# Used water resource recovery using green microalgae

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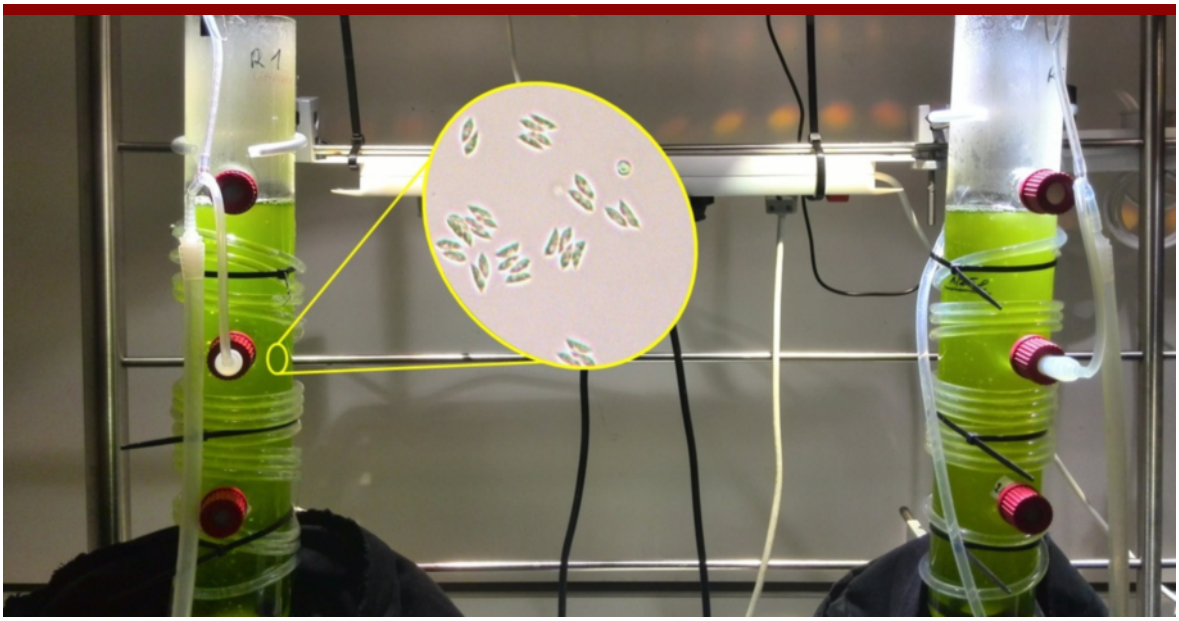


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PhD Thesis
December 2016

DTU Environment
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# Preface

This thesis is based on the work carried out at the Department of Environmental Engineering at the Technical University of Denmark from September 2013 to October 2016. This thesis was prepared as part of the EU project E4WATER (FP7-NMP-2011.3.4-1 grant agreement 280756). The research was performed under the main supervision of Associate Professor Benedek Gy. Plósz (DTU Environment) and co-supervision of Professor Barth F. Smets (DTU Environment).

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the text by their paper number written with the Roman numerals **I-IV**.

**I** **Wágner, D.S.**, Valverde-Pérez, B., Sæbø, M., Bregua de la Sotilla, M., Van Wagenen, J., Smets, B.F., Plósz, B.Gy., 2016. Towards a consensus-based biokinetic model for green microalgae – The ASM-A. *Water Research*, **103**, 485-499.

**II** **Wágner, D.S.**, Radovici, M., Smets, B.F., Angelidaki, I., Valverde-Pérez, B., Plósz, B.Gy., 2016. Harvesting microalgae using activated sludge can decrease polymer dosing and enhance methane production via co-digestion in a bacterial-microalgal process. *Algal Research*, **20**, 197-204.

**III** **Wágner, D.S.**, Valverde-Pérez, B., Plósz, B.Gy., 2016. Light attenuation in photobioreactors and algal pigmentation under different growth conditions – model identification and complexity assessment. Manuscript in preparation.

**IV** **Wágner, D.S.**, Cazzaniga, C., Steidl, M., Dechesne, A., Valverde-Pérez, B., Plósz, B.Gy., 2016. Re-definition of the optimal N-to-P ratio concept and its importance for stable microalgal cultivation and water treatment. Submitted Manuscript.

In this online version of the thesis, paper **I-IV** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from DTU Environment, Technical University of Denmark, Miljoevej, Building 113, 2800 Kgs. Lyngby, Denmark, info@env.dtu.dk.

In addition, the following publications, not included in this thesis, were also concluded during this PhD study:

- Valverde-Pérez, B., **Wágner, D.S.**, Steidl, M., Villez, K., Plósz, B.Gy., 2016. On-line monitoring of open pond algal reactors treating wastewater using a spectral sensor. In preparation for Algal Research.
- Valverde-Pérez, B., **Wágner, D.S.**, Lóránt, B., Gülay, A., Smets, B.F., Plósz, B.Gy., 2016. Short-sludge age EBPR process – Microbial and biochemical process characterisation during reactor start-up and operation. *Water Research*, **104**, 320-329.
- **Wágner, D.S.**, Ramin, E., Szabo, P., Dechesne, A., Plósz, B.Gy., 2015. *Microthrix parvicella* abundance associates with activated sludge settling velocity and rheology – Quantifying and modelling filamentous bulking. *Water Research*, **78**, 121-132.
- Ramin, E., **Wágner, D.S.**, Yde, L., Binning, P.J., Rasmussen, M.R, Mikkelsen, P.S., Plósz, B.Gy., 2014. A new settling velocity model to describe secondary sedimentation. *Water Research*, **66**, 447-458.

This PhD study also contributed to international conferences with the following proceeding papers:

- Valverde-Pérez, B., Penkarski-Rodon, E., Zhang, X., **Wágner, D.S.**, Plósz, B.Gy., 2016. Protocol for settling velocity model calibration using an innovative batch settling test– focus on identifiability analysis of the hindered-transient-compression model. Oral presentation, WEFTEC, New Orleans, United States.
- **Wágner, D.S.**, Valverde-Pérez, B., Cazzaniga, C., Steidl, M., Dechesne, A., Plósz, B.Gy., 2016. Impact of influent quality on green microalgal cultivation with used water resources – experimental assessment combined with image analysis. Poster presentation, MEWE and biofilms IWA specialist conference, Copenhagen, Denmark.

- Valverde-Pérez, B., **Wágner, D.S.**, Lóránt, B., Gülay, A., Radovici, M., Angelidaki, I., Smets, B.F., Plósz, B.Gy., 2016. Microbial and biochemical process characterization of a low-sludge age EBPR process for resource recovery. Poster presentation, MEWE and biofilms IWA specialist conference, Copenhagen, Denmark.

- Valverde-Pérez, B., **Wágner, D.S.**, Lóránt, B., Gülay, A., Radovici, M., Angelidaki, I., Smets, B.F., Plósz, B.Gy., 2016. Low-sludge age EBPR process for resource recovery – microbial and biochemical process characterization. Poster presentation, IWA-WEF Nutrient Removal and Recovery, Denver, CO, United States.

- Valverde-Pérez, B., Penkarski-Rodon, E., **Wágner, D.S.**, Plósz, B.Gy., 2016. Secondary settling sensor setup development – testing prototypes and compression models via practical model parameter identifiability assessment. Oral presentation, Particle Separation, Oslo, Norway.

- Radovici, M., **Wágner, D.S.**, Angelidaki, I., Valverde-Pérez, B., Plósz, B.Gy., 2016. Bioflocculation of green microalgae using activated sludge and potential for biogas production. Poster presentation, 13$^{th}$ IWA Leading Edge Conference on Water and Wastewater Technologies, Jerez da la Frontera, Spain.

- Valverde-Pérez, B., **Wágner, D.S.**, Fuentes-Martínez, J.M., Steidl, M., Dechesne, A., Flores Alsina, X., Gernaey, K., Huusom, J.K., Plósz, B.Gy., 2016. Optimal algal cultivation for used water resource recovery. Poster pitch presentation, 13$^{th}$ IWA Leading Edge Conference on Water and Wastewater Technologies, Jerez da la Frontera, Spain.

- **Wágner, D.S.**, Radovici, M., Angelidaki, I., Valverde-Pérez, B., Plósz, B.Gy., 2016. Co-digestion of microalgae and activated sludge following a novel bioflocculation method. Poster pitch presentation, YAS2016: Young Algaeneers Symposium, Malta.

- **Wágner, D.S.**, Radovici, M., Valverde-Pérez, B., Plósz, B.Gy., 2016. A novel bioflocculation method to separate microalgal biomass cultivated on wastewater resources. Oral presentation, 2$^{nd}$ Young Water Professionals Denmark Conference and Workshop, Aarhus, Denmark.

- **Wágner, D.S.**, Valverde-Pérez, B., Sæbø, M., Bregua de la Sotilla, M., Van Wagenen, J., Smets, B.F., Plósz, B.Gy., 2015. Wastewater resource recovery with green microalgae – modelling the microalgal growth, nutri-

ent uptake and storage using ASM-A. Poster presentation, 1st IWA Resource Recovery Conference, Ghent, Belgium.

- Valverde-Pérez, B., **Wágner, D.S.**, Cecchin, F., Jensen, C.K., Smets, B.F., Plósz, B.Gy., 2015. Impact of operational conditions and reactor configuration on process performance and microbial community in short solid retention time EBPR systems. Poster presentation, 1st IWA Resource Recovery Conference, Ghent, Belgium.

- **Wágner, D.S**., Valverde-Pérez, B., Sæbø, M., Bregua de la Sotilla, M., Van Wagenen, J., Smets, B.F., Plósz, B.Gy., 2015. Modeling green microalgal growth, nutrient uptake and storage in the ASM framework. Oral presentation, 9th IWA Symposium on Systems Analysis and Integrated Assessment, Gold Coast, Queensland, Australia.

- Valverde-Pérez, B., Fuentes-Martínez, J.M., Flores Alsina, X., **Wágner, D.S.**, Gernaey, K., Huusom, J.K., Plósz, B.Gy., 2015. Control structure design for an EBP2R process operated as a sequencing batch reactor. Poster presentation, 9th IWA Symposium on Systems Analysis and Integrated Assessment, Gold Coast, Queensland, Australia.

- **Wágner, D.S.**, Valverde-Pérez, B., Sæbø, M., Van Wagenen, J., Angelidaki, I., Smets, B.F., Plósz, B.Gy., 2014. The effect of light on mixed green micro-algal growth: experimental assessment and modelling. Poster presentation, IWA World Water Congress & Exhibition, Lisbon, Portugal.

- **Wágner, D.S.**, Ramin, E., Szabo, P., Dechesne, A., Smets, B.F., Plósz, B.Gy., 2014. Effects of filamentous bulking on activated sludge rheology and compression settling velocity. Oral presentation, IWA World Water Congress & Exhibition, Lisbon, Portugal.

- Bregua de la Sotilla, M., **Wágner, D.S.**, Valverde-Pérez, B., Van Wagenen, J., Angelidaki, I., Smets, B.F., Plósz, B.Gy., 2014. Modelling and assessment of the storage of nutrients in a mixed green microalgae culture. Oral presentation, 2nd International Conference on Algal Biorefinery, Lyngby, Denmark.

- **Wágner, D.S.**, Valverde-Pérez, B., Sæbø, M., Van Wagenen, J., Angelidaki, I., Smets, B.F., Plósz, B.Gy., 2014. An activated sludge model for mixed green microalgae (ASM-A): model identification and calibration. Oral presentation, YAS2014: Young Algaeneers Symposium, Montpellier-Narbonne, France.

- Ramin, E., **Wágner, D.S.**, Yde, L., Szabo, P., Rasmussen, M.R., Dechesne, A., Smets, B.F., Mikkelsen, P.S., Plósz, B.Gy., 2014. Modelling the impact of filamentous bacteria abundance in a secondary settling tank: CFD sub-models optimization using long - term experimental data. Oral presentation, 4th IWA/WEF Wastewater Treatment Modelling Seminar, Spa, Belgium.
- Valverde-Pérez, B., **Wágner, D.S.**, Sæbø, M., Van Wagenen, J., Angelidaki, I., Smets, B.F. Plósz, B.Gy., 2014. A green micro-algal growth model developed in the activated sludge modeling framework. Poster presentation, 4th IWA/WEF Wastewater Treatment Modelling Seminar, Spa, Belgium.

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# Summary

A paradigm shift is promoted in wastewater treatment whereby wastewater is considered as a source of nutrients, water and energy, rather than waste and it is referred to as used water. Microalgae cultivation on used water resources offers the potential to recover nitrogen, phosphorus, water and energy. When coupling with used water treatment, microalgae is mostly considered to produce energy through biofuel production. A novel used water resource recovery approach was presented earlier, referred to as TRENS – a fully biochemical process for the removal, recovery and reuse of used water resources promoting sustainable urban water management. The system consists of a low solids retention time (SRT) enhanced biological phosphorus removal and recovery (EBP2R) system that can provide optimal cultivation medium – in terms of nutrients and water – for downstream microalgal cultivation. The microalgal suspension cultivated in the photobioreactor (PBR) can be then used for e.g., "fertigation" on agricultural land whereby the water and the nutrients are recovered. Alternatively, the algal biomass can be harvested and can be used for co-digestion in existing anaerobic digesters, whereas the water content can be used for aquifer recharge.

Design and optimization of bacterial-microalgal systems requires process models that can be readily combined with consensus used water treatment models, e.g. the activated sludge models (ASM). Previous microalgal process models cannot be used for such purposes as a result of their deficiencies. Some lack e.g., accounting for the storage of nitrogen and phosphorus and for the potential for microalgae to grow heterotrophic on organic carbon that are relevant processes for used water resource recovery systems.

Therefore, the first objective of this thesis is to develop a consensus-based microalgal process model (ASM-A) accounting for photoautotrophic and heterotrophic microalgal growth, the uptake and storage of nitrogen and phosphorus and decay. The model was developed in the ASM framework as an extension to ASM-2d, thus it can be readily connected to bacterial unit processes. The process rates of the microalgal model were identified based on extensive literature review. Laboratory experiments in differently scaled batch PBRs were conducted in order to provide proper measurement data for model identification, comprising the selection of process rate equations as well as the estimation of the stoichiometric and kinetic model parameter distribution. The model identifiability analysis was conducted using the Latin Hypercube Sampling based Simplex (LHSS) method, adapted from the litera-

ture. The process model identified can effectively describe microalgal biomass concentration, soluble ammonium and phosphate concentrations as well as the phosphorus storage. The nitrogen storage is found to be affected by substrate availability, whilst the soluble nitrate concentration depends on the culture history, thereby requiring scenario specific model calibration. One of the most important factors affecting microalgal growth is the available light. Thus, for predicting the light distribution, the effect of using different simulation model structures on the model accuracy and uncertainty was assessed. Moreover, the effects of light scattering, biomass concentration and pigmentation on light attenuation in PBRs were investigated, using laboratory-scale experimental data. The light attenuation coefficient was estimated using the Lambert-Beer equation. Results suggest that light attenuation depends primarily on the pigmentation of the microalgae and also on the biomass concentration. Moreover, using a discretized layer-model to describe the light distribution in PBRs can result in more accurate prediction of the microalgal growth as well as the reduction of the uncertainty of the model predictions.

Furthermore, the effect of the variation of influent N-to-P ratio on the reactor performance was assessed in a mixed consortium of *Chlorella* and *Scenedesmus sp*. as well as in a monoculture of *Chlorella sp*. (both commonly used in used water treatment systems) in continuous cultivation using the treated used water from the upstream EBP2R system. When the N-to-P ratio in the influent was lowered to a sub-optimal level diatoms proliferated in the PBR cultivating the mixed green microalgal consortium. Once the ratio was increased again, the diatoms could be washed out of the system. Model predictive accuracy deteriorated as a result of the changes in culture composition due to the possible change in microalgal kinetics. The variation of the N-to-P ratio did not have an effect on the composition of the monoculture of *Chlorella sp.*, no contamination was encountered during the 85 days of cultivation on used water. The upstream bacterial unit process in the second case was operated at a higher SRT (16 d), suggesting that longer SRT might be able to mitigate the potential of contamination by other microalgal species.

Lastly, an innovative method was developed to harvest microalgal biomass grown in suspended cultures in the TRENS system. A two-step flocculation was applied, whereby in the first step cationic polymer was added to the microalgae to destabilize the cells, then in the second step the aggregation of flocs was enhanced by the addition of bacterial biomass wasted in the upstream short-SRT EBPR process. Effective recovery was obtained (97%), by the significant (40%) reduction in the amount of cationic polymer required

compared to the case when only cationic polymer was used for the flocculation without the addition of bacteria, thus further reducing harvesting costs. The biomethane potential of the harvested microalgal-bacterial biomass was estimated at mesophilic conditions, obtaining synergistic effect when co-digesting the two substrates and resulting in a maximum methane yield of 560±24 ml$CH_4$/gVS.

# Dansk sammenfatning

En paradigmeskift er på vej indenfor spildevandsrensning, spildevandet betragtes ikke længere som affald, men som en kilde til næringssalte, vand og energi, og omtales i denne sammenhæng som brugt vand. Brugt vand kan bruges til dyrkning af mikroalger, hvilket giver mulighed for at genindvinde kvælstof, fosfor, vand og energi. Når mikroalger kobles til behandling af brugt vand, så er det mest for at producere energi gennem produktion af biobrændstoffer. Et nybrud i ressource genindvinding er blevet præsenteret tidligere, omtalt som TRENS – en fuldt ud biokemisk proces til fjernelse, genindvinding og genbrug af ressourcer i brugt vand, der fremmer bæredygtig urban vandforvaltning. Systemet består af en forbedret biologisk fosforfjernelse og genindvinding (EBP2R) med lav slamopholdstid (SRT), der kan give et optimalt dyrkningsmedie – i form af næringssalte og vand – for nedstrøms dyrkning af mirkoalger. Opløsningen med mikroalger dyrket i fotobioreaktor (PBR) kan bruges som gødning på landbrugsjord, hvorved vand og næringsstoffer genanvendes. Som et alternativ, kan algebiomassen høstes og anvendes til udrådning i eksisterende anaerobe rådnetanke, hvorimod vandindholdet kan bruges til at genopfylde grundvandsmagasiner.

Design og optimering af bakterie-mikroalge systemer kræver procesmodeller, som let kan kombineres med konsensusprægede vandbehandlingsmodeller, f.eks. ASM-modeller for aktiv slam. Tidligere procesmodeller med mikroalger kan ikke bruges til sådanne formål pga. deres mangelfuldhed. Nogle mangler for eksempel at redegøre for mikroalgers lagring af kvælstof og fosfor samt heterotrof vækst på organisk kulstof, der begge er relevante processer for ressource genanvendelsessystemer for brugt vand.

Det første formål med denne afhandling var at udvikle en konsensusbaseret mikroalge procesmodel (ASM-A), der redegør både for fotoautotrof og heterotrof vækst, optagelsen og lagring af kvælstof og fosfor samt nedbrydning. Modellen blev udviklet i en ASM struktur, en udvidelse af ASM-2d, således at den let kan forbindes til bakterielle enhedsprocesser. Procesraterne for mikroalge modellen er baseret på en omfattende gennemgang af publicerede artikler. Laboratorieforsøg med forskellige batch PBR'r blev udført for at få passende måledata til model identifikation, hvilket også inkluderer valg af procesrate, reaktionsligninger samt en vurdering af den støkiometriske og kinetiske fordeling af modelparametre. Analyse af modellens identificerbarhed blev udført ved hjælp af "Latin Hypercube Sampling based Simplex (LHSS)" metoden, tilpasset fra litteraturen. Den identificerede procesmodel-

len kan effektivt beskrive biomassekoncentration af mikoalger, den opløste koncentration af ammonium og fosfat samt fosfor lagring. Lagring af kvælstof var påvirket af substrattilgængelighed, mens nitratkoncentrationen afhænger af mikroalgekulturens historie, hvilket kræver situationsspecifik modelkalibrering. En af de vigtigste faktorer, der påvirker væksten af mikroalger, er tilgængeligheden af lys. For at kunne forudsige lysfordeling blev effekten af brugen af forskellige simulationsmodelstrukturer på modellens nøjagtighed og usikkerhed vurderet. Ligeledes blev effekterne af lysspredningen, biomassekoncentrationen og pigmentering på lys dæmpningen i PBR'r undersøgt i laboratoriet. Dæmpningskoefficient for lys blev beregnet ved brug af Lambert-Beer ligningen. Resultater antyder, at lysdæmpning afhænger af mikroalgernes pigmentering og biomassekoncentrationen. En diskretiseret lag-model til at beskrive lysfordelingen i PBR kan resultere i mere præcise forudsigelser af mikroalgervækst samt føre til en reduktion af usikkerheden på modellens forudsigelser.

Endvidere blev reaktor ydelsen som effekt af varierende N-til-P-forhold i indstrømningen undersøgt. Dette blev undersøgt i en blandet kultur af *Chlorella sp*. og *Scenedesmus sp.* samt i en renkultur af *Chlorella sp*. Begge disse alger er anvendt i behandlingsanlæg for brugt vand. Der blev brugt kontinuerlig dyrkning med behandlet brugt vand fra et opstrøms EBP2R system. Når N-til-P forholdet i tilløbet blev sænket til et sub-optimalt niveau, formerede kiselalgerne sig i PBR'en med blandingskulturen. Når forholdet blev forøget igen, blev kiselalgerne vasket ud af systemet. Model nøjagtigheden blev forværret som følge af ændringerne i kultursammensætning på grund af den mulige ændring i kinetikken til mikroalgerne. Ændringen i N-til-P-forholdet havde ikke nogen indvirkning på sammensætningen af renkulturen (*Chlorella sp.*), da der ikke forekom nogen forurening under de 85 dages dyrkning på spildevand. Den opstrøms bakterielle enhedsproces i det andet tilfælde blev drevet ved en højere SRT (16 d). Dette antyder at en længere SRT kunne være medvirkende til at man undgår en potential forurening af andre arter af mikroalger.

Til sidst blev en innovativ metode til høstning af mikroalgebiomassen, der dyrkes som opløste kulturer i TRENS systemet, udviklet. En to-trins flokkulering blev brugt. I det første trin blev kationiske polymer tilsat til mikroalgerne for at destabilisere cellerne. I det andet trin blev udviklingen af flokaggregater forøget ved at tilsætte bakteriel biomasse, som blev taget fra den opstrøms Bio-P (EBPR) proces med lav slamopholdstid (SRT). Der blev opnået en effektiv genvinding (97%) ved at reducere mængden af kationiske polymer

væsentlig (40%) sammenlignet med tilfældet, hvor kun kationisk polymer blev anvendt til flokkulering uden tilsætning af bakterier, hvilket yderligere reducerer omkostningerne til algehøst. Biometanpotentialet af den høstede mikroalge- og bakteriebiomasse blev under mesofile betingelser, med en opnået synergieffekt af at udrådne de to typer biomasse, beregnet til et maksimal metan udbytte på 560 ± 24 ml $CH_4$ / gVS.

# Table of contents

# Abbreviations

| | |
|---|---|
| AIC | Akaike's information criterion |
| ATP | Adenosine triphosphate |
| ASM | Activated sludge models |
| BMP | Biomethane potential |
| EBPR | Enhanced biological phosphorus removal |
| EBP2R | Enhanced biological phosphorus removal and recovery |
| N-to-P | Nitrogen to phosphorus molar ratio |
| LCA | Life cycle assessment |
| LHS | Latin Hypercube Sampling |
| LHSS | Latin Hypercube Sampling based Simplex |
| PAO | Phosphorus accumulating organisms |
| PBR | Photobioreactor |
| PDADMAC | Poly(diallyldimethylammonium chloride) |
| PHA | Polyhydroxyalkanoates |
| RMSNE | Root mean square normalised error |
| SBR | Sequencing batch reactor |
| SRT | Solids retention time |
| TAG | Triacylglycerides |
| VFA | Volatile fatty acids |

# 1 Introduction and objectives

## 1.1 Motivation

Historically, the role of wastewater treatment was to provide sanitation. Later, the removal of nutrients became important due to environmental protection, related to eutrophication. Thus, the aim of conventional wastewater treatment systems was to remove the organic carbon and nutrients from the water, releasing the treated effluent to the receiving water bodies. Due to the rapid increase of world population, the industrialization, the rise in living standards, agricultural growth and climate change, water became a scarce resource globally (Verstraete et al., 2009). By 2050 half of the global population could face water shortages (Verstraete and Vlaeminck, 2011). Thus, water should be considered as a valuable resource and new technologies should aim to reuse of the water. The main source of phosphorus is the non-renewable phosphate rock. 90% of the phosphate rock that is mined is used in mineral fertilizers in agriculture. It is hard to predict the exact amount of available phosphate reservoirs (Mehta et al., 2015); however research should be focused on ways to recover this limited resource (Solovchenko et al., 2015). Nitrogen for industrial and agricultural applications is mainly obtained from the energy-intensive Haber-Bosch process, whereby nitrogen gas is fixed into ammonia, contributing to 1-2 % of the world's total energy consumption (Batstone et al., 2015). Moreover, inorganic NPK fertilizers supply nitrogen and phosphorus in unbalanced ratio to plants and the excess nutrients can end up in soil deposits or in the water bodies as contaminants, contributing to eutrophication (Mehta et al., 2015). Thus, to secure food supply, for an increasing global population, technologies are sought for to recover nutrients. Nitrogen present in wastewater can cover 30% of the global fertilizer demand (Verstraete et al., 2009). Furthermore, it is estimated that about 20% of the global demand for phosphorus is excreted by humans and end up in sewage (Mehta et al., 2015). The conventional wastewater treatment processes are energy extensive, due to the need for aeration to mineralize organic carbon (Meerburg et al., 2015). Thus, recently there is a paradigm shift present in wastewater treatment, promoting the recovery of nutrients (nitrogen and phosphorus), water and energy (Guest et al., 2009; Verstraete and Vlaeminck, 2011). Indeed, the term wastewater is proposed to be replaced with used water (Verstraete et al., 2009), in order to help change common perception related to this resource. In this thesis the term used water is applied from here on.

Anaerobic processes for used water treatment are considered to reduce the need for aeration, thereby reducing the energy costs (Shoener et al., 2014). High rate used water treatment processes have been proposed to recover energy, whereby the loss of organic carbon is minimized by maximizing biomass production applying short solids retention times (SRT) (Jimenez et al., 2015; Meerburg et al., 2015). Common processes for biological nutrients recovery include the enhanced biological phosphorus removal (EBPR) employing phosphorus accumulating organisms (PAO) whereby up to 90% of the phosphorus can be removed by the bacteria (Yuan et al., 2012). Purple non-sulphur bacteria and cyanobacteria are also considered as ideal candidates for the recovery of nutrients from used water resources (Mehta et al., 2015). Chemical-physicochemical nutrients recovery from used water is predominantly achieved by struvite precipitation (Batstone et al., 2015). However, due to its fixed chemical composition only 30% of the ammonium is recovered from used water, leaving substantial amount in the soluble form.

Although it is not a new technology, the cultivation of microalgae on used water resources recently gained interest (Shoener et al., 2014). Cultivation of microalgae on used water resources offers the potential to recover water, nitrogen and phosphorus, providing an opportunity for nutrient recycling (Cai et al., 2013; Mehta et al., 2015; Samorì et al., 2013). Microalgal biomass can be used as slow-leaching fertilizer (Matassa et al., 2015; Mulbry et al., 2005). Moreover, algal biomass can be used for biogas or biodiesel production (Mata et al., 2010; Wijffels and Barbosa, 2010). Microalgal biomass does not compete with agricultural land used for food production, qualifying it as a third generation biofuel (Clarens et al., 2010). However, due to the high water and nutrients demand, large-scale microalgal cultivation for biofuel production appears neither energetically nor economically favourable, unless coupling with used water resource recovery (Chen et al., 2015; Markou et al., 2014; Pittman et al., 2011).

Effective used water resource recovery with microalgae can be challenging. Variation of the nutrient composition of influent water is reported to effect the nutrient removal and thus the effluent quality (Arbib et al., 2013). The available nutrients for microalgal cultivation are often expressed as the nitrogen to phosphorus molar ratio (N-to-P ratio). Additionally, using different used water streams for microalgal cultivation can affect the nutrients removal (Wang et al., 2010). Hence, under sub-optimal cultivation conditions the effluent water quality might be deteriorated. Moreover, the culture composition can change due to environmental factors (Samorì et al., 2013),

potentially affecting the nutrients recovery. Furthermore, efficient light supply within the reactor is reported to be crucial to obtain efficient microalgal growth (Sutherland et al., 2014). Light limitation or light supplied in inhibiting levels can affect the composition of microalgae, thus limiting further use of the biomass (Aburai et al., 2015).

For used water resource recovery a novel, completely biochemical process is proposed by Valverde-Pérez et al. (2015), whereby an innovative short-SRT enhanced biological phosphorus removal and recovery (EBP2R) process is combined with green microalgal cultivation, providing optimal cultivation media for algal cultivation. The EBP2R combined with an algal photobioreactor (PBR) is referred to as the TRENS system. The system is able to produce an algal suspension where nutrients are stored in the algal biomass, which can be used for fertigation. Alternatively, the biomass can be used to recover energy through anaerobic digestion while the water can be reused in aquifer recharge. However, an effective harvesting method should be tested as suggested by a life-cycle assessment study (Fang et al., 2016). To maintain stable downstream algal cultivation, Valverde-Pérez et al. (2016) designed the control structure for the EBP2R system. However, under highly dynamic conditions, the N-to-P ratio presented some variability around the optimal ratio.

Design, operation and control of PBRs require process models able to predict microalgal growth, as well as the nutrient uptake and storage from used water. A consensus model already exists for used water processes, i.e. the Activated Sludge Models (ASMs) (Henze et al., 2000), whilst for microalgal cultivation there was still a lack of a consistent modelling approach that allows combining microalgal and bacterial systems.

## 1.2 Objectives

The main aim of this thesis is to develop and evaluate a model for photoautotrophic and heterotrophic microalgal growth that can be used in bacterial-microalgal used water resource recovery systems, such as the TRENS system. Moreover, the potential effect of the varying influent N-to-P ratio on downstream microalgal cultivation is assessed at laboratory-scale. Finally, an effective harvesting method is proposed to recover the microalgal biomass. The main goals of the thesis are:

- To identify and evaluate a biokinetic process model for photoautotrophic and heterotrophic microalgal growth and nutrient uptake and storage in the ASM framework, after an extensive literature review of microalgal process models (**Paper I**).
- To assess the model identifiability using data obtained from laboratory-scale experiments and to assess the impact of culture history and substrate availability on parameter estimates (**Paper I**).
- To assess factors affecting the distribution of light intensity inside PBRs, i.e. reactor diameter, biomass concentration and to assess the changes in pigment concentration during batch cultivation and the potential effect on the light attenuation in the PBR (**Paper III**).
- To compare and evaluate different simulation model complexity levels used to predict light distribution in PBRs (**Paper III**).
- To assess the effect of the variation of influent N-to-P ratio on culture composition in open cultivation, using mixed and mono microalgal consortium via continuous cultivation and to assess the potential effect on culture kinetics (**Paper IV**).
- To develop and optimize an effective method of harvesting microalgae via a two-step flocculation using cationic polymer for destabilisation of microalgae and bacterial biomass from the upstream short-SRT EBPR system to enhance the aggregation of the algae (**Paper II**).
- To assess the potential to co-digest the harvested bacterial-algal biomass (**Paper II**).

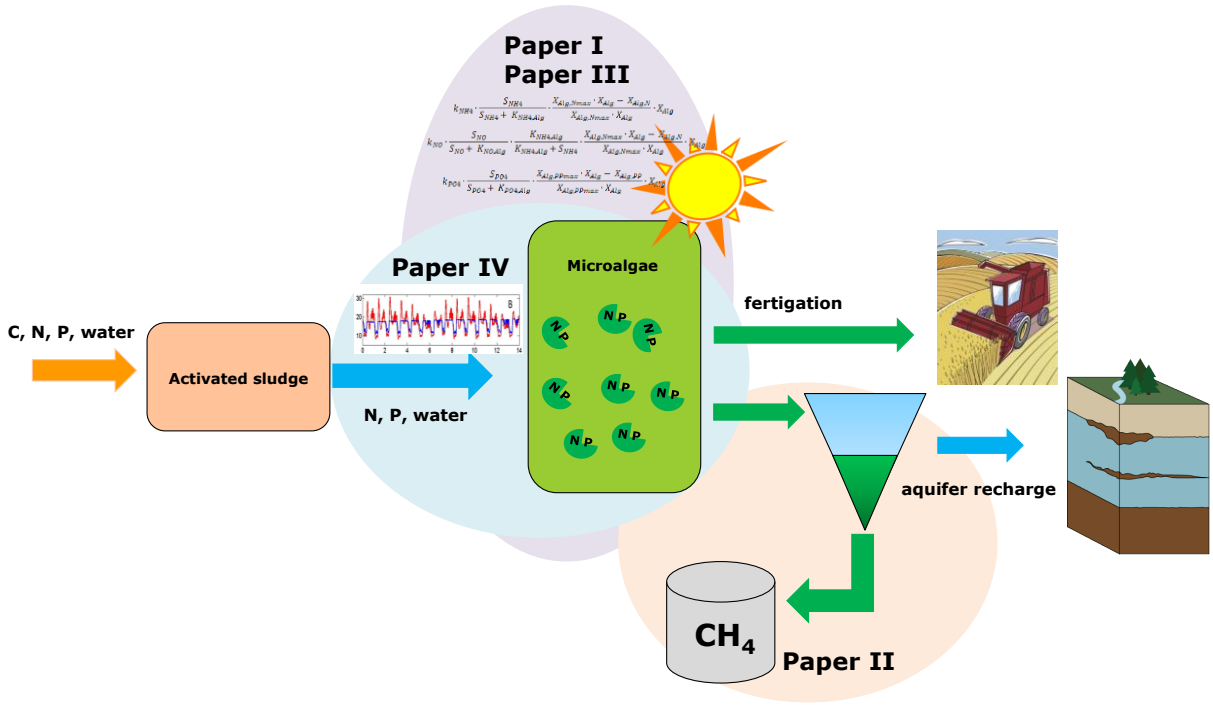



**Figure 1:** The outline of the thesis work done in the TRENS framework, referring to each journal paper appended to the thesis.

# 2 Microalgal cultivation

## 2.1 Microalgal physiology

Green algae or *Chlorophyta* are part of the *Plantae* kingdom. Green algae include both microscopic organisms and macroscopic seaweed. Green algae can be found in freshwater, as well as in the marine environment and on terrestrial land, e.g. on trees or rocks (van den Hoek et al., 1995). Some species can live in extreme environments such as the arctic or desert areas. Microalgae can have unicellular, colonial and filamentous cell organization; can be motile, with the presence of flagella, or non-motile. Both motile and non-motile cell types can form colonies with either fixed or variable number of cells (Richmond, 2004). Green algae are photosynthetic organisms, i.e. they use the energy obtained from sunlight to convert inorganic carbon ($CO_2$) to organic material. Their main photosynthetic pigments include chlorophyll a and b, contributing to their green colour. *Chlorella sp*. include small, unicellular, coccoid and nonmotile cells. *Scenedesmus sp.* are small nonmotile cells that form coenobic colonies, of fixed number of cells (Richmond, 2004). Both *Chlorella sp*. and *Scenedesmus sp.* have wide industrial applications and can be grown on used water resources. Both species are commonly used for e.g. biofuel production (Brennan and Owende, 2010).

Through photosynthesis the light energy is used to convert carbon dioxide and water to carbohydrates and oxygen. The process has two phases, comprising light and dark reactions (Fig. 2, Baroukh et al., 2015). During the light reactions, the light energy (photons) is converted into chemical energy in the form of $NADPH_2$ and ATP (Eq. 1, Richmond, 2004). The light antenna harvests the incoming light and transports it to the reaction centres of the photosystem II and I (Wilhelm and Jakob, 2011). When the cell is illuminated photophosphorylation takes place. Two electrons are extracted from water and one molecule of $NADPH_2$ is produced in the reaction centres, whilst protons are transported from the stroma into the thylakoid, thus forming a pH gradient driving the ATP synthesis (Richmond, 2004).

$$2\,NADP + 3\,H_2O + 2\,ADP + 2P_i \xrightarrow{hv+Chla} 2\,NADPH_2 + 3\,ATP + O_2 \quad (1)$$

$$CO_2 + 4\,H^+ + 4\,e^- \xrightarrow{2\,NADPH_2,3ATP} (CH_2O) + H_2O \quad (2)$$

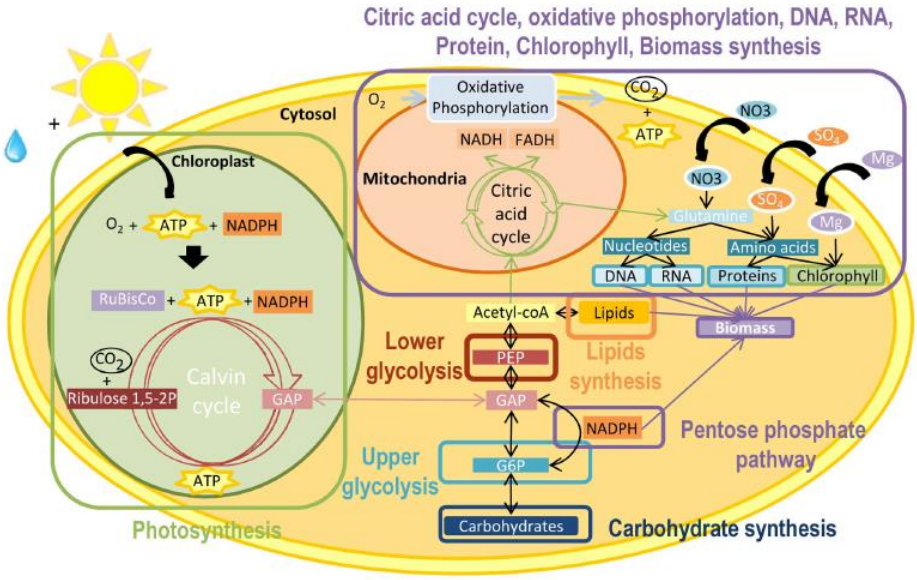



**Figure 2:** Carbon metabolic network of unicellular photoautrotophic microalgae. Figure taken from Baroukh et al. (2014).

The dark reaction consists of the Calvin-cycle, whereby the chemical energy, produced in the light reactions, is used to reduce carbon dioxide to phosphoglycerate that is then used for carbohydrate synthesis (Eq. 2, Baroukh et al., 2015).

There are two major groups of photosynthetic pigments in green algae: chlorophylls – green pigment; and carotenoids – yellow pigment (Carvalho et al., 2011). Chlorophylls absorb light in two spectrum bands: blue (450-475 nm) and red (630-675 nm). Carotenoids absorb at 400-550 nm, thereby potentially improving the light absorbance and the light utilization (Wang et al., 2014). Carotenoids consist of hydrocarbons, i.e. carotenes (e.g. β-carotene) and oxygenated hydrocarbons, i.e. xanthophylls, e.g. lutein, violaxanthin (Richmond, 2004). Carotenoids can also serve as protective pigments against high irradiance and reactive oxygen species (Seyfabadi et al., 2011).

## 2.2 Cultivation requirements

Light is essential for photoautotrophic microalgal cultivation. Under light limited conditions, at low light intensity, photosynthesis is affected linearly by the light intensity (Fig. 3). The maximum rate of photosynthesis is reached at saturation light intensity at which the photosynthetic rate is limited by the dark reactions (Wilhelm and Jakob, 2011). Light intensity that is higher than the saturation level causes photoinhibition, where the photosynthetic rate declines (Béchet et al., 2013).

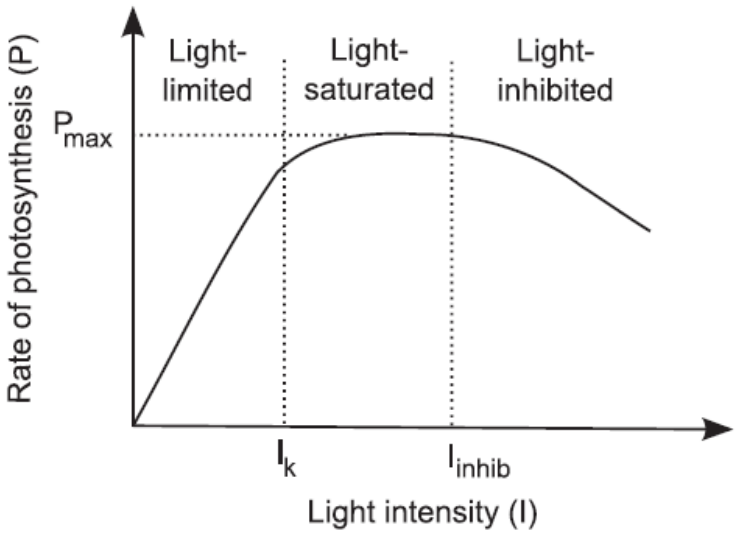



**Figure 3:** The light limited, light saturated and light inhibited regimes of photosynthesis. Figure was taken from Béchet et al. (2013).

The photosynthetically active radiation (PAR) corresponds to the visible spectrum of light (from 380nm to 750 nm) that is utilized during photosynthesis (Borowitzka and Moheimani, 2013). During mass algal production one of the main challenges is light limitation of the culture. 90% of the incoming light intensity is absorbed by the first few centimetres of the culture, causing light inhibition and an inefficient use of photons. The rest of the culture uses the photons much more efficiently, however they are light limited (Borowitzka and Moheimani, 2013). Proper mixing of the culture can be used to optimize the utilization of photons better in the culture. Light attenuation in the PBR is caused by the absorption of photosynthetic pigments, the shading by the cells and scattering within the culture (Wang et al., 2014). Reflection of light from the reactor wall is found to impact light attenuation under low biomass concentrations (Pandey et al., 2015). Due to constant changes in

the light regime, microalgae have developed some acclimation mechanisms (Carvalho et al., 2011). At high irradiance, to avoid photoinhibition, microalgae can reduce their light-harvesting capacity or the number of reaction centres (García-Camacho et al., 2012). Moreover, the light harvesting antenna can dissipate the excess light as heat to avoid photoinhibiton (Wilhelm and Jakob, 2011). The production of photo-protective pigments, e.g. lutein increases under high irradiances to reduce the effect of active oxygen species (Xie et al., 2016). In light limiting conditions microalgae increase the amount of chlorophyll to enhance the light harvesting capacity (Béchet et al., 2013).

Both organic and inorganic carbon can be utilized by microalgae. Inorganic carbon is used during photosynthesis in the Calvin-cycle (Baroukh et al., 2015). $CO_2$ is the preferred form of inorganic carbon supply (Decostere et al., 2013) that is assimilated using the Rubisco enzyme (Markou et al., 2014). However, microalgae have developed processes to be able to use other inorganic carbon species. Using bicarbonate as inorganic carbon source requires the conversion to $CO_2$ that produces $OH^-$, resulting in the increase of pH in the medium (Markou et al., 2014). $CO_2$ can be supplied through aeration with air or with $CO_2$ enriched air; however this can be costly. Alternatively, $CO_2$ can be supplied through the addition of flue gas (Gao et al., 2014). Organic carbon supply can be used when cultivating algae under heterotrophic or mixotrophic conditions. Organic carbon can be supplied in the form of glucose, glycerol or acetate (Perez-Garcia et al., 2011).

Macronutrients, nitrogen and phosphorus are essential for microalgal cultivation. Nitrogen is used in the synthesis of e.g., chlorophylls, amino acids and nucleic acids (Markou et al., 2014). Nitrogen content of the algae varies between 1% and 14%. Nitrogen can be supplied in inorganic form, i.e. nitrate, nitrite and ammonium and in organic form, i.e. urea (Perez-Garcia et al., 2011). The assimilation of ammonium is less energy consuming than utilizing the other nitrogen sources. Nitrate first has to be reduced to ammonium, thus ammonium is the preferred nitrogen source over nitrate (Cai et al., 2013). Nitrite is an intermediate between the reduction processes of nitrate to ammonium. Nitrite can be used as a nitrogen source, however at high concentrations it is toxic (Markou et al., 2014). When both nitrate and ammonium is present in the system, the uptake of nitrate will be repressed until ammonium is depleted (Cai et al., 2013). Care should be taken when utilizing different nitrogen species as the pH might drop if ammonium is applied as the nitrogen source due to the release of protons, while the pH might rise when nitrate is used (Nguyen and Rittmann, 2015). Phosphorus is an essential component of

e.g., nucleic acids, phospholipids and ATP (Cai et al., 2013). The biomass phosphorus content varies from 0.05% to 3.3% (Markou et al., 2014). Many studies report that under nutrients starvation, microalgal growth continues (Li et al., 2008; Ördög et al., 2012; Powell et al., 2009). Thus it is suggested that there is an intracellular storage pool of nitrogen and phosphorus. Phosphorus is reported to be stored intracellularly as polyphosphate either through over-compensation (or overshoot phenomenon) or luxury uptake (Powell et al., 2009). Prior to over-compensation, microalgae are exposed to phosphorus starvation, resulting in storing phosphorus once re-exposed to it. Luxury uptake of phosphorus does not require starvation period and is reported to be triggered by high soluble phosphate concentrations. Under nitrogen starvation microalgae degrade intracellular molecules, e.g. chlorophylls or proteins, to support growth (Li et al., 2008; Ördög et al., 2012).

Other requirements for microalgal cultivation include the presence of micronutrients and operation at optimal temperature. Essential micronutrients include Mg, S, Ca, Fe, Zn, Cu, Mn and Co that are mostly used for metabolic processes (Markou et al., 2014). The optimal temperature is shown to be species specific (Ras et al., 2013) and diverting from the optimal conditions can result in lower growth rate. However, other environmental factors, e.g. light or $CO_2$ might be able to compensate for changes in temperature, thus reducing temperature effects on growth. Moreover, some species are capable of acclimating to changes in the temperature in a wide range (Ras et al., 2013).

## 2.3 Cultivation methods

Two types of reactor configurations are typical in microalgal cultivation. Raceway ponds (see example Fig. 4 c and d) are the most commonly used open cultivation systems. They are typically built with 0.2 and 0.5 m depth and use mixing and circulation with a paddlewheel to optimize microalgal growth and prevent sedimentation of the biomass (Brennan and Owende, 2010). Open pond systems are a cheap form of large-scale algal cultivation (Ugwu et al., 2008). Since, they take up comparably large land space, they can ideally be built on non-agricultural land (Brennan and Owende, 2010). They have low energy requirements, and maintenance is simple. However, maintaining a stable culture composition is challenging in such systems, thus the potential downstream uses of the biomass and water might be limited (Novoveská et al., 2016; Safi et al., 2016). Microalgal species that can grow in extreme conditions can be used in open systems without potential contamination. Moreover, the use of native microalgal species or a mixed microalgal consortia offers the potential for a more robust cultivation (Novoveská et al., 2016). Microalgae grown on used water resources might be more exposed to contamination by bacteria or protozoa present in the used water (Henze et al., 2008). Due to more challenging control of environmental factors, e.g. temperature, inorganic carbon and light limitation, productivity is lower than in closed reactors (Ugwu et al., 2008).

Closed PBRs are designed to overcome some of the issues associated with the open pond cultivation. These systems include the tubular (see example Fig. 4 a and b), flat plate or column PBRs (Posten, 2009). The control of contamination can be better achieved resulting in stable cultivation of monocultures (Ugwu et al., 2008). Closed systems are reported to have higher productivities than open systems. However, the operational and capital costs are higher than in open pond systems (Brennan and Owende, 2010). The reactors are made from transparent materials, have short light paths and have comparably larger surface area exposed to light, to maximize the light harvesting of the system (Posten, 2009). However, some drawbacks are related to their operation, e.g. bio-fouling, oxygen accumulation and overheating (Mata et al., 2010).

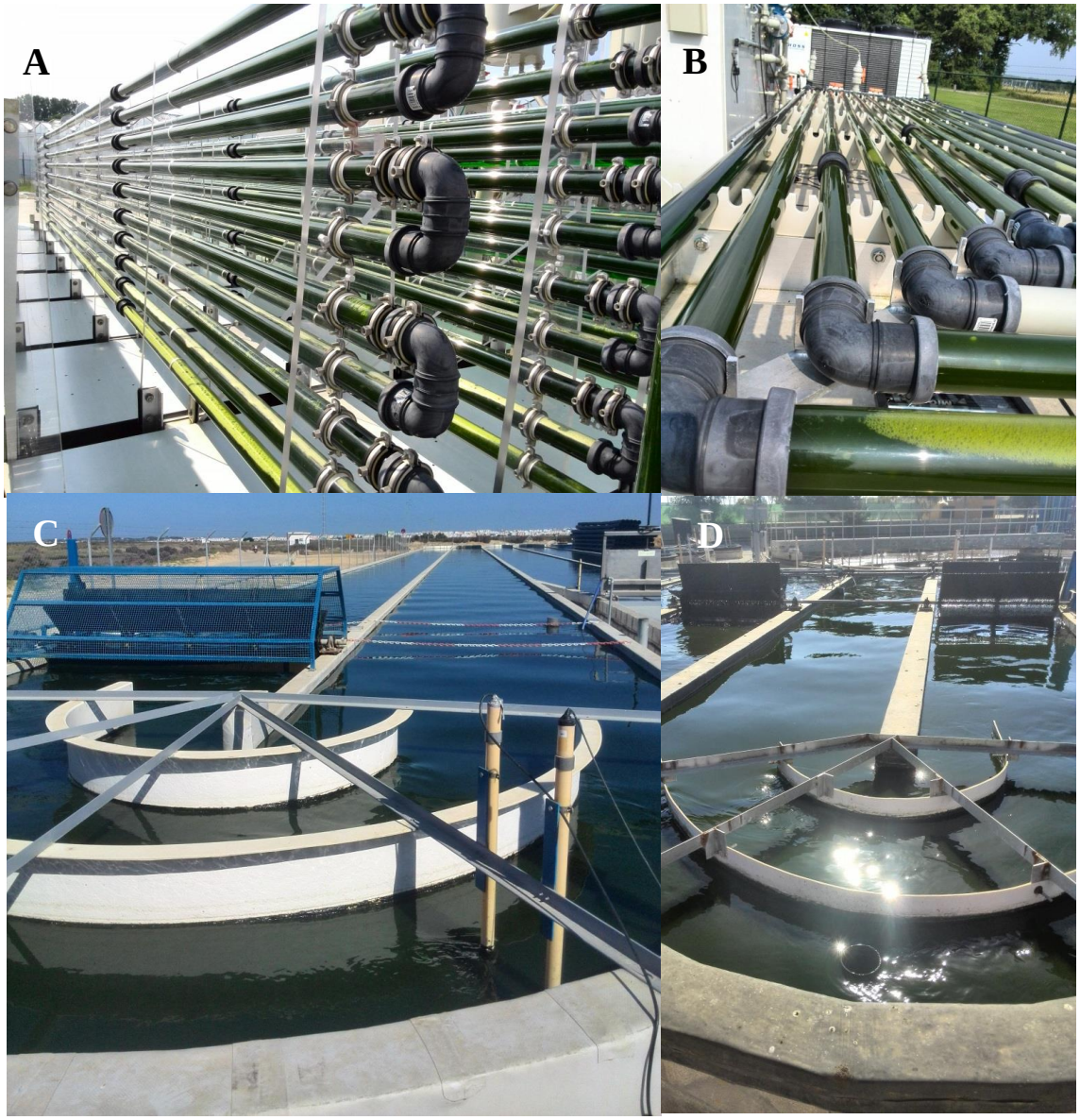



**Figure 4:** Pilot-scale closed tubular PBRs in AlgaePARC (Wageningen, The Netherlands) (**a** and **b**). Open raceway ponds in Chiclana de la Frontera (Cádiz, Spain) (**c** and **d**).

Microalgae can be cultivated under photoautotrophic, heterotrophic and mixotrophic conditions. The requirements of photoautotrophic cultivation have been discussed in detail in the previous chapters. The main driver of photoautotrophic cultivation is photosynthesis. Thus sufficient light and inorganic carbon supply is needed (Richmond, 2004). Heterotrophic cultivation has also been used for algal biomass production (Perez-Garcia et al., 2011). Microalgae are grown in darkness on organic carbon substrates such as glucose or acetate. The advantage of this system is that light is not needed to be supplied, thus the system design becomes much simpler (Brennan and Owende,

2010). Heterotrophic cultivation of microalgae might favour the production of biomass for biodiesel production, due to higher lipid yields (Miao and Wu, 2006). However, its limitations include the need for aeration to supply oxygen to support growth and the potential contamination by e.g. heterotrophic bacteria (Perez-Garcia et al., 2011). Mixotrophic cultivation means that both metabolism processes (autotrophic and heterotrophic) are applied in the culture. Thus apart from light and inorganic carbon, organic carbon is supplied (Cai et al., 2013). Mixotrophic microorganisms can utilize organic carbon under light limited conditions resulting in more flexible cultivation (Brennan and Owende, 2010). Moreover, the oxygen that is produced during autotrophic growth can be used during the heterotrophic growth. Growth rates of mixotrophic cultivation are reported to be higher than of photoautotrophic growth (Van Wagenen et al., 2015a).

## 2.4 Resource recovery using microalgae

Microalgal biomass grown on used water has a high potential to produce biofuels. The composition of the microalgae can change based on the type of used water chosen for cultivation as well as the microalgal species. High lipid content and high productivity usually cannot be achieved due to the characteristics of lipid production (Gao et al., 2014). Algae can accumulate energy dense lipids such as triacylglycerides (TAG) that can be used to produce biodiesel. However, lipid accumulation is enhanced by nitrogen limitation in the culture (Adams et al., 2013). In used water systems usually there is sufficient amount of nutrients to support algal growth and carbohydrates and protein production rather than synthesis of lipids. The lipid content of algae grown on used water is typically 10% (Shoener et al., 2014). Biorefinery approach is proposed to be applied in biodiesel production, whereby the residual biomass can be used for e.g., feed or anaerobic digestion (Chisti, 2007). Anaerobic digestion or co-digestion with activated sludge is preferred over biodiesel production as a means to recover energy from used water systems (Mehrabadi et al., 2015). It is more suitable to apply anaerobic digestion when the lipid concentration is lower than 40% (Sialve et al., 2009). Furthermore, anaerobic digestion can be used to treat biomass with high moisture (90-99%) content (Brennan and Owende, 2010). The methane yield produced from microalgae is typically ranging from 200-400 ml $CH_4$/gVS (Mehrabadi et al., 2015). Nonetheless, not all microalgal species are suitable for anaerobic digestion, due to their high nitrogen content (e.g. proteins) and cell wall structure (Kumar et al., 2016). The C/N ratio of the biomass can be a limiting factor for the digestion. A C/N ratio of 20 (g/g) is ideal for anaerobic digestion (Dębowski et al., 2013), while in freshwater microalgae the C/N ratio is typically around 10 (Sialve et al., 2009). Thus, co-digestion with other high carbon content biomass (e.g. waste or sludge) can improve digestibility. Anaerobic digesters are often available in the existing used water treatment facilities and biogas production can be enhanced by co-digestion of microalgae and activated sludge (Sahu et al., 2013). The digestate can be recycled to promote microalgae cultivation (Uggetti et al., 2014) or the residue of anaerobic digestion can be applied for fertilizer production for agriculture, further improving environmental performance due to the reduction of the production costs related to mineral fertilizers (Shimako et al., 2016). Alternatively, bioethanol can be produced through fermentation of carbohydrates (Uggetti et al., 2014), whereby, after the extraction of carbohydrates a biomass rich in lipids and proteins is left

that can be used as e.g. animal feed (Mehrabadi et al., 2015). Hydrothermal liquefaction (HTL) can be an alternative to biogas production. HTL does not require drying of the biomass and can be used on high moisture content (75-98%). Moreover, it can be applied with low lipid content as carbohydrates and proteins contribute to the formation of bio-crude oil (Gao et al., 2014).

Nutrient rich microalgal biomass can be applied in bio-fertilizer production (Gao et al., 2014) or the production of high-value products (Uggetti et al., 2014). High value products from microalgae include e.g., omega-3 fatty acids for food supplements, antioxidants for medicine and pigments for cosmetics or food additive (Borowitzka, 2013). Carotenoids are sought after as microalgal pigments that can be applied as food or feed supplements or colorants, or applied in cosmetics or as natural antioxidants (Araya et al., 2014; Perez-Garcia et al., 2011; Safafar et al., 2015). Most commonly β-carotene (from *Dunaliella salina*), lutein (from *Chlorella* or *Scenedesmus sp.*) and astaxanthin (from *Haematococcus pluvialis*) are produced through microalgal cultivation (Borowitzka, 2013). Lutein is the carotenoid found most commonly in *Chlorella* and *Scenedesmus sp.*, however, β-carotene and violaxanthin are also reported to be produced by these species (Paliwal et al., 2016; Safafar et al., 2015; Van Wagenen et al., 2015a). The presence and fate of emerging contaminants, i.e. pharmaceuticals in used water are reported to be relevant in the literature (Plósz et al., 2012). Pharmaceuticals can be removed by microalgae (Escapa et al., 2015; Matamoros et al., 2016), potentially affecting the downstream applications. Thus, further assessment and specific regulations are needed when considering high value products from microalgae cultivated on domestic used water streams.

The use of algal biomass as bio-fertilizer offers the opportunity for nutrients recovery. Mulbry et al. (2005) reported the potential to use microalgal biomass as slow-leaching fertilizer. Coppens et al. (2015) reported the potential improvement of plant nutritional level using microalgal flocs as bio-fertilizer, cultivated on used water resources (Van Den Hende et al., 2014a). The main advantage of microalgal fertilizers is that the release of nutrients is slow, thus reducing the oversupply of nutrients that occurs when using mineral fertilizers (Solovchenko et al., 2015), thereby making the process more sustainable and reducing the risk of groundwater contamination. It is reported in the literature that xenobiotics emerging from used water resources can accumulate in plants (Polesel et al., 2015). Hence, the potential risks of the presence of emerging contaminants and pathogens in bio-fertilizers from microalgal biomass cultivated on used water resources should be further investigated.

Combined algal-bacterial processes (Fig. 5) received renewed attention in recent years (e.g. Alcántara et al., 2015; Arbib et al., 2013; Van Den Hende et al., 2014b). $O_2$ produced by algae reduces the aeration requirements for aerobic treatment processes. Moreover, heterotrophic bacteria provide the algae with $CO_2$ while removing organic matter (Muñoz and Guieysse, 2006). Furthermore, bacteria can support algae with vitamin B12 (Wirth et al., 2015). However, algae and heterotrophs might compete for organic carbon under dark conditions and for ammonium (e.g. with nitrifier bacteria, Delgadillo-Mirquez et al., 2016) and bacterial excretion of algicidal chemicals might inhibit algal growth (Muñoz and Guieysse, 2006).

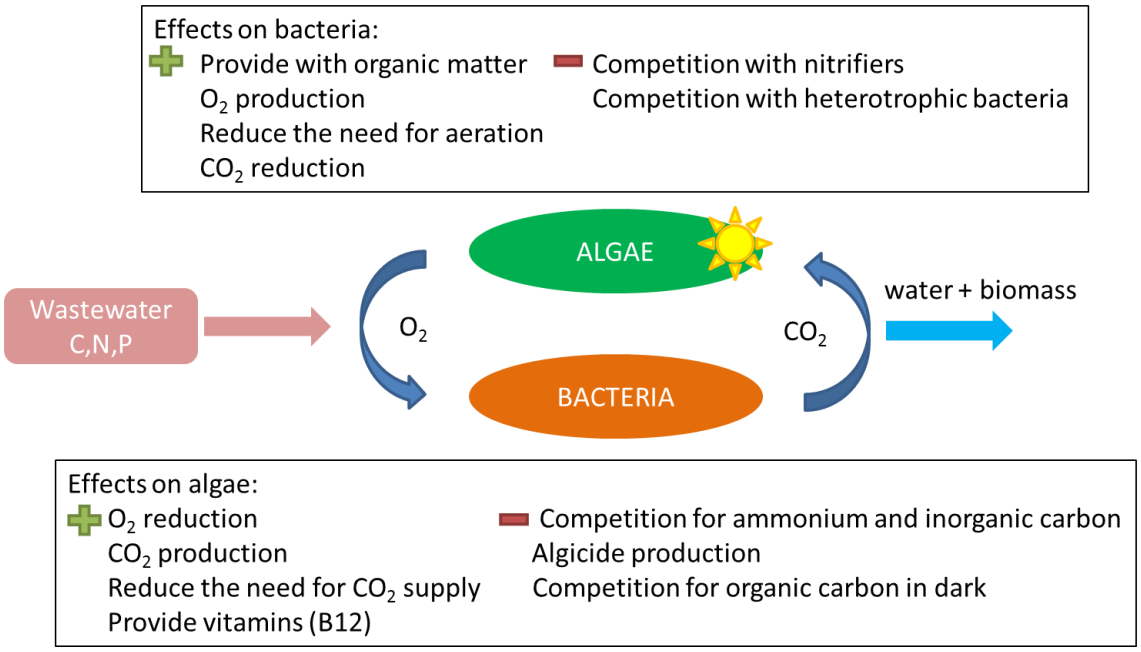



**Figure 5:** Positive and negative interactions between bacteria and algae during used water treatment processes (figure taken from **Paper I**, Supporting Information).

Harvesting of algal biomass could be promoted by e.g. floc formation between bacteria and algae (Van Den Hende et al., 2014b). Operational costs can be further reduced by decreasing the external supply of $CO_2$ by using flue gas produced in other industrial activities, or by upgrading the biogas produced through anaerobic digestion, whereby $CO_2$ is removed and $CH_4$ is concentrated in the biogas (Bahr et al., 2014; Gao et al., 2014; Serejo et al., 2015; Uggetti et al., 2014). However, due to the heavy metal (e.g. As, Cd, Co, Cr, Cu and Zn) content of flue gas, the downstream use of the biomass might be limited (Napan et al., 2016).

As used water contains microorganisms, e.g., algal species and protozoa (Henze et al., 2008), there is a potential risk of contamination, especially in open cultivation systems, which may compromise algal cultivation (Montemezzani et al., 2015). The question arises whether such contamination can be reduced downstream to bacterial processes, e.g., activated sludge. Generally, mixed microalgal consortia or robust microalgal species are preferred to conduct successful long term microalgal cultivation (Novoveská et al., 2016). Mixed cultures are reported to be more advantageous over monocultures, and the species selection for the specific used water is important (Gao et al., 2014). The use of native species are suggested that would outperform other microorganisms (Lynch et al., 2015; Olguín, 2012). Furthermore, optimizing algal cultivation to promote microalgal growth (e.g. sufficient light availability and supply of inorganic carbon source) can help to avoid contamination (Borowitzka and Moheimani, 2013). Nevertheless, high variance in species composition is reported in the literature during microalgal cultivation (Alcántara et al., 2015a; Krustok et al., 2016; Marcilhac et al., 2015; Samorì et al., 2013).

The optimal N-to-P ratio has been an interest since the 1950s, when Redfield suggested that the N-to-P ratio in marine phytoplankton was 16. Many researchers has suggested since then that the N-to-P ratio in microalgae is species specific (Anbalagan et al., 2016; Beuckels et al., 2015; Rhee and Gotham, 1980; Whitton et al., 2016). The N-to-P ratio for algal cultivation is also reported to vary depending on cultivation conditions. Microalgae are reported to be able to adapt their N-to-P ratio to the culture conditions (Arbib et al., 2013; Beuckels et al., 2015; Boelee et al., 2011; Dickinson et al., 2013; Geider and La Roche, 2002). Moreover, the influent N-to-P ratio might also affect the synthesis of storage products, e.g. proteins, lipids, chlorophyll and polyphosphate, (Geider and La Roche, 2002; Mayers et al., 2014; Rhee, 1978) and the potential for nutrient removal might be deteriorated outside the optimal range for cultivation (Arbib et al., 2013; Wang et al., 2010).

Cultivation of microalgae on different used water streams is reported in the literature, showing the potential to use microalgae in a wide range of treatment processes. Van Den Hende et al. (2014a) showed the potential to cultivate microalgal-bacterial flocs in industrial used water streams (i.e. in used water from aquaculture). Ruiz-Martinez et al. (2012) combined microalgal cultivation with an anaerobic membrane bioreactor treating domestic used water, whereby algae successfully recovered the nitrogen and phosphorus left after the anaerobic treatment. Van Wagenen et al. (2015b)

showed the potential to combine microalgal cultivation with anaerobic internal circulation reactor treating industrial used water. Tuantet et al. (2014) cultivated algae on human urine, showing the potential to use this source separated waste stream as cultivation medium. Benavente-Valdés et al. (2016) applied a two-stage microalgal cultivation strategy, to enhance the accumulation of high-value products. They operated reactors in series whereby heterotrophic microalgal cultivation was followed by photoautotrophic cultivation condition, enhancing lipids production and growth. Zamalloa et al. (2013) proposed a decentralized two-stage domestic used water process, whereby a chemical biological adsorption (A-stage) process is used to remove organic carbon and in a downstream microalgal biofilm process the nutrients are assimilated. Alcántara et al. (2015) showed the potential to use a two-stage bacterial-algal process for used water treatment, implementing an anoxic reactor (using nitrate as terminal electron acceptor) as first stage, whereby organic carbon was removed and denitrification occurred and a photobioreactor downstream was used to assimilate a fraction of nutrients while supporting bacterial growth with oxygen. The $N_2O$ production was assessed in a high rate algal pond (Alcántara et al., 2015b), showing the potential to reduce $N_2O$ production compared to conventional used water treatment processes. However, more research is needed as the literature is inconclusive as to whether bacteria or algae contribute to the observed $N_2O$ production (Fagerstone et al., 2011; Guieysse et al., 2013).

As mentioned before, in a novel wastewater resource recovery approach, an EBP2R process, provides optimal culture media for downstream microalgal cultivation (Valverde-Pérez et al., 2015). The TRENS system consists of a modified low-SRT EBPR process where an additional solid-liquid separation is included after the anaerobic reactors. Under anaerobic conditions, PAO accumulate volatile fatty acids (VFA) from the used water, storing them as polyhydroxyalkanoates (PHA) intracellularly while releasing intracellular polyphosphate (Oehmen et al., 2007). In the following step, under aerobic conditions the PHA storage is used to produce energy to support biomass growth and phosphorus uptake and storage (Oehmen et al., 2007). Thus, the water after the solid-liquid separation of the anaerobic phase is rich in phosphorus, whilst the water after the secondary sedimentation after the aerobic phase is low in phosphorus and rich in nitrogen. Due to the low-SRT kept in the EBP2R, nitrifiers are washed out of the system, thus nitrogen is mostly present as ammonium – the preferred nitrogen source for microalgae.

Thus, by controlling the ratio of mixing the phosphorus and nitrogen rich effluent streams, the low-SRT EBP2R can provide cultivation medium to downstream microalgal cultivation. The system can be designed for a chosen used water stream and it has the flexibility to provide optimal cultivation medium to different microalgal species. The EBP2R process can be implemented as a sequencing batch reactor (SBR) and as a continuous flow system. However, through the continuous flow scheme, under highly dynamic influent conditions, the N-to-P ratio presented some variability around the optimal ratio even after a control structure was implemented (Valverde-Pérez et al., 2016a). An LCA study, conducted on the TRENS system suggests two possible resource recovery strategies through the system (Fang et al., 2016). In the first case, the microalgal suspension together with the water is sent to fertigation on agricultural land, whereby recovering the nitrogen and phosphorus and the water content of the used water. Secondly, the microalgal biomass is considered to be separated from the water, whereby the water is used for aquifer recharge and the biomass is sent to the incineration at an existing used water treatment plant. In the former application, the positive effects are highlighted when using algae as bio-fertilizer, through the reduction of mineral fertilizer production. However, the LCA study finds some significant negative environmental effects in terms of uncertainty related to the fate of heavy metals originating from the used water, thus prompting further research. The latter application highlights the negative effects related to the coagulation-flocculation using $AlCl_3$ and alternative biomass harvesting options are suggested to be sought for. However, in this scenario, the costs related to mineral fertilizer-use on land instead of the microalgal suspension are higher, due to the production of fertilizer.

# 3 Microalgal process modelling

Effective reactor design, operation and control of used water resource recovery systems requires process models that can predict microalgal cultivation. Consistent mathematical models developed for algal processes can also facilitate the simulation of combined algal-bacterial systems. This requires models accounting for processes able to predict microalgal growth and the uptake and storage of nitrogen and phosphorus. The activated sludge modelling framework was used in this thesis for the novel ASM-A process model development. This chapter is based on **Paper I and III** whereby the main aim is to identify and evaluate process rate equations, based on extensive literature review, for photoautotrophic and heterotrophic microalgal growth.

## 3.1 State of the art modelling of microalgal processes

### 3.1.1 Biokinetic processes

Process modelling approaches found in the literature range in complexity, accounting for the influence of a single variable on growth, e.g. light availability (Blanken et al., 2016; Huesemann et al., 2013; Molina Grima et al., 1994), or the combination of multiple variables, e.g. the availability of nutrients, temperature or pH (Adesanya et al., 2014; Ambrose et al., 2006; Broekhuizen et al., 2012; Coppens et al., 2014; Decostere et al., 2013; Fachet et al., 2014; Guest et al., 2013; Huesemann et al., 2016; Muñoz Sierra et al., 2014; Quinn et al., 2011; Solimeno et al., 2015; Wolf et al., 2007; Zambrano et al., 2016). The more complex approaches lack some structural components to properly predict microalgal cultivation on used water resources. The PHOBIA biofilm model (Wolf et al., 2007) includes the growth of heterotrophs, nitrifiers and microalgae on inorganic carbon, light and nitrogen, but neglects the effect of phosphate, a key aspect for applications in used water treatment. Broekhuizen et al. (2012) model the effects of pH, inorganic carbon, oxygen, nitrogen, phosphate and light on microalgal growth. However, growth and nutrient uptake are considered directly coupled based on the Monod kinetics, and storage of nutrients is not considered. Droop (1973) proposed an approach to model microalgal growth on stored nutrients. The Droop model predicts growth in the absence of external bulk nitrogen or phosphorus – shown to be relevant during microalgal cultivation (Coppens et al., 2014; Ferreira et al., 2015; Powell et al., 2009) – utilising the internally stored nitrogen and phosphorus. As nutrients become limiting, the minimum

internal nutrient quota is reached gradually and the growth rate converges to zero. When nutrients in the bulk medium become available again, microalgae replenish their internal cell quota until the maximum quota is reached, whereby algal growth becomes independent from the nutrient availability and the maximum growth rate is reached (Bernard, 2011). Models applying Droop's approach can be found in the literature (Ambrose et al., 2006; Bernard, 2011; Fachet et al., 2014; Guest et al., 2013; Quinn et al., 2011).

Heterotrophic microalgal growth is widely applied (Brennan and Owende, 2010; Mata et al., 2010; Perez-Garcia et al., 2011; Van Wagenen et al., 2015a), however, the above mentioned models do not describe mixotrophic and heterotrophic growth. Moya et al. (1997) propose a model for photoautotrophic growth as a function of light and heterotrophic growth on acetate, expressed using the Haldane kinetics. As this model does not account for the uptake and storage of nitrogen and phosphorus it has limited applicability in used water systems.

### 3.1.2 Light distribution

The prediction of the light distribution in PBRs is required. The Lambert-Beer expression is used most commonly to account for light distribution. It includes the attenuation of light based on the absorbance by the biomass concentration (Koller et al., 2016) or by the biomass and pigments concentration (Bernard, 2011) and does not account for scattering. Schuster's law can be applied to predict the effects of light scattering on light attenuation in PBR (Koller et al., 2016). When absorbance by the pigments is considered, predicting the pigments concentration in the model and the inclusion of pigments as a state-variable is necessary. The chlorophyll concentration can be predicted by relating it to the intracellular nitrogen quota (Bernard, 2011) or to the nitrogen uptake rate (Geider et al., 1998). Photo-acclimation can be considered as the driving force for chlorophyll synthesis (García-Camacho et al., 2012). Moreover, the chlorophyll synthesis can be related to carbon uptake (Adesanya et al., 2014). Microalgal growth dependence on light can be modelled following three complexity levels (Béchet et al., 2013). Type 1 models consists of biokinetic models that employ incident or average light intensity, i.e. the algal cells receive the same light intensity in the entire reactor, having the same photosynthetic rate, and are not affected by photoinhibition closer to the light source and light limitation in the deeper layers. This approach was used in **Paper I**. Type II models account for the distribution of light by applying e.g. the Lambert-Beer expression (e.g., Blanken et al., 2016; Koller et al., 2016) to predict the light intensity at a given reactor depth. Finally, type

III models take into consideration the light story of the cells as the they move around in the reactor (e.g., Wu and Merchuk, 2004). The effect of light on microalgal growth can be accounted for by including the effect of photo-inhibition using the Steele, Peeters-Eilers and Haldane kinetics (Ambrose et al., 2006; Bouterfas et al., 2002), or neglecting photoinhibition using the Monod, Platt-Jassby, Poisson single-hit and Smith models (Ambrose et al., 2006; Bouterfas et al., 2002; Skjelbred et al., 2012).

## 3.2 The ASM-A process model

### 3.2.1 Model development

The development of a biokinetic process model for green microalgae is presented in **Paper I**. The aim was to develop a tool that can be used to simulate and predict the performance of used water resource recovery systems, e.g. the TRENS system. The model was developed as an extension to the well-established Activated Sludge Model, ASM-2d (Henze et al., 2000), facilitating the integration of the microalgal model into the existing benchmark models. ASM-2d models the bacterial activity in the EBPR system, i.e. ordinary heterotrophs, nitrifiers and PAO. Thus the model expressions included in detail in this thesis do not consider the bacteria, but only the microalgal processes (Gujer matrix shown in Table 1). Processes R1-R6 were identified and used for parameter estimation and identifiability analysis in **Paper I**. Process R7 was identified to account for light dynamics based on the chlorophyll content, in **Paper III**. Furthermore, the chlorophyll content as a state variable was introduced in **Paper III**. The units are expressed as in the ASM framework, i.e., as chemical oxygen demand (g-COD), g-N and g-P per cubic metre. Moreover, the ASM nomenclature is used (Corominas et al., 2010).

**Table 1:** The Gujer matrix of ASM-A model including the state-variables, the stoichiometric coefficients and the process rate equations identified.

| Component | $NH_4$ | $NO_3$ | Internal quota N | $PO_4$ | Internal quota P | Inorganic carbon | Acetate | $O_2$ | Algal Biomass | Inert Particulates | Slowly biodegradable Particulate | Chlorophyll content | Process rate equations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Symbol | $S_{NH4}$ | $S_{NO}$ | $X_{Alg,N}$ | $S_{PO4}$ | $X_{Alg,PP}$ | $S_{Alk}$ | $S_A$ | $S_{O2}$ | $X_{Alg}$ | $X_I$ | $X_S$ | $X_{Chl}$ | |
| Unit | $gN/m^3$ | $gN/m^3$ | $gN/m^3$ | $gP/m^3$ | $gP/m^3$ | $gC/m^3$ | $gCOD/m^3$ | $gCOD/m^3$ | $gCOD/m^3$ | $gCOD/m^3$ | $gCOD/m^3$ | $gN/m^3$ | |
| Process | Stoichiometric Matrix | | | | | | | | | | | | |
| Uptake and storage of nitrogen from NH4 | $-1$ | | $1-fXN_{Chl}$ | | | | | | | | | $fXN_{Chl}$ | R1 |
| Uptake and storage of nitrogen from NO3 | | $-1$ | $1-fXN_{Chl}$ | | | | | | | | | $fXN_{Chl}$ | R2 |
| Uptake and Storage of PO4 | | | | $-1$ | 1 | | | | | | | | R3 |
| Autotrophic growth | | | $-iN_{Xalg}$ | | $-iP_{Xalg}$ | $-1/Y_{Xalg,SAlk}$ | | $2.67/Y_{Xalg,SAlk}$ | 1 | | | | R4 |
| Heterotrophic growth | | | $-iN_{Xalg}$ | | $-iP_{Xalg}$ | $0.4/Y_{Ac}$ | $-1/Y_{Ac}$ | $-(1/Y_{Ac}-1)$ | 1 | | | | R5 |
| Decay | $iN_{Xalg}-fX_I\cdot iN_{XalgI}-(1-fX_I)\cdot iN_{XalgS}$ | | | $iP_{Xalg}-fX_I\cdot iP_{XalgI}-(1-fX_I)\cdot iP_{XalgS}$ | | | | $-(1-fX_I)$ | $-1$ | $fX_I$ | $1-fX_I$ | | R6 |
| Decay of $X_{Chl}$ | | | 1 | | | | | | | | | $-1$ | R7 |

| | Process rate equations |
|---|---|
| R1 [g N $m^{-3}$ $d^{-1}$] | $k_{NH4,Alg}\cdot\frac{S_{NH4}}{S_{NH4}+K_{NH4,Alg}}\cdot\frac{X_{Alg,Nmax}\cdot X_{Alg}-X_{Alg,N}}{X_{Alg,Nmax}\cdot X_{Alg}}\cdot X_{Alg}$ |
| R2 [g N $m^{-3}$ $d^{-1}$] | $k_{NO,Alg}\cdot\frac{S_{NO}}{S_{NO}+K_{NO,Alg}}\cdot\frac{K_{NH4,Alg}}{K_{NH4,Alg}+S_{NH4}}\cdot\frac{X_{Alg,Nmax}\cdot X_{Alg}-X_{Alg,N}}{X_{Alg,Nmax}\cdot X_{Alg}}\cdot X_{Alg}$ |
| R3 [g P $m^{-3}$ $d^{-1}$] | $k_{PO4,Alg}\cdot\frac{S_{PO4}}{S_{PO4}+K_{PO4,Alg}}\cdot\frac{X_{Alg,PPmax}\cdot X_{Alg}-X_{Alg,PP}}{X_{Alg,PPmax}\cdot X_{Alg}}\cdot X_{Alg}$ |
| R4 [g COD $m^{-3}$ $d^{-1}$] | $\mu_{A,max}\cdot(1-\frac{X_{Alg,Nmin}X_{Alg}}{X_{Alg,N}})\cdot(1-\frac{X_{Alg,PPmin}X_{Alg}}{X_{Alg,PP}})\cdot\frac{S_{Alk}}{S_{Alk}+K_{Alk}}\cdot\frac{I_{Av}}{I_S}\cdot e^{1-\frac{I_{Av}}{I_S}}\cdot X_{Alg}$ |
| R5 [g COD $m^{-3}$ $d^{-1}$] | $\mu_{H,max}\cdot(1-\frac{X_{Alg,Nmin}X_{Alg}}{X_{Alg,N}})\cdot(1-\frac{X_{Alg,PPmin}X_{Alg}}{X_{Alg,PP}})\cdot\frac{S_A}{S_A+K_A}\cdot\frac{S_{O2}}{S_{O2}+K_{O2}}\cdot\frac{K_I}{K_I+I_{Av}}\cdot X_{Alg}$ |
| R6 [g COD $m^{-3}$ $d^{-1}$] | $b_{Xalg}\cdot X_{Alg}$ |
| R7 [g N $m^{-3}$ $d^{-1}$] | $b_{XChl}\cdot X_{Chl}$ |

***Uptake and storage of nitrogen (R1 and R2):*** ASM-A includes the uptake and storage of both ammonium (R1) and nitrate (R2) nitrogen by the microalgae (Table 1). R1 and R2 depend on the available external nitrogen sources ($S_{NH4}$ or $S_{NO}$), as well as on the internal cell quota of nitrogen ($X_{Alg,N}$). Nitrogen uptake rate slows down as the nitrogen cell quota approaches its maximum, $X_{Alg,Nmax}$, in the biomass ($X_{Alg}$). As described earlier, ammonium is preferred over nitrate for most microalgae. Hence, a competitive inhibition term is included in the nitrate uptake process rate dependent on the level of ammonium (R2).

The chlorophyll content ($X_{Chl}$, introduced as a state-variable in **Paper III**) is proportional to the internal nitrogen quota ($X_{Alg,N}$) and can be predicted by relating it to the storage and uptake of nitrogen using the stoichiometric coefficient of the fraction of chlorophyll-to-nitrogen ($fXN_{Chl}$).

***Uptake and storage of phosphorus (R3):*** The uptake and storage of phosphorus (R3, Table 1) depend on the external soluble orthophosphate ($S_{PO4}$) availability, and on the internal cell quota of phosphorus ($X_{Alg,PP}$). As the phosphorus storage approaches the maximum cell quota, $X_{Alg,PPmax}$, the phosphorus uptake rate decreases.

***Photoautotrophic growth (R4):*** Droop's model is used to account for nutrient limitations, whereby as the internal cell quota approaches the minimum ($X_{Alg,Nmin}$ or $X_{Alg,PPmin}$), the specific growth rate decreases. The consumption of inorganic carbon ($S_{Alk}$) is modelled using Monod kinetics. In **Paper I**, the available light intensity was assumed to be a constant average value (type I light model) denoted as $I_{Av}$. Six different model equations were fitted to the experimental data, to identify a suitable model structure to describe the light influence on microalgal growth. Light dependence was chosen to be modelled using the Steele equation (Fig. 6) as it was found to most accurately (R2=0.995) describe the light dependence of algal growth. The Steele equation accounts for the photoinhibition, a factor not fully supported by the measured data, and hence, further assessment at higher light intensities is necessary to understand better the inhibition by light.

***Heterotrophic algal growth (R5):*** Acetate is used as the organic carbon substrate ($S_A$) that is included in the ASM-2d as state-variable. The heterotrophic growth is expressed with the Monod kinetics as a function of the substrate concentration. Oxygen is a terminal electron acceptor for heterotrophic growth ($S_{O2}$), modelled by Monod kinetics. Light availability inhibits the heterotrophic growth and it is modelled using the competitive inhibition term.

The nutrient consumption associated with algal growth is analogous to photoautotrophic growth.

***Algal decay (R6):*** The algal decay process rate includes the biomass loss during dark respiration and death and lysis, including reduction in biomass due to predator grazing. The decay process is modelled following the dead-regeneration principle, stating that fractions of the products from decay become available for microbial growth.

***Chlorophyll synthesis (R7):*** This term was only used in **Paper III**. Chlorophyll is an easily accessible nitrogen source from the internal nitrogen pool that is used for nitrogen supply under nitrogen limitation. Thus, an independent decay term for the chlorophyll content was introduced (R7, Table 1) assuming that it is degraded faster than the other constituents in the internal nitrogen content.

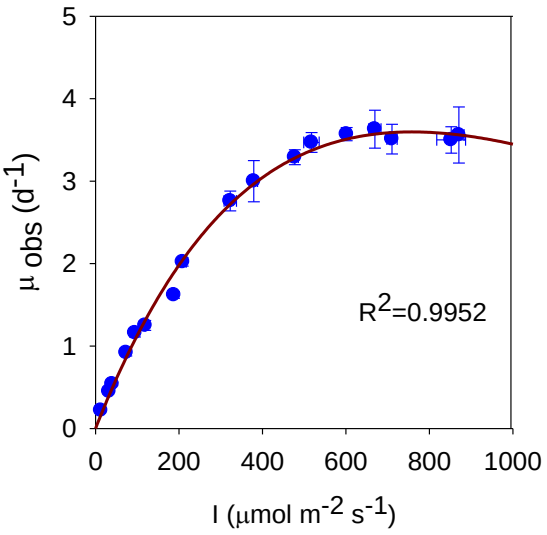



**Figure 6:** Specific photoautotrophic growth rate of microalgae plotted as a function of incident light intensity. The solid line denotes the fitting obtained using the Steele equation (**Paper I**).

### 3.2.2 Model calibration and evaluation

A mixed green microalgal consortium was cultivated during the experiments. The culture consists of *Chlorella sorokiniana* (Fig. 7, identification made by the PCR method as described in **Paper I**) and *Scenedesmus sp.* (Fig. 7, based on microscopic observations). The mixed consortium was cultivated using the

MWC+Se synthetic medium (Guillard and Lorenzen, 1972), where the amounts of nutrients were modified in the experiments.

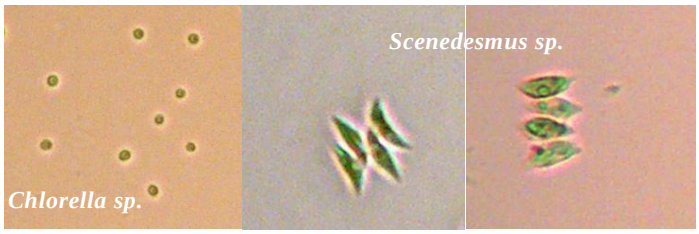



**Figure 7:** Microscopic images of the microalgal species present in the mixed consortium.

Laboratory-scale batch experiments were set up in three scales to obtain experimental measurements for model calibration. To assess the effect of light intensity on the photoautotrophic microalgal growth, microbatch experiments were set up in 2 ml 24 well microbatches (Fig. 8a). Neutral density filters (Fig. 8b) were attached to the bottom of the microbatches to create different light intensities (Van Wagenen et al., 2014). Moreover, the effect of light availability on the heterotrophic microalgal growth was assessed using microbatch experiments. 1-L batch experiments (Fig. 8c) were set up and three parallel batch reactors were run where the effect of nutrient limitation on photoautotrophic growth was assessed by limiting only one nutrient at a time. Heterotrophic growth and the acetate uptake were assessed in 1-L batches under dark conditions. 24-L laboratory-scale airlift PBR was set up to collect experimental data for model calibration and evaluation (Fig. 8d). In the first four cycles (Descending cycles), the initial ammonia and nitrate concentration decreased in sequential cycles, whilst in the following four cycles (Ascending cycles), the initial ammonia and nitrate concentration were increased (Fig. 9).

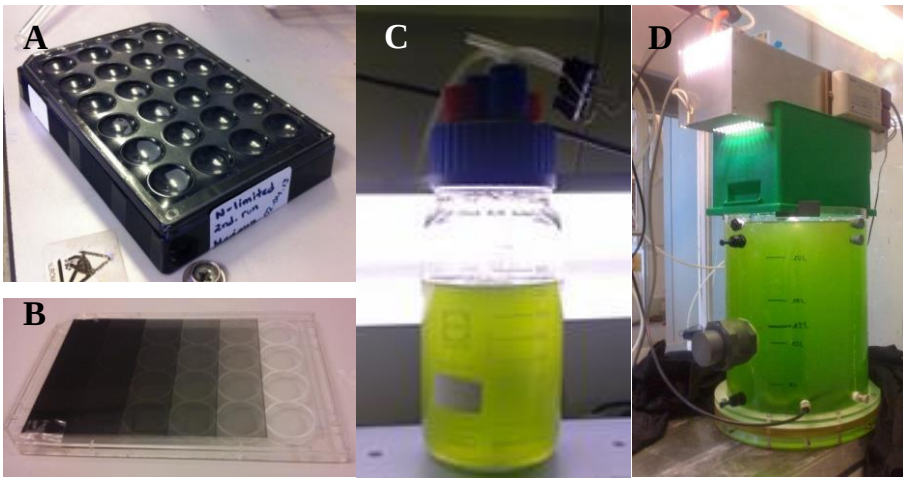



**Figure 8:** The 24-well microbatch (**a**) and the neutral filter used to be attached on the bottom (**b**). The 1-L batch reactor (**c**) and the 24-L airlift photobioreactor (**d**) used for obtaining experimental data for model calibration. The 24-L reactor was covered from the side with a black cloth to avoid light entering from the side of the reactor.

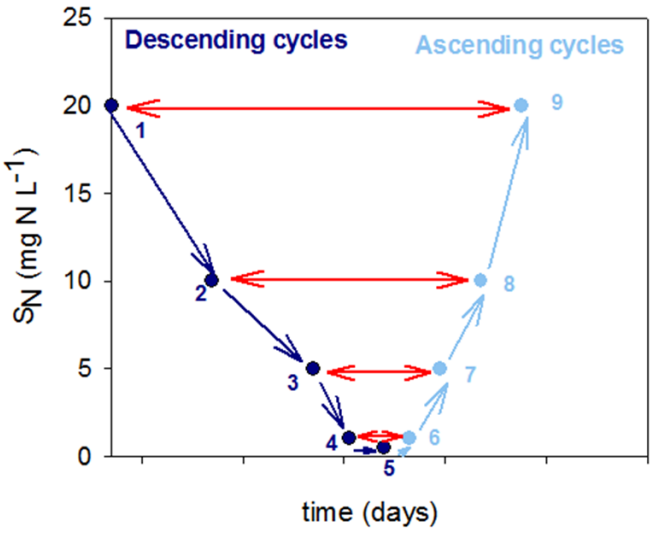



**Figure 9:** Experimental design of the 24-L batch experiment. On the Y axis the total initial nitrogen (ammonium and nitrate) concentration is shown (**Paper I**).

Model identifiability analysis was carried out to determine if the information gathered from the 1-L and 24-L batches was rich enough to estimate parameters. The identifiability analysis was conducted using the Latin Hypercube

Sampling based Simplex (LHSS) method. LHSS relies on the Simplex optimisation, employing priors selected using Latin Hypercube Sampling. LHSS includes 5 steps (Fig. 10): Step 1: the parameter space is defined; Step 2: Latin Hypercube Sampling (LHS) is used to select prior values from the parameter space; Step 3: the parameter sets obtained using LHS are used as initial values for the local optimisation algorithm, Simplex, thereby resulting in a global optimisation approach. Step 4: Thresholds are set by visualization of the distribution of the RMSNE (histogram) for the estimated parameter subsets, where parameter subsets having an error higher than the threshold are omitted; Step 5: The distribution of the optimal parameter set values, combined with the average parameter values, standard deviations and correlation matrix are used for identifiability assessment (Fig. 10). For more details on the method, the reader is referred to **Paper I**.

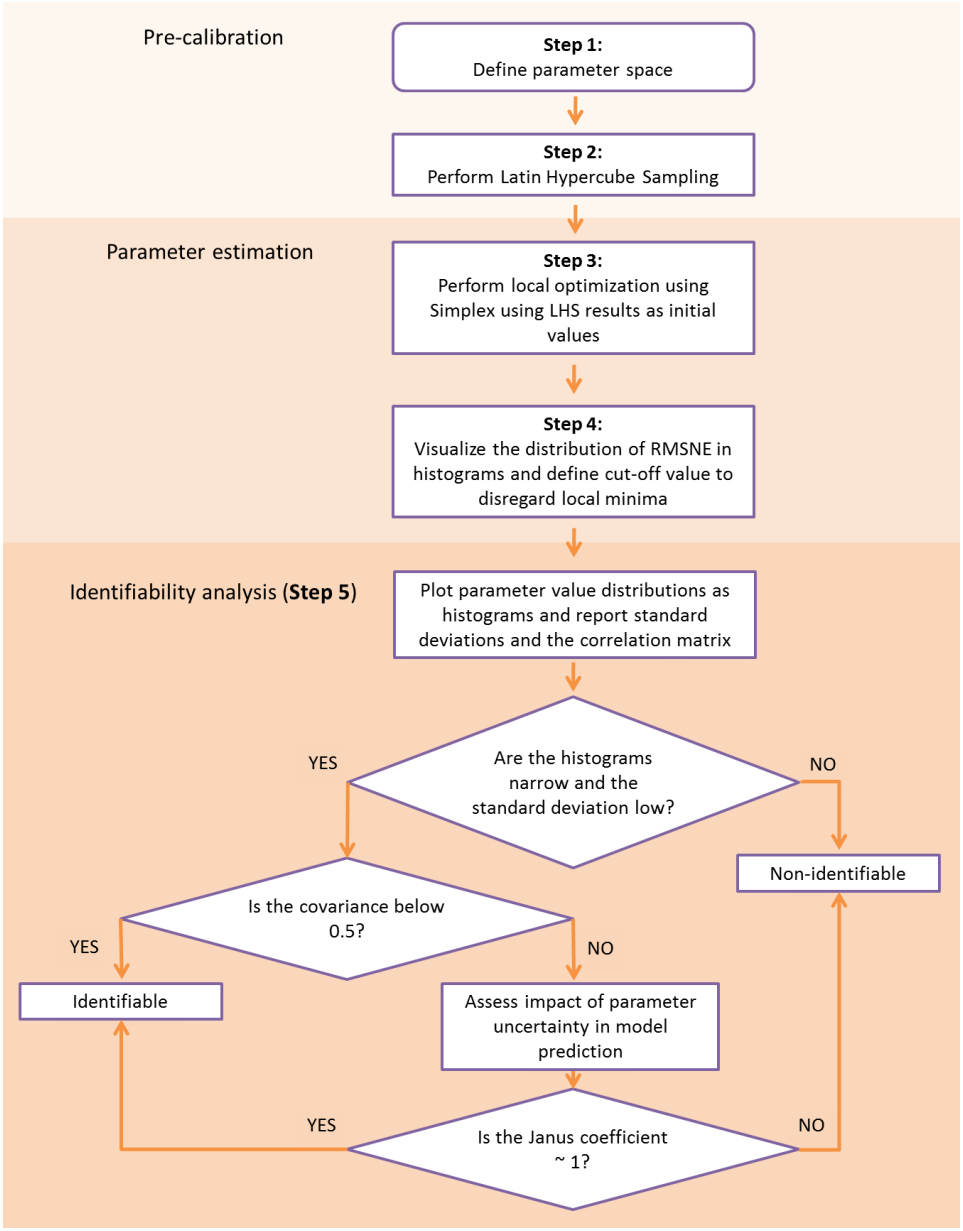



**Figure 10:** Overview of the LHSS method proposed for parameter estimation and identifiability assessment (**Paper I**).

Moreover, a two-step model evaluation was conducted using the experimental design of the 24-L batch experiments. In the first step, hypothesis tests were conducted to assess if culture history and/or substrate availability have an influence on parameter estimates. To test this, the experimental design used in the 24-L batch experiments with different initial substrate to biomass ratios in each cycle, allowed decoupling the culture history from the substrate availability impact. Parameter sets obtained through the descending cycles were compared (using the Janus coefficient, J) with those obtained in the cor-

responding ascending cycles. Furthermore, in the second step, it was tested if a mean parameter set could be used to predict microalgal processes and if there are any inaccuracies in the model prediction, can it be the result of parameter variability. Monte Carlo simulations were performed to obtain a confidence interval of model predictions to answer the previous questions (Sin et al., 2009). For those state-variables that failed both evaluation steps global sensitivity analysis (GSA) was carried out.

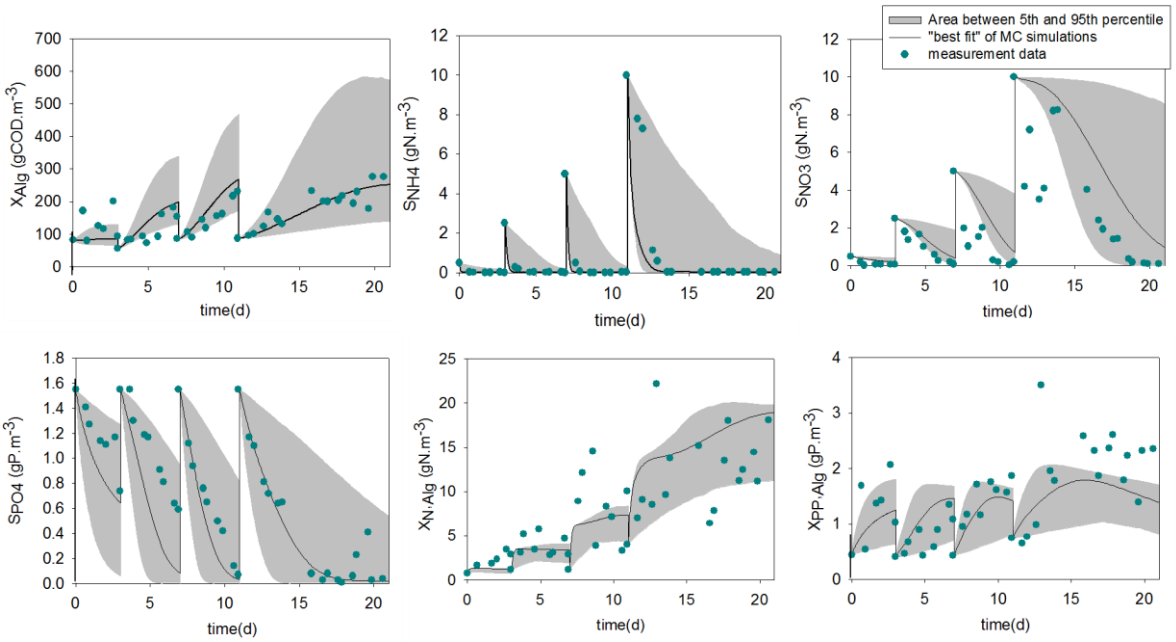



**Figure 11:** Model evaluation of the prediction of microalgal biomass concentration, bulk ammonium concentration, bulk nitrate concentration, bulk phosphate concentration, internal nitrogen quota and internal phosphorus quota (**Paper I**).

Results obtained suggest that, in the absence of dissolved nitrogen species and phosphate, microalgal growth is sustained by accessing intracellularly stored nitrogen and phosphorus (Fig. 11). This highlights the importance of using the Droop model in ASM-A that can uncouple nutrient uptake from microalgal growth. A default parameter set is selected from the model calibrations in different scales (**Paper I**). Through model evaluation, it was found that for the parameters sensitive to microalgal biomass concentration, ammonium and phosphate bulk concentrations and the nitrogen and phosphorus internal quota, the source of parameter variability is not the culture history (J~1). The measurement values of microalgal biomass concentration, bulk ammonium and phosphate concentration and phosphorus storage are in the

proximity of the best fit of the Monte Carlo simulation results (Fig. 11). This suggests that the mean parameter values with their associated uncertainty can be used to predict algal cultivation in PBRs, operated with *Chlorella* and *Scenedesmus sp.* This, does not apply for predicting the nitrate concentration and the internal nitrogen storage. Nitrogen storage can be predicted using the estimated parameters from the descending cycle (i.e. J~1). In the second evaluation step the discrepancy between the prediction and measured values cannot be explained through parameter variability (i.e. most data falls outside the confidence interval). Thus, substrate availability is assumed to affect the prediction of nitrogen storage, indicating the need for case-specific calibration of the nitrogen storage process. The prediction of the bulk nitrate concentration fails for both steps (J>>1 and most of the measured values are outside the confidence interval). Hence, values of the parameters affecting this model output depend on the culture history. The most sensitive model parameter affecting the bulk nitrate concentration is the maximum uptake rate of nitrate ($k_{NO,Alg}$). This parameter affects the nitrogen storage as well. It was found (using the LHSS method) that $k_{NO,Alg}$ is identifiable. Thus the case specific calibration of $k_{NO,Alg}$ is suggested. $k_{NO,Alg}$ was estimated for each cycle, showing hysteresis in the parameter value (**Paper I**).

### 3.2.3 Modelling light distribution in PBRs

Different factors affecting the light distribution inside the PBR were assessed in **Paper III**. Moreover, the consequences of choosing different model complexities to predict the light distribution inside the reactor were assessed.

Three reactors (see e.g. Fig. 12a) of different diameters were used to test the effect of multiple factors on the light distribution in PBRs. The effect of cultivation conditions, i.e. nutrient availability and type of cultivation medium, the effect of reactor diameter, bubble size during aeration and the biomass concentration was tested. Light intensity was measured inside the reactors to predict the light distribution curves. A batch experiment was carried out in an 8-L PBR (Fig. 12b). Light intensity was measured inside the reactor twice a day together with soluble nutrients concentration, algal biomass concentration, internal nitrogen and phosphorus content and pigments (including chlorophyll a and b, violaxhantin, lutein and β-carotene) concentration.

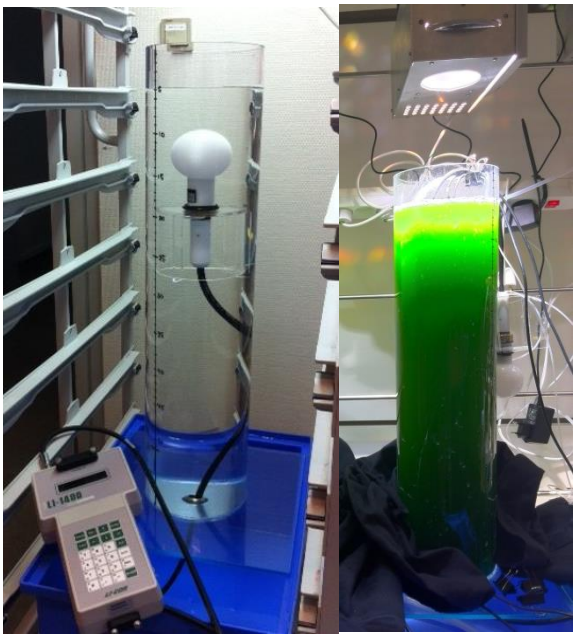
a  b

**Figure 12:** The 8-L reactor and the light sensor used to measure the light attenuation inside the reactor (**a**). The reactor during microalgal cultivation with a custom built light source providing light from above (**b**) (**Paper III**).

As discussed earlier, different model complexities are used to account for light intensity in the PBR. Three different assumptions were tested to account for light intensity during model simulations based on the Lambert-Beer law. In the first case (complexity 1), constant average light intensity is assumed to be available in the reactor throughout the simulation. In the second case (complexity 2) includes an average light intensity is calculated for each time-step of the simulation, thereby accounting for the dynamics of the biomass concentration in the reactor. The third case (complexity 3) includes the one-dimensional discretization of the culture volume into n equal layers orthogonal with the light source, entering from the top discretization layer. The light intensity is calculated in the middle of each layer using the Lambert-Beer equation and assumed to be equal in each layer. The model complexity was then compared based on four criteria: (1) model accuracy assessment based on the root mean square normalised error (RMSNE) and Akaike's information criterion (AIC); (2) parameter uncertainty based on the comparison of mean value and standard deviation; (3) parameter correlation; (4) model prediction uncertainty, assessed based on the 95% confidence bands using average relative interval length (ARIL) together with the coverage. The detailed description of each criterion can be found in **Paper III**.

The light attenuation coefficient was estimated by fitting the Lambert-Beer equation on the light distribution curves obtained at three different biomass concentrations. It was found that the attenuation coefficient varies with changing biomass concentrations (Fig. 13) and an exponential relation can be fitted on the obtained correlations (Eq. 3):

$$k_a = a * e^{-b*X_{Alg}} \qquad (3)$$

where $k_a$ ($m^2$ $g^{-1}$) is the attenuation coefficient a ($m^2$ $g^{-1}$) and b ($m^3$ $g^{-1}$) are the estimated parameters and $X_{Alg}$ (g $m^{-3}$) is the biomass concentration.

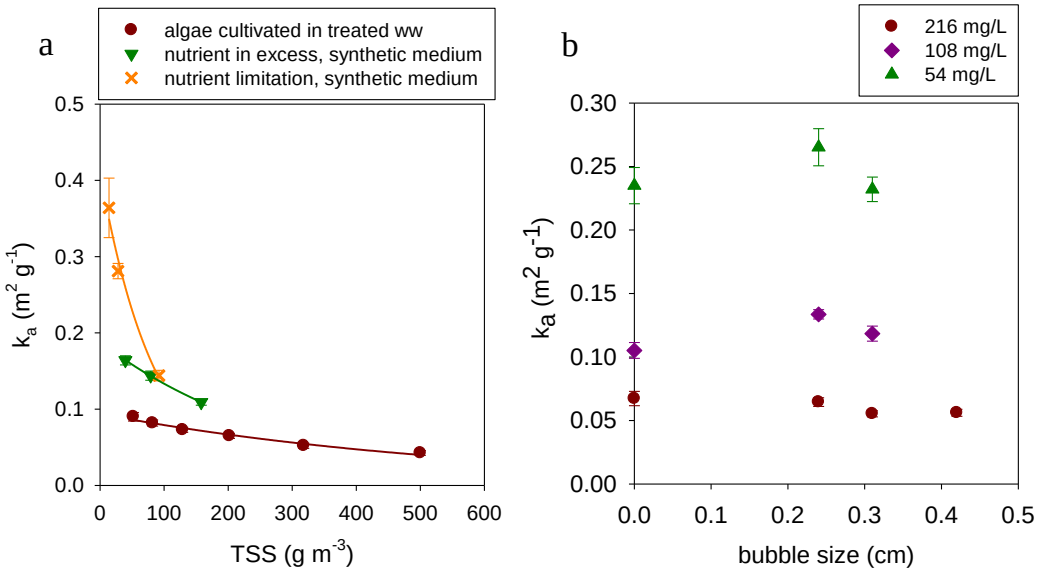



**Figure 13:** The attenuation coefficient as a function of the biomass concentration inside the reactor (**a**). The figure shows results obtained with cultivation with synthetic medium and treated used water (**Paper III**). The attenuation coefficient presented as function of bubble size (**b**). The measurement was done in synthetic medium (**Paper III**, Supporting Information).

The cultivation medium, i.e. synthetic medium and treated used water, is found to affect the light attenuation and thus light distribution in the PBR (Fig. 13a). The treated wastewater might contain chromophores and particulate matter that can interfere with the light attenuation. The bubble size did not have a significant effect on the light attenuation in the PBR, even under low biomass concentration (Fig. 13b). The nutrient availability was found to have an impact on the light attenuation in the reactor (Fig. 13a). The nutrient availability can affect the microalgal physiology (e.g. pigments composition). Under nitrogen limitation, the chlorophyll content is reported to be reduced

(Ördög et al., 2012). Furthermore, high light intensities the production of carotenoids are promoted (Vaquero et al., 2014). Thus, it is hypothesized that the change in light attenuation is affected by the pigment composition and it was further analysed.

Results obtained in the 8-L batch experiments show that the chlorophyll a and b concentration inside the cells decreased from the beginning of the experiment, reaching a minimum concentration after 4 days (Fig. 14). Lutein is the most abundant carotenoid. Carotenoids were accumulated in the first 2 days and then depleted. As it was discussed earlier, under high light intensities, the chlorophyll production is suppressed and carotenoids are synthesized to avoid photoinhibition (García-Camacho et al., 2012; Vaquero et al., 2014; Xie et al., 2016). Thus, the sudden increase of light intensity in the beginning of the cultivation could potentially result in photoinhibition. The total chlorophyll content (expressed as nitrogen) is found to be maximum 2% of the internal nitrogen quota similar as to found in the literature (Geider and La Roche, 2002).

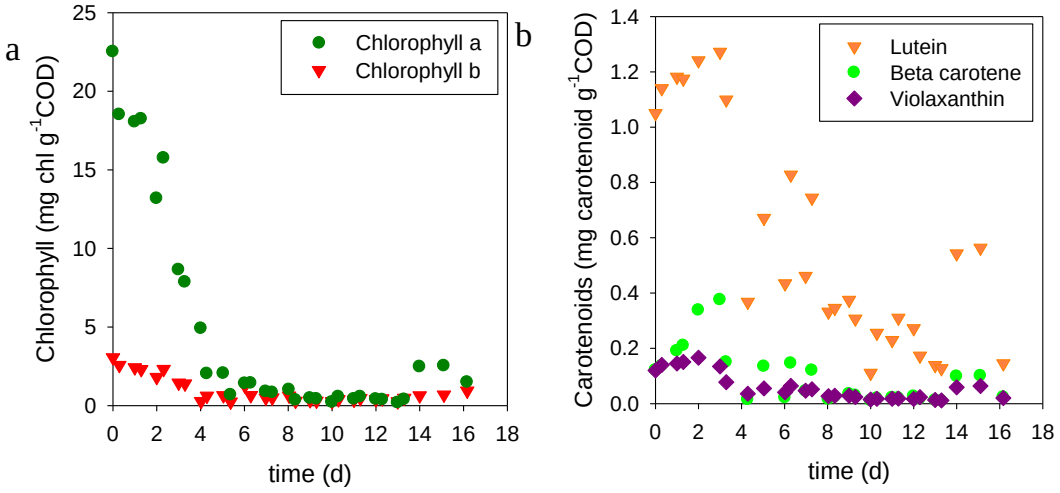



**Figure 14:** Chlorophyll a and b content (a) and carotenoids content (b) of the microalgae during the batch cultivation (**Paper III**).

Similarly to the previous results presented, the attenuation coefficient is function of the TSS concentration in the 8-L batch cultivation (**Paper III**). Thus to effectively predict the light distribution in the PBR the attenuation coefficient should be expressed as a variable during the cultivation period and not as a single value. The chlorophyll content was found to be mostly affecting the attenuation coefficient (found based on PCA analysis, **Paper III**), and

thus it was considered for model identification. Consequently, the dependence of the attenuation coefficient on the total chlorophyll concentration was assessed. A trend different from that obtained as a function of TSS was found between the attenuation and the pigments concentration (Fig. 15). The dependence of the light attenuation coefficient ($k_{a,p}$) on the total pigment concentration ($X_{Chl}$) is described as :

$$k_{a,p} = \frac{d}{X_{Chl}} - c \qquad (4)$$

where $k_{a,p}$ is the attenuation coefficient specific for total chlorophyll concentration, c and d are the estimated parameters and $X_{Chl}$ is the total chlorophyll concentration.

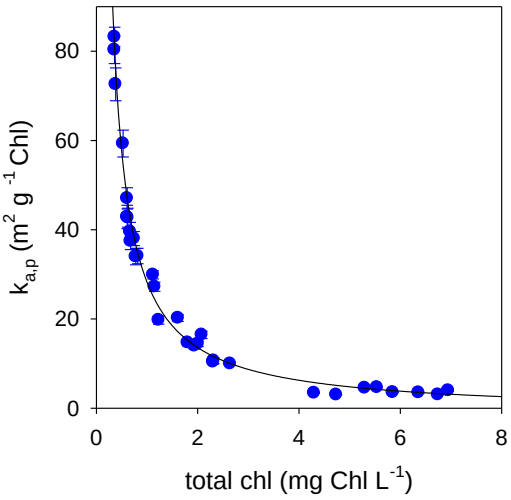



**Figure 15:** The chlorophyll specific attenuation coefficient as a function of the total chlorophyll concentration (**Paper III**).

The model complexity was compared based on four criteria, as described earlier. The model prediction of the biomass concentration ($X_{Alg}$) improved by using a model structure with higher complexity, i.e. model with the discretized layers, due to the more realistic prediction of light availability for algal growth (Fig. 16 and Table 2 in **Paper III**). However, regarding the prediction of the bulk nutrients and internal cell quota, there is no clear improvement. Based on the estimated parameter values and their standard deviation, the different model structures of the prediction of light distribution model affects the maximum specific growth rate ($\mu_{A,max}$), i.e. it is significantly higher when

the estimation was done using the discretized model structure. The model prediction uncertainty was assessed based on the 95% confidence bands and it was found that the model performance is improved with increasing modelling complexity due to the reduction of the width of the uncertainty bands (Fig. 16). Based on the parameter correlation analysis presented in the LHSS method and the reduction of the uncertainty, more complex model structures might improve parameter identifiability.

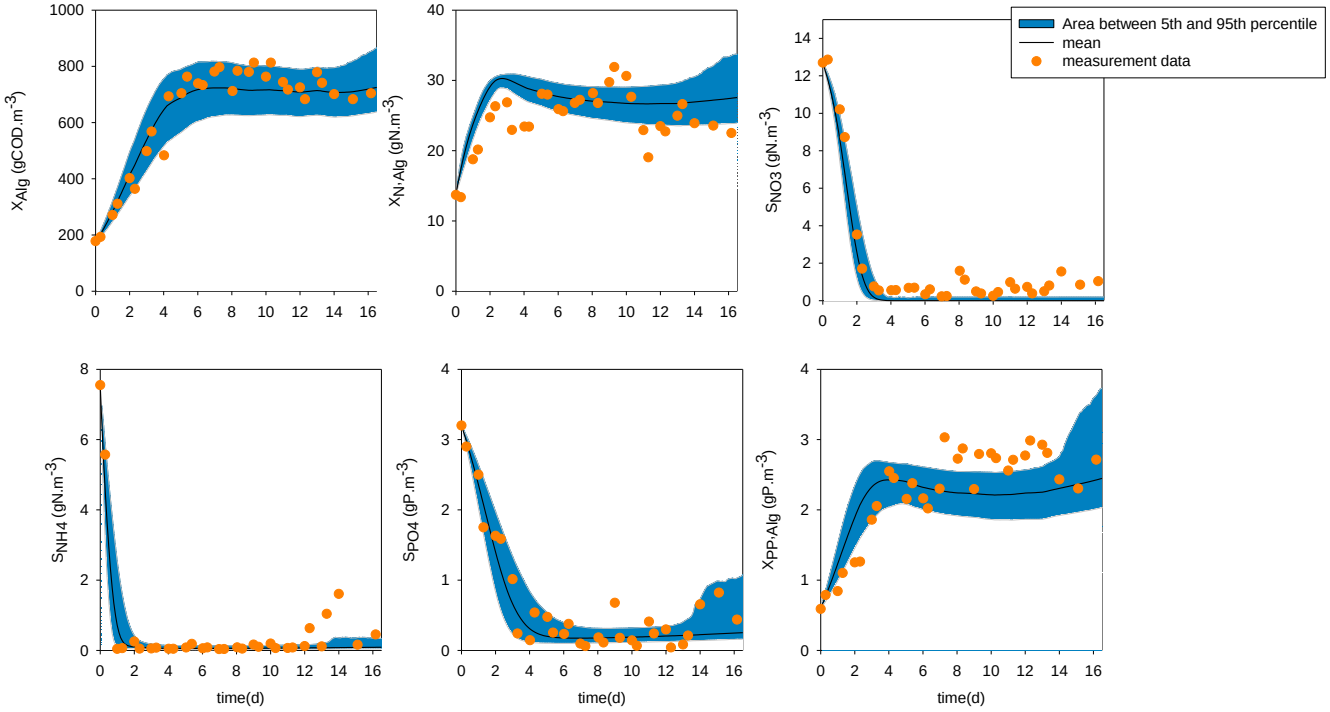



**Figure 16:** Model simulation using the one-dimensional layer model. The simulation using the mean values of the parameter set is shown in black line. The 95% uncertainty bands are shown in blue (**Paper III**).

# 4 Microalgal cultivation

As discussed earlier, the variation of the nutrient composition of influent water or the used water stream used for the microalgal cultivation might affect the nutrient removal and thus the effluent quality. Moreover, under highly dynamic conditions, the control structure designed for the EBP2R system could not supply the required N-to-P ratio for the microalgal cultivation. Therefore, the effects of using sub-optimal cultivation conditions in terms of nutrient availability are assessed in this chapter and in **Paper IV**.

Apart from the mixed green microalgal culture, a monoculture of *Chlorella sp.* was used. The mixed consortium was cultivated in continuous operation, on used water treated by a laboratory scale low-SRT EBPR system (Valverde-Pérez et al., 2016b) operated as a sequencing batch reactor (SBR) at 3 days SRT (referred to as Case 1). The monoculture of *Chlorella sp.* was cultivated with treated used water collected from a laboratory scale continuous EBPR system operated at 16 days SRT (referred to as Case 2). A glass cylindrical PBR, with a working volume of 1.4 L (Fig. 17), was used to cultivate the cultures. The details about the cultivation can be read in **Paper IV**. The N-to-P ratio of the influent to the PBR was varied during the cultivation. During the cultivation of the mixed consortium starting at 17, the N-to-P ratio was lowered to 5 and then back to 17 by varying the nitrogen supply in the influent. During cultivation with the monoculture, also starting from 17, the N-to-P ratio was lowered to 10, then back to 17 and then up to 25 by varying the nitrogen supply. The culture composition was monitored using an image analysis method, developed during this study (details can be found in **Paper IV**). The method is based on the identification and quantification of the different types of algae based on their morphology, i.e. *Chlorella sp*. (shape: round and small individual cells), *Scenedesmus sp*. (shape: elongated cells grown in two-to-four-cell colonies) and diatoms (that appeared during the cultivation; shape: elongated cells, larger than the previous two species). The method can serve as an automated tool to distinguish between the genera.

**Figure 17:** The 1.4-L PBRs used in continuous cultivation. The light was supplied only from the top of the reactor and the reactor walls were covered with black cloths to avoid light entering from the side.

The image analysis tool was used to monitor the culture composition in the mixed microalgal consortium and the monoculture of *Chlorella sp*. At the start of the experiment the mixed consortium contained mostly *Scenedesmus sp.*, about 83% of the total cell count (Fig. 18), whilst *Chlorella sp*. were present in 9%. The composition did not vary significantly in the first 6 days of the cultivation, at 17 N-to-P ratio. As the N-to-P ratio was lowered to 5, there was a sudden appearance of diatoms belonging to the *Nitzschia sp.*, identified from microscopic observation. Microscopic observations suggested that the diatoms were seeded from the influent water to the PBR that proliferated in the altered cultivation conditions. By day 10, the number of cell fraction of diatoms increased up to 8% in the culture. Their ratio, however, was considerably higher when accounting for the cell area, up to 34% (Fig. 18). Thus, diatoms constitute a relevant fraction of the biomass concentration, due to their cell size that is 3-5 times higher relative to *Chlorella sp.* and *Scenedesmus sp.* Moreover, the number of ciliates increased in the reactor (accounted for in the fraction of other species), increasing the relative cell area of other species (66%). Importantly, diatoms were washed out of the system, soon after the N-to-P ratio was set back to 17, a nutrient availability favourable for *Chlorella* and *Scenedesmus sp*. Furthermore, the relative ratio of *Chlorella* and *Scenedesmus sp.* has shifted by the end of the experiment with *Chlorella sp.* reaching 77% at day 21. Similar observations have been made else-

where (Alcántara et al., 2015a), whilst Beuckels et al. (2015) find that *Chlorella sp.* are capable of accumulating more nitrogen than *Scenedesmus sp.* Thus, the selection of *Chlorella sp.* was possibly natural and is not related to the changes in cultivation conditions. The monoculture of *Chlorella sp.* cultivated in continuous PBR operation did not show variation in the culture composition throughout the 85 days of cultivation. *Chlorella sp.* remained the single microalgal species in the culture. The used water used in the experiments was not autoclaved. Difference in the influent water quality was triggered by the operation of the upstream EBPR system. In Case I and II, the EBPR was operated at 3 d SRT and 16 d, respectively. Taken together, these results suggested that in short-SRT bacterial systems some phototrophic organisms, e.g. diatoms might be able to persist and potentially contaminate the downstream algal cultivation. In contrast, at high-SRT, the diatoms or other phototrophic organisms might be removed from the system. Hence, the control of the N-to-P ratio is a powerful tool to regulate and stabilize PBR combined with bacterial systems operated at short-SRTs, such as in the TRENS system (Fang et al., 2016).

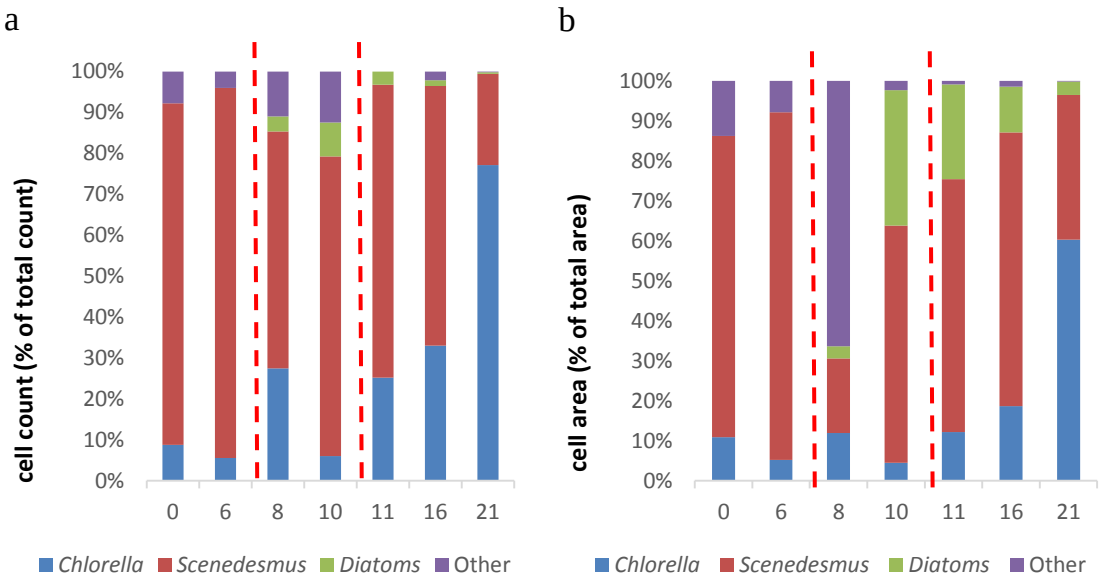



**Figure 18:** Variation in the culture composition of the cultivation with the mixed microalgal species. (**a**) The cell count is presented as the fraction of the total cell count. (**b**) The cell area is presented as the fraction of the total cell area (**Paper IV**).

Model simulations were used to assess the effects of the change in the culture composition on the kinetics of the culture. Model simulations (shown in **Paper IV**) could successfully predict the measurement results in Case 2, the

monoculture of *Chlorella sp*., when no contamination by other species was observed. Simulation results show that when diatoms proliferated in the mixed culture of *Chlorella* and *Scenedesmus sp.* the nutrient removal kinetics changes significantly (Case 1), and the simulation model fails to predict the measurements (Fig. 19). Taken together, the observations suggest that varying nutrient availability can potentially lead to the opportunistic selection of algal species, not present originally in the culture and it does not seem to cause alterations in metabolic activities of the dominant cultured species. Invasion of an algal culture by alien species seems to occur primarily via the PBR influent flow. Furthermore, calibration scenarios accounting for the differences in kinetics between microalgal species may correct for the deficiencies in predicting variability in process performance. Such solutions would be especially useful when short-SRT upstream systems are used to produce the cultivation medium.

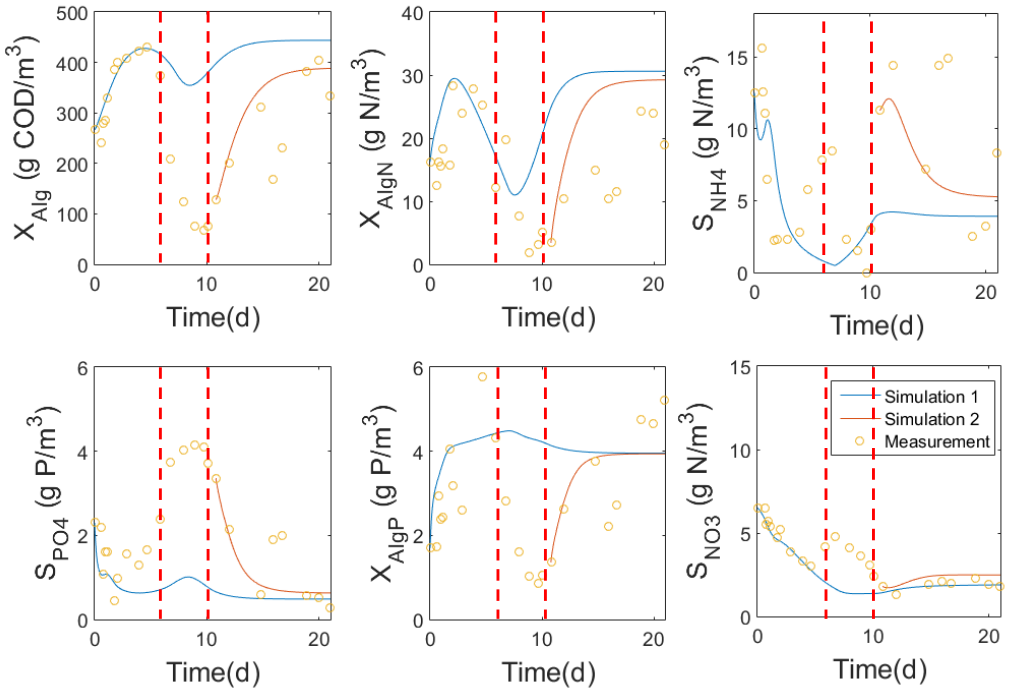



**Figure 19:** Simulation results of Case 1, the mixed microalgal species cultivation. The red vertical dashed lines represent the time when the N-to-P ratio was changed. Simulation 1 (blue line) represents the simulation of the whole cultivation period. Discrepancy after the decrease of N-to-P ratio is due to change in the culture composition. Simulation 2 (red line) represents the simulation of the second 17 N-to-P ratio period (**Paper IV**).

# 5 Biomass harvesting and biogas potential

Anaerobic digestion or co-digestion of algal biomass cultivated on used water resources is more energetically favourable than biodiesel production, due to its simple technology and the characteristics of the biomass. Thus, in this thesis and in **Paper II** as an alternative scenario to using the algal suspension for fertigation, co-digestion with bacterial biomass produced in the upstream EBP2R system is considered.

## 5.1 Harvesting of microalgae

One of the major bottlenecks of microalgal cultivation for biogas production is the cost related to harvesting that can contribute to 20-30% of production costs (Gerardo et al., 2015; Roselet et al., 2015). Methods, such as, centrifugation or membrane technologies are expensive and require energy input (Gerardo et al., 2015) and applicable when high value products are produced. Hence, simple harvesting methods are sought for to support safe downstream applications (Gao et al., 2014). Coagulation-flocculation can be used as cheap harvesting method (Gouveia et al., 2016). Microalgae have negative surface charge that can be destabilized through coagulation. This is followed by the aggregation of particles, promoting more effective gravity sedimentation (Gutiérrez et al., 2015). Iron or aluminium salts, are successfully applied as coagulants promoting microalgal biomass harvesting (Vandamme et al., 2013). Nonetheless, metal salts require high dosage and the downstream usage of the biomass and water is limited due to toxicity (Roselet et al., 2015). Cationic polymers can be applied as an alternative to harvest algal biomass by surface charge neutralization or by inter-cellular bridging (Vandamme et al., 2013). Polymers usually require lower dosages compared to metal salts. However, flocculation efficiency at high dosages of polymers declines due to restabilisation (Gerardo et al., 2015) thus care should be taken when applying this technology. Alternatively, bioflocculation has been proposed whereby, bacteria, fungi or algae promote flocculation (Manheim and Nelson, 2013).

## 5.2 Innovative two-step flocculation method

First, a pre-screening of possible inorganic coagulants was performed in **Paper II**. The coagulation aids included $AlCl_3$, the cationic biopolymer Greenfloc 120 and the cationic polymer Poly(diallyldimethylammonium chloride) (PDADMAC). The coagulants were compared based on the price and effectiveness of the flocculation in jar tests and the optimal coagulant was chosen to be the cationic polymer (PDADMAC). An optimum recovery of the microalgal biomass of 92% was found at the intermediary dose of ca. 27 mg polymer/g algae. Higher polymer dosages than this value resulted in restabilisation of the aggregates, whereby reducing the recovery.

Second, an innovative two-step flocculation method was tested to recover the algal biomass. In the first step the algae were coagulated first with the cationic polymer (PDADMAC, as chosen previously) and then bacterial biomass was added in the second step to enhance the flocculation (Fig. 20).

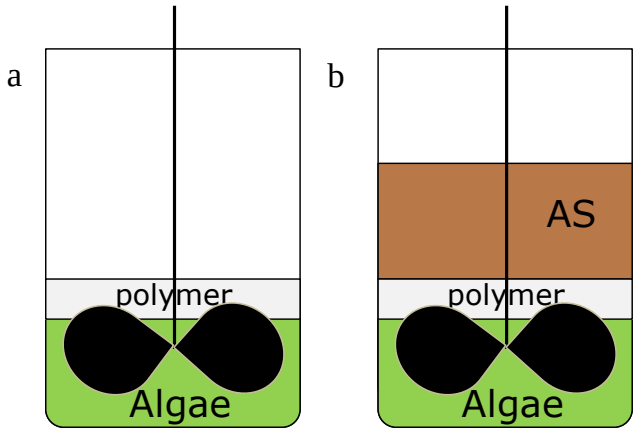



**Figure 20:** The set-up of the innovative two-step flocculation. Polymer is mixed with the algae in a first step (a), then bacterial biomass (AS) is added in a second step (b).

The ratio of microalgal and bacterial biomass was kept constant, whereas the polymer dosage was increased to assess the optimal polymer dosage. With increasing polymer dosage the microalgal recovery improved, suggesting that as larger aggregates are formed the probability of collision with the bacterial biomass flocs increase. Recovery rate of microalgae of ca. 97% was achieved using a dosage of 16 mg polymer/g algae at a 0.1 g algae/g bacterial biomass ratio (Fig. 21). Thus, using bacterial biomass can improve the flocculation and the polymer dosing can be reduced by 40% compared to the scenario when only cationic polymer was used and harvesting costs can be reduced

(Fig. 21). No restabilisation effect was observed at higher dosing, likely due to the bacterial biomass addition, thus making the process more stable.

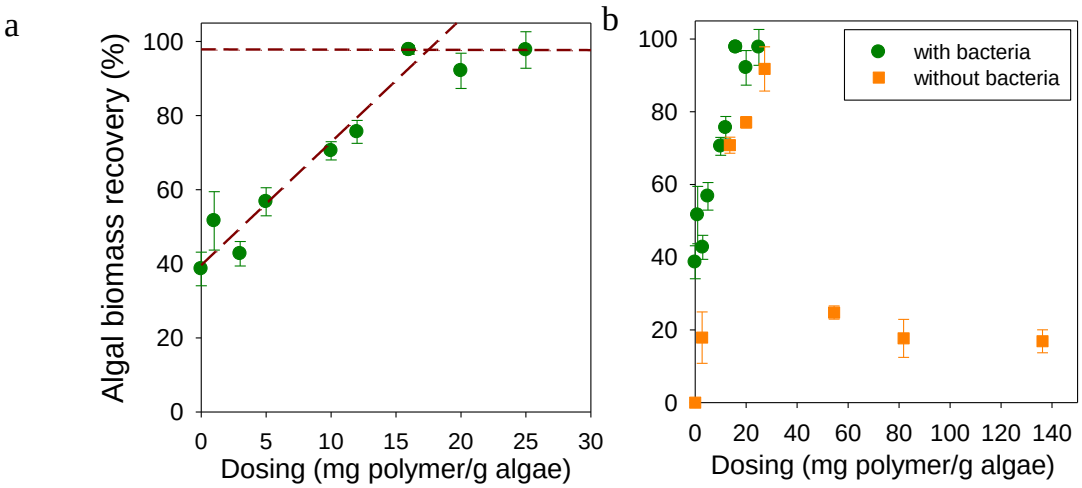



**Figure 21:** Choosing the optimal polymer dosing of the two-step flocculation (a), and comparison of the flocculation efficiency with and without bacterial biomass dosing (b) (**Paper II**).

## 5.3 Co-digestion with bacterial biomass

The harvested biomass was assessed in biomethane potential (BMP) tests to compare the single and the co-digestion of microalgae and bacterial biomass. The bacterial biomass was taken from a laboratory-scale EBP2R system. The BMP obtained after 27 days of digestion of the microalgal biomass is 331±76 ml $CH_4$/gVS (Fig. 22), corresponding to the methane yield reported in the literature (Ward et al., 2014). This result is similar to those that are reported with different pre-treatment options in the literature (Passos et al., 2014), thus pre-treatment in this case is not necessary. Furthermore, the addition of polymer does not significantly affect the biomethane potential of the microalgae (Fig. 22).

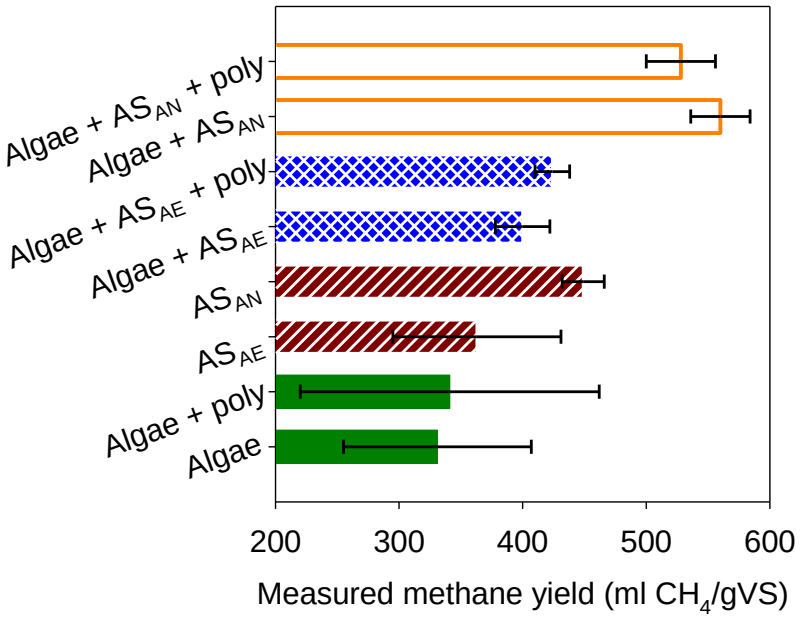



**Figure 22:** Comparison of the single and co-digestion of microalgae and bacterial biomass (**Paper II**).

Two sludge wastage strategies were considered from the EBP2R system, i.e. (i) bacterial biomass wasted from the secondary settler after the aerobic reactors, (ii) the solid-liquid separation after the anaerobic phase. The BMP of the biomass removed after the aerobic phase is 363±68 ml $CH_4$/gVS, whereas, for biomass removed after the anaerobic phase is 449±17 ml $CH_4$/gVS (Fig. 22). The difference between these two digestion scenarios is not significant. Liter-

ature is relatively limited in assessing the BMP of short-SRT bacterial biomass. Ge et al. (2013) reports similar results to those obtained with the biomass removed after the aerobic phase in this thesis. The co-digestion of algae with bacterial biomass wasted from the solid-liquid separation after the anaerobic reactor yielded significantly higher BMP compared to the single digesting of the algal and bacterial biomass and synergistic effect of the co-digestion was found. Values of the BMP obtained with and without polymer dosing are 528±28 ml $CH_4$/gVS (Algae + ASAN + poly) and 560±24 ml $CH_4$/gVS (Algae + ASAN), respectively. The co-digestion with bacterial biomass wasted after the aerobic phase did not yield significantly higher BMP and only additive effect was found. Furthermore, the BMP of the co-digestion yielded significantly higher with bacterial biomass taken after the anaerobic phase than with biomass taken from the aerobic phase. In the anaerobic phase of the EBP2R system the biomass contains PHA storage by the PAO. PHA is an easily available substrate for the digestion than other organic materials, e.g. the cell wall. Thus this storage of PHA can improve the BMP of the biomass. There is no significant difference between the digestion of solely the bacterial biomass taken after the anaerobic and aerobic phase. Thus, the single digestion of the bacterial biomass taken after the anaerobic phase may be nutrient limited. Whereas, co-digestion with microalgae, could provide the nutrients (both macro and micronutrients) required to digest the increased organic carbon content of the biomass.

Results suggest that the microalgal biomass can be successfully harvested from the water using a minimal polymer dosage and bacterial biomass taken from the upstream EBP2R. The harvested biomass shows the potential to produce methane through anaerobic digestion. Furthermore, the bacterial biomass wasted from the solid-liquid separation after the anaerobic reactor can further enhance the biogas potential of the co-digestion by providing an easily available substrate, PHA, while, the microalgal biomass can provide the essential nutrients needed for the co-digestion.

# 6 Conclusions

This thesis presents the identification and evaluation of a model for photoautotrophic and heterotrophic microalgal growth, nutrient uptake and storage, developed in the activated sludge modelling framework (ASM-A). Furthermore, factors affecting the light distribution in PBRs were assessed in laboratory-scale batch reactors, together with the implications on the modelling of light distribution. The effect of varying N-to-P ratio was assessed in continuous reactor operation in the laboratory, where microalgae were cultivated on used water resources, treated by an upstream EBPR system, in open PBRs. Finally, an innovative two-step flocculation method is presented to harvest the algae, together with the potential for co-digestion with bacterial biomass. The main conclusions are:

- A biokinetic process model for photoautotrophic and heterotrophic microalgal growth and nutrient uptake and storage was developed in the ASM framework, based on an extensive literature review of microalgal process models. Based on a specific experimental design and data treatment, the ASM-A model parameters were estimated and were found identifiable. An average parameter set can be used to predict microalgal biomass concentration, bulk ammonium and phosphate concentrations and phosphorus storage. However, the nitrogen storage is affected by substrate availability, whilst the soluble nitrate concentration depends on the culture history. Thus, the case specific re-estimation of $k_{NO,Alg}$ is needed to predict the soluble nitrate and nitrogen storage.
- The light attenuation depends on the primarily on the pigmentation as well as the biomass concentration of the microalgae and the light scattering in the reactor. The Lambert-Beer equation can be used to model the light attenuation in the PBR. The light attenuation coefficient estimated was found to be a variable rather than a single value. Elevated light intensity promoted the synthesis of carotenoids and the reduction of chlorophyll was observed. The chlorophyll content can be predicted by relating it directly to the internal nitrogen quota.
- Using a model with discretized layers to predict the light distribution in PBRs resulted in more accurate prediction of the microalgal biomass concentration as well as the reduction of the uncertainty of the model output.
- The influent N-to-P ratio is found to affect the culture composition during continuous microalgal cultivation. Diatoms proliferated in the reactor in a

mixed green microalgal consortium when the N-to-P ratio was below optimum. This was found to deteriorate model prediction accuracy due to the potential change in culture kinetics. The diatoms could be washed out of the system once the N-to-P ratio was increased back to an optimal level. It was found that the SRT of the upstream bacterial unit process might be able to mitigate the potential of contamination by other microalgal species, at high SRT.

- An innovative bioflocculation method was introduced to harvest microalgal biomass. The microalgae were destabilised with cationic polymer in a first step, then in a second step bacterial biomass was used as a flocculant. Up to 97 % recovery was reached with 16 mg polymer /g algae and 0.1 g algae/g bacterial biomass ratio. The cationic polymer dosage could be reduced by 40% compared to the scenario when only polymer was used as a flocculant to harvest algae, thus harvesting costs are reduced.
- The highest methane yield was found at 560±24 ml$CH_4$/gVS when microalgae and bacterial biomass rich in easily accessible organic carbon (PHA) were co-digested.

# 7 Future perspectives

The possibility of cultivating microalgae on used water resources was shown in this thesis under laboratory conditions. There are further points that need to be addressed both in the TRENS system and in general in microalgal cultivation on used water resources.

- It was shown in this thesis that microalgal cultivation is possible on used water resources from the up-stream bacterial treatment process under laboratory conditions. The focus now should be put on the scale-up of the system and testing TRENS in pilot-scale operation. On-line sensors should be tested to monitor the pilot scale application. This is addressed in an ongoing study whereby a UV/VIS sensor is tested to be used to monitor microalgal biomass, nitrate and pigments concentration.
- The ASM-A model could be extended with tools that further improve applicability in open cultivation systems on used water resources. By using methods for the proper estimation of pH, a more accurate estimation of the carbon speciation can be achieved, which might additionally affect the prediction of microbial growth. Furthermore, the model currently does not consider the effects of temperature, which is particularly important when considering open cultivation.
- One of the aims of TRENS is to apply the produced microalgae on agricultural land for fertigation. There are a limited number of publications on using the microalgae as bio-fertilizer. Hence, research should be focused on the use microalgae as fertilizer. Moreover, the removal of heavy metals and pharmaceuticals through the TRENS system is yet to be assessed. This could affect the downstream application. It is important to show to the consumers that microalgal biomass cultivated on used water resources can be used as an alternative of the conventional mineral fertilizer. The advantages of a slow-leaching fertilizer over a mineral fertilizer need to be shown in order to make the product.
- In general, the perception about using a product obtained from used water should be changed. Examples can be found all around the world, where used water is reused, e.g. as drinking water. These good examples should be promoted among the public, to make the acceptance towards these technologies. Proper legislations should be made to be able to use microalgae grown on used water resources as bio-fertilizers or other high value products for e.g. animal feed.

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# 9 Papers

**I** **Wágner, D.S.**, Valverde-Pérez, B., Sæbø, M., Bregua de la Sotilla, M., Van Wagenen, J., Smets, B.F., Plósz, B.Gy., 2016. Towards a consensus-based biokinetic model for green microalgae – The ASM-A. *Water Research*, **103**, 485-499.

**II** **Wágner, D.S.**, Radovici, M., Smets, B.F., Angelidaki, I., Valverde-Pérez, B., Plósz, B.Gy., 2016. Harvesting microalgae using activated sludge can decrease polymer dosing and enhance methane production via co-digestion in a bacterial-microalgal process. *Algal Research*, **20**, 197-204.

**III** **Wágner, D.S.**, Valverde-Pérez, B., Plósz, B.Gy., 2016. Light attenuation in photobioreactors and algal pigmentation under different growth conditions – model identification and complexity assessment. Manuscript in preparation.

**IV** **Wágner, D.S.**, Cazzaniga, C., Steidl, M., Dechesne, A., Valverde-Pérez, B., Plósz, B.Gy., 2016. Re-definition of the optimal N-to-P ratio concept and its importance for stable microalgal cultivation and water treatment. Submitted Manuscript.

In this online version of the thesis, **paper I-IV** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from.

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