to nitrate (NO₃) in the presence of oxygen (O₂). This process is called the nitrification process and it is strictly aerobic. In the presence of additional carbon sources (e.g. methanol or glycol), oxidised nitrogen is reduced to nitrogen (N₂) gas. This step is called denitrification. This process is strictly anoxic, and nitrate is used for respiration. Finally, the nitrogen and carbon depleted water is polished and released into water streams.

Removal of P is not an efficient process in the activated sludge process. Hence, 90-95% of the P is removed during the primary step through chemical precipitation and the remaining P is removed after the secondary step (SEPA, 2012). Moreover, the bound P cannot be recovered due to complex formation.
Figure 2. Overview of the activated sludge process.

**PRIMARY STEP**
- Screening
- Floculant
- Pre-sedimentation tank

**SECONDARY STEP**
- Activated sludge tank
- Post-sedimentation tank
- Flocculant
- Clarifier
- Air
- Recirculation
- Anaerobic digestion

**TERTIARY STEP**
- TREATED WATER
- Biogas
- Centrifuge
- Reject water (Supematant)
- Sludge (Pellet)

Flow arrows:
- Wastewater
- Sludge
- Gas
- Precipitant
2.2 Microalgae in WT

The use of microalgae in sewage treatment was first reported by Oswald in 1957 (Oswald and Gotaas, 1957). Simply stated, in bacterial decomposition of organic matter in wastewater, carbon dioxide (CO₂) is released while O₂ is consumed. Algae utilise sunlight in consuming ammonium (NH₄) and CO₂ to synthesise organic molecules using chlorophyll (green pigment), and release ammonia (NH₃) and O₂ (Oswald and Gotaas, 1957). In addition, there is simultaneous phosphorous precipitation due to the alkaline pH caused by the growth of the algae. According to the earliest sources, algal bacterial consortia were part of wastewater treatment in extended lagoons (1963) in tertiary wastewater treatment (Hoffmann, 1998). Since then, micro-algal communities have been extensively evaluated in high rate algal ponds/raceway ponds (HRAPs). Therefore, microalgae based wastewater treatment is not new in sewage treatment.

However, HRAPs were not immediately installed as the primary biological wastewater treatment in centralised municipal wastewater treatment plants. This is because microalgae grow slowly and demand a large footprint and energy consumption to capture the sunlight or artificial light (Muñoz and Guieysse, 2006). In most cases, they were considered for municipal wastewater treatment for removing nitrogen and phosphorous (N and P) after the activated sludge process (Park and Craggs, 2011; Posadas et al., 2015). Gutzeit et al. (2005) and Weinburger et al. (2012) have demonstrated the importance of the microalgae and activated sludge combination in closed PBRs using a monoalgal culture (e.g. *Chlorella sp.*) in pulp and paper wastewater, and pre-treated municipal wastewater using discontinuous lighting (i.e. light and dark hours). Alcántara et al. (2015) demonstrated a complex modified closed PBR configuration. However, a continuous wastewater treatment needs a constant light supply for the cultivation of microalgae because growth stops during the dark hours (Edmundson and Huesemann, 2015).

2.3 Microalgae cultivation in activated sludge system – why?

The bottlenecks of activated sludge and micro-algal process are discussed in sections 2.3.1 and 2.3.2.

2.3.1 Activated sludge process

- Chemical step: the consumption of phosphorous precipitation chemicals such as ferric chloride (FeCl₂) or ferrous Sulphate (FeSO₄) is very high (Gutzeit et al., 2005). Later in the process, cationic electrolytic polymers are
widely used to concentrate the suspended biomass and to increase the removal of sludge from treated wastewater.

- Oxygen supplementation: the wastewater generates biochemical oxygen demand through the chemical reactions performed by bacteria (see also section 2.4.1). Therefore a constant supply of oxygen is needed for bacterial respiration in the activated sludge tank to overcome the oxygen deficit (Amand et al., 2013).

- Handling of sludge: the sludge from the WWTPs contains excess water that needs to be conditioned ahead of the thickening process. Moreover, the excess sludge production during the primary and biological steps requires a strict sludge management practice to avoid GHG emissions during storage (Liu, 2003).

- Settling of sludge: a large sedimentation unit and a long time are essential to settle the final sludge. Cationic polymers are mandatory after the biological step to improve dewatering of the sludge (Wood et al., 1992).

- External carbon source: addition of an external carbon source (i.e. methanol) is often required for reduction of the oxidised nitrogen (i.e. denitrification, conversion of NO₃ to N₂ gas). This adds operational complexity to the process (Teixeira et al., 2010).

- CO₂ emissions: globally, CO₂ emission is not a high priority for WWTPs. CO₂ is released as a result of bacterial oxidation of organic substrates in wastewater (Chai et al., 2015).

2.3.2 Micro-algal process

- Stability of the process: micro-algal photosynthesis is not a stable process. The volatilisation of N in the form of NH₃ and the precipitation of P are very common due to the alkaline pH during algae growth (Muñoz and Guieysse, 2006). These elevated pH conditions are very harmful to the activated sludge microorganisms.

- Design of the reactors: microalgae reactors or PBRs are used for the cultivation method. In general, HRAPs have been widely reported but these require a large land area and surface lighting throughout the year. However, the depth of HRAPs varies between 0.1 and 0.3 m (Muñoz and Guieysse, 2006). The design of PBRs is moving towards tubular configurations to reduce the land requirement. The tubular constructions are expensive due to the installation cost and the settling characteristics are poor due to the rapid liquid flow inside the tubular section (Muñoz and Guieysse, 2006).
Harvesting of microalgae: microalgae have poor settleability (i.e. gravity sedimentation) due to their small size, which varies from 1–8 µm depending on the algal species. This is in spite of different cultivation strategies where the pH plays an important role in charge based settling during the sedimentation process (Liu et al., 2014). Several methods other than gravity sedimentation have been suggested for harvesting the biomass, such as dissolved air flotation, chemical coagulation, centrifugation and filtration (Christenson and Sims, 2011; Larsdotter, 2006). However, these processes not only increase the cost but also increase the complexity of the wastewater treatment.

It may be possible to eliminate the above bottlenecks by combining MAAS with optimal artificial lighting in the activated sludge units. The advantages of this are

- Reduction of precipitation chemicals and sludge: the combined biological nutrient removal and recovery of N and P opens the possibility of avoiding part of the chemical sludge production and extensive usage of the precipitation chemical (Anbalagan et al., 2016; Gutzeit et al., 2005; Medina and Neis, 2007).

- pH of the process: the process can be controlled by maintaining pH values above 8 through addition of external CO₂. This helps to avoid carbon limitation during the process by increasing the mass ratios of carbon:nitrogen:phosphorous. Through this method, the treatment days can be reduced further from 3 to 2 days or even to 1 day in comparison to the HRAP (Tiron et al., 2015), which usually requires a longer treatment. Therefore, the optimisation step can be defined for fewer treatment days once a pH control is established over the process (Posadas et al., 2015).

- Settleability: there is a wide knowledge gap between cultivation and the settleability in the pilot scale demonstrations (Paper III) (Muñoz and Guieysse, 2006). In contrast to these studies, MAAS can be a good alternative for self-flocculation if the operation is pH controlled in an activated sludge environment (Gutzeit et al., 2005; Medina and Neis, 2007). The cyanobacterial or fungal filaments present in the activated sludge can help the microalgae to aggregate as floc instead of remaining as suspended micro-algal communities (Gultom and Hu, 2013). Moreover, extra polymeric substances (EPS) manufactured from activated sludge organisms (i.e. on the cell surface) play a major role in interaction of the MAAS (Gultom and Hu, 2013). Microalgae are negatively charged and activated sludge bound EPS are positively charged, which can result in binding of the charged particles. The combined sludge may be hydrophobic at neutral pH, which can increase its dewatering ability (Nouha et al., 2015). Overall, settling characteristics of microalgae and activated sludge can be improved in a cost effective manner without external processes.
2.4 MAAS process

2.4.1 State of art

MAAS is a biological process that occurs by the interaction of two different processes, micro-algal photosynthesis and bacterial respiration. These two processes occur simultaneously in the wastewater environment with exchange of nutrients as shown in Figure 2.1. The balanced exchange of nutrients between two processes is dependent on the pH of the micro-algal process.

![Figure 2.1](image)

In micro-algal photosynthesis, the dissolved form of inorganic carbon, N and P are utilised in the presence of light for micro-algal growth (photosynthesis) to manufacture proteins, carbohydrates, lipids and photosynthetic pigments (e.g. chlorophyll, phaeophytin and carotenoids, etc.). The inorganic carbon may originate from bacterial respiration, flue gas, pure CO₂ or biogas. Figure 2.2 shows a simplified sketch of the photosynthetic pathway.

The photosynthetic photosystems (PS) are classified into two types, PSI (700 nm) and PSII (680 nm) based on the excitation wavelength of the chlorophyll-a (chl. a) molecule. The pigment excites two electrons as it receives light energy as photons. As a result, a series of electron transfers take place in different protein complexes across PSII and PSI in tandem, which powers proton movement from stroma to the thylakoid lumen. The transport of electrons is based on the movement of protons. At the same time, the released electrons in the pigment are replaced by electrons gained from the photolysis of water by water splitting protein complex (an oxygen oxidising complex with a manganese – calcium active centre) in the PS II (Renger, 2012). During the photolysis, oxygen is released as by-product. The proton movement from stroma to thylakoid lumen causes a gradient during this step, which is used by the enzyme ATP (Adenosine triphosphate) synthase to synthesise ATP from ADP and inorganic phosphorus (Pᵢ) in the medium. Nicotinamide diphosphate (NADP) gains a free proton to
form NADPH and acts as terminal electron acceptor during the light cycle (Lodish et al., 2000).

As a result of photosynthesis, oxygen is released as by-product and the recovered NADPH and ATP are used for conversion of CO₂ into carbohydrate through a series of light-independent reactions called the Calvin cycle (Lodish et al., 2000). The process takes place in the space between the thylakoid membrane and the internal membrane of the chloroplast. Additionally, in parallel, microalgae can take up nitrogen either in the form of NH₄ or NO₃. NH₄ is preferred by microalgae over NO₃ due to the loss of energy when reducing NO₃ to NH₄ (Chen et al., 2012), which is a NADPH dependent process (Cresswell et al., 1989).
Figure 2.2 An overview of photosynthesis based nutrient removal
In the bacterial process, the activated sludge bacteria mainly perform the nitrification and respiration steps, producing carbon dioxide and oxidised nitrogen. Apart from this, ammonia oxidising archaea (i.e. single celled organisms) also perform the oxidation of ammonia. These conditions are favourable for photosynthesis (section 2.1). Excess CO₂ is released as a by-product from COD removal and acts as a carbon source for nitrifiers and algae. In this case, nitrate is used as nitrogen for the photosynthesis process. Bacteria thriving in the activated sludge also help to manufacture vitamins that are not synthesised during micro-algal photosynthesis.

In summary, the microalgae and activated sludge process involves the following simplified reaction scheme (1) to (5),

**Photosynthesis**

\[
\text{CO}_2 + \text{H}_2\text{O} + \text{NH}_4 + \text{NO}_3 + \text{PO}_4 + \text{Light} + \text{Algae} \rightarrow \text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} + \text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \tag{1}
\]

**Oxidation of organic matter/exogenous respiration**

\[
\text{Organic matter} + \text{O}_2 + \text{Bacteria} \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{NH}_4 + \text{endproducts} + \text{Energy} \tag{2}
\]

**Synthesis of new cells**

\[
\text{Organic matter} + \text{O}_2 + \text{Bacteria} + \text{energy} \rightarrow \text{C}_5\text{H}_7\text{O}_2\text{N} \text{ (Biomass)} \tag{3}
\]

**Oxidation of cellular matter/Endogenous respiration**

\[
\text{C}_5\text{H}_7\text{O}_2\text{N} + 5\text{O}_2 \rightarrow 5\text{CO}_2 + \text{NH}_4 + \text{H}_2\text{O} \tag{4}
\]

**Nitrification**

\[
\text{Bacteria} + \text{NH}_4 + 1.815\text{O}_2 + 0.1304\text{CO}_2 \rightarrow 0.0261\text{C}_5\text{H}_7\text{O}_2\text{N} \text{ (new cells)} + 0.973\text{NO}_3 + 0.921\text{H}_2\text{O} + 1.973\text{H ions} \tag{5}
\]

The current state of knowledge of the micro-algal and bacterial processes clearly defines the major advantages such as nutrient removal and CO₂ consumption, with the main disadvantage being the increase in pH to alkaline (Figure 2.1). Most of the reported studies on wastewater treatment based on this concept use HRAPs (Muñoz and Guieysse, 2006; Park and Craggs, 2011; Posadas et al., 2013). In these conditions, microalgae will be the dominant communities during the wastewater treatment. The question remains how to overcome this disadvantage. The solution may involve cultivating microalgae in the activated sludge tank under controlled pH conditions. Control of the operational pH might be achieved by establishing a balance in buffering capacity between the two processes (Posadas et al., 2015). External CO₂ buffering can be provided based on the CO₂ demand, which has to be verified at shorter HRT (Park and Craggs, 2011; Posadas et al., 2015).
2.5 Biogas potential of MAAS

Microalgae has the empirical formulae $C_{106}H_{263}O_{110}N_{16}P$, thus each molecule of biomass has the potential to remove 41% of C, 7.2% of N and 1% of P by mass of nutrients in wastewater (Xin et al., 2010). Hence, the nutrients accumulated in the biomass can be used directly as a substrate for a wet anaerobic digestion process. The digested sludge can also be used as a slow fertiliser in the field if it complies with the regulations. For instance, according to Yuan et al., (2012) (6), stoichiometric conversion of 1 mole of algae alone during anaerobic digestion should theoretically produce 47.17 moles of methane. The degradability of biomass depends on the cell wall composition and the inhibitory compounds of the investigated species (Dalrymple et al., 2013). By applying the ideal gas law equation (i.e., $PV = nRT$), 508 L of biogas can be produced from one kilogram of algae biomass (based on COD) from combined sludge containing algae alone.

$$C_{106}H_{263}O_{110}N_{16}P + 6.67H_2O \rightarrow 13.67NH_4 + 33.50CO_2 + 47.170CH_4 + 2.33C_2O_5H_7N + 13.67HC_3O_3^- + HPO_4^- + H^+ \quad (6)$$

Additionally, the synergistic effect of microalgae and activated sludge ($C_5H_7O_2N$) during co-digestion versus digestion of algae alone has been reported in recent years for anaerobic digestion. This represents another advantage for the MAAS process (Liu, 2003; Olsson et al., 2014). In contrast to traditional treatment, MAAS provides an opportunity to close all loops in the wastewater treatment plant to treat wastewater and reject water. Therefore, the removal of CO$_2$ and the boosting of C to N mass ratio from biogas during the treatment of wastewater should also be considered.
3 Methodology

3.1 Wastewater collection

Kungsängen wastewater treatment plant (KWWTP) is situated in a bay of lake Mälaren, the third largest lake in Sweden. The plant receives sewage from more than 140 000 inhabitants of Västerås, Sweden. Wastewater is treated using conventional activated sludge for nitrogen and biological oxygen demand (BOD for 7 days (BOD₇)) to achieve a removal efficiency of 65–75 and 97% respectively. The plant also removes 95% of the total phosphorous using iron sulphate based precipitation. After the removal of nutrients, treated water is released into the lake. The discharge limits for total nitrogen and phosphorous in the treated wastewater are 15 and 0.3 mg L⁻¹ (Mälarenergi AB, 2014).

Figure 3.1 shows a simplified sketch of the KWWTP. The wastewater in our studies was collected at two different locations (1 and 2) at the KWWTP; Location 1: inflow pipe, before the screening and precipitation step (Figure 3.1); Location 2: pre-sedimentation tank after the iron sulphate based precipitation step (Figure 3.1).

3.2 Inoculum collection

The indigenous mixed micro-algal communities from Lake Mälaren were investigated for the MAAS process. Lake water used for pre-culturing and other preliminary studies was collected from the surface of the lake during the early autumn (2014) (Paper I and II). The inoculum obtained during middle and late spring (2015) from the pilot scale PBR (1 m³) was also investigated in paper III.
Figure 3.1 A simplified overview of wastewater treatment in Kungsängen wastewater treatment plant (KWWTP) (UWW/UW represents treated wastewater and TWW/TW represents treated wastewater)
3.3 Experimental setup

3.3.1 MAAS process

**A) Influence of LEDs and fluorescent light**

The experimental set up presented in paper I was a preliminary or indicator study designed to determine the influence of the light spectrum using mixtures of light of various wavelengths. Thus, the experiment was performed with reference to the fluorescent light in optimal growth conditions without preculturing of algae. The effect of pulse width modulation (PWM) of light emitting diodes (LEDs) was also analysed in this study. Figure 1 A shows the experimental setup of a simple flask study in the presence of PWM–LED 1 and fluorescent light as described in paper I. The following wavelengths were considered: red (632 nm), orange (612 nm), blue (470 and 430 nm) and white (380–670 nm). These wavelengths were considered to cover the spectral wavelength of chl. a pigment (i.e. blue and red light spectrum). The additional white light can be regarded as similar to the excitation wavelengths of PSI and PSII (see also section 2.4.1). PWM–LED 1 and PWM–LED 2 were designed with and without reduction of white light intensity respectively. Wastewater to lake water ratios of 70 to 30% were used in this study based on the previous investigation by Krustok et al. (2016). Three different test groups were investigated: PWM–LED 1 (high intensity), PWM–LED 2 (low intensity) and fluorescent light with intensity similar to PWM–LED 1. Each group contained untreated (Location 1) and treated (Location 2) wastewater in triplicates, and the flasks were shaken manually twice every day.

![Figure 3.2](image)

*Figure 3.2  Experimental set up for LED and fluorescent light experiment. A) PWM–LED 1 and B) fluorescent light*
B) Influence of HRT on photo–bioreactors
The previous section addressed the importance of light intensity, penetration and wavelengths. With this in mind, paper II reports on PBR studies to verify the favourable conditions for MAAS. As a reference, the knowledge gained on the micro-algal cultivation period (i.e. time required for increase in chl. a content) from the previous batch study was taken into consideration. Fluorescent light with an intensity of 150 photosynthetic active radiation (PAR) units was used as the light source. The reactors in this study had a working volume of 22 L. Figure 4 shows the PBR (Reactor I (RI) and Reactor II (RII)) set up in duplicates. Instead of a 30:70 (Lake water: wastewater) ratio (in this case, the preliminary batch conditions failed the reproducibility test due to the increase in volume and depth), a 50:50 ratio was used to avoid the magnetite or pyrite formation of iron flocculated wastewater. In addition, the pH was controlled and reactors were stirred during the experiment to avoid dead or dark zones. Additionally, 50% of wastewater was replaced manually to achieve a semi–continuous operation mode, where the HRT was equivalent to the sludge retention time (SRT). This was done to determine the adaptability (acclimatisation) of the MAAS to a rapid shift in operational conditions.

C) Availability of phosphorous in pre–treated wastewater
The precipitation of iron sulphate consumes most of the available residual phosphorous, which in turn reduces the dissolved oxygen and the uptake of the nitrogen by microalgae. Hence, the fed batch study was conducted with
controlled pH using CO₂ to determine the impact of total phosphorous (P) (paper III). The experimental setup presented in paper I was used in this study, with a working volume of 250 ml and fluorescent light as the light source.

**D) Influence of light on dissolved oxygen (DO) concentration**

The experimental study presented in paper III aimed to answer the following questions:

1. What is the relationship between chl. a concentration (i.e., as an indicator for the growth dynamics) and light intensity? And how does it influence the DO evolution in wastewater?
2. How does the DO change with the biomass concentration in the PBRs?
3. Does lower concentration of microalgae require more light for the photosynthetic DO?

Figure 3.4 shows the experimental procedure used to determine the influence of light intensity on different concentrations of MAAS using a cylindrical photo-bioreactor (1.5 L). The light intensity was expressed in PAR (µ mol. m⁻² s⁻¹). Light plays a crucial role in oxygen synthesis in microalgae, and oxygen plays a key role in bacterial oxidation of organic matter present in the wastewater. Therefore, oxygen evolution rate was evaluated at different light intensities. Five different light intensities of 50, 75, 100, 250 and 500 µ mol. m⁻² s⁻¹ were studied against MAAS concentrations of 111, 258 ± 3.5 and 573 ± 11 mg L⁻¹ of total suspended solids (TSS) (paper III). These MAAS concentrations corresponded to 0.475, 1.048 and 1.91 mg L⁻¹ of chl. a respectively.

![Figure 3.4](image)  
*Figure 3.4 Influence of light on the evolution of oxygen. 1) Low (111 mg L⁻¹), 2) Intermediate (258 ± 3.5 mg L⁻¹) and 3) High MAAS (573 ± 11 mg L⁻¹) based on the total suspended solids measurements*
**F) MAAS in phosphate precipitated condition**

Paper III aimed to investigate whether phosphorous addition had any impact on nitrogen removal during the semi-continuous MAAS process.

The MAAS process was examined for COD and nitrogen removal in the presence of effective \((3 \text{ mg L}^{-1})\) and higher \((6 \text{ mg L}^{-1})\) concentrations of P in wastewater (See section 3.3.1 C). The initial concentration of MAAS used in this study was similar to a previous study (section 3.3.1 E) \((\text{i.e. } 10\% \text{ of the PBR volume, 2 L})\). The PBR setup discussed in paper II was used in this study without any duplication. A PBR working volume of 20 L was used. The collected periodical wastewater of 5 L from the KWWTP was replaced every day to reach a HRT of 4 days after complete settling of the wastewater (i.e. as in the sequential batch reactors (SBRs)). A 0.5 L sample of uniformly mixed effluent was removed at each retention time point and used for the analysis.

### 3.4 Settleability of MAAS

The dewaterability of microalgae is one of the bottlenecks during the harvesting stage of microalgae cultivation and it varies from species to species (Uduman et al., 2010). Therefore, the settling characteristics of the MAAS were determined in the PBRs (paper II and III) using Imhoff sedimentation cones.

![Imhoff cone sedimentation experiment in PBRs after the acclimatisation phase](image)

**Figure 3.5** Imhoff cone sedimentation experiment in PBRs after the acclimatisation phase. A) Sedimentation at HRT of 6 days in UW. B) Sedimentation at HRT of 4 days in pre-treated wastewater

In wastewater treatment, the sludge volume index (SVI) is used as a parameter to analyse the settleability of sludge. In practice, 30 minutes of settling is widely used. Due to the rapid settleability of MAAS, 10 and 5 minutes settling times were investigated in papers II and III.

SVI \((\text{mL g}^{-1})\) was calculated as follows:
SVI = \frac{SV}{TSS} \quad (7)

TSS or dry weight of MAAS used in paper II and III was calculated from the PBR liquid sample subjected to oven drying at a constant 105°C for 24 hours. The settling velocity of the MAAS was calculated in paper III using the Imhoff cone experiment as follows

\[ V_s = \frac{H}{t} \quad (8) \]

### 3.5 Biogas potential of MAAS

Biogas potential tests were performed in 120 ml serum bottles as described in paper II. The tests were conducted in two different groups: MAAS without pre-treatment and with pre-treatment. Each group contained three sets of samples. The samples were obtained after 6, 4 and 2 days HRT. Each set was investigated in triplicate. The pre-treatment was performed at 120°C for 2 hours. The experimental setup was implemented according to Schwede et al. (2013). The bottles contained an inoculum to MAAS ratio of 2:1. This contributed to a volatile solids (VS) ratio of 7.5 g L\(^{-1}\) inoculum to 3.5 g L\(^{-1}\) MAAS in each bottle (Olsson et al., 2014; Schwede et al., 2013).

![Figure 3.6 Biogas potential tests of MAAS](image)

VS measurement was performed using crucibles made of aluminium and china clay. VS (mg g\(^{-1}\)) was calculated according to APHA standard methods according to (9) and (10).

\[ TS = \frac{TS_{105} - E_w}{W_w} \quad (9) \]

\[ VS = \frac{TS - FS_{550}}{W_w} \quad (10) \]
The gas production was determined by measuring the excess pressure exerted by biogas in the headspace of the bottles using a digital pressure gauge. The gas volume was calculated and normalised at standard temperature and pressure based on (11).

\[ V = \frac{V_h(p_a-p_m)}{p_a} - V_h \] (11)

\[ V_0 = V \frac{(p-p_w)T_o}{p_oT_a} \] (12)

### 3.6 Other calculations

Removal efficiencies (\% RE) of the nutrients organic carbon (OC), total nitrogen (N) and P were determined according to (13). The same equation was used to determine the characteristics of wastewater by evaluating the removal efficiency of COD (13).

\[ \text{RE, \%} = \frac{C_i - C_f}{C_i} \times 100 \] (13)

In paper I, the total COD and total organic carbon (TOC) were used to determine the increase in the MAAS. In addition, the total inorganic carbon (TIC) was also used to determine the TIC concentration during the cultivation. The chl. a dynamics were determined in papers I, II and III based on the acetone extracted chl. a content, according to the method described in Bellinger and Sigee (2010) (14),

\[ C = (A_{665} - A_{750}) \times \left( \frac{V_{sl}}{V_{sol}} \right) \times \left( \frac{f}{L} \right) \] (14)

In addition, dissolved oxygen concentration was also measured as an indicator of photosynthesis during the experiment using sensors (papers II and III).

The rate of biogas production (RBP) was calculated for untreated and treated biomass based on (15).

\[ \text{BPR (\%)} = \frac{Y_{BG(1,2,3...25 \text{ days})} \times 100}{Y_{BG25^{th} \text{ day}}} \] (15)
4 Results and Discussion

Two different types of wastewater were investigated for the MAAS process in this thesis. They are referred as untreated and treated wastewater (UW and TW). The UW was considered as having a rich medium N to P mass ratio in comparison to treated wastewater since P in TW was limited due to the precipitation step. The heterogenic profile of wastewater in the appended papers is shown in Table 1.

<table>
<thead>
<tr>
<th>Papers</th>
<th>Average concentration of bio-nutrients and COD in the influent (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
</tr>
<tr>
<td>I</td>
<td>UW</td>
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<tr>
<td></td>
<td>TW</td>
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<tr>
<td>II</td>
<td>UW</td>
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<tr>
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</tbody>
</table>

§ and ⊙ – 6 and 3 mg L\(^{-1}\) of P addition; NM – Not measured.

4.1 Chl. a dynamics

The dynamics of chl. a were monitored as a measure of photosynthetic growth in all experiments. Chl. a development was directly related to light capturing and the carbon fixation process during the oxidation by bacteria (COD removal) (refer also to section 2.1). Therefore, the inorganic carbon forms (CO\(_2\) or H\(_2\)CO\(_3\) or HCO\(_3\)) were directly converted to carbohydrates (Bellinger and Sigee, 2010; Hosikian et al., 2010). Hence, chl. a content provides an overall measure of green microalgae and cyanobacterial proliferation during the MAAS process. In any case, a decrease in chl. a content was considered either as a decrease in the biomass or as an instability in the process due to the limitation of light and the C/N/P ratio in wastewater. However, other factors such as
overgrowth of bacteria or incoming organic content in the wastewater may also increase the absorption of light or decrease the chl. a content.

4.1.1 Batch experiment

A preliminary study was performed in paper I with three different light sources (PWM–LED 1, PWM–LED 2 and FL) using TW and UW. The chl. a analysis from a preliminary study is shown in Figure 4.1 A and B.

In TW, the FL showed a significant increase in chl. a content after 6 days (0.6 ± 0.2 mg L$^{-1}$) of cultivation in comparison to PWM–LED 1 (0.4 mg L$^{-1}$) and PWM–LED 2 (0.3 mg L$^{-1}$) (Figure 4.1 A; Orange bars). The pH increased above 8 after day 6, and the system became unstable. This may be due to NH$_3$ volatilisation and bio-precipitation of available P (Muñoz and Guieysse, 2006). In addition, the light spectrum of the PWM–LED 1 was augmented with an additional white light shifting it towards the wavelength of the PS (refer also to section 2.1). Therefore, the intensity of white light directly influenced the development of chl. a in PWM–LED 1 after day 6 and continued to increase until day 14 (Figure 4.1 A).

However, the PWM–LED 1 showed an increase in chl. a after day 6 and 8 (0.5 ± 0.4 and 1 ± 0.3 mg L$^{-1}$) in comparison to FL (day 6 and 8: 0.3 mg L$^{-1}$) and PWM–LED 2 (day 6 and 8: 0.4 mg L$^{-1}$) in the UW (Figure 4.1 B). A larger deviation in the UW in comparison to the TW was due to the alignment of the LEDs. A shift in pH from 6.8 to 8 occurred between days 6 and 8, where most of the carbon was fixed due to bacterial oxidation in both cases (paper I). This might have supported the buffering ability of the wastewater even after day 6 of the cultivation phase. The batch study was considered as an indicator study. The uncertainties were considered in order to improve the design of the later experiments with controlled pH (papers II and III).

Altogether, a high chl. a concentration was observed in UW (1–1.3 mg L$^{-1}$) in comparison with TW (0.6–0.8 mg L$^{-1}$). In both cases, red and blue wavelength LED mixtures registered slow growth in comparison to increased white light intensity regardless of wastewater composition. Hence, white LED lights should be considered for closed PBR cultivation instead of FL and LEDs of specific wavelength.
Figure 4.1  Influence of different light on chl. a. A) TW. B) UW.
4.1.2 Fed batch experiment

Following the previous study (paper I), the dynamics of chl. a were studied in paper III as shown in Figure 4.2. The influence of different phosphorous supplements: 0.5, 1, 2, 3, 4 and 5 mg L\(^{-1}\) on the TW was studied using a fed-batch approach. The pH of the system was maintained at 7.5 using CO\(_2\) to avoid the detrimental effects of high pH on bacterial activity. 50% of the reactor volume was replaced (after settling of the biomass) daily for 3 days to mimic the retention time of the pilot PBR, i.e. 6 days HRT.

The chl. a dynamics revealed that the effective P concentrations were 3 and 4 mg L\(^{-1}\) (i.e. in addition to the residual P). The maximum chl. a content of 1.2 mg L\(^{-1}\) was achieved in both P concentrations after 3 days of cultivation. These results were in agreement with the study conducted by Chen et al. using a culture obtained from lake Taihu, China (2010). They noticed that an increase in phosphorous concentration increased the chl. a content during the photosynthetic growth of *Scenedesmus obliquus* and *Microcystis aeruginosa* (Chen et al., 2010).

![Figure 4.2 Chl. a dynamics during the fed-batch study of micro-algal cultivation in treated wastewater](image-url)
4.1.3 Semi-continuous

Figure 4.3 A and B shows the acclimatisation phase (6 days HRT) and the semi-continuous phase (6, 4 and 2 days HRT) of the MAAS process in the PBR treating UW from the KWWTP as described in paper II. Table 2 shows the relationship between chl. a and dry weight of the MAAS.

The chl. a content gradually decreased with the retention time in both reactors due to low SRT. Chl. a concentrations of 1.4 (RI) and 1.1 (RII) mg L⁻¹ were obtained during the acclimatisation phase (Figure 4.3 A). The dynamics show fluctuating chl. a concentration with reduction in the HRT from 6, 4 and 2 days (Figure 4.3 B). In RI and RII, the average chl. a of 0.9 ± 0.4 (6 days), 0.3 ± 0.1 (4 days) and 0.2 ± 0.1 (2 days HRT) was achieved due to the heterogenic profile of wastewater and light limitation.

Chl. a to dry weight ratio was stable throughout the study in RII but in the case of RI, chl. a content fluctuated and was unstable (Figure 4.3 B and Table 2). A similar study from Guzeit et al. (2005) showed a higher chl. a to dry weight content (>10 mg g⁻¹) at 2 to 7 days HRT (based on 10: 14 and 12: 12 (light: dark) hours) with Chlorella sp. in laboratory and pilot scale reactors with 2000 and 500 PAR using pre-treated wastewater.

Medina and Neis (2007) report a similar study to Gutzeit et al. (2005), with the same configuration using synthetic wastewater at laboratory scale, showing a lower chl. a content at 2.7 days (5.8 mg g⁻¹, avg.) and 4 days (3.12 mg g⁻¹, avg.) of HRT. They also observed higher chl. a content in suspended form during the sedimentation (2.51 mg g⁻¹). This needs to be verified in future investigations.

Table 2. Trend of chl. a to dry weight during the semi-continuous PBR operation

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>UW</th>
<th>TW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 days HRT</td>
<td>4 days HRT</td>
</tr>
<tr>
<td>RI</td>
<td>RII</td>
<td>RI</td>
</tr>
<tr>
<td>Chl. a to dry weight (mg g⁻¹)</td>
<td>4.36 ± 0.61</td>
<td>3.75 ± 0.50</td>
</tr>
</tbody>
</table>

Figure 4.3 C shows the development of chl. a at 4 days HRT during the acclimatisation step, the luxury uptake of phosphorous (LUP) and the P stress condition (paper III). The acclimatisation of the high P condition contributed to an increase in chl. a content from 0.43 to 1.04 mg L⁻¹. Average chl. a contents of 1.4 ± 0.3 and 0.91 ± 0.04 mg L⁻¹ were observed in the respective conditions. However, the P stress condition showed a decrease in chl. a content in
comparison to LUP. This is in agreement with the chl. a to dry weight ratio (see also section Figure 4.7 and Table 1). The decrease in chl. a may be a result of either the limitation of light or the fungal or cyanobacterial colonies observed on fat particles during the cultivation period (Paper III; supplementary Figures).
Figure 4.3  Chl. a dynamics during the semi-continuous study on MAAS process. A) Acclimatisation phase of the MAAS in UW. B) At different HRTs in UW. C) At 4 days HRT in treated wastewater (Dash dotted lines in B and C separate each cycle during the HRT study)

4.2 Bio-nutrient and COD removal

The removal of bio-nutrients such as N and P were studied in papers I, II and III as shown in Figure 4.4 A, B and C. In addition, organic carbon was studied in paper II. The average initial concentrations of N, P, COD and OC in papers I, II and III are shown in Table 1. In general, N and P were used to determine the removal efficiency of N and P in the results and discussion sections.

4.2.1 Nitrogen, phosphorus and organic carbon removal

Differences in light intensity and wavelength influenced the chl. a dynamics. Here nutrient removal is used to indicate the performance of the process. Figure 4.4 A shows the results of nutrient removal obtained from indigenous microalgae cultivation with different lighting (paper I). The removal of N after the preliminary experiment showed similar response with the FL, 68 ± 6 % and PWM-LED 1, 72 ± 3 %. The residual concentrations ($r_0$) were 9.60 ± 1.70 and 8.60 ± 0.84 mg L⁻¹ in UW, as presented in paper I (Figure 4.4 A). On the other hand, the PWM-LED 1 (74 ± 6 %) achieved a higher N removal in comparison
to FL (52 ± 2%) in TW. The remaining N concentrations after the treatment period were 6.64 ± 0.50 and 17.65 ± 0.64 mg L⁻¹ in PWM LED 1 and FL respectively.

In both cases, the PWM–LED 2 with lack of white light (46 ± 6%, TW and 58 ± 10%, UW) showed a lower removal of N regardless of wastewater. The corresponding $r_d$ values were 21.77 ± 3.76 and 11.43 ± 2.94 mg L⁻¹. Overall, it can be reasoned that bacterial oxidation of organic compounds and nitrification favoured an increase in micro-algal concentration as observed in the previous section (see section 4.1.1). The nitrate based micro-algal growth may increase the pH due to the consumption of $H_2CO_3$ in the flasks as discussed in paper I (Krustok et al., 2016). Also, the intensity of white light in the PWM–LEDs played a vital role in the assimilation of nutrients. Therefore, an intense white LED could be a good candidate for microalgae cultivation in the UW. Kim et al. (2013) reported a similar study where white light increased the micro-algal growth in wastewater with the addition of blue and red light.

Further, the shift in pH showed 80–93% ($r_d$ of 0.26–0.4 mg L⁻¹) removal of P with UW (paper I), whereas in the case of TW similar removal was observed with PWM–LED 1 (73.66 ± 7.92 %, $r_d$ = 0.21 ± 0.10 mg L⁻¹) and FL (70.35 ± 5.76 %, $r_d$ = 0.29 mg L⁻¹). The removal was lower with PWM–LED 2 (45.77 ± 1.31 %, $r_d$ = 0.53 mg L⁻¹), although the initial concentrations were very low (Table 1). This was due to the slow growth as observed from chl. $a$ dynamics (Figure 4.1 A and B).
Figure 4.4 Removal of bio nutrients. A) Removal of TN/N and TP/P during different light regime. B) Removal of N, P and OC at different HRTs in UW and C) Removal of N and P in iron flocculated wastewater.
In paper II, the removal of N was studied in semi-continuous reactors (RI and RII) with similar conditions in a controlled environment using UW. A stable N and P removal was achieved at 6 and 4 days HRT in RI and RII as shown in Figure 4.4 B. The residual concentrations of nutrients remaining in treated wastewater at all HRTs is shown in Figure A.1 and A.2 (Appendix). Similar nitrogen removal was observed by Gutzeit al. (2005) and Medina and Neis (2007) at 2-3 days HRT under discontinuous lighting. Most importantly, the shift from 4 to 2 days HRT facilitated an unstable nutrient removal during the second cycle in RI in comparison to RII. Hence, this contributed to a nitrogen removal efficiency of 34 ± 24% and r_d of 16 ± 5 mg L^{-1}. However, RII showed a removal efficiency of 43 ± 7% and 16 ± 0.80 mg L^{-1} of residual nitrogen in the treated wastewater. RI and RII showed a P removal of 54 ± 5 and 56 ± 5% with a similar r_d of 1.9 ± 0.1 and 1.8 ± 0.3 mg L^{-1}. At all HRTs, bio-mediated precipitation (i.e. due to calcium ions) and elevated pH contributed to a P removal efficiency of 50-60% (Mañas et al., 2012).

As shown in figure 4.4 B, a predominantly bacterial oxidation was observed in different HRT regimes. This resulted in stable organic carbon (OC) removal efficiencies of 83 ± 8 (r_d = 27 ± 10.45 mg L^{-1}), 80 ± 6 (r_d = 35.56 ± 4.03 mg L^{-1}) and 78 ± 5 (r_d = 33.80 ± 4.72 mg L^{-1}) % at 6, 4 and 2 days HRT in RI. The removal efficiencies in RII were 73 ± 10 (r_d = 40.75 ± 9.63 mg L^{-1}), 80 ± 1 (r_d = 31.76 ± 4.29 mg L^{-1}) and 73 ± 12 (r_d = 34.33 ± 7.10 mg L^{-1}) % respectively (see also section 4.2.2 for COD removal).

As shown in Figure 4.4 C, the removal of nitrogen at the expense of P was stable during the MAAS process in iron flocculated wastewater (paper III). The addition of P at 6 (LUP) and 3 (P stress) mg L^{-1} resulted in nitrogen removal of 88 ± 1 and 92 ± 1 % with residual nitrogen in wastewater of 3.78 ± 0.85 and 2.21 mg L^{-1}. Phosphate removal was consistent with the LUP stage (68 ± 7 %) and decreased from 74 to 43% under the P stress conditions in the flocculated wastewater. The remaining P concentrations were 2.31 ± 0.14 (LUP) and 1.74 ± 0.53 (P stress stage) mg L^{-1}.

4.2.2 COD removal in wastewater

Figure 4.5 shows the overall COD removal efficiency during the semi-continuous PBR operation. Figure 4.5 A shows the average COD removal of 3 cycles at different HRT regimes as discussed in paper II. The average COD removal efficiency was stable at all HRTs: 6 days (81 ± 5 %, RI and 82 ± 3%, RII), 4 days (74 ± 4 %, RI and 70 ± 2 %, RII) and 2 days (76 ± 6 %, RI and 78 ± 3 %, RII) (paper II). These results were in agreement with the OC removal where bacterial oxidation favoured the biological oxidation of organic matter in raw wastewater.
Figure A.3 (Appendix) shows the residual concentration in treated wastewater with different HRT regimes. The corresponding concentrations 51–106, 68–96, and 54–95 mg L\(^{-1}\) were found in the treated wastewater.

![Graph A](image)

**Figure 4.5** COD removal in PBRs. A) Influence of different HRTs in PBRs with duplicates and B) Influence of P.
Figure 4.5 B shows the removal of COD during the luxury uptake of phosphorous (LUP) and P stress (i.e. stress due to decrease in P) conditions as described in paper III. The results were obtained from the average of 2 cycles, where LUP and P stress conditions achieved a stable COD removal of 63 ± 10 and 78 ± 1% and the residual concentrations of 67.20 ± 7.80 and 55.5 ± 15 mg L\(^{-1}\), respectively.

Overall, the COD results were in agreement with the study conducted by Gutzeit et al. (2005), although that study included discontinuous lighting and light intensity of 2000 PAR. In both cases, the COD removal was stable and varied slightly due to seasonal weather and the heterogenic profile of the wastewater at the different locations, 1 and 2 (also refer Table 1). Most importantly, bacterial assimilation and COD oxidation were dominant at shorter HRT (Ge et al., 2013).

4.3 Characteristics of the biomass

4.3.1 Dry weight

The dry weight was used for estimating the MAAS content in PBRs. Figures 4.6 and 4.7 show the measured dry weight during the batch and the semi-continuous experiments.
In the batch experiments, effective nutrient utilisation from wastewater was observed based on the dry weight obtained at different spectral compositions of light. Overall, the PWM-LED with higher light intensity showed higher biomass concentration in comparison to low intensity PWM-LED and fluorescent light (Figure 4.6).

Figure 4.7 A shows the effect of HRT on dry weight during the semi-continuous experiment. In the first part, the HRT had a significant influence on the biomass production using UW as shown in Figure 4.7 A. There was a higher dry weight of MAAS with a HRT of 6 days in comparison to 4 and 2 days in both reactors. This was in agreement with measured chl. a content to dry weight (see Table 2). The biomass concentration increased with increased HRT in this study where the HRT is equivalent to SRT.

Bacterial concentrations were very high in comparison to the micro-algal concentration due to bacterial proliferation based on the COD, OC and dry weight. Therefore, it was important to maintain a optimal balance between the micro-algal and activated sludge organisms in the PBRs by operating with an optimal sludge retention time (Santiago et al., 2013). On the other hand, the HRT equivalent to SRT will either decrease micro-algal concentration or increase activated sludge organisms, which affect the photosynthesis by introducing turbidity in the system. Gutzeit et al. (2005) showed higher biomass (>1.5 g L⁻¹) growth with synthetic and pretreated wastewater in comparison to
this study without any control of the operation with higher sludge age (>15 days). Overall, the batch study (paper I) showed similar dry weight in comparison to 6 days HRT of PBRs, even though the process was not controlled and the working volume was very small.

In the second part of the semi-continuous experiment, the HRT was kept constant at 4 days and the MAAS was observed to be high in both test conditions, LUP and P stress. The average maximum of 408.35 ± 2.60 mg L\(^{-1}\) was achieved with the P stress condition, compared to LUP (373 ± 2.50 mg L\(^{-1}\)). Further study may provide evidence of a constant biomass maintenance in a continuous PBR at low HRT. Overall, the dry weight obtained during this study (paper III) was higher compared to the raw waste water treatment (paper II) due to the difference in the pre-culturing strategy and the wastewater characteristics.

![Graph showing dry weight comparison with HRT]

A)
4.3.2 Sludge volume index

Imhoff cone experiments using UW showed rapid settleability during the semi-continuous PBR operation at different HRTs even though the dry weight varied with the HRT (Figure 4.7 C). SVI was determined from the ratio of settled volume in the cones to the measured dry weight. Overall, the average measured SVI values were <117 and <85 mL g⁻¹ in RI and RII respectively in the HRT study in UW (Figure 4.7 C; Paper II). Higher SVI values were observed during the first cycle of all HRTs, and a slight decrease in SVI was subsequently observed for all treatment conditions (Paper II, supplementary information). The obtained results were in agreement with the previous study from Gutzeit et al. (2005), which reported complete settling after 10 minutes of settling time with one part of *Chlorella* and two parts of activated sludge. However, the conditions were different to those used in the present study, for instance, Gutzeit et al. (2005) used light and dark hours (12:12), high sludge age (i.e., 15–25 days) and pre-treated wastewater.

SVI₅ of LUP and P stress condition are shown in Figure 4.7 D. The phosphorous study on the PBRs using chemically processed wastewater showed <45 mL g⁻¹ SVI at 4 days HRT in both conditions. In addition to the SVI, the settling velocity was calculated to determine the rapid settleability of the MAAS.
(Table 3 and Paper III). Table 3 shows the velocity of settled sludge in Omhoff cones. In general, cone shaped sedimentation vessels are used in most WWTPs. The settling velocity was not comparable with a similar study from Tiron et al. (2015) with activated algae granules because their settling velocity was determined in volumetric cylinders. The sludge obtained during the MAAS process in pre-treated wastewater showed a rapid settling velocity in the cones with complete settling within 5 minutes. It can be reasoned that residual iron may assist flocculation of MAAS.

Overall, bulking of sludge was not observed after the MAAS process. Bulking is a very common problem in the activated sludge process due to the presence of filamentous bacteria which creates a web-like structure by retaining gas bubbles and floc particles, forming floating stacks (Madoni et al., 2000).

Table 3. Settling velocity of MAAS flocs obtained at 4 days HRT during the semi-continuous study on PBR.

<table>
<thead>
<tr>
<th>Acclimatisation phase</th>
<th>LUP</th>
<th>P stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Settling velocity (m hr⁻¹)</td>
<td>130</td>
<td>91.8±1.8*</td>
</tr>
</tbody>
</table>

* Average of two cycles

4.4 Oxygen evolution of MAAS with different light intensities and biomass concentration.

Figure 4.8 shows oxygen evolution of the MAAS under continuous lighting with different light intensities (see also the experimental setup in section 3.3.1). The rate of oxygen evolution increased with increasing light intensity (>100 PAR) when the fixed initial biomass concentrations in the PBRs were 258 ± 2.5 and 573 ± 11 mg L⁻¹, as described in paper III. However, the oxygen evolution reached a saturation point at low MAAS (110 mg L⁻¹) in the PBR. The photosynthetic activity proliferated between 200 to 400 PAR and was lower above 400 PAR. The literature indicates that this effect is due to the saturation of the light apparatus (i.e. photosystems) (Muñoz and Guieysse, 2006; Ogbonna and Tanaka, 2000). For instance, this state was clearly observed with lower biomass concentration (110 mg L⁻¹) during the DO evolution study. In contrast to low and intermediate MAAS, the high concentration of the MAAS showed a rapid evolution of DO with light intensities above 100 PAR (Figure 4.9 A).

Figure 4.9 B shows the trend of final DO to initial chl. a mass ratio in the PBRs. The low and high MAAS showed a similar trend which could be due to the saturation (i.e. low chl.a content, 0.475 mg L⁻¹) of light receiving antennae
in the pigment and overcrowding (Muñoz and Guieysse, 2006). This trend was
dominant although the evolution of oxygen increased with the intensity of light
(Cuaresma et al., 2011; Van Wagenen et al., 2012) (Figure 4.8).

Figure A. 4 shows the oxygen evolution rate at different MAAS in the PBRs.
The oxygen evolution rate increased with increase in the concentration of the
MAAS at higher light intensities. The rate approached a stationary phase at low
MAAS. These results suggest that higher MAAS during the cultivation requires
more lighting due to the resulting turbidity.

Altogether, the DO evolution study demonstrates that a constant supply of
PAR between 250 and 500 is required for the MAAS process in closed PBRs.
Additionally, the TSS should be maintained between 250 and 573 mg L\(^{-1}\) to
achieve optimal growth and to avoid excessive power consumption due to
excess lighting.

![Figure 4.8](image)

*Figure 4.8 Oxygen evolution from different biomass concentrations at
different light intensities in PBRs*
4.5 Rate of biogas production from MAAS

The rates of biogas production in response to the MAAS at different HRTs based on the biogas potential tests are shown in Figure 4.10. More than 80% of the biogas accumulation was achieved between 9 and 11 days following pre-treatment, and after 13 days for untreated samples. The final biogas yields for untreated MAAS were 268 ± 2, 258 ± 11 and 349 ± 10 mL gVS⁻¹ at 6, 4 and 2 days HRT respectively (Figure A. 5, Appendix). The corresponding biogas yields after the thermal pre-treatment were 263 ± 10, 266 ± 16 and 308 ± 19 mL gVS⁻¹ (Figure A. 5).

The rapid gas production observed in this study was in agreement with previous work by Olsson et al. (2014) on the anaerobic mono-digestion of microalgae cultivated in flue gas. However, the MAAS from 6 and 4 days HRT did not show any significant variation with the biogas yield regardless of the pre-treatment (Paper II). Overall, the MAAS obtained from 2 days HRT showed a higher biogas production rate in comparison to 6 and 4 days HRT. This may due to an increase in the activated sludge organisms and the solids in comparison to micro-algal content and SRT as observed in the previous study by Ge et al. (2013).
Wang et al. (2013) showed that biogas yield was lower with mono-digestion of Chlorella alone in comparison to digestion with waste activated sludge. The biogas yield (262 mL g VS$^{-1}$) observed in that study after 45 days was similar to that observed in the present study. In addition, the observed gas production is consistent with the decrease in biomass with decrease in HRT (See also section 4.1.3). The presence of cell wall components (i.e. cellulose, hemicellulose, pectin and glycol proteins) and inhibitory compounds may be responsible for lower biogas production with the MAAS after the pre-treatment (Carrere et al., 2016). In addition, a recent study showed that a combination of thermal (80°C) and low dose of acid or alkali treatment (0.5%, V/V) improved the methane yield (82–86% increase) with algae grown in municipal wastewater (Passos et al., 2016). Therefore, biogas production may be enhanced in future with an alternative pre-treatment of microalgae.

**Figure 4.10** Rate of biogas production during mesophilic anaerobic digestion of the MAAS obtained at different HRTs (final biogas yields after 25 days are shown in the legends with respective HRTs)
5 Conclusions and outlook

The experiments performed in lab scale photo-bioreactors (Paper II and paper III) were based on the preliminary batch studies (Paper I and paper III), and showed the applicability of the MAAS process. The preliminary batch study (Paper I) showed that intensity of white LED light had a positive effect on microalgae chlorophyll development and nutrient removal. Hence, white LED light can be considered as a good candidate for closed PBR operation.

HRT studies (Paper II) in the semi-continuous PBRs demonstrated that removal of nitrogen and phosphorous increased with increased HRT during treatment of UW. Hence, most of the nutrients can be recovered as C, N and P in the sludge with buffering of CO₂ at 4 and 6 days HRT. In addition, the biogas potential of sludge through anaerobic mono-digestion showed an increase in biogas yield with sludge obtained from 2 days HRT in comparison with sludge from 4 and 6 days HRT. Further studies are required to confirm the reproducibility of these results in a continuous PBR. The results from the lab scale studies may provide the opportunity to reduce the retention time further during pilot scale operation using UW.

The fed batch study showed that the phosphorous remaining in wastewater after the chemical precipitation step was not available for micro-algal chl. a development (Paper III). In addition, the semi-continuous PBR showed improved micro-algal growth and nitrogen removal in the presence of added phosphorous in the TW. Most importantly, the inoculum preparation presented in UW (Paper II) is key for maximum nitrogen removal efficiency, in comparison to that of paper III (TW).

In addition, the cultivation of microalgae in the activated sludge process resulted in outstanding sludge settleability after 10 minutes in UW and TW throughout these investigations (papers II and III). However, this has to be verified in the pilot scale studies.

During the experiments, fluorescent lights were employed, but the replacement with LEDs will be key to the operation of the full scale reactors. On the other hand, other technological solutions such as solar reflected lighting technology (i.e. used in buildings) can also be used to augment the sunlight conditions in Sweden. This creates a possibility for solar based LED operation in the future. Economic evaluation of the studies has to be verified for long-term operation. The process has added benefits, such as photo-degradation of harmful viruses and emerging contaminants such as oestrogens, aromatic compounds from personal care products, and antibiotics that may be reduced.
during the process. Furthermore, these cultivation conditions also enable the production of biomass for biofuel production, such as exhaust gas, and biogas upgrading for carbon capture and biomass production.

Overall, the energy allocated to the aeration process can be reallocated to the MAAS process if it is operated with optimal light conditions in the long run. However, the energy demand for lighting in PBRs can be compensated for since the excess chemical consumption is reduced in comparison with the conventional process. Moreover, impurities such as trace metals associated with flocculants, accumulation of precipitated solids and corrosion can be avoided by using the MAAS process. The carbon, nitrogen and phosphorous can be captured in the biomass with less impact on environment (i.e., release of CO$_2$ and N$_2$, and chemical sludge) in comparison to the conventional process.
6 Future works

Future studies will focus on a continuous photo-bioreactor in pilot scale operation (from 20 L to 600-800 L) using LEDs in winter conditions. The possible outcome of this study will be a complete process, where the recirculation of sludge is a vital part of the process to maintain optimal sludge age based on the HRT. Also, the maximum removal of COD, N and P with shorter HRT ($\leq 2$ days HRT) to achieve discharge limits stricter than the Swedish regulations will be the main objective.

The future objectives can be categorised as follows:

- **Other application**: ongoing work on a continuous system with removal of CO$_2$ and biomass production using diesel engine exhaust gases and reject water as a nutrient source. This study includes optimisation of nutrient removal using similar dominant microbial communities in a saline environment (*Chlorella* and *Scenedesmus sp.* ) using tubular photo-bioreactors. It also includes the integration of reject water as a nutrient source for increasing biomass in wastewater application and carbon capture.

- **Pilot study**: the nutrient mass balance of the continuous MAAS process and its settling characteristics has to be verified, also including energy efficient lighting and mixing strategies in the PBRs.

- **Stress conditions on the MAAS**: an extended study on stress conditions such as temperature and shorter HRT on the integrity of algal bacterial flocs will be a forthcoming study from a process perspective. Flocs or granules have a large influence on light penetration and thus influence the number of photons that hit the micro-algal cells.
References


Glossary

**Anoxic**: A condition where molecular oxygen is not available for bacterial respiration. Anoxic bacteria prefer NO₃ for respiration.

**Activated sludge**: A mass of aerated precipitated portion of sewage that contains bacteria, archaea and protozoa as dominant microbes along with other organisms. This mass is active during the degradation of the organic matter in the sewage.

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

**Biogas/Bio–methane potential tests**: A bottle assay used to measure the maximum amount of biogas or bio–methane per gram of volatile solids from the organic substrate during the anaerobic digestion.

**Batch operation**: During the operation, wastewater is only added at the beginning and not during the treatment.

**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 (NADPH)**: Nicotinamide adenine di nucleotide phosphate is a reducing agent for most of the biosynthetic reactions, e.g. during the Calvin cycle (i.e. assimilation of carbohydrate).

**Chemical Oxygen demand (COD)**: The chemical oxygen demand 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. The oxygen required to degrade (oxidise) the pollutants completely in wastewater is determined during this step.

**Eutrophication**: A condition where lakes or rivers acquire high concentration of nitrates and phosphates. This apparently promotes excessive growth of algae. As the algae grow and decompose on the surface, high levels of organic matter and the decomposing organisms deplete the dissolved oxygen in the water. This causes the death of other oxygen-dependent organisms such as fish. Eutrophication is in nature, but human emissions can significantly speed up the process.

**High rate algal ponds (HRAPs)**: Shallow, open raceway ponds 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 the photo-bioreactor/any storage unit.

**Imhoff Cone:** The name Imhoff comes from German engineer Karl Imhoff, who designed this cone for determining sand content in wells and settleable matter in water and waste effluents. In principle, the sliding of substances takes place in the cone shaped vessels.

**Light emitting diodes (LEDs):** Light emitting diodes are defined as semiconductor devices that emit monochromatic light when an electric current passes through them. LEDs with wavelengths between red (650 nm) and blue-violet (480-400 nm) are commercially available.

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

**Pigments:** Substances that absorb light in the visible spectrum of electromagnetic radiation.

**Photosynthetic active radiation (PAR):** For photosynthesis, plants (including algae) require visible light of wavelength between 400 to 700 nm from electromagnetic radiation (Sun light) or artificial light. The radiation in this range of wavelengths is called photosynthetic active radiation (PAR). It is expressed in photosynthetic photon flux density (PPFD) as $\mu$ mol m$^{-2}$ s$^{-1}$ or $\mu$ Einstein m$^{-2}$ s$^{-1}$ (i.e. photon flux per unit area). In general, during the photosynthesis process, two photons excite the electron of chloroplast.

**Sludge retention time (SRT):** The SRT is the average time the solids (MAAS) are in the system.

**Sludge volume index (SVI):** The SVI is used to evaluate the settling properties of sludge and it can be related to poorer settleability of the sludge. SVI above 150 mL g$^{-1}$ is considered as poor sludge.
Appendix

Figure A.1 Influence of HRT on nitrogen and phosphorous removal

Figure A.2 Influence of HRT on suspended organic carbon removal
Figure A.3 Influence of HRT on COD removal

Figure A.4 Oxygen evolution rate (OER) of MAAS in response to different light intensities
Figure A.5  Accumulated biogas yield of MAAS, before and after pre-treatment.