Assessment of microalgal growth kinetics through respirometry and titrimetry: data collection and model analysis

Ing. Bjorge Decostere
“To succeed, you need to find something to hold on to, something to motivate you, something to inspire you” (Tony Dorset)
Promoters

Prof. dr. ir. Stijn Van Hulle
Department of Industrial Biological Sciences
Faculty of Bioscience Engineering
Ghent University
Graaf Karel de Goedelaan, 5
8500 Kortrijk, Belgium
Stijn.VanHulle@Ugent.be

Prof. dr. ir. Ingmar Nopens
Department of Modelling, Statistics and Bioinformatics
Faculty of Bioscience Engineering
Ghent University
Coupure Links, 653
9000 Ghent, Belgium
Ingmar.Nopens@Ugent.be

Examination Board

Prof. dr. ir. Wim Soetaert
Prof. dr. ir. Andres Alvarado
Prof. dr. ir. Raf Dewil
Prof. dr. ir. Peter Goethals
Prof. dr. ir. Diederik Rousseau
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Ghent University, Belgium
Ghent University, Belgium
KU Leuven, Belgium

Dean: Prof. dr. ir. Marc Van Meirvenne
Rector: Prof. dr. Ann De Paepe
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DANKWOORD

Toen men mij in mijn kinderjaren vroeg wat ik later wilde worden, antwoordde ik steevast: “vliegtuigpiloot”. Na mijn secundaire opleiding, waar ik finaliseerde in de richting Wiskunde - Wetenschappen, was ik echter sterk aangetrokken tot de wetenschapsvakken. Het logische gevolg, namelijk een opleiding tot industrieel ingenieur heb ik het daaropvolgende jaar met goede moed aangevat. Ik bleek toen echter nog niet “klaar” voor het serieuze studentenleven en heb deze studies na mijn graduatsjaren moeten afbreken en mij heroriënteren. Na verloop van tijd (reeds gehuwd en gezin), heb ik dan toch nog de opleiding tot industrieel ingenieur milieukunde terug aangevat. Hierin moest ik in het laatste jaar van de opleiding onderzoek doen in het kader van mijn masterproef. Daarbij kwam ik het tot de vaststelling dat ik graag onderzoek deed. Meer specifiek naar afvalwaterbehandeling, mede door de wetenschappelijke en maatschappelijke relevantie van deze problematiek. Ook de eindwerken die ik begeleidde tijdens mijn mandaat als assistent waren hoofdzakelijk gericht op afvalwaterbehandeling.

Toen 5 jaar geleden mij de mogelijkheid werd geboden om te doctoreren op een onderwerp rond afvalwaterbehandeling met microalgen, heb ik die kans dan ook met beide handen gegrepen. Dit was voor mij een echte uitdaging, omdat mijn kennis rond microalgen toen nog beperkt was. Wanneer ik mijn onderwerp toelichtte in mijn kenniskring was meestal de reactie “microalgen heb ik mijn zwembad of vijver en ze moeten er uit …”. Maar niets is minder waar. Immers, in Nieuw - Zeeland, worden (micro)algen “The Green Gold” genoemd.

Het werk dat ik heb verricht en de resultaten die ik heb bekomen gedurende deze intense periode zijn gebundeld in deze eindverhandeling. Dit werk is natuurlijk niet tot stand kunnen komen zonder de medewerking van studenten Master Milieukunde die het onderwerp “Modelleren van de groei van microalgen” hadden gekozen voor hun masterproef. Mijn dank gaat dan ook uit aan Natascha, Mathieu en Johannes. Dank om dit toch niet zo eenvoudig thema steeds met enthousiasme tot een goed einde te brengen. Ook heb ik gebruik gemaakt van het werk van Ying en Paula, buitenlandse studenten in het kader van Erasmus uitwisseling.

Ook wil ik graag het Fonds Voor Wetenschappelijk Onderzoek bedanken. Dank zij hun toekenning van mijn persoonlijk krediet voor langdurig verblijf in het buitenland, kon ik gedurende 16 weken onderzoek doen rond de algenbiomassa van een Waste Stabilization Pond te Cuenca, Ecuador. Dit was een zeer mooie ervaring in een prachtig land, met prachtige
mensen. Dank aan Prof. dr. Andres Alvarado om dit onderzoek te willen steunen en mij te ontvangen in het Laboratoria de Sanatoria van de Universiteit van Cuenca. Dank aan Esteban, Daniella, Gabriella, Guiermina Pauta, Carlos, Juan, Katja, Ellias en Zara voor het vele werk dat zij voor mij hebben verricht en de vele mooie momenten samen. Ook hiervoor dank aan mijn moeder, die tijdens mijn langdurige afwezigheid, de zorg voor mijn kindjes voor zich nam.

Uiteraard zou dit doctoraat niet tot stand zijn gekomen zonder de steun van mijn promotoren, Prof. dr. ir. Stijn Van Hulle (LIWET) en Prof. dr. ir. Ingmar Nopens (BIOMATH). Dank om in mij te blijven geloven en mij te blijven steunen, ook in mindere periodes.

Graag wou ik ook een dankwoordje richten aan personen die ik heb leren kennen bij BIOMATH. Hoewel ik in het kader van mijn doctoraatsonderzoek ook tot die vakgroep behoorde, was ik er slechts sporadisch aanwezig. Door mijn drukke lesopdracht aan de Campus Kortrijk, was het voor mij niet evident om daar aanwezig te zijn. De zovele BIOMATH activiteiten die meestal in het weekend plaats vonden moest ik ook aan mij laten voorbijgaan. Graag had ik erbij geweest. Deze evenementen blijken legendarisch en er wordt met blijde herinneringen aan terug gedacht tot in het verre Ecuador. Een dankwoord voor Thomas, Stijn Van Hoey en Yoeri, die me bij stonden rond problemen over simulatie technieken, vragen rond kinetiek van algen en problemen met het o zo gebruiksvriendelijke software programma WEST … 😊. Dank u Tine voor de ondersteuning in het BIOMATH labo.

Met het finaliseren van mijn doctoraatsonderzoek, sluit ik ook het hoofdstuk UGent af. Ik wil dan ook de collega’s van de vakgroep Industriële Biologische Wetenschappen van harte bedanken voor de jarenlange samenwerking. Ik heb hieraan goede ervaringen en herinneringen over gehouden. Ik wens jullie het allerbeste in jullie verdere (academische) carrière.

Gent, mei 2016
<table>
<thead>
<tr>
<th>Chapter 1: Introduction, aims, objectives and thesis outline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Introduction .................................................................................................................. 2</td>
</tr>
<tr>
<td>1.2 Aims and objectives ........................................................................................................ 3</td>
</tr>
<tr>
<td>1.3 Outline ............................................................................................................................ 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 2: Literature review</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Introduction .................................................................................................................. 8</td>
</tr>
<tr>
<td>2.2 The use of microalgae for wastewater treatment .......................................................... 9</td>
</tr>
<tr>
<td>2.2.1 Introduction ................................................................................................................ 9</td>
</tr>
<tr>
<td>2.2.2 Microalgal reactor systems .......................................................................................... 10</td>
</tr>
<tr>
<td>2.2.2.1 Open microalgal cultivation system ......................................................................... 11</td>
</tr>
<tr>
<td>2.2.2.2 Closed photobioreactor for microalgae cultivation ............................................... 12</td>
</tr>
<tr>
<td>2.2.2.3 Immobilized algal systems ...................................................................................... 12</td>
</tr>
<tr>
<td>2.2.2.4 Waste stabilization ponds ....................................................................................... 13</td>
</tr>
<tr>
<td>2.3 Methods to measure microalgal kinetics ......................................................................... 15</td>
</tr>
<tr>
<td>2.3.1 Introduction ................................................................................................................ 15</td>
</tr>
<tr>
<td>2.3.2 Continuous cultures .................................................................................................... 16</td>
</tr>
<tr>
<td>2.3.3 Batch cultures ............................................................................................................. 16</td>
</tr>
<tr>
<td>2.3.4 Respirometry and titrimetry ....................................................................................... 17</td>
</tr>
<tr>
<td>2.3.4.1 Respirometry .......................................................................................................... 17</td>
</tr>
<tr>
<td>2.3.1.2 Titrimetry ............................................................................................................... 19</td>
</tr>
<tr>
<td>2.3.5 Conclusion ................................................................................................................... 22</td>
</tr>
<tr>
<td>2.4 Factors influencing the microalgal growth rate ................................................................ 23</td>
</tr>
<tr>
<td>2.4.1 Introduction ................................................................................................................ 23</td>
</tr>
<tr>
<td>2.4.2 Light intensity ............................................................................................................. 23</td>
</tr>
<tr>
<td>2.4.3 Temperature ............................................................................................................... 24</td>
</tr>
</tbody>
</table>
3.4.4.1 Standardized Regression Coefficients ................................................................. 53
3.4.4.2 Monte Carlo Filtering Method................................................................................. 54
3.5 Parameter estimation .................................................................................................... 54
3.6 Goodness – of – fit........................................................................................................ 55

Chapter 4: A novel methodology to measure the microalgal growth kinetics

4.1 Introduction .................................................................................................................... 58
4.2 Methods and materials .................................................................................................. 59
  4.2.1 Cultivation of microalgae ......................................................................................... 59
  4.2.2 The algal respirometer ............................................................................................ 59
  4.2.3 Data interpretation .................................................................................................... 60
  4.2.4 Modelling approach ................................................................................................. 61
  4.2.3.1.1 Algal growth and decay kinetics ...................................................................... 62
  4.2.3.1.2 Inorganic carbon species ................................................................................. 63
  4.2.3.1.3 Oxygen production and oxygen transfer ............................................................. 64
  4.2.3.1.3 Parameter values .............................................................................................. 65
4.3 Results and discussion ................................................................................................. 67
  4.3.1. Data collection and derived information ................................................................. 67
  4.3.2 Model calibration ..................................................................................................... 70
4.4 Conclusions .................................................................................................................. 72

Chapter 5: Assessing the effect of environmental conditions on the microalgal growth rate

5.1 Introduction .................................................................................................................... 76
5.2 Materials and methods ................................................................................................. 77
  5.2.1 Experimental design ............................................................................................... 77
  5.2.2 Analytical methods ................................................................................................. 79
5.3 Results and discussion ................................................................................................. 79
5.3.1 Exploration of the influence of biomass concentration ........................................... 79
5.3.2 Assessment of the influence of pH on the availability of inorganic carbon .......... 81
5.3.3 Influence of inorganic nitrogen limitation on oxygen production and proton addition .................................................................................................................. 82
5.3.4 Preferential uptake of inorganic nitrogen species ............................................. 84
5.3.5 Influence of inorganic phosphorus on oxygen and proton addition ..................... 85
5.3.6 Influence of temperature, light intensity on oxygen production and proton addition 86
5.3.7 Overall kinetic expression ......................................................................................... 88
5.4 Conclusions ................................................................................................................. 89

Chapter 6: Assessment of nutrient removal by microalgal biomass

6.1 Introduction .................................................................................................................. 92
6.2 Materials and methods ............................................................................................... 94
  6.2.1 Experimental data collection ............................................................................... 94
  6.2.2 Model development .............................................................................................. 94
  6.2.3 Model parameter values ....................................................................................... 95
  6.2.4 Global sensitivity analysis .................................................................................... 97
6.3 Results and discussion ............................................................................................... 100
  6.3.1 Data selection ....................................................................................................... 100
  6.3.2 Global sensitivity analysis ................................................................................... 100
  6.3.3 Model calibration .................................................................................................. 104
  6.3.4 Model validation .................................................................................................. 107
6.4 Conclusions ................................................................................................................. 109

Chapter 7: Kinetic exploration of intracellular nitrate storage

7.1 Introduction .................................................................................................................. 112
7.2 Material and methods ............................................................................................... 114
  7.2.1 Cultivation of the strains ...................................................................................... 114
7.2.2 Quantification of intracellular nitrate ....................................................... 115
7.2.3 Kinetic experiments .................................................................................. 115
7.2.4 Analytical techniques .............................................................................. 116
7.2.5 Model development .................................................................................. 116
7.2.6 Model parameter values .......................................................................... 119
7.2.7 Global sensitivity and parameter identifiability ....................................... 120
7.3 Results and discussion ................................................................................ 121
7.3.1 Intracellular nitrate storage of the different species .............................. 121
7.3.2 Global sensitivity analysis and parameter identifiability ...................... 123
7.3.3 Identifiability of parameter subsets: the collinearity index .................... 125
7.3.4 Model calibration ..................................................................................... 126
7.3.4 Nitrate storage evolution ......................................................................... 127
7.3.5 Model validation ...................................................................................... 128
7.3.6 Scenario analysis ..................................................................................... 129
7.4 Conclusions ................................................................................................. 130

Chapter 8: Kinetics of microalgae residing in a WSP

8.1 Introduction .................................................................................................. 134
8.2 Methods and materials ............................................................................... 135
8.2.1 Cultivation of the microalgae ................................................................. 135
8.2.2 Experimental protocol ............................................................................ 136
8.2.3 Analytical methods .................................................................................. 137
8.2.4 Modelling software .................................................................................. 138
8.3 Results and discussion ................................................................................. 138
8.3.1 Data interpretation and derived information .......................................... 138
8.3.2 Determination of significance of factors ................................................ 140
8.3.3 Model development ................................................................................ 141
8.3.3.1 Microalgal growth........................................................................................................141
8.3.3.2 Temperature dependent oxygen transfer rate.........................................................142
8.3.4 Model parameter values............................................................................................143
8.3.5 Model calibration ......................................................................................................144
  8.3.5.1 Experiments with *Chlorella vulgaris*................................................................144
  8.3.5.2 Experiments with *Scenedesmus obliquus* .........................................................147
8.3 Model validation ..........................................................................................................148
  8.3.1 Experiments with *Chlorella vulgaris* ................................................................148
  8.3.2 Experiments with *Scenedesmus obliquus* .............................................................149
  8.3.3 Validation of the extended model with additional experimental data ....................150
8.4 Conclusions ..................................................................................................................151

Chapter 9: General conclusions and future perspectives

  9.1 Introduction ................................................................................................................156
  9.2 Development of a new technique to measure algal growth kinetics .........................158
  9.3 Optimization of nutrient removal in wastewater by microalgal biomass based on
      combined respirometric and titrimetric data................................................................159
  9.4 Kinetic exploration of intracellular nitrate storage......................................................160
  9.5 Growth kinetics of microalgae residing in WSP..........................................................160
  9.6 Perspectives and opportunities for future research ....................................................161
Appendix.............................................................................................................................163

Samenvatting......................................................................................................................171
Summary..............................................................................................................................176
Bibliography.........................................................................................................................181
Curriculum Vitae..................................................................................................................197
# LIST OF SYMBOLS AND ABBREVIATIONS

## SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>Temperature constant (-)</td>
</tr>
<tr>
<td>$b_{max}$</td>
<td>Maximum algal decay rate (d$^{-1}$)</td>
</tr>
<tr>
<td>$I$</td>
<td>Light intensity (lux or $\mu$E m$^{-2}$s$^{-1}$)</td>
</tr>
<tr>
<td>$I_c$</td>
<td>Light compensation point (lux or $\mu$E m$^{-2}$s$^{-1}$)</td>
</tr>
<tr>
<td>$I_h$</td>
<td>Light inhibition point (lux or $\mu$E m$^{-2}$s$^{-1}$)</td>
</tr>
<tr>
<td>$I_s$</td>
<td>Light saturation point (lux or $\mu$E m$^{-2}$s$^{-1}$)</td>
</tr>
<tr>
<td>$K_{a1}$</td>
<td>First acidity constant (mol l$^{-1}$)</td>
</tr>
<tr>
<td>$K_{a2}$</td>
<td>Second acidity constant (mol l$^{-1}$)</td>
</tr>
<tr>
<td>$k_1$</td>
<td>Reaction constant for hydration of CO$_2$ (d$^{-1}$)</td>
</tr>
<tr>
<td>$k_2$</td>
<td>Reaction constant for dissociation of HCO$_3^-$ (d$^{-1}$)</td>
</tr>
<tr>
<td>$K_{CO_2}$</td>
<td>Half saturation constant for growth on CO$_2$ (g CO$_2$ m$^{-3}$)</td>
</tr>
<tr>
<td>$K_H$</td>
<td>Henry coefficient for carbon dioxide (g m$^{-3}$ atm$^{-1}$)</td>
</tr>
<tr>
<td>$K_{HCO_3}$</td>
<td>Half saturation constant for growth on HCO$_3^-$ (g HCO$_3^-$ m$^{-3}$)</td>
</tr>
<tr>
<td>$K_I$</td>
<td>Half saturation constant for light intensity (lux or $\mu$E m$^{-2}$s$^{-1}$)</td>
</tr>
<tr>
<td>$K_{NO_3}$</td>
<td>Half saturation constant for growth on NO$_3^-$ (g N m$^{-3}$)</td>
</tr>
<tr>
<td>$K_{NH_4^+}$</td>
<td>Half saturation constant for growth on NH$_4^+$ (g N m$^{-3}$)</td>
</tr>
<tr>
<td>$K_{PO_4^{3-}}$</td>
<td>Half saturation constant for growth on PO$_4^{3-}$ (g P m$^{-3}$)</td>
</tr>
<tr>
<td>$K_{CO_2}$</td>
<td>Inhibition constant for growth on HCO$_3^-$ (g CO$_2$ m$^{-3}$)</td>
</tr>
<tr>
<td>$K_{cap}$</td>
<td>Half saturation constant for nitrate storage (mg N g$^{-1}$ DW)</td>
</tr>
<tr>
<td>$k_{STO}$</td>
<td>Nitrate storage rate (m$^3$ g$^{-1}$ DW d$^{-1}$)</td>
</tr>
<tr>
<td>$k_{ix}$</td>
<td>Self-shading biomass constant (kg m$^{-3}$)</td>
</tr>
<tr>
<td>$K_{iNH_4^+}$</td>
<td>Inhibition constant for growth on NO$_3^-$ (g N m$^{-3}$)</td>
</tr>
<tr>
<td>$K_{i,a}$</td>
<td>Oxygen transfer coefficient at certain temperature (d$^{-1}$)</td>
</tr>
<tr>
<td>$K_{i,a, ref}$</td>
<td>Oxygen transfer coefficient at 293 K (d$^{-1}$)</td>
</tr>
<tr>
<td>$k_Q$</td>
<td>Minimum intracellular nutrient amount needed for growth (g g$^{-1}$)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Light attenuation coefficient (m)</td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>Maximum specific growth rate (d$^{-1}$)</td>
</tr>
<tr>
<td>$n_x$</td>
<td>Self-shading biomass constant (-)</td>
</tr>
<tr>
<td>$P_{H}$</td>
<td>Specific proton addition rate (g H$^+$ g$^{-1}$ DW d$^{-1}$)</td>
</tr>
<tr>
<td>$P_{O_2}$</td>
<td>Photosynthetic activity (g O$_2$ g$^{-1}$ DW d$^{-1}$)</td>
</tr>
<tr>
<td>$P_{O_2,max}$</td>
<td>Maximum photosynthetic activity (g O$_2$ g$^{-1}$ DW d$^{-1}$)</td>
</tr>
<tr>
<td>$Q$</td>
<td>Total amount of nutrients that can be stored intracellular (g g$^{-1}$)</td>
</tr>
<tr>
<td>$r_{max}$</td>
<td>Maximum respiration rate (d$^{-1}$)</td>
</tr>
<tr>
<td>$\rho_{Alg}$</td>
<td>Algal growth rate (g DW m$^{-3}$ d$^{-1}$)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>( \rho_{\text{Alg}(HCO_3^-, NH_4^+, PO_4^{3-})} )</td>
<td>Algal growth rate on HCO(_3^-,) NH(_4^+) and PO(_4^{3-}) (g DW m(^{-3}) d(^{-1}))</td>
</tr>
<tr>
<td>( \rho_{\text{Alg}(HCO_3^-, NO_3^-, PO_4^{3-})} )</td>
<td>Algal growth rate on HCO(_3^-,) NH(_4^+) and PO(_4^{3-}) (g DW m(^{-3}) d(^{-1}))</td>
</tr>
<tr>
<td>( \rho_{\text{Alg}(CO_2, NH_4^+, PO_4^{3-})} )</td>
<td>Algal growth rate on CO(_2,) NH(_4^+) and PO(_4^{3-}) (g DW m(^{-3}) d(^{-1}))</td>
</tr>
<tr>
<td>( \rho_{\text{Alg}(CO_2, NO_3^-, PO_4^{3-})} )</td>
<td>Algal growth rate on CO(_2,) NO(_3^-) and PO(_4^{3-}) (g DW m(^{-3}) d(^{-1}))</td>
</tr>
<tr>
<td>( S_{CO_3^{2-}} )</td>
<td>Dissolved carbonate concentration (g CO(_3^{2-}) m(^{-3}))</td>
</tr>
<tr>
<td>( r_{\text{red} CO_2} )</td>
<td>Reduction factor for CO(_2) mass transfer coefficient ((-))</td>
</tr>
<tr>
<td>( S_{CO_2} )</td>
<td>Dissolved carbon dioxide concentration (g CO(_2) m(^{-3}))</td>
</tr>
<tr>
<td>( S_{CO_2\text{sat}} )</td>
<td>Dissolved carbon dioxide concentration at saturation (g CO(_2) m(^{-3}))</td>
</tr>
<tr>
<td>( S_{H^+} )</td>
<td>Proton addition (g H(^+) m(^{-3}))</td>
</tr>
<tr>
<td>( S_{HCO_3^-} )</td>
<td>Dissolved bicarbonate concentration (g HCO(_3^-) m(^{-3}))</td>
</tr>
<tr>
<td>( S_{NH_4^+} )</td>
<td>Dissolved ammonium concentration (g N m(^{-3}))</td>
</tr>
<tr>
<td>( X_{\text{STN}} )</td>
<td>Intracellular stored nitrate (g N g(^{-1}) DW)</td>
</tr>
<tr>
<td>( Y_1 )</td>
<td>Algal growth yield on HCO(_3^-) (g DW g(^{-1}) HCO(_3^-))</td>
</tr>
<tr>
<td>( Y_2 )</td>
<td>Algal growth yield on CO(_2) (g DW g(^{-1}) CO(_2))</td>
</tr>
<tr>
<td>( Y_3 )</td>
<td>Oxygen production yield (g O(_2) g(^{-1}) DW)</td>
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<tr>
<td>( Y_4 )</td>
<td>Algal growth yield on nitrogen (g DW g(^{-1}) N)</td>
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<tr>
<td>( Y_5 )</td>
<td>Algal growth yield on phosphorus (g DW g(^{-1}) P)</td>
</tr>
<tr>
<td>( Y_6 )</td>
<td>Oxygen production yield with growth on NH(_4^+) (g O(_2) g(^{-1}) DW)</td>
</tr>
<tr>
<td>( Y_7 )</td>
<td>Oxygen production yield with growth on NO(_3^-) (g O(_2) g(^{-1}) DW)</td>
</tr>
<tr>
<td>( Y_K )</td>
<td>Collinearity index ((-))</td>
</tr>
</tbody>
</table>

**ABBREVIATIONS**

- **ASM**: Activated sludge models
- **BSAR**: Background signal addition rate (g H\(^+\) m\(^{-3}\)d\(^{-1}\))
- **CDF**: Cumulative density function
- **CO2TR**: Carbon dioxide transfer rate (g CO\(_2\) m\(^{-3}\) d\(^{-1}\))
- **CSTR**: Continuous stirred tank reactor
- **DO**: Dissolved oxygen (g O\(_2\) m\(^{-3}\))
- **DW**: Dry weight (g DW m\(^{-3}\))
- **FME**: Flexible modelling environment
- **GSA**: Global sensitivity analysis
- **HAR**: Proton addition rate (g H\(^+\) m\(^{-3}\) d\(^{-1}\))
- **HRAP**: High rate algae pond
- **LHS**: Latin hyper cube sampling
- **LSA**: Local sensitivity analysis
- **MCR**: Monte Carlo run
- **MSL**: Model specification language
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPR</td>
<td>Oxygen production rate (g O₂ m⁻³ d⁻¹)</td>
</tr>
<tr>
<td>OTR</td>
<td>Oxygen transfer rate (g O₂ m⁻³ d⁻¹)</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen uptake rate (g O₂ m⁻³ d⁻¹)</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetic active radiation</td>
</tr>
<tr>
<td>PDF</td>
<td>Probability density function</td>
</tr>
<tr>
<td>TIC</td>
<td>Theil’s Inequality Coefficient (-)</td>
</tr>
<tr>
<td>TPAR</td>
<td>Total proton addition rate (g H d⁻¹)</td>
</tr>
<tr>
<td>RMSE</td>
<td>Root mean squared errors</td>
</tr>
<tr>
<td>RSA</td>
<td>Regional sensitivity analysis</td>
</tr>
<tr>
<td>SRC</td>
<td>Standardized regression coefficients</td>
</tr>
<tr>
<td>SSE</td>
<td>Sum of squared errors</td>
</tr>
<tr>
<td>WEST</td>
<td>Wastewater treatment plant engine for simulation and training</td>
</tr>
<tr>
<td>WSP</td>
<td>Waste stabilization pond</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction, aims, objectives and thesis outline
Chapter 1

1.1 Introduction

Wastewater treatment systems with use of microalgal biomass represents an increasingly attractive strategy. Indeed, microalgae have the capacity for intensive nutrient removal from wastewater. Further green microalgae have the capacity to fix inorganic carbon source (Sydney et al., 2010) and in combination with nutrients uptake (nitrogen and phosphorus), convert it into biomass and highly valuable molecules. In this regard, the use of microalgae for removal of nutrients and carbon dioxide uptake (Aslan and Kaplan, 2006) is an emerging technology which offers several advantages over conventional technologies for wastewater treatment. In particular, as microalgae produce oxygen by photosynthetic activity, the dissolved oxygen in the aqueous phase increases. This could be interesting when stringent standard discharge limits for dissolved oxygen are imposed or in case when a consortium of microalgae and bacteria is used for aerobic wastewater treatment (Van den Hende et al., 2011) as this could significantly decrease the aeration cost of waste water treatment. The latter is the major operational cost within conventional wastewater treatment systems.

A major drawback and even a possible obstruction to the implementation of microalgal systems on industrial scale, is the high harvesting cost. Indeed, due to the dilute nature of harvested microalgae cultures, the dewatering is a huge operational cost rendering microalgal systems less economic attractive (Uduman et al., 2010). Furthermore, in the absence of light, microalgal species will consume oxygen by respiration.

Optimization of this biological process is achieved by determining the optimal growth conditions for microalgal biomass. A cost-effective and efficient method is the use of kinetic growth models. With such models “in silico” experiments can be performed to assess the behavior of the microalgal biomass. However, setting up and performing such experiments in the virtual world alone is not enough. Indeed, next to model development and use, experimental data generation and mining in view of model calibration and validation is very important. Such experiments that aim at obtaining suitable data regarding microalgal growth, often require proxy measurements, for example chlorophyll content and lipid content. In general the analysis protocol for these measurements are very time consuming and require good technical practice. In view of this, respirometry offers a low cost alternative and is rather easy to perform.
1.2 Aims and objectives

Accurate representation of algal growth is one of the most difficult and poorly understood areas in water quality modelling. Algal growth is inherently complex, in general showing non-linear responses to various environmental parameters such as temperature, light and several nutrients, as well as demonstrating poorly understood interactions among these separate factors (Sandnes et al., 2005). Site-specificity also makes extrapolation from lab or other field studies inherently problematic. As such accurately describing the microalgal kinetic growth kinetics remains a significant challenge.

As already stated, the methods to measure the microalgal growth kinetics are mainly off-line measurements of parameters that are related to the growth rate. Moreover, some of these features are correlated to the growth rate after a certain adaptation period to the experimental conditions that are used. Therefore, the main objective of this dissertation is the development of a methodology to measure the microalgal kinetics that overcomes these drawbacks. This methodology involves the combined measurement of respirometric and titrimetric data. Such titrimetric and respirometric measurements were previously used for assessing the kinetics of activated sludge (Gernaey et al., 2001).

The generated respirometric and titrimetric data was then used to set-up, calibrate and validate a mathematical model describing microalgal kinetics. The model implementation was based on already existing activated sludge models (ASM) (Henze et al., 2000). This makes future combination of the microalgal model and other waste water treatment models straightforward. Once a simple model based on a single factor limitation was successfully implemented it was extended with different environmental conditions limitations. This allowed for model based optimization of microalgal systems for nutrient removal and nutrient recovery in wastewater treatment.

It should also be emphasized that in order to get a feeling for the microalgal kinetics, it was chosen to perform the kinetic experiments under autotrophic conditions in the presence of light. To this end, batch experiments with defined cultures of microalgae species were pursued. In view of (full scale) installations for wastewater treatment other aspects should be considered. Such as for example the different processes that occur during the night cycle. Also wastewater streams can contain loads of organic dissolved matter that can be assimilated by for example
bacteria or microalgae under heterotrophic and mixotrophic conditions. This was however not in the scope of this research but are very interesting aspects to assess in future research.

1.3 Outline

Next to the introduction, objectives and outline (Chapter 1), this dissertation consists of four major parts, namely literature review, simulation methods, a part including all performed research and a general conclusion and future perspective part. A brief description of the several chapters is given below.

Chapter 2 is a literature review in which several aspects are treated. At first the different systems for wastewater treatment in which microalgal biomass is implemented are given. Next, methods used to measure the microalgal kinetics are discussed. This is followed by the different environmental factors influencing the microalgal growth rate and the different mathematical equations used to describe the resulting microalgal kinetics. Finally some examples of models are summarized.

Chapter 3 provides information about the different simulation methods used during the research.

Chapter 4 describes the development of a combined respirometric and titrimetric method to measure the kinetics of microalgal systems for wastewater treatment. Further a simple model based on only inorganic carbon limitation was developed and used.

In Chapter 5 this methodology was further used to assess the effect of different environmental factors on the microalgal growth rate. Experiments with the respirometric and titrimetric technique were performed according to an optimal experimental design scheme. Based on the experimental results additional kinetic equations for inorganic nitrogen and phosphorus in view of further modelling were suggested.

Chapter 6 is the implementation of the additional kinetic equations in an extended model structure. Moreover a parameter identifiability study was performed based on a global
sensitivity analysis. Based on this, the model was calibrated and validated by using the experimental data of Chapter 5.

**Chapter 7** describes a model based comparative study on intracellular nitrate storage in two marine microalgae. For this, additional kinetic equations were developed and experimental data generated by batch wise experiments were used.

**Chapter 8** involves the implementation of the combined respirometric and titrimetric methodology on different microalgal strains that were isolated from a waste stabilization pond in order to assess the behavior of this species when different environmental conditions were imposed. Specifically, the influence of light and temperature was assessed as these factors were not further considered in Chapter 5 and 6.

**Chapter 9** contains the final conclusions of this dissertation combined with opportunities and perspectives for future research.

The relation between the chapters dealing with investigation is schematically presented in Figure 1.1. These chapters involve mathematical simulations, experimental work or a combination of both.
Figure 1.1: Outline of this dissertation.
Chapter 2

Literature review
Chapter 2

2.1 Introduction

Algae are considered as one of the oldest life forms of this planet that can reside in either fresh water, salt water and brackish water environments. The term microalgae refers to all algae that are too small to be seen properly without a microscope and they mainly consist of eukaryotic (microalgae) and prokaryotic (cyanobacteria) microorganisms. The most important common feature between the eukaryotic and prokaryotic microorganisms is that their growth is mainly based on photosynthetic reactions where available light intensity is converted into energy for growth (Barsanti and Gualtieri, 2005). Furthermore nutrients are essential such as an inorganic carbon source, an inorganic nitrogen source (e.g. ammonium or nitrate), inorganic phosphorus source and some trace elements (Juneja et al., 2013).

Next to photosynthesis, respiration and photorespiration are important processes in the microalgal growth that occur simultaneously when light is available. These processes are schematically presented in Figure 2.1 (Kliphuis et al., 2010).

The photosynthesis involves the fixation of the light energy in the chloroplast with the release of oxygen and production of adenosine triphosphate (ATP) and nicotinamide dinucleotide phosphate (NADPH) in order to fix carbon dioxide into glyceraldehyde 3-phosphate (GAP). This can then be converted into biomass building blocks.

Respiration mainly takes place in the mitochondria where NADPH is oxidized to generate extra energy as ATP to support biomass production and maintenance processes. During this process, oxygen is consumed (Graham, 1980).

In case of high extracellular oxygen concentrations or low carbon dioxide concentrations, oxygen is fixated by the oxygenase activity of rubisco with the production of glycolate. This glycolate is converted into GAP so it can be re-used in biosynthesis. This process is called photorespiration and only occurs when the O₂/CO₂ ratio exceeds a certain value (Peltier and Thibault, 1985).
Microalgal growth can occur under different conditions. These can be autotrophic conditions while using light and carbon dioxide, heterotrophic conditions while using organic compounds as energy and carbon source or mixotrophic conditions while using both light and organic substrate as energy sources and CO$_2$ and organic substrate as carbon sources (Mata et al., 2012). This dissertation will only focus on the autotrophic microalgal conditions, which can be further used as solid basis for future research with alternative microalgal growth conditions.

2.2 The use of microalgae for wastewater treatment

2.2.1 Introduction

This section mainly focusses on removal of nutrients in wastewater with microalgal biomass. Many species of microalgae are able to effectively grow in wastewater conditions by their ability to use abundant inorganic phosphorus and nitrogen in wastewater. More specifically,
microalgae have been shown to be very efficient in removing these nutrients from sewage based wastewater either in suspension or in an immobilized form.

Studies reported very high removal (>80%) of ammonium, nitrate and total phosphorus from secondary treated wastewater by various species of Chlorella and Scenedesmus (Pittman et al., 2011). Also a removal efficiency over 90% of total nitrogen and 80% of total phosphorus from primary settled wastewater was reached by a microalgal system containing Chlorella vulgaris (Lau et al., 1995). Agricultural wastewater streams are in general derived from manure and contain higher amounts of nitrogen and phosphorus compared to municipal wastewater. Microalgae have also been used for treatment of such streams and this resulted in efficient removal of these nutrients. Moreover, benthic freshwater algae such as Microspora willeana and Rhizoclonium hierglyphicum that have a higher nutrient uptake rate demonstrated a nutrient removal similar to the removal of nutrients from municipal wastewater (Mulbry et al., 2001).

Although there is significant interest in the use of microalgae for treatment of industrial wastewater, mostly for the removal of specific components such as heavy metal pollutants and organic compounds, some industrial wastewaters have less potential in view of large scale algal biomass cultivation. This is due to the low content of nitrogen and phosphorus and the presence of toxins at high level concentrations (Pittman et al., 2011). Nevertheless some use of microalgae for industrial wastewater treatment has been reported in literature (Pitmann et al., 2011).

2.2.2 Microalgal reactor systems

There are 4 major configuration systems with microalgal biomass or microalgae in combination with bacteria. This includes the open reactor systems, the closed photobioreactor systems, the waste stabilization ponds (WSP) and the immobilized microalgal systems. Open systems are in general simpler to conduct and are cheaper. However, open systems are more sensitive to environmental conditions such as light intensity and temperature compared to the closed systems that allow optimal control with respect to the growth conditions. Therefore the implementation of these microalgal cultivation system is restricted to tropical and subtropical regions of low rainfall and low cloud cover (Cai et al., 2013).
2.2.2.1 Open microalgal cultivation system

One of the advantages of an open system is that it can be implemented on large scale and is rather easy to manage. Moreover it is more durable than large closed photobioreactors (Cai et al., 2013). In general open systems are carried out in natural or artificial lakes or ponds. Open systems are typically developed as shallow raceway ponds or circular ponds with a rotating arm to mix the microalgal biomass. The raceway pond (Figure 2.2) also known as high rate algae pond (HRAP) has a meandering configuration with in general paddle wheels to mix the microalgal biomass. The fresh wastewater is added to the raceway pond in front of the wheels, whilst the microalgal biomass is harvested behind the paddle wheels. Although these open systems are cost effective, they have some disadvantages. Amongst them the fact that in order to obtain high microalgal biomass yield, a large surface area is needed. Ponds areas range from 1 ha to more than 200 ha with an average depth of 20 to 30 cm (Cai et al., 2013). Furthermore, the systems are influenced by water evaporation and rain fall. In addition, due to the fact that these systems can be contaminated by unwanted algal species or algae predators, only few species are resistant enough in open pond systems. Species that are commonly known to be cultivated in large open raceway ponds are *Chlorella spp.*, *Spirulina platensis* and *Spirulina maxima* (Lee, 2001).

![High rate algae pond (Octaform).](image)

Figure 2.2: High rate algae pond (Octaform).
2.2.2.2 Closed photobioreactor for microalgae cultivation

Closed photobioreactors (Figure 2.3) usually have better light penetrating characteristics than open ponds (Andersen, 2005), which make it possible to sustain high biomass and productivity with less retention time than is possible in open ponds. Typical reactor configuration of closed photobioreactor systems are flat plate reactors, tubular photobioreactors and bag systems (Borowitzka, 1999). The flat plate and tubular photobioreactors are designed to allow maximum light availability and an optimal gas exchange. Moreover, the arrangement of the reactor tubes can be changed depending on the orientation of the sun (Cai et al., 2013). However, there are some major drawbacks regarding these systems. They are more complex compared to the open systems and need a higher energetic input and as such higher operating cost. The bag systems use large plastic bags with a diameter of 0.5 m fitted with aeration systems. A major drawback of the bag systems is the inadequate mixing, which can induce system failure (Cai et al., 2013).

![Tubular closed photobioreactor](image)

Figure 2.3: Tubular closed photobioreactor (Chempur Technologies).

2.2.2.3 Immobilized algal systems

Due to the fact that the size of microalgal cells is very small and the cultures are usually quite diluted, harvesting or separating them from the treated wastewater or culture medium is a major drawback for full scale implementation. In general, the harvesting methods include chemical, biological, electrical and mechanical techniques with a high consumption of energy or dosed
chemic als (Cai et al., 2013). One way to overcome this drawback is the immobilization of microalgae, which prevents the microalgae from moving freely within the system. Hoffmann (1998) reported higher removal rates with immobilized systems compared to suspended systems. This could be explained by the fact that no washout of the microalgal biomass occurs. In addition, it is easier to control the microalgal biomass as washout of the cultivated species is avoided or minimized. Furthermore, the effluent is cell-free and can be re-used for other purposes (Hoffmann, 1998). As reported in literature, most research on immobilized microalgal systems is conducted at laboratory scale and entrapment is the most frequently immobilization technique used for these experiments. The cells are confined in a three-dimensional matrix, but can move freely within their compartment. The matrix material is in general a synthetic (polyvinyl, acrylamide) or natural polymer (collagen, cellulose) (Cai et al., 2013).

2.2.2.4 Waste stabilization ponds

Waste stabilization ponds (WSP) are a series of large, shallow basins treating raw wastewater through natural processes involving bacteria and algae. They are used to treat different kinds of wastewater, ranging from industrial wastewater to municipal wastewater. The most important advantage of this type of treatment is the simplicity in construction and operation (Alvarado, 2013).

The use of WSPs is one of the most cost-effective methods for treating domestic and industrial waste water, because sunlight is the only energy requirement for its operation (unless aeration is applied). This in contrast to conventional aerobic wastewater treatment, in which mechanical aeration accounts for approximately 50% of the energy consumption (Tchobanoglous et al., 2003). Moreover, WSPs improve energy efficiency through the use of algae for oxygen production. With this kind of wastewater treatment systems, typically Biological Oxygen Demand (BOD) removal efficiencies up to 80% can be achieved. In addition, treatment through the use of WSPs provides removal of pathogens compared to other treatment systems (Kayombo et al., 2004). Considering total nitrogen, net removal efficiencies as high as 80% have been reported. This removal has been attributed to the assimilation of inorganic nitrogen by microalgal biomass, sedimentation and volatization of ammonia gas from the pond surface (Ferrara and Avci, 1982). For total phosphorus net removal efficiencies of 50% have been
reported, mainly by sedimentation and assimilation by the microalgal biomass residing in the system.

Since WSP technology highly depends on photosynthetic activity, a large surface exposed to solar energy is needed and as such high land area is required. Moreover because biological reactions are influenced by the prevailing temperature, WSP treatment systems depend on the climate (Von Sperling, 2007). All this implies that WSP treatment is more suitable in cases where land is inexpensive, climate is favorable, a low energy cost is wanted and no special training of the operators is desired (Arceivala, 1981). Therefore, WSPs are appropriate for low-income tropical countries. However, there are thousands of WSPs in Europe as well and one third of the treatment plants in the USA are WSPs (Alvarado, 2013).

In Figure 2.4 a schematic configuration of a WSP is illustrated. In general, it consists of a combination of facultative and maturation ponds where aerobic or anaerobic lagoons can be added for pretreatment purposes (Alvarado, 2013).

Facultative ponds are the most common in pond treatment. The bottom layers of such ponds are anaerobic with similar characteristics as anaerobic ponds. The upper layer is oxygenated due to the presence of a high concentration of algae, which produce oxygen through photosynthesis (Von Sperling, 2007).

The photosynthetic activity depends on the availability of light. As such, with increasing depth the oxygen production will decrease due to the lack of light penetration. Furthermore, photosynthesis does not take place during the night and the absence of oxygen can prevail (Von Sperling, 2007).

Maturation ponds usually follow treatment in the facultative pond and serve as a tertiary treatment. Their primary function is to remove pathogens and they can also achieve a significant amount of nutrient removal (Shilton, 2005).
2.3 Methods to measure microalgal kinetics

2.3.1 Introduction

In view of microalgal system optimization it is essential to have insight in the kinetics related to the microalgal growth. The latter can be measured as an increase of biomass in the algal culture or it can also be measured with a surrogate parameter which is proportional to cell amount (Andersen, 2005). Measuring an increase in biomass or a related surrogate parameter in general gives insight in the microalgal growth rate. However, it is not evident to determine other biokinetic parameters such as for example half saturation coefficients for nutrients.

With respect to the experimental set-up used, in general two methods can be distinguished, namely by means of continuous cultures or batch cultures.
2.3.2 Continuous cultures

In continuous cultures, a fresh supply of wastewater is added to the culture at the same rate at which it is withdrawn. This allows the culture to remain in the exponential growth phase. Here the steady state concentration of the algae is determined by either a limiting nutrient or by a certain dilution rate that is implemented to maintain the cell concentration constant in the culture. In this case the specific growth rate can be calculated as:

\[ \mu = \frac{F}{V} = D \]  

(2.1)

With \( F \) (m\(^3\) d\(^{-1}\)) the flowrate of the medium and \( V \) (m\(^3\)) the reactor volume. \( D \) (d\(^{-1}\)) represents the dilution rate. In this equation no microalgal decay is included since the assumption is made that the microalgal growth rate is much higher than the decay rate. However this assumption only stands if the microalgae did not suffer physiological stress from the environment during the experiment (Andersen, 2005). Furthermore, when using continuous cultures to determine the microalgal growth rate, a uniform mixing in the reactor is assumed. However this is an assumption that is difficult to maintain (in larger reactors).

2.3.3 Batch cultures

Compared to continuous cultures where the specific growth rate is determined by the dilution rate, in case of batch cultures a time series of measurements is needed to assess the rate of change in biomass (amount of cells). The specific growth rate can be calculated by quantifying the increase in number of cells within a certain time interval. The latter time interval is defined by the beginning and end of the logarithmic growth rate during a batch experiment (Binaghi et al., 2003). The growth rate can then be calculated as:

\[ \mu = \frac{\ln N_f - \ln N_0}{\Delta t} \]  

(2.2)

With \( N_0 \) and \( N_f \) the cell number at the start and in the end of the logarithmic phase of the growth experiment. \( \Delta t \) is the time interval of the experiment.
Instead of assessing the change in cell number also other parameters can be measured that are related to the biomass concentration. Typical proxy measurements are organic particulate matter, by e.g. in vivo fluorescence, optical density or volatile suspended solids. Also the amount of chlorophyll, carotenoids, proteins, lipids or carbohydrates are used as proxy measurements, however only if these methods are linearly correlated to cell number or biomass. The latter is a major drawback of these proxy measurements. Hence for many parameters it is essential to know under which growth conditions these parameters are linearly correlated to cell number or biomass and what are the detection ranges. As such prior experiments need to be conducted to verify if there is an existing linear relationship. Wingard et al. (2002) for example demonstrated the non-linearity between in vivo fluorescence and cell number at high cell densities. This was probably due to changes in fluorescence yield by microalgal self-shading. Furthermore, under each growth condition the relation between parameters with respect to cellular content and cell number or biomass is variable during a certain time. This acclimation time can last for 20 or more generations (Andersen, 2005). Further, due to the photosynthetic activity of the microalgae it is necessary to devise a sampling strategy that takes into account the difference between light period and dark period to minimize the scatter in the time series measurements (Andersen, 2005).

2.3.4 Respirometry and titrimetry

2.3.4.1 Respirometry

Respirometry is a well-known technique to measure the kinetics of activated sludge and composition of wastewater. It involves the measurement and interpretation of the respiration rate of activated sludge when specific experimental conditions are implemented. It is expressed as the amount of oxygen that is consumed by activated sludge per unit of volume and per unit of time. The obtained respirometric data is directly related to the growth rate of the microorganisms residing in activated sludge and the corresponding substrate consumption. For this it is generally known as a very accurate method to measure the kinetics of activated sludge (Carvalho et al., 2001).
Chapter 2

The basic measurement principles for respirometry depend on two major criteria, namely in which phase the oxygen concentration is measured (gas phase or liquid phase) and whether a static or flowing regime for the gas phase or liquid phase is used (Gernaey et al., 2001).

A respirometric set-up that is often used, is a batch wise reactor with constant volume and continuous aeration, also known as a flowing gas-static liquid respirometer (Gernaey et al., 2001). The respiration rate of the activated sludge is calculated by making a mass balance of oxygen in the liquid phase. In case of a flowing gas-static liquid respirometer this mass balance (Equation (2.3)) consists of two terms, namely an oxygen transfer rate (OTR) due to aeration and an oxygen uptake rate (OUR) due to respiration of activated sludge (Gernaey et al., 2001).

\[
\frac{dO_2}{dt} = OTR - OUR
\]  

(2.3)

The OTR (g O$_2$ m$^{-3}$ d$^{-1}$) is defined by the oxygen mass transfer between the liquid and gas phase ($K_La$) (d$^{-1}$) and the difference between the dissolved oxygen concentration at saturation $O_2^{sat}$ (g O$_2$ m$^{-3}$) and the prevailing dissolved oxygen concentration in the liquid phase $O_2$ (g O$_2$ m$^{-3}$) and can be denoted as:

\[
OTR = K_La (O_2^{sat} - O_2)
\]  

(2.4)

Moreover the value of the oxygen mass transfer coefficient depends on several factors such as for example temperature, operational conditions and geometry of the reactor (Garcia-Ochoa and Gomez, 2009). Even the biomass concentration and the medium composition present in the reactor influence these parameters. This was also observed when performing the experimental runs.

Considering the biological processes that influence the dissolved oxygen concentration in the liquid phase, it should be noted that the metabolism of microalgal biomass is different compared to the metabolism of bacteria in activated sludge. Microalgae produce oxygen through photosynthetic activity by using an inorganic carbon source and nutrients with abundant light intensity. As such, the OUR term changes sign and becomes an oxygen production rate (OPR) term. Given the similarities, the determination of microalgae kinetics from OPR curves, in analogy with bacterial respirometry experiments, is an elegant method to measure microalgae kinetics. Since the features of the dissolved oxygen are related to the gross microalgae oxygen.
production, the oxygen respiration to sustain the population is included. This is however a small percentage compared to the oxygen production due to photosynthetic activity (Kliphuis, 2010).

The use of respirometry to assess the effect of certain environmental factors is already reported in literature. For example Hancke et al. (2008b) compared the use of oxygen measurements with pulse-amplitude-modulated fluorescence (PAM) and $^{14}$C assimilation measurements to determine the effect of temperature on the photosynthetic activity in different monocultures of marine phytoplankton. These three methods were compared because they measure the photosynthetic pathway differently and as such generate different responses on environmental variables. The oxygen level was monitored with a micro-electrode. Further Li et al. (2003) used online dissolved oxygen measurements for online state estimation of *Duniella salina* cultures grown in a stirred tank photobioreactor. With this they successfully implemented a method to improve the operational process control in the photobioreactor.

### 2.3.1.2 Titrimetry

Next to respirometry, measurements of titrimetry are used to obtain information about the biological processes in activated sludge. More specific the pH value of a biological system is influenced by the biological reactions which take place. In case of wastewater treatment systems with activated sludge several biological reactions such as nitrification, denitrification and the degradation of organic carbon source influence the pH (Gernaey, 2001). Furthermore, the pH is influenced by the stripping of for example carbon dioxide. However changes in pH in the liquid phase by biological reactions are difficult to observe due to the presence of several acid-base buffer systems with pH depending buffer capacity (Stumm and Morgan, 1996). This makes accurate calculation of the consumed or released protons difficult. Thus by controlling the pH at a certain level through acid and base addition, the rate of proton consumption or production due to biological reactions can be provided (Gernaey et al., 2001).

With respect to microalgal growth also changes in the value of pH by biological reactions are induced. Indeed, according to Stumm and Morgan (1996) the photosynthetic reactions can be denoted as:

$$106CO_2 + 122H_2O + 16NO_3^- + 18H^+ + HPO_4^{2-} \leftrightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2$$ (2.5)
Chapter 2

\[106HCO_3^- + 16H_2O + 16NO_3^- + 124H^+ + HPO_4^{2-} \leftrightarrow C_{106}H_{263}O_{110}N_{16}P + 138O_2\] (2.6)

As can be deducted, the photosynthetic activity leads to an increase of pH in the liquid phase. Further it should be noted that when bicarbonate is used as inorganic carbon source, more protons are consumed compared to when carbon dioxide is used.

Another aspect that influences the pH of the liquid phase during microalgal monitoring is the chemical equilibrium of inorganic carbon. This can simplified be denoted as:

\[CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+\] (2.7)

with \(K_{a1} = [HCO_3^-][H^+] / [CO_2] = 10^{-pK_{a1}}\)

\[HCO_3^- \leftrightarrow CO_3^{2-} + H^+\] (2.8)

With \(K_{a2} = [CO_3^{2-}][H^+][HCO_3^-] = 10^{-pK_{a2}}\)

When inorganic carbon is used for microalgal growth, this chemical equilibrium will be disturbed resulting in proton production or proton consumption.

The rate at which CO\(_2\) is transferred between the liquid phase and gas phase depends on the saturated CO\(_2\) concentration and the mass transfer coefficient for CO\(_2\), which can be calculated from the mass transfer coefficient for O\(_2\) multiplied with a reduction factor based on the diffusivity (Alex et al., 2010; Sin, 2004). The CO\(_2\) transfer rate (\(\rho_{CO_2,trans}\) (g m\(^{-3}\) d\(^{-1}\))) can be calculated as:

\[\rho_{CO_2,trans} = K_L a red_{CO_2} (S_{CO_2}^{sat} - S_{CO_2})\] (2.9)

Where \(red_{CO_2} = \frac{D_{CO_2}}{D_{O_2}}\) (2.10)

With \(D_{O_2}\) the diffusion coefficient of oxygen in water and \(D_{CO_2}\) the diffusion coefficient of carbon dioxide in water, respectively 1.65 \(10^{-4}\) m\(^3\) d\(^{-1}\) and 1.73 \(10^4\) m\(^3\) d\(^{-1}\) (Sin, 2004). \(S_{CO_2}^{sat}\) is the saturation concentration (g m\(^{-3}\)) and \(S_{CO_2}\) the concentration of carbon dioxide in the solution (g m\(^{-3}\)). Moreover the saturation concentration is governed by Henry’s Law:

\[S_{CO_2}^{sat} = p_{CO_2} K_H\] (2.11)
In this equation $K_H \, (g \, m^{-3} \, atm^{-1})$ represents the Henry coefficient for carbon dioxide and $p_{CO_2} \, (atm)$ the partial pressure of carbon dioxide in the gas phase. The saturation concentration of carbon dioxide in air at a temperature of 298 K and atmospheric pressure is 0.32 g m$^{-3}$. When carbon dioxide gas is used as sparging gas with 2 % volumetric carbon dioxide concentration, the saturation concentration becomes 32 g m$^{-3}$.

The above mentioned effects result in a titrimetric background signal, i.e. the background signal addition rate (BSAR) (Sin et al., 2006). The amount of protons consumed by this BSAR needs to be corrected for when calculating the net proton addition rate due to carbon dioxide consumption by microalgae (i.e., proton addition rate or HAR). This can be clearly observed in Figure 2.5. The first part of the curve (before the “knee”) corresponds to the period where carbon dioxide is consumed by microalgae, whereas the second part is only due to the BSAR. The slope obtained from the first part of the curve represents the total rate of proton addition (TPAR), including the BSAR. The latter can be determined from the second part of the curve. Subtracting this from the TPAR yields the HAR.

![Figure 2.5: Typical titrigram for microalgal growth with indication of TPAR and BSAR.](image)

Combining titrimetric data with respirometric data would allow to understand the biological processes that take place more accurately. Certain processes that cannot be observed by one specific data set could be explained by the other one. In Table 2.1 different processes in case of microalgal growth and which datatype they will affect are summarized.
Chapter 2

Table 2.1: Influence of different processes on specific datatype

<table>
<thead>
<tr>
<th>Data</th>
<th>Respirometric</th>
<th>Titrimetric</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ production</td>
<td>Proton addition by photosynthetic activity</td>
<td>Stripping CO₂</td>
</tr>
<tr>
<td>PROCES</td>
<td>Respiration</td>
<td>Chemical equilibrium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inorganic carbon</td>
</tr>
</tbody>
</table>

2.3.5 Conclusions

Compared to batch experiments to measure the microalgal kinetics, the major drawback of continuous systems is the fact that the microalgal cultures should be kept in the exponential growth phase. As such it is not possible to determine the kinetics when certain stress conditions (for example light intensity) are implemented. This makes this method not suitable in view of mimicking the natural environment.

Considering the measurements used in batch experiments, in general proxy measurements are used. However the features of this proxy measurements are not always correlated to the microalgal kinetics. When different conditions are imposed, an adaption period is needed before the features can be directly related to the kinetics.

Therefore, it was chosen to use the combined respirometric and titrimetric methodology in the dissertation to determine the microalgal kinetics. This is a known methodology proven to be easy and accurate to measure the kinetics of activated sludge and can now be transferred to measure the kinetics of microalgae.
2.4 Factors influencing the microalgal growth rate

2.4.1. Introduction

For successful treatment of wastewater with microalgal biomass, a thorough knowledge of the various parameters that effect the microalgal growth and as such the system efficiency is a prerequisite.

These various parameters can be respectively physical, chemical and biological factors. Abiotic factors such as light intensity and temperature are the most important parameters affecting the microalgal growth. Examples of chemical factors are the availability of nutrients (nitrogen and phosphorous) and inorganic carbon source. Biological factors can be the competition between microalgal species residing in the system. In addition operational factors such as mixing, reactor configuration, the rate of dilution and harvesting frequency can affect the microalgal growth rate.

2.4.2 Light intensity

The availability of light is essential for microalgal growth. Figure 2.6 illustrates the effect of light intensity on the photosynthetic activity of algae.

With light intensities lower than the light compensation point ($I_c$) respiration occurs and there is no gross oxygen production. Once this point is passed, the oxygen production is higher than the respiration. The initial slope of the curve represents the maximal efficiency of growth in response to light. A maximal growth rate is achieved by a certain light intensity ($I_s$). As such, the light intensity is no longer limiting the overall photosynthesis. Above the light saturation point, the light-dependent reactions are producing more ATP (adenosine-5’-triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate) than can be used by the light independent reactions for CO₂ fixation and the availability of CO₂ becomes the limiting factor. A further increase in light intensity will not result in a further increase in growth rate, but may even cause damage to the photosynthetic complex, which results in photoinhibition. Photoinhibition mainly occurs in the electron transfer chain located at photosystem II. Its
mechanism is directly related to protein damage that is responsible for the electron transfer at the photosystem II. As such the production of ATP is interrupted. This causes a decrease in growth rate and can even lead to cell death (Richmond, 2004).

The light energy is converted into chemical energy by photosynthetic activity, however large parts are lost as heat. It has been reported for outdoor microalgal ponds that more than 90 % of the total incident solar energy is converted into heat and only less than 10 % is converted in chemical energy.

![Figure 2.6: Light response curve (Richmond, 2004).](image)

### 2.4.3 Temperature

Next to light, temperature is the most important factor influencing microalgal growth in non-nutrient limiting conditions. Muñoz et al. (2004) and Bordel et al. (2009) reported that higher growth rates with increasing temperatures could be observed. This could be explained by the fact that augmentation of temperature shifts the light saturation point to higher light intensities and as such also the intensity at which photo-inhibition occurs. This was observed by Sorokin and Krauss (1962) for *Chlorella pyrenoidosa*. Each temperature seemed to have a specific light intensity at which maximum growth rate was reached. For example, at a temperature of 15 °C light intensity at which photo-inhibition occurred, equaled 242 µE m⁻² s⁻¹, while at 20 °C photo-inhibition only occurred at 484 µE m⁻² s⁻¹ (Sorokin and Krauss, 1962).
Furthermore, the microalgal growth as function of temperature is mainly based on the Van ’t Hoff rule that stipulates that biological reaction rates double for each temperature increase by 10 °C. This is due to the fact that the temperature influences the activation energy needed for biological reactions. However, this rule can only be validated in a narrow temperature range (Goldman, 1974; Henze et al., 2000). Once a certain temperature level has been exceeded, essential proteins are damaged and the growth rate decreases. In Figure 2.7 the growth rate of four different microalgal species is illustrated. As can be seen, the optimal temperature for growth depends on species. This can be explained by difference in cell size and in the difference in photosynthetic pigments concentration within the cells (Eppley and Sloan, 1966).

![Figure 2.7: Microalgal growth rate as function of temperature for four different microalgal species (Ras et al., 2013).](image)

### 2.4.4 Inorganic carbon

Inorganic carbon, more specifically carbon dioxide and bicarbonate are the most important nutrients for microalgal growth. Microalgae biomass contains approximately 50 % of carbon on a dry weight basis. Some microalgal species are only able to assimilate either one of the mentioned inorganic carbon sources (Moss, 1973). Other species can use both inorganic carbon sources, however with a preferential uptake of carbon dioxide compared to bicarbonate. Moroney and Somanchi (1999) explained that this preferential uptake is due to the fact that the
carbon dioxide molecule is smaller and as such the diffusion into the microalgal cell occurs faster. According to Van den Hende et al. (2012) the main reason for this preferential uptake of carbon dioxide is that carbon dioxide is zero valent allowing an uptake by the cell without the need of active transporters.

Furthermore the pH of the aquatic environment determines the concentration of different inorganic carbon species present in the water. At values of pH < 6.36 \((pK_{a1} = 6.36)\) the most dominant inorganic carbon source is carbon dioxide, while at values of pH higher than pH = 10.33 \((pK_{a2} = 10.33)\) almost all inorganic carbon prevails as carbonate (Reichert et al., 2001).

In Figure 2.8 the relative amount of the different inorganic species as function of the pH and certain temperature of the aqueous phase is illustrated.

Figure 2.8: Relative amount of inorganic carbon species as function of the pH of the aqueous phase (LAWR, 2013).
2.4.5 Inorganic nitrogen and inorganic phosphorus

Nitrogen and phosphorus are the most abundant nutrients in environmental water systems. These two nutrients play a major role in the cell metabolism since they are a part of several biochemical processes.

2.4.5.1 Inorganic nitrogen

Ammonium and nitrate are the most important sources of inorganic nitrogen for microalgal growth. These molecules are synthesized into glutamine which is needed for the production of more complex molecules as mentioned before. Further, Schuler et al. (1952) stated that ammonium is preferred to nitrate by green microalgae. Furthermore, cyanobacteria and diatoms are not able to assimilate ammonium. The uptake of nitrate is inhibited when both nitrogen species are present in the environment (Broekhuizen et al., 2012) and the ammonium concentration is at high level. This can be explained by the different way of assimilation of both inorganic nitrogen sources. Ammonium is intracellularly synthesized into glutamine, while in case of nitrate, a prior reduction by respectively nitrate reductase and nitrite reductase is needed where the nitrate is converted into ammonium before assimilation (Flynn et al., 1997). This extra reduction requires more energy, respectively 385 kJ mol\(^{-1}\) and as such ammonium is preferred compared to nitrate for microalgal growth (Bienfang, 1975). Therefore wastewater streams with high ammonium concentrations can be effectively used to rapidly grow microalgae. In contrast excess of ammonium can have a growth inhibiting effect. The ammonium tolerance of different algae species varies from 0.22 g N m\(^{-3}\) to 14 g m\(^{-3}\) (Collos et al., 2004).

Next to inorganic nitrogen assimilation, certain microalgal species have the capacity of intracellular nitrate storage. In marine ecosystems specific microalgae occur that have the capacity to store nitrate intracellularly in transitory cytoplasmic pools in concentrations up to several grams per liter of nitrogen (Bode et al., 1997; Dortch et al., 1984; Kamp et al., 2011; Lomas and Glibert, 2000; Needoba and Harrison, 2004). With nitrogen limited conditions, the intracellular nitrate is reduced and used as nitrogen source for growth.
Chapter 2

2.4.5.2 Inorganic phosphorus

Concerning the assimilation of phosphorus only the uptake of inorganic phosphorus will be considered in this dissertation. Inorganic phosphorus has a significant role in microalgal cell growth and metabolism. It is preferably taken up in the form of \( \text{H}_2\text{PO}_4^- \) or \( \text{HPO}_4^{2-} \) and is converted into organic compounds by phosphorylation. Then these organic compounds are involved in the production of ATP from adenosine di phosphate (ADP) accompanied by a form of energy input, such as light, by oxidation of respiratory substrates or by the electron transport in mitochondria.

The growth rate of algae on phosphorus is more dependent on the internal cellular concentrations than on the external quantities (Richmond, 2004). However, this was not considered in this dissertation because of the used experimental features. In literature, experimental results have proved that P-starved cells could attain much higher nutrient uptake rates than saturated cells and may uptake phosphate by 8–16 times the minimum cell-quota in phosphate repletion medium, which were stored as polyphosphate bodies (internal P pool) and could sustain 3-4 generations of growth in phosphate-depleted conditions theoretically (Yao et al., 2010).

Furthermore phosphorus uptake may be affected by other phosphorus pools on microalgal cells caused by phosphorus adsorption. According to Yao et al. (2010), 60-70% of the total phosphorus content in different microalgal species is made up by cell surface adsorption. This indicates that the kinetics of phosphorus involves a two stage kinetic process.

2.4.5.3 Microalgal species dependent nutrient removal

Next to influencing the microalgal growth kinetics, nitrogen and phosphorus are also removed from the liquid phase.

The nutrient removal efficiency of microalgal systems can depend on the microalgal species used. In Table 2.2 the removal efficiencies for nitrogen and phosphorus with different
microalgal species (chlorophytes, cyanobacteria and diatoms) in case of different wastewater streams are summarized. It should also be stressed that in case of simultaneous uptake of nitrogen and phosphorus, the optimal N/P ratio varies among cultures due to different metabolic pathways within species. Podola et al. (2007) reported an optimal N/P ratio of 7/1 (w/w) for *Chlorella vulgaris* which is similar to the molar N/P ratio of 16/1 as described by Stumm and Morgan (1996). Considering the chlorophyte *Scenedesmus sp* Rhee (1978) reported a N/P ratio of 30/1 (w/w) is needed to grow without nutrient limitation. When this microalgal species was cultivated in an environment with N/P ratios between 12 to 18 (w/w) it was nitrogen limited, which caused an increased use of the internal phosphate pool. Thus the dissolved nitrogen removal was always higher than the dissolved phosphorus removal.

**Table 2.2: Nutrient removal in case of different microalgal species and different wastewater streams (Cai et al., 2013)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Specie</th>
<th>Wastewater</th>
<th>Total N Initial g m⁻³</th>
<th>Total N Removal %</th>
<th>Total P Initial gm⁻³</th>
<th>Total P Removal %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyte</td>
<td><em>C. pyropecta</em></td>
<td>Industrial</td>
<td>267</td>
<td>87-89</td>
<td>56</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td><em>C. vulgaris</em></td>
<td>Industrial</td>
<td>13-410</td>
<td>23-100</td>
<td>23-100</td>
<td>46-94</td>
</tr>
<tr>
<td></td>
<td><em>C. vulgaris</em></td>
<td>Industrial</td>
<td>20</td>
<td>30-95</td>
<td>112</td>
<td>20-55</td>
</tr>
<tr>
<td></td>
<td><em>C. vulgaris</em></td>
<td>Municipal</td>
<td>48-1150</td>
<td>55-88</td>
<td>25</td>
<td>12-100</td>
</tr>
<tr>
<td></td>
<td><em>C. reinhardtii</em></td>
<td>Artificial</td>
<td>129</td>
<td>42-83</td>
<td>120</td>
<td>13-14</td>
</tr>
<tr>
<td></td>
<td><em>S. obliquus</em></td>
<td>Municipal</td>
<td>27</td>
<td>79-100</td>
<td>12</td>
<td>47-98</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td><em>A. platensis</em></td>
<td>Industrial</td>
<td>3</td>
<td>96-100</td>
<td>18-21</td>
<td>87-99</td>
</tr>
<tr>
<td></td>
<td><em>Oscillatoria sp.</em></td>
<td>Municipal</td>
<td>498</td>
<td>100</td>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>Diatoms</td>
<td><em>P. tricornutum</em></td>
<td>Municipal</td>
<td>498-835</td>
<td>80-100</td>
<td>76-116</td>
<td>50-100</td>
</tr>
</tbody>
</table>

**2.4.6 Acidity of the environment**

The pH of the medium in which microalgae are cultured is very important because it affects the solubility and availability of carbon dioxide and other essential nutrients. Moreover the pH has a significant impact on the microalgal metabolism. Most microalgal species grow maximally around neutral pH values (7-7.6) (Juneja et al., 2013).
At higher pH, the inorganic carbon is available in form of carbonates and as such suppresses the microalgal growth. Also, alkaline pH, where the external pH is higher than the internal pH, increases the flexibility of the cell wall of mother cells. This means that rupture of the cell wall will be prevented and the release of autospores will be inhibited. Thus the time for cell cycle completion will be increased (Juneja et al., 2013). Akin to alkaline pH, low pH conditions can affect the nutrient uptake or induce metal toxicity which will affect the microalgal growth (Juneja et al., 2013).

However some microalgal species have tolerance to high or low pH levels. For example *Spirulina platensis* has tolerance for high pH values (pH = 9), whilst *Chlorococcum littorale* is an example of microalgal species that has tolerance for low pH values (pH = 4) (Alsyah, 2012).

### 2.4.7 Salinity of the environment

High salinity levels have a negative effect on several stages of the biochemical pathway for photosynthetic activity (Satoh et al., 1983). Therefore the salinity of the reactor in which cultivation is conducted should be adapted to the level of salinity of the natural environment in which the microalgae are residing (Kaplan et al., 1986). Although microalgae have developed the possibility to adapt to a wide range of salinity levels, their growth is inhibited when the salinity exceeds the concentration of 200 mM (Satoh et al., 1983). However this growth inhibition depends on microalgal species. This is illustrated in Fig 2.9 where an augmentation of salinity from 100 – 200 µM caused a decrease of 50 % of growth in case of *Chlorella vulgaris* compared to 40 % in case of *Chlorococcum humicotta* (Abdel-Rahman et al., 2005).

![Figure 2.9: Influence of salinity, expressed as NaCl on the growth in case of Chlorococcum humicotta and Chlorella vulgaris (Abdel-Rahman et al., 2005).](image)
2.5 Kinetic modelling of the microalgal growth rate

Several models for algal growth modelling have been described in literature. Some of the models take into account only limitation of one factor, for example light intensity (Cornet et al., 1995; Molina Grima et al., 1999; Martinez et al., 1997; Ogbanna et al., 1995; Yeh et al., 2010) and inorganic carbon (Hsueh et al., 2009; Goldman et al., 1974; Tang et al., 2011; Nouals, 2000). A basic assumption governing the use of these kinetic models related to a single factor is that microalgal growth rate solely depends on this factor. Their applicability is thereby restricted to describe the response of growth to a specific range of environmental conditions such as natural waters. Furthermore a simplified model based on one single factor permits no consideration of possible interdependency between different factors.

Some models are based on co-limitation. For example co-limitation by light intensity and inorganic carbon (Filali et al., 2011), co-limitation by nitrogen and phosphorus (Bougaran et al., 2010) and co-limitation of light and temperature (Bernard and Rémond, 2012).

Next to describing the microalgal growth based on one or multiple factors, two major approaches can be distinguished. One assumption with Monod kinetics is that the microalgal growth is not limited by high concentrations of nutrients, high temperature or high light intensities (Monod, 1940). This assumption seems however not trustworthy. For example at certain temperature, denaturation of proteins can occur causing microalgal decay. This compared to the assumption that microalgal growth is inhibited by for example high nutrient concentration, certain temperature or level of light intensity at which photoinhibition occurs. Such models seem to be able to describe more accurately natural systems. In addition also models are developed to describe the microalgal growth taking into account interactions between different factors, for example light intensity and temperature (Carvalho and Malcata, 2003).

Other authors developed detailed metabolic models by accounting for all available, yet still partial, knowledge about the metabolic pathways of specific microalgal species (Cogne et al., 2011).

Furthermore, biokinetic equations describing the microalgal growth have also been combined with hydrodynamic models in view of modelling full scale installations for wastewater
treatment systems. Alvarado (2013) combined a hydrodynamic model based upon the compartmental model approach with two different complete biokinetic models (Alex et al., 2010; Sah et al., 2011) to describe the system performance of a maturation pond. The results of that research indicated good similarities between predicted and experimental values with respect to chemical oxygen demand removal. However, the biomass concentration was predicted dissimilar by both biokinetic models, suggesting that default parameter values or processes needed to be reconsidered.

Beran and Kargi (2004) also used the combination of a biokinetic model with a two dimensional hydrodynamic model for predicting the effluent quality of a facultative pond in a WSP in terms of microalgal and bacterial biomass, nutrient concentrations and chemical oxygen demand. Different experimental results taken at different locations in the pond were used for model calibration. The results of that research confirmed the need of introducing the two dimensional hydrodynamic model to obtain good similarities between model predictions and experimental values.

The different modelling approaches mentioned implemented on the factors influencing the microalgal growth rate will be discussed below.

### 2.5.1 Maximum specific growth rate

The knowledge of microalgal growth rate is essential to control the efficiency of the wastewater treatment and removal of nutrients. Furthermore it is interesting to make a selection of the microalgae with highest growth rates for the valorization of biomass and/or nutrient recuperation. It should be noted that, the growth rate depends on the metabolism and availability of nutrients, on the operating conditions provided by the system under operation, and on the produced biomass for the effective nutrient removal (Mata et al., 2012). In Table 2.3 maximum specific growth rates of different microalgal species are summarized with their residing environment and prevailing temperature. Most of the data obtained are in the temperature range between 15 – 30 °C for both marine microalgae and freshwater microalgae. Minor differences between the $\mu_{\text{max}}$ for freshwater and marine algae, respectively 1.55 ± 0.82 d$^{-1}$ and 1.19 ± 0.46 d$^{-1}$ in this temperature range could be observed.
Table 2.3: Maximum specific growth rates of different microalgal (marine and fresh water) species

<table>
<thead>
<tr>
<th>Value (d$^{-1}$)</th>
<th>°C</th>
<th>Algae</th>
<th>fresh/marine</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>20</td>
<td><em>Chlorella vulgaris</em></td>
<td>F</td>
<td>Gutzeit, 2006</td>
</tr>
<tr>
<td>2.5</td>
<td>20</td>
<td>algal ponds</td>
<td>x</td>
<td>Alex et al., 2010</td>
</tr>
<tr>
<td>3.26</td>
<td>20</td>
<td><em>Pseudochlorococcum sp</em></td>
<td>F</td>
<td>Packer, 2011</td>
</tr>
<tr>
<td>0.6</td>
<td>23</td>
<td><em>Nannochloropsis oculata</em></td>
<td>M</td>
<td>Quinn 2011</td>
</tr>
<tr>
<td>0.9</td>
<td>20</td>
<td>Algal ponds</td>
<td>x</td>
<td>Broekhuizen et al., 2012</td>
</tr>
<tr>
<td>1.92</td>
<td>25</td>
<td><em>Chlorella vulgaris</em></td>
<td>F</td>
<td>Filali, 2011</td>
</tr>
<tr>
<td>1.3</td>
<td>30</td>
<td><em>Chlorella vulgaris</em></td>
<td>F</td>
<td>Dauta et al., 1990</td>
</tr>
<tr>
<td>0.58</td>
<td>25</td>
<td><em>Fragilaria crotonensis</em></td>
<td>F</td>
<td>Dauta et al., 1990</td>
</tr>
<tr>
<td>0.77</td>
<td>27</td>
<td><em>Staurastrum pingue</em></td>
<td>F</td>
<td>Dauta et al., 1990</td>
</tr>
<tr>
<td>1.32</td>
<td>32</td>
<td><em>Synechocystis minima</em></td>
<td>F</td>
<td>Dauta et al., 1990</td>
</tr>
<tr>
<td>0.4</td>
<td>1.8</td>
<td><em>Asterionella formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
</tbody>
</table>
Table 2.3: Maximum specific growth rates of different microalgal (marine and fresh water) species (continued)

<table>
<thead>
<tr>
<th>Value (d⁻¹)</th>
<th>°C</th>
<th>Algae</th>
<th>fresh/marine</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.65</td>
<td>20</td>
<td><em>Asterionella formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
<tr>
<td>1.34</td>
<td>25</td>
<td><em>Asterionella formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td><em>Asterionella formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
<tr>
<td>1.68</td>
<td>37.7</td>
<td><em>Chlorella pyrenoidosa</em></td>
<td>F</td>
<td>Sorokin and Krauss, 1962</td>
</tr>
<tr>
<td>2</td>
<td>38.7</td>
<td><em>Chlorella pyrenoidosa</em></td>
<td>F</td>
<td>Sorokin and Krauss, 1962</td>
</tr>
<tr>
<td>2.15</td>
<td>39.6</td>
<td><em>Chlorella pyrenoidosa</em></td>
<td>F</td>
<td>Sorokin and Krauss, 1962</td>
</tr>
<tr>
<td>1.68</td>
<td>27</td>
<td><em>Selenastrum minutum</em></td>
<td>M</td>
<td>Bourgaran et al., 2010</td>
</tr>
<tr>
<td>1.5</td>
<td>27</td>
<td><em>Isochrysis affinis galbana</em></td>
<td>M</td>
<td>Bourgaran et al., 2010</td>
</tr>
<tr>
<td>1.55</td>
<td>25</td>
<td><em>Chlorella vulgaris</em></td>
<td>F</td>
<td>Concas et al., 2012</td>
</tr>
<tr>
<td>1.36</td>
<td>19</td>
<td><em>Chlorella pyrenoidosa</em></td>
<td>F</td>
<td>Goldman, 1974</td>
</tr>
<tr>
<td>0.65</td>
<td>5</td>
<td><em>Asterionella formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
<tr>
<td>0.8</td>
<td>7.8</td>
<td><em>Asterionella formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
<tr>
<td>1.08</td>
<td>10.7</td>
<td><em>Asterionella formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
<tr>
<td>1.45</td>
<td>13.75</td>
<td><em>Asterionella Formosa</em></td>
<td>F</td>
<td>Bernard and Rémond, 2012</td>
</tr>
</tbody>
</table>
2.5.2. Modelling of microalgal processes

Dochain et al. (2003) modelled the influence of the microalgae present in the system from the following considerations:

Microalgal growth: \( CO_2 + S_1 \rightarrow X_{Alg} + O_2 \)

Microalgal respiration: \( O_2 + X_{Alg} \rightarrow X_{Alg} + CO_2 \)

Microalgal decay: \( X_{Alg} \rightarrow S_2 \) \hspace{1cm} (2.12)

In these reactions the microalgae are represented by \( X_{Alg} \) (g DW m\(^{-3}\)) which use soluble influent substrate (g m\(^{-3}\)) (soluble nitrogen and phosphorus) and CO\(_2\) (g m\(^{-3}\)) for growth. Their decay leads to soluble substrate \( S_2 \) (g m\(^{-3}\)).

In general the specific microalgal growth rate \( \rho_{Alg} \) (g DW m\(^{-3}\)d\(^{-1}\)) can be denoted as:

\[ \rho_{Alg} = \mu_{max} X_{Alg} \] \hspace{1cm} (2.13)

With \( \mu_{max} \) the maximum specific growth rate (d\(^{-1}\))

From these considerations the dynamical mass balance equations of the algae-based processes can be deduced:

\[ \frac{dX_{Alg}}{dt} = \mu_{max} X_{Alg} - b_{Alg} X_{Alg} \] \hspace{1cm} (2.14)

\[ \frac{dCO_2}{dt} = -y_1 \mu_{Alg} X_{Alg} + K_{L1}a_1 (S_{CO_2}^{sat} - S_{CO_2}) + y_2 f_{resp} X_{Alg} \] \hspace{1cm} (2.15)

\[ \frac{dO_2}{dt} = y_3 \mu_{max} X_{Alg} - K_{L2}a_2 (S_{O_2}^{sat} - S_{O_2}) - y_4 f_{resp} X_{Alg} \] \hspace{1cm} (2.16)

\[ \frac{dS_1}{dt} = -y_5 \mu_{Alg} X_{Alg} \] \hspace{1cm} (2.17)

\[ \frac{dS_2}{dt} = y_6 b_{ALG} X_{Alg} \] \hspace{1cm} (2.18)

In these equations the microalgal decay rate is represented by \( b_{Alg} \) (d\(^{-1}\)). \( K_{L1} \) (d\(^{-1}\)) and \( K_{L2} \) (d\(^{-1}\)) are the mass transfer coefficients between aqueous phase and gas phase for carbon dioxide and oxygen respectively. A microalgal respiration function is denoted by \( f_{resp} \). \( S_{CO_2}^{sat} \) (g CO\(_2\) m\(^{-3}\)) and \( S_{O_2}^{sat} \) (g O\(_2\) m\(^{-3}\)) are the concentrations of carbon dioxide and oxygen at
saturated, whilst \( S_{CO_2} \) (g CO\(_2\) m\(^{-3}\)) and \( S_{O_2} \) (g m\(^{-3}\)) represent the dissolved concentration of carbon dioxide and oxygen. The yield coefficients are denoted as \( y_i \) with \( i = 1 \) to 6.

A classical way to describe the growth kinetics is the Monod model (Monod, 1949). This approach assumes a constant yield for nutrients where substrate utilization rate changes proportionally with the organisms growth rate. While a constant yield may be assumed for carbon, it may not be valid for nutrients such as nitrogen and phosphorus. As such, using this model could prove inadequate to explain the microalgal growth kinetics (Palabhanvi et al., 2014). This drawback could be overcome by segregating the overall yield coefficient for nitrogen and phosphorus in a variable fraction and a non-variable fraction. The non-variable fraction corresponds to the minimum yield coefficient which is achieved when extracellular nutrient concentration tends to zero, whereas the variable yield coefficient depends on the extracellular nutrient concentration that changes in time (Palabhanvi et al., 2014).

Since the microalgal growth is often limited by different factors such as light intensity, availability of nutrients and temperature this should be taken into account in the model. For this the specific growth rate \( \rho_{Alg} \) of organisms is generally modelled by multiplying the maximum growth rate \( \mu_{max} \) with some limiting factors \( f_i \) (Kayombo et al., 2000). These limiting functions will be discussed in the following sections. Further taking into account nutrients will have as consequence that dynamic mass balances for these components are additionally needed.

\[
\rho_{Alg} = \mu_{max} \prod_i f_i X_{Alg}
\] (2.19)

### 2.5.3. Kinetic models with one factor

#### 2.5.3.1 Kinetic models related to light intensity

There can be several approaches distinguished to describe the growth rate as function of light or radiation intensity. The most simplistic way to describe the availability of light is a modified Monod relationship (2.20) without taking into account light inhibition (Kayombo et al., 2000; Lee and Shen, 2004; Bordel et al., 2009; Sasi et al.; 2011). In this equation, the prevailing field light intensity is denoted by \( I \) (\( \mu E \) m\(^{-2}\) s\(^{-1}\)), \( K_I \) represents the half saturation coefficient
(µE m\(^{-2}\) s\(^{-1}\)) which corresponds to that light intensity required to reach half of the maximum specific growth rate. In literature often instead of light intensity, irradiation is mentioned expressed as W m\(^{-2}\) or as lux, depending on which type of sensor is used to quantify light.

\[ f(I) = \frac{I}{K_I + I} \]  

(2.20)

In more recent models, the effect of light saturation at low intensities and inhibition at high intensities can be described by a Haldane equation (Keesman and Stichter, 2003). The Haldane model was initially developed for growth on nutrients to overcome the drawback of Monod kinetics, namely the fact that there is no inhibition included at high substrate level. The model implemented for light intensity can be denoted as follows:

\[ f(I) = \frac{1}{I + K_I + \frac{I^2}{K_2}} \]  

(2.21)

With I (lux) the prevailing light intensity, \(K_I\) (lux) half saturation coefficient for light and \(K_2\) (lux) the inhibition coefficient. In Figure 2.10 the difference between the Monod model and Haldane model is illustrated. It should be stressed that the higher the \(K_2\) value, the lesser the inhibition effect which is also illustrated in Figure 2.10. In this illustration \(K_I\) was set at 3150 lux and \(K_2\) was set at 15000 lux and 75000 lux. As can be deducted that when the Monod equation is used, the specific growth rate tends to a maximum value beyond the saturation intensity. While in case of the Haldane relationship a maximum specific growth rate can be observed at the saturation intensity and beyond this value it decreases due to photoinhibition. With high values of the inhibition parameter, the function tends to a Monod function.
In Figure 2.10: Comparison between Monod (full line) and Haldane (dashed lines) model with different inhibition coefficients, respectively 15000 lux (……) and 75000 lux ( - - - ) implemented.

In Table 2.3 examples of parameters used in the Haldane equation for different microalgal cultures are given.

An other equation often used in literature to model the microalgal growth as function of light intensity, is the Steele relationship (Equation(2.22)) (Alex et al. 2010; Gehring et al., 2010) where light limited microalgal growth is given by a saturation type of responses at low light intensities and a light inhibition at high intensities. In this equation $K_I$ (lux) represents the light inhibition constant.

Table 2.3: Parameter values used in Haldane equation for different microalgal cultures

<table>
<thead>
<tr>
<th>Par.</th>
<th>Value</th>
<th>Unit</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>42-43</td>
<td>$\mu$Em$^{-2}$s$^{-1}$</td>
<td><em>Chlorella pyrenoidosa</em></td>
<td>Sorokin and Krauss, 1962</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>$\mu$Em$^{-2}$s$^{-1}$</td>
<td><em>Nanochloropsis oceanic</em></td>
<td>Sandnes et al., 2005</td>
</tr>
<tr>
<td>$K_2$</td>
<td>275</td>
<td>$\mu$Em$^{-2}$s$^{-1}$</td>
<td><em>Chlorella pyrenoidosa</em></td>
<td>Sorokin and Krauss, 1962</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^8$</td>
<td>$\mu$Em$^{-2}$s$^{-1}$</td>
<td><em>Nanochloropsis oceanic</em></td>
<td>Sandnes et al., 2005</td>
</tr>
</tbody>
</table>

In Figure 2.11 the Haldane and Steele relationship are illustrated when an equal value for the inhibition parameter ($K_I = K_2 = 35000$ lux) was used. The value for the half saturation coefficient in case of the Haldane relation was set at 3150 lux. As can be seen, the Haldane relationship reaches a maximum value faster at low light intensities, due to the low value of the
half saturation coefficient, and then starts to decrease. However, the decrement of the function, starts already at a light intensity smaller than the value of the inhibition parameters. Whilst in case of the Steele relationship, the function reaches a maximum value, and once the light intensity exceeds the value of the inhibition parameter, a swift decrement of the function can be observed. Higher light intensities can be observed. This relationship corresponds to the observations made by Richmond (2004) and as such suggests that the Steele relationship would be more accurate at high light intensities.

Moreover the Steele relationship is often combined with the exponential function of Lambert-Beer (Equation (2.23)) to express light attenuation over a certain distance in the aqueous phase or due to mutual shading of the microalgal biomass. Here the light intensity at the water surface is represented by $I_0$ (µE m$^{-2}$ s$^{-1}$), while $h$ (m) represent the depth of a certain distance of the water and the light attenuation factor is denoted by $\gamma$ (m). The light attenuation factor is determined by the absorption properties of water and the biomass concentration in the water.

\[
f(I) = \frac{I}{K_I} e^{\left(1 - \frac{I}{K_I}\right)} \quad (2.22)
\]

\[
I = I_0 e^{-h \gamma} \quad (2.23)
\]

Other models described in literature that take into account the different phases in the microalgal photosynthetic activity are Platt (1980) and Eilers and Peeters (1988). However these relations contain parameters related to the chlorophyll content of microalgal cells and are nowadays not commonly used to describe the microalgal growth rate.

![Figure 2.11: Comparison between the Haldane (full line) relationship and Steele relationship (dashed line)](image)

with $K_1 = K_2 = 35000$ lux. The vertical line corresponds to light intensity $I = 35000$ lux
In more recent models, another aspect that is considered when modelling the microalgal growth rate in outdoor systems, where the light irradiance varies greatly, is photoacclimation. In the latter process, the microalgae adjust their pigment content to light intensity, which could affect the photosynthetic rate. In contrast to photoinhibition, that occurs on a timescale of minutes, photoacclimation acts on a time scale of days (Nikolaou et al., 2016). The dynamic coupling of photoinhibition and photoacclimation has already been described in literature (Garcia-Camacho et al., 2012; Nikolaou et al., 2016), however because of the experimental set-up used in this dissertation, namely batch experiments with a limited duration time, this was not taken into account.

2.5.3.2. Kinetic models related to temperature

Kinetic models to describe the effect of only temperature on the microalgal growth rate are mainly based on the exponential Arrhenius relation. This relation describes the maximum specific growth rate at certain temperature related to a specific maximum growth rate at a reference temperature. Reichert et al. (2001) adopted this Arrhenius equation to describe the effect of temperature on the growth. The equation can be denoted as follows:

\[ f(T) = e^{\beta_{Alg}(T - T_0)} \]

In this relationship \( T_0 \) represents a reference temperature equal to 293 K. In the model of Alex et al. (2010) \( \beta_{Alg} \) equals a value of 0.046.

Another approach to describe the effect of temperature on the microalgal growth is reported in literature as the Cardinal Temperature Model with Inflection (CTMI) that was originally developed to describe the effect of temperature on bacteria (Rosso et al., 1993). The principle of this relationship is that the microalgae have a maximum specific growth rate in a certain temperature range. If the temperature is lower than the lower limit or higher than the upper limit of this temperature range the specific growth rate becomes zero. This relationship can be denoted as:

\[ \mu_{max} = \begin{cases} 
0 & \text{for } T < T_{min} \\
\mu_{opt} \cdot \phi(T) & \text{for } T_{min} < T < T_{max} \\
0 & \text{for } T > T_{max}
\end{cases} \]
With $\phi(T) = \frac{(T - T_{\text{max}})(T - T_{\text{min}})}{(T_{\text{opt}} - T_{\text{min}})(T_{\text{opt}} - T_{\text{min}})^2(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\text{max}})(T_{\text{opt}} + T_{\text{min}} - 2T)}$ \hspace{1cm} (2.25)

In this equation, $T_{\text{min}}$ ($^\circ$C) represents the temperature below which the growth is assumed to be zero, $T_{\text{max}}$ ($^\circ$C) the temperature at which there is no growth. The maximal growth rate $\mu_{\text{opt}}$ ($^\circ$C) occurs at $T_{\text{opt}}$ ($^\circ$C). However, it should be noted that this relationship includes 3 cardinal temperatures that need to be calibrated to experimental data and for this it can be stated that this equation is difficult to use in practice.

2.5.3.3 Kinetic models related to nutrients

To describe the kinetics related to uptake of nutrients two models are commonly used, respectively Monod model and Droop Model. In Equation (2.26) the Monod equation is denoted. In this function the growth is described as function of the ambient dissolved concentration of a certain substrate, respectively inorganic nitrogen, inorganic phosphorus or inorganic carbon. In this equation $S$ is the ambient nutrient concentration (g m$^{-3}$) and $K_S$ the half saturation coefficient (g m$^{-3}$) which is the nutrient concentration that corresponds to 50% of the maximum specific growth rate. The parameter $K_S$ is specific for the microalgal species and specific for the substrate. The lower this value, the better the ability to grow on low environmental concentration of this substrate. However, it should be stressed that in the numerous published manuscripts regarding measured or calibrated half saturation coefficients, there is a lot of variability for this parameter in case of one substrate and one species. Possible reasons for this that are mentioned in literature, are the hydraulics of the used reactor, physical conditions such as medium viscosity or temperature (Arnoldos et al., 2005).

$$f(S) = \frac{S}{K_S + S} \hspace{1cm} (2.26)$$

Compared to the Monod equation, Droop (2.27) describes the microalgal growth as function of intracellular concentration of a certain substrate.

$$f(S) = (1 - \frac{k_Q}{Q}) \hspace{1cm} (2.27)$$
Chapter 2

Here $k_Q (g \ g^{-1})$ represents the minimum intercellular nutrient amount needed for growth and $Q (g \ g^{-1})$ is the total amount of the nutrient that can be stored in the total microalgal biomass.

In general researchers would prefer to use the Monod model because the external substrate concentration is easily measured. However, the applicability of the Monod model is doubtful, because luxury uptake of nutrients and storage for later growth may lead to a temporal uncoupling between reproductive rates and dissolved nutrient concentrations. Under unsteady state conditions and when intracellular storage happens, the cell quota of the limiting nutrient, (expressed as the total amount of nutrient per cell) is considered to be a better indicator of the nutritional status than ambient concentrations. However the cell quota of individual species cannot be measured easily under natural conditions. This difficulty arises from the fact that, when changes occur of environmental conditions, a certain adaptation period is needed before the features can be directly related to the kinetics.

In Table 2.4 some half saturation coefficients for nutrients in case of different microalgal cultures are summarized. A big difference between the half saturation coefficient for ammonium in case of two Chlorella species was noted. This is due to the different environments in which the microalgae were residing. Overall it can be concluded that the affinity coefficients for nutrients are low. Although a difference between microalgal cultures can be observed, within this literature study 75% of the values found for ammonium were lower than 0.1 g N m$^{-3}$. For nitrate this was 0.05 g N m$^{-3}$. Concerning inorganic phosphorus the 75% percentile value was 5.27 g P m$^{-3}$ whilst the 50% percentile value was 0.05 g P m$^{-3}$.

**Table 2.4: Half saturation coefficients for ammonium and phosphate for different microalgal cultures**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
<th>Unit</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.5</td>
<td>g N m$^{-3}$</td>
<td><em>Chlorella vulgaris</em></td>
<td>Aslan and Kapdan, 2006</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>gN m$^{-3}$</td>
<td><em>Chlorella sp</em></td>
<td>Moreno-Grau et al., 1996</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>gN m$^{-3}$</td>
<td><em>Algal ponds</em></td>
<td>Broekhuizen et al., 2012</td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>gP m$^{-3}$</td>
<td><em>Chlorella vulgaris</em></td>
<td>Aslan and Kapdan, 2006</td>
</tr>
</tbody>
</table>
2.5.4 Kinetic models with interdependent factors

Kinetic models with interdependent factors describe the microalgal growth rate as function of multiple variables based on experimental data where the conditions of these variables are changed simultaneously and interdependency between these variables is observed. These models are needed in view of good modelling for microalgal system optimization since there is evidence of interdependency of certain environmental factors influencing the microalgal growth rate.

Carvalho and Malcata (2003) adopted the Arrhenius equation in order to describe the microalgal growth rate as function of the simultaneous effect of light and temperature, as there is evidence of interaction between these two factors. Basic assumption for this modification is that for a given temperature, there is a direct relation between light intensity and activation energy and as such a light dependency of the activation energy should be included. Furthermore the light saturation level is influenced as mentioned before by the temperature, next to the prevailing light intensity. For this an equation was proposed that fitted the experimental data very good. This equation could be denoted as (Carvalho and Malcata, 2003):

\[
f(I, T) = \frac{K_1}{K_2} \frac{I}{T+I} e^{-\beta \frac{I}{T}}
\]

(2.28)

With \( I \) (lux) and \( T \) (K) respectively the prevailing light intensity and temperature. \( \beta \) (-) represents a constant related to the activation energy and ideal gas constant.

2.5.5 Examples of modelling microalgal autotrophic growth accounting multiple factors

Bernard and Rémond (2012) proposed a model accounting for light and temperature with non-limiting nutrient conditions. The growth function as function of light and temperature was denoted as:

\[
f(I, T) = \mu_{opt} (I) \varphi(T)
\]

(2.29)
In which $\mu_{opt}$ (I) represents the optimal growth rate at a certain light intensity described following the Haldane relationship (Equation (2.21)). Considering the temperature function the CTMI function (Equation (2.25)) was used. Experimental data was extracted from previously published experimental studies, with this constraint that the number of observations made had to be greater than the number of parameters used in the combined equation.

Although good model prediction was observed, the parameter estimation resulted in an average 95% confidence interval width for $T_{min}$, $T_{opt}$ and $T_{max}$ of respectively 19.2 °C, 13.6 °C and 19.0 °C. This could indicate a shortcoming of parameter identifiability of this model structure.

Filali et al. (2011) developed a model for *Chlorella vulgaris* taking into account the simultaneous effect of light intensity and inorganic carbon on the microalgal growth rate. The model included dynamic equations with respect to the CO$_2$ mass transfer between the liquid phase and gaseous phase, the equilibrium of inorganic carbon species in the liquid phase, a kinetic expression for the growth on inorganic carbon and a light transfer model depending on the reactor geometry and the incident and outgoing light intensity which was mainly determined by the biomass concentration. Filali et al. (2011) calibrated the model to data of biomass evolution during batch experiments of *Chlorella vulgaris* when non-limiting conditions of nutrients were applied. Next to the maximum specific growth rate, the affinity coefficients for growth on inorganic carbon and light intensity were considered for model calibration. A value for the maximum specific growth rate $\mu_{max} = 1.92$ d$^{-1}$ was the result. Also experimental biomass data coincided within the confidence interval of the calculated biomass concentration, indicating good model performance.

Mennaa et al. (2015) used the Verhulst logistic model to compare the microalgal growth rate and the nutrient removal kinetics in urban wastewater of different microalgal species and algal bloom next to the harvestability of these species. The experiments were performed on lab scale by using a batch wise photobioreactor. The temperature was maintained at 20 ± 3 °C and light intensity was set at 90 µE m$^{-2}$s$^{-1}$. The different strains were cultured in artificial medium and maintained in the exponential growth phase before they were seeded to the batch wise experimental set-up. Results demonstrated that the proposed model was able to describe the microalgal biomass evolution and the nutrient removal very accurate. The difference of the maximum specific growth rate between species was according to the authors due to the difference in adaptation from culture medium to the urban wastewater between species. In Table
2.5 this maximum specific growth rates used in the simulations and the correspondence between model simulations and experimental biomass evolutions (by means of $R^2$-values) are summarized.

Table 2.5: Maximum specific growth rate in model simulations (Menaa et al., 2015)

<table>
<thead>
<tr>
<th>Microalgal species</th>
<th>$\mu_{\text{max}}$ (d$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>0.38</td>
<td>0.99</td>
</tr>
<tr>
<td><em>C. sorokiniana</em></td>
<td>0.37</td>
<td>0.98</td>
</tr>
<tr>
<td><em>B. braunii</em></td>
<td>0.42</td>
<td>0.98</td>
</tr>
<tr>
<td><em>S. obliquus</em></td>
<td>0.28</td>
<td>0.98</td>
</tr>
<tr>
<td><em>A. falcatus</em></td>
<td>0.1</td>
<td>0.99</td>
</tr>
<tr>
<td><em>Bloom</em></td>
<td>0.52</td>
<td>0.99</td>
</tr>
</tbody>
</table>

2.6 Conclusions and perspectives

Since microalgae have the capacity to assimilate inorganic carbon and nutrients in their biomass, the use of this biomass for wastewater treatment offers a promising alternative for conventional wastewater treatment systems. Moreover the biomass can be valorized as feedstock for biofuel production, or down-stream processing such as anaerobic digestion. However the microalgal growth is inherently more complex compared to activated sludge. Several environmental factors such as temperature, prevailing light intensity, availability of nutrients, salinity and pH can have a significant influence on the microalgal growth. For this a good insight of these different aspects is needed in view of system performance and system optimization.

Considering system optimization the use of virtual in silico experiments offers a promising methodology to reduce experimental costs. Despite the fact that several models exist to describe the microalgal growth, in general they only take into account one environmental factor or a combination of a few factors. Mathematical models developed mimicking the natural environment are until today only scarcely reported.

Also for model development, a thorough knowledge of growth kinetics is needed. Although the measurement of microalgal growth kinetics is well documented in literature, in general this is
determined by proxy measurements which have some drawbacks. Most of these proxy measurements are time consuming and are in need of expensive analytical equipment. Furthermore the results of these proxy measurements are very dependent on the conditions at which the experiments were performed and are difficult to translate to other environmental conditions.

Therefore, this dissertation seeks the development of a novel methodology to measure the microalgal growth kinetics. Further a model to describe the microalgal growth and removal and storage of nutrients taking into account several factors in view of mimicking the natural environment is proposed.
Chapter 3

Simulation methods
Chapter 3

3.1 Introduction

For the simulations described in this dissertation, two software packages were used, namely WEST ® (Vanhooren et al., 2003) and the Flexible Modelling Environment (FME) package (Soetaert and Herman, 2009). In this chapter, the software and mathematical tools that were used will be discussed.

3.2 WEST modelling platform

WEST®, acronym for Wastewater Treatment Plant Engine for Simulation and Training (mikebydhi.com) is a modelling and simulation package especially designed for the modelling of wastewater treatment processes. Although it provides a default set of wastewater treatment models that can be readily used for simulation, it is possible to alter the provided models or create new ones (Benedetti et al., 2008). Thus each model that consists of a set of differential and algebraic equations can be implemented in the software. Since the microalgal growth models which are presented in this dissertation were not available in WEST® by default, they were first implemented in the model editor in a matrix format. This matrix is the so-called Gujer matrix (Figure 3.1) that consists of the different processes, model state variables, different process rates and the stoichiometric coefficients. For each process a corresponding process rate will be determined. Finally, the stoichiometric coefficients corresponding to the reactions between different components are introduced as central matrix elements. For each process defined in the matrix a mass balance in a continuous stirred tank reactor (CSTR) can be generated which results in an ordinary differential equations.
Simulation methods

<table>
<thead>
<tr>
<th>Process</th>
<th>( X_{\text{alg}} ) (g DW m(^{-3}))</th>
<th>( S_{\text{HCO}_3^-} ) (g HCO(_3^-) m(^{-3}))</th>
<th>( S_{\text{CO}_2} ) (g CO(_2) m(^{-3}))</th>
<th>( S_{\text{CO}_3} ) (g CO(_3^2^-) m(^{-3}))</th>
<th>( S_{\text{O}_2} ) (g O(_2) m(^{-3}))</th>
<th>Process Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Growth on HCO(_3^-)</td>
<td>1</td>
<td>( Y_1 )</td>
<td>( Y_3 )</td>
<td>( \rho_1 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Growth on CO(_2)</td>
<td>1</td>
<td>( Y_2 )</td>
<td>( Y_3 )</td>
<td>( \rho_2 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.1: Schematic presentation of a Gujer Matrix including process rates and a stoichiometrie.

Parameters that are implemented in the matrix as a symbol are defined in the parameter section. Also additional algebraic equations can be implemented in a separate section. When the model is implemented in the Gujer matrix, the new model is transformed into a model specification language (MSL) and added to the model base. In this model base all physical units, default parameter values and mass balances are declared (Vanhooren et al., 2003).

To implement the model, the modelling environment is used. In case of a batch wise experiment, the used configuration consists of a single activated sludge unit building block.

The actual simulation is performed in the experimentation environment, where all initial conditions and simulation time are defined.

### 3.3 The Flexible Modelling Environment

The Flexible Modelling Environment (FME) is an available package of R, an open access package originally developed for statistical data analysis. Contrary to WEST ® this software package does not include a graphical interface. Recent years this package has been more intensively used in view of ecological modelling (Haario et al., 2009; Mannina et al., 2012).
Also, each separate equation describing the dynamic mass balance needs to be typed in the software console.

With the FME package several simulation methods can be performed, namely a local and global sensitivity analysis based on the methodology of Brun et al. (2001) and Soetaert and Herman (2009). Also parameter estimation and a parameter identifiability analysis according to Brun et al. (2001) can be performed.

### 3.4 Parameter identifiability

#### 3.4.1 Introduction

An important aspect regarding a certain model structure is the identifiability of parameters included in the model given the available experimental data. In other words if it is possible by model calibration to find a unique value for a parameter. Two different kinds of identifiability can be distinguished, respectively the theoretical and practical identifiability. In case of theoretical identifiability the assumption is made that the obtained experimental data is perfect, whilst with practical identifiability the quality of the experimental data is considered as well. As such theoretical identifiable parameters can be considered as practically non-identifiable parameters due to occurring errors in the experimental data (Agathos et al., 2003).

In case of more complex model structures, the theoretical identifiability of parameters is assessed by complex calculations.

#### 3.4.2 Local sensitivity analysis

A local sensitivity analysis (LSA) was used to determine the influence of model parameters on certain variables calculated. To compare the sensitivity functions of different variables, relative
sensitivity functions (RSA) were used, rather than absolute sensitivity functions (ASF). The ASFs were calculated by using the finite forward difference method, that could be denoted as:

\[
\frac{dy_i}{d\theta_k} = \frac{y_i(t,\theta_n + \varepsilon \theta_n) - y_i(t,\theta_n)}{\varepsilon \theta_n}
\]  

(3.1)

In which \(y_i(t,\theta_n)\) represents the output variable, \(\theta_n\) represents the nominal parameter value and \(\varepsilon\) is the perturbation factor.

The RSF can be calculated by:

\[
RSF = \frac{ASF_{\theta_n}}{y_i(t,\theta_n)}
\]  

(3.2)

A RSF less than 0.25 indicates a non-influential parameter. Parameters are moderately influential when RSF is in the range of 0.25 to 1. Values higher than 1 and 2 indicate influential and very influential parameters respectively (Audenaert et al., 2010).

3.4.3 Collinearity index

The identifiability of the model parameters can be further investigated according to Brun et al. (2001). Brun et al. (2001) present an appropriate method to tackle the problem of models with a lot of parameters that often lead to poorly identifiable or non-identifiable parameters. This method uses local sensitivity functions and the resulting collinearity index is based on the joint influence of parameters in a random parameter subset on the model output. More specific this is done by assessing the degree of near-linear dependence of the column subsets of the normalized scaled sensitivity matrix (Brun et al., 2001). In case of near-linear dependence, a change in the model output caused by the modification of one specific parameter in the parameter subset can be compensated by changes of other parameters in the parameter. As such the parameters of this parameter subset cannot be uniquely identified.

To assess this near-linear dependency a collinearity index \(Y_K\) is defined by Brun et al. (2001). This collinearity index is a measure for the calculated determinant of the normalized scaled sensitivity matrix. High value of \(Y_K\) indicate that this determinant tends to zero. As such indicating linear dependency between the scaled sensitivity functions. This means a shift of one
Chapter 3

parameter will be almost completely compensated by appropriate changes of the other parameters and thus indicating a poorly identifiable parameter subset. Brun et al. (2001) stipulate a threshold value $Y_K = 20$ to indicate a good identifiable parameter subset with no correlation between the parameters in the parameter subset.

3.4.4 Global sensitivity analysis

Compared to a LSA a global sensitivity analysis (GSA) is performed over a broader predefined range in the parameter space with all parameters varying simultaneously. In particular the Monte Carlo Simulation (MCS) technique is used to perform a GSA (Schonkwiler and Medvill, 2009). In general a MCS consists of four steps. In a first step the parameter uncertainty is determined. For this the parameter range and the parameter distribution, or in other words the probability density function (PDF) of the parameter within this range is defined. Since in this work the PDF of the parameters was not known, a uniform distribution was assumed (Saltelli et al., 2005). Next a method to sample the parameter space is chosen. In this work Latin Hypercube Sampling (LHS) was used. This sampling method involves a stratification of the parameter space at which every level contains the same number of sampling points. This results in a homogenous sampling of the parameter space (Saltelli et al., 2005). In a third step the number of simulations that need to be performed is defined. According to Saltelli et al. (2005) the accuracy of the MCS increases with increasing number of simulations. However Audenaert (2013) proposed a total of 150 simulations per parameter as a rule of thumb.

In a final step the results of the MCS are analyzed. Different methods are reported in literature, however two methods were used in this work and will be discussed, namely the Standardized Regression Coefficient method and the Monte Carlo Filtering method.
3.4.4.1 Standardized Regression Coefficients

The method of the Standardized Regression Coefficients is often used. For this a value at a certain time for the calculated model variable is taken and a linear regression is performed with the variable and the corresponding parameters. The resulting variability in the model output was then analysed using a linear regression which resulted in regression coefficients that are an indication of the linear dependency between output variables and parameters. In this study, SPSS (IBM, Armonck, NY USA) was used for linear regression. After standardization of the regression coefficients (Saltelli, 2005), the t-statistic value of the latter was calculated from the standard errors of the regression coefficients. The impact of parameters on the model was evaluated by means of the absolute t-value. For example if the t-statistic value exceeds 1.96, the parameter has a significant influence on the model output at the 5 % confidence level (Saltelli, 2005). The results of such an analysis are represented in a tornado plot (Figure 3.2). Here the parameters are given with decreasing order of t-SRC value. In this example the maximum photosynthetic rate and the maximum growth rate are the most influential parameters.

![Tornado plot example](image)

Figure 3.2: Example of a tornado plot as result of a GSA for a microalgal growth model (Quin et al., 2011). Parameters are ranked from more influential (top) to less influential (bottom)
3.4.4.2 Monte Carlo Filtering Method

In order to get a qualitative idea about the identifiability of the different parameters, regional sensitivity analysis was applied (Camacho and Gonzalez, 2008). In contrast to the SRC mentioned above, where the focus was on a specific point in time, the entire simulation output is taken into account. By assessing the effect of the parameters on the Sum of Squared Errors (SSE), the impact of the parameter on the model fitting is taken into account. This sum of squared errors, can be denoted as:

\[ \text{SSE} = \sum (y_i - y)^2 \]  

where \( y_i \) represents the calculated respirometric and titrimetric values and \( y \) the measured values, both at \( t = i \)

Further the set of simulations was divided into 10 classes, with increasing SSE. Then, the marginal cumulative distribution function of the parameters within each of the 10 classes was depicted.

Clustered lines indicate non-sensitive parameters. As such, the degree of dispersion of the 10 lines gives a qualitative measure, according to Camacho and Gonzalez (2008), for a first indication of the identifiability of the parameters. As such, variations in those parameter values will have a profound effect on the model performance.

3.5 Parameter estimation

Parameter estimation was performed by the minimization of an objective function by using an optimization algorithm. The objective function was defined as SSE between model prediction and measurements and could be denoted as Equation (3.3).

To minimize the objective function, the Simplex algorithm (Nelder and Mead, 1965) in WEST® was used.
In case of the parameter estimation was performed with the FME package, the Levenberg–Marquardt algorithm was used for objective function minimization.

### 3.6 Goodness-of-fit

When model calibration and validation was performed, the goodness-of-fit between measured and calculated was quantified by calculating the Theil’s inequality coefficient (TIC) (Theil, 1961) which can be denoted as follow:

\[
TIC = \frac{\sqrt{\sum (y_i - y_{im})^2}}{\sqrt{\sum y_i^2} \sqrt{\sum y_{im}^2}}
\]  

(3.4)

in which \( y_i \) represents simulated data and \( y_{im} \) represents measured data points. A TIC value lower than 0.3 (Audenaert et al., 2010) thereby indicates a good agreement with measured data.

This criterium was preferred to assess the model performance, because it uses a relative number. This in contrast to other criteria, such as for example Root Means Squared Errors (RMSE), where only an absolute number is used.

In Table 3.1 an overview of the different simulation methods and the chapters in this dissertation in which they were used is given.

<table>
<thead>
<tr>
<th>Simulation method</th>
<th>Chapter 4</th>
<th>Chapter 5</th>
<th>Chapter 6</th>
<th>Chapter 7</th>
<th>Chapter 8</th>
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<td>Local sensitivity analysis</td>
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<tr>
<td>Monte Carlo simulation with SRC</td>
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<td>X</td>
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<td>Monte Carlo simulation with filtering</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<tr>
<td>Collinearity study</td>
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<td>-</td>
<td>X</td>
<td>-</td>
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<tr>
<td>Goodness-of-fit</td>
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</table>
Chapter 4

A novel methodology to measure the microalgal growth kinetics

Redrafted from

Chapter 4

Abstract

The potential of microalgae for wastewater treatment has recently led to significant surge in research towards economically more viable and technologically optimised systems. In this context, mathematical modelling has not been used to its full capacity. In this work, a novel approach, namely the combined respirometric-titrmetric methodology for the determination of microalgal kinetics and an experimental protocol are proposed. It was found that the overall oxygen production was lower than stoichiometrically expected, which could be attributed to CO₂-transfer to the gas phase. A basic model for microalgae growth on inorganic carbon and oxygen production is proposed and was successfully calibrated using several respirometric datasets. The model structure was based on the activated sludge models (ASM) and can now be extended with impact of additional degrees of freedom.

4.1 Introduction

The efficiency of the use of algal processes in environmental technologies is rather low (especially in view of upscaling) and optimisation is required to make them cost-effective. For this accurate knowledge of microalgal kinetics is of crucial importance.

A method that is often used to accomplish this in the context of activated sludge waste water treatment is respirometry (Vanrolleghem and Spanjers, 1998). This method measures the consumption rate of O₂ and translates this into an oxygen uptake rate which is then coupled to the kinetics of the organisms.

The metabolism of algal biomass is somewhat different, i.e., algae produce oxygen through photosynthesis, hereby using an inorganic carbon source (CO₂ or HCO₃⁻) and the energy of light. With abundant light, a respirometric batch setup will then result in a negative oxygen uptake rate, or in other words an oxygen production rate.

In literature, respirometry is in some cases accompanied by titrimetry for activated sludge, providing an independent measure of biological activity, which is helpful when calibrating models (Petersen et al., 2001). This titrimetric approach exploits a pH-effect that is governed
by the organism’s metabolism (Gernaey et al., 2002). Since microalgae use carbon in its inorganic form, the carbonaceous equilibrium and, hence, the pH will be influenced. Whether the rate of the latter is directly related to microalgae kinetics will be tested.

By combining titrimetry with respirometry the different aspects occurring during the microalgal photosynthetic activity will be accounted for. In this chapter a respirometer setup including a titrimetric approach is proposed along with a protocol to successfully perform respirometric-titrimetric experiments that provide a maximum of information. Furthermore, a kinetic model taking into account inorganic carbon limitation is proposed and calibrated.

4.2 Methods and materials

4.2.1 Cultivation of microalgae

The strain of microalgae used for the respirometric experiments was *Chlorella vulgaris*. This strain was cultured in a 10 L breeding reactor. The growth medium used was a variant of the BG-11 medium (Stanier et al., 1971). In order to prevent phosphorus limitation, the medium was slightly modified, i.e. the phosphorus concentration was increased for the N:P ratio to comply to the Redfield ratio, defined as 106C:16N:1P (Grobbelaar, 2004). The pH of the culture was controlled by adding pulses of CO$_2$ into the culture and was carried out by a pH control algorithm implemented in LabView (www.ni.com). At the same time this provided carbon source to the system to obtain high growth yields. Mixing through air sparging prevented the microalgae to settle or attach to the reactor wall.

4.2.2 The algal respirometer

A schematic of the microalgal respirometer is given in Figure 4.1. The 1 l reactor vessel was heat-jacketed to allow temperature control (Alpha R8, www.lauda.de) enabling the exploration of system behavior at different temperatures (default at 293 K). The light cage enclosing the
reactor entirely consisted of eight fluorescent lamps (Grolux T8 18 W, Sylvana). Light intensity was measured using a photosynthetic active radiation (PAR) light sensor (PAR mini, PP-systems). The light intensity measurements were performed on different locations in the reactor. The mean value of these measurements were then considered as the light intensity value mentioned in the text. The spectrum of the lamps used in the light cage ranged from 400 to 700 nm.

Dissolved oxygen (DO) and pH were measured online with an oxygen (Inpro 6100, Mettler Toledo) and pH electrode (Inpro 4250, Mettler Toledo) and the data logged using a PCI-MIO-16XE-50 data acquisition card using LabView (www.ni.com). The DO sensor delay (determined to be 0.53 s) was taken into account according to Vanrolleghem and Spanjers (1998). The pH was controlled online at a user defined set-point using a banded (+/- 0.05 pH) on-off feedback control algorithm implemented in LabView by dosing HCl or NaOH through two 3-way pinch valves (Z530A, SIRAI, Italy). The rate and amount of 0.5 M HCl and 0.5 M NaOH dosed into the reactor vessel constitutes the titrimetric data.

Figure 4.1: Schematic overview of the combined respirometric – titrimetric setup.

4.2.3 Data interpretation

The dynamic dissolved oxygen concentration is determined from a balance between the oxygen production rate (OPR) and oxygen transfer rate (OTR) as was discussed before (Equation (2.3)).
Stoichiometrically, 1.24 g of oxygen is produced for the production of 1 g of biomass (Equation (2.5) and Equation (2.6)). This value represents the oxygen production yield ($Y_O$) and is used to calculate the OPR from the biomass concentration present in the system. Moreover it should be stressed that at this stage of the investigation no microalgae respiration is included in the model, due the fact that not enough information was present in the experimental data to estimate the contribution of this process.

The rate of proton addition is determined by the removal rate of carbon source spiked to the algal respirometer. As can be deducted from Equation (2.5) and Equation (2.6), consumption of one gram of bicarbonate leads to a removal of $19.2 \times 10^{-3}$ g of protons, whereas consumption of one gram of carbon dioxide removes $3.9 \times 10^{-3}$ g of protons. Hence, the proton addition rate can be modeled from the consumption of bicarbonate and carbon dioxide. Moreover the BSAR due to the chemical equilibria of the different carbon species in the water and the diffusion of $\text{CO}_2$ between the atmosphere and the liquid phase (Ifrim et al., 2012) influences the proton balance.

### 4.2.4 Modelling approach

#### 4.2.3.1. Modelling of the respirometric data

To describe the respiration behavior of microalgae, a first basic kinetic model was set up based on the experimental observations. It contains five state variables: microalgae biomass concentration, concentrations of the different carbon species in the aqueous system ($\text{HCO}_3^-$, $\text{CO}_2$ and $\text{CO}_3^{2-}$) and dissolved oxygen concentration. The model was inspired by Gehring et al. (2010) and Alex et al. (2010), which are similar to the River Water Quality Model by Reichert et al. (2001). The next sections describe the model in more detail through the seven processes that it accounts for. The final model presentation is for the first time based on the activated sludge type models (ASM) (Henze et al., 2000), allowing (1) straightforward interchange with existing waste water treatment models and (2) extension of the presented model. Further the kinetic model presents a trade-off between detailed metabolic models (e.g. Kliphuis et al. (2010)) and oversimplified kinetic models (e.g. Nedbal et al. (2010)).
Chapter 4

4.2.3.1.1 Algal growth and decay kinetics

Since the microalgae used in the respirometric tests were suspended in the growth medium with a sufficient amount of macro- and micronutrients, the nutrients were assumed not to be limiting for their growth in the current experimental setting. Because temperature and light intensity were kept constant in the different experiments studied here, no factor for the temperature dependency nor light intensity dependency for the growth rate of microalgae was included in the model at this stage. The inorganic carbon source (C-substrate), however, is consumed in the respirometer and it becomes limiting for the algae growth. The availability of carbon dioxide and bicarbonate is therefore modelled by a Monod function. As already mentioned in Chapter 2, carbon dioxide is able to cross cell membranes and enters directly into the cell by diffusion. Contrarily, the uptake of bicarbonate requires a transporter system or its prior conversion to carbon dioxide (Van den Hende et al., 2012). Therefore, carbon dioxide will be preferentially taken up by the microalgae. Given this, an inhibition term in the bicarbonate kinetics has been incorporated in the model. As such the growth rate on the two inorganic carbon sources can be denoted as:

\[ \rho_{\text{Alg}(\text{HCO}_3^-)} = \mu_{\text{max}} \left( \frac{S_{\text{HCO}_3^-}}{K_{\text{HCO}_3^-} + S_{\text{HCO}_3^-}} \right) \left( \frac{K_{\text{CO}_2}}{K_{\text{CO}_2} + S_{\text{CO}_2}} \right) X_{\text{Alg}} \]  

(4.1)

\[ \rho_{\text{Alg}(\text{CO}_2)} = \mu_{\text{max}} \left( \frac{S_{\text{CO}_2}}{K_{\text{CO}_2} + S_{\text{CO}_2}} \right) X_{\text{Alg}} \]  

(4.2)

With \( K_{\text{HCO}_3^-} \) and \( K_{\text{CO}_2} \) (g m\(^{-3}\)) the half saturation coefficients for bicarbonate and carbon dioxide respectively, \( S_{\text{HCO}_3^-} \) and \( S_{\text{CO}_2} \) (g m\(^{-3}\)) the ambient inorganic carbon species concentration in the aqueous phase. \( K_{i\text{CO}_2} \) (g m\(^{-3}\)) represents the inhibition coefficient for growth on bicarbonate, indicating the preferential uptake of carbon dioxide. When the carbon dioxide concentration is significantly higher than \( K_{i\text{CO}_2} \), Equation (4.1) tends to zero.

As such the dynamic balance for microalgal biomass can be denoted as:

\[ \frac{dX_{\text{Alg}}}{dt} = \rho_{\text{Alg}(\text{CO}_2)} + \rho_{\text{Alg}(\text{HCO}_3^-)} - \rho_{\text{decay}} \]  

(4.3)
Where:

\[ \rho_{\text{decay}} = b_{\text{max}} \times X_{\text{Alg}} \]  

(4.4)

In this Equation \( b_{\text{max}} \) (\( \text{d}^{-1} \)) represents the maximal decay rate.

**4.2.3.1.2 Inorganic carbon species**

The concentration of inorganic carbon species are related to each other by the governing chemical equilibrium as already mentioned in Chapter 2 by Equations (2.7) and (2.8). Bicarbonate is dosed to the system and carbonate and carbon dioxide are formed in the aqueous environment by dissociation and dehydration of bicarbonate, respectively. To calculate the rate at which chemical conversion between the three carbon species takes place, the concentrations of the carbon species need to be converted into mol l\(^{-1}\), as the dissociation constants \( K_{a1} = S_{HCO_3^-} \frac{S_{H^+}}{S_{CO_2}} = 10^{-pK_{a1}} \) and \( K_{a2} = S_{CO_2^-} \frac{S_{H^+}}{S_{HCO_3^-}} = 10^{-pK_{a2}} \) are expressed in this unit and the concentrations of the different species in the model are expressed in g m\(^{-3}\). From the chemical equilibria the equilibrium concentrations of the three species are calculated and subtracted from the actual concentration of the inorganic carbon source. Consequently, the value that is obtained is proportional to a driving force determined by the difference between the equilibrium and the actual concentration. This value is then multiplied by a rate constant \( k_1 \) or \( k_2 \) to obtain a process rate to express the change in the concentrations of bicarbonate, carbon dioxide and carbonate, because the system strives for a chemical equilibrium (Wolf et al., 2007). Thus the rate of dissociation and hydration is denoted as:

\[ \rho_{\text{dissoc}} = k_2 \left( \frac{S_{HCO_3^-}}{61} - \frac{10^{-pH} S_{CO_2}}{60 K_{a1}} \right) \]  

(4.5)

\[ \rho_{\text{hydrat}} = k_1 \left( \frac{S_{CO_2}}{44} - \frac{10^{-pH} S_{HCO_3^-}}{61 K_{a2}} \right) \]  

(4.6)

Next also transfer of carbon dioxide between the liquid phase and gas phase occurs. The rate at which this occurs can be denoted as:

\[ \rho_{\text{CO}_2,\text{trans}} = K_L a \frac{\partial C_{\text{CO}_2}}{\partial z} \left( S_{\text{sat}} - S_{\text{CO}_2} \right) \]  

(4.7)
With $D_{O_2}$ the diffusion coefficient of oxygen in water and $D_{CO_2}$ the diffusion coefficient of carbon dioxide in water, respectively $1.65 \times 10^{-4}$ m$^3$ d$^{-1}$ and $1.73 \times 10^{-4}$ m$^3$ d$^{-1}$ (Sin, 2004). $S_{CO_2}^{sat}$ is the saturation concentration (g m$^{-3}$) and $S_{CO_2}$ the concentration of carbon dioxide in the solution (g m$^{-3}$).

Considering the microalgal assimilation of inorganic carbon, the chemical equilibria of inorganic carbon in the liquid phase and the transfer of carbon dioxide, the dynamic mass balances of the different inorganic carbon sources can be expressed as:

\[
\frac{dCO_2}{dt} = - \frac{1}{Y_2} \rho_{Alg(CO_2)} + \rho_{CO_2,trans} - 0.721 \rho_{hydrat} \tag{4.8}
\]

\[
\frac{dHCO_3^-}{dt} = - \frac{1}{Y_1} \rho_{Alg(HCO_3^-)} + \rho_{hydrat} - 1.016 \rho_{dissoc} \tag{4.9}
\]

\[
\frac{dCO_3^2-}{dt} = + \rho_{dissoc} \tag{4.10}
\]

With $Y_1$ (g DW g$^{-1}$ HCO$_3$) and $Y_2$ (g DW g$^{-1}$ CO$_2$) yield coefficients for growth on bicarbonate and carbon dioxide respectively.

### 4.2.3.1.3 Oxygen production and oxygen transfer

The dynamic mass balance of dissolved oxygen, including oxygen production and oxygen transfer can be expressed as:

\[
\frac{dO_2}{dt} = + Y_3 \rho_{Alg(CO_2)} + Y_3 \rho_{Alg(HCO_3^-)} + \rho_{O_2,trans} \tag{4.11}
\]

With $Y_3$ (g O$_2$ g$^{-1}$ DW) the oxygen produced per gram of biomass.

In Table 4.1 an overview of the different processes (Gujer matrix) is given.
### Table 4.1: Gujer matrix of the microalgal growth model (=Algcarb model)

<table>
<thead>
<tr>
<th>Process</th>
<th>$S_{HCO_3^-}$ (g HCO$_3$- m$^{-3}$)</th>
<th>$S_{CO_2}$ (g CO$_2$ m$^{-3}$)</th>
<th>$S_{CO_3^{2-}}$ (g CO$_3^{2-}$ m$^{-3}$)</th>
<th>$S_{O_2}$ (g O$_2$ m$^{-3}$)</th>
<th>$X_{Alg}$ (g DW m$^{-3}$)</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on HCO$_3$-</td>
<td>(-\frac{1}{Y_1})</td>
<td></td>
<td>$Y_3$</td>
<td></td>
<td></td>
<td>$\rho_{Alg(HCO_3^-)}$</td>
</tr>
<tr>
<td>Growth on CO$_2$</td>
<td>(-\frac{1}{Y_2})</td>
<td></td>
<td>$Y_3$</td>
<td></td>
<td></td>
<td>$\rho_{Alg(CO_2)}$</td>
</tr>
<tr>
<td>Decay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{decay}$</td>
</tr>
<tr>
<td>O$_2$ transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{O_2,trans}$</td>
</tr>
<tr>
<td>CO$_2$ transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{CO_2,trans}$</td>
</tr>
<tr>
<td>CO$_2$ hydration</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{hydrat}$</td>
</tr>
<tr>
<td>HCO$_3$- dissociation</td>
<td>-1.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{dissoc}$</td>
</tr>
</tbody>
</table>

### 4.2.3.1.3 Parameter values

Default values for the parameters are summarized in Table 4.2 and were obtained from literature (Alex et al., 2010; Aseada and Van Bon, 1997; Dochain et al., 2003; Kayombo et al., 2000; Omlin et al., 2001; Reichert et al., 2001; Wolf et al., 2007). In case parameters were used in an estimation (see further) the range that was used is provided.

The dissociation constants (pKa) for the hydration of carbon dioxide and the dissociation of bicarbonate were taken as 6.36 and 10.33, respectively (Stumm and Morgan, 1996). The rate constants for these reactions were chosen to be 10000 d$^{-1}$ for $k_1$ and $k_2 = 100000$ d$^{-1}$ (Gehring et al., 2010), respectively indicating very fast reactions.

The values for the yields for the production of biomass from bicarbonate or dissolved carbon dioxide and the yield of oxygen production were determined stoichiometrically from Equations...
Chapter 4

(2.5) and (2.6). The maximum decay rate $b_{max}$ was set to a value of 0.001 d$^{-1}$ because tests were short and the decay rate was considered not to play a significant role.

At this stage, to have insight in the methodology, only the respirometric profile was considered for model calibration, similar to activated sludge respirometry. At first, the model was tested by manually changing the values of different parameters. This illustrated that $\mu_{max}$ and $K_La$ had strong influence on the respirometric profile. This was not the case for other parameters such as $K_{HCO_3^-}$ for example. As such it was decided to use these 2 parameters for further model calibration.

Table 4.2: Parameter values used for simulations with the Algcarb model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Literature range</th>
<th>Assigned value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{max}$</td>
<td>0.1-11</td>
<td>*</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$b_{max}$</td>
<td>0.003-0.1</td>
<td>0.01</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$Y_1$</td>
<td>0.549</td>
<td>0.549</td>
<td>g DW g$^{-1}$ HCO$_3^-$</td>
</tr>
<tr>
<td>$Y_2$</td>
<td>0.761</td>
<td>0.761</td>
<td>g DW g$^{-1}$ CO$_2$</td>
</tr>
<tr>
<td>$Y_3$</td>
<td>1.24</td>
<td>1.24</td>
<td>g O$_2$ g$^{-1}$ DW</td>
</tr>
<tr>
<td>$K_{HCO_3^-}$</td>
<td>0.061-6.1</td>
<td>3</td>
<td>g HCO$_3^-$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_{CO_2}$</td>
<td>0.044-4.4</td>
<td>0.2</td>
<td>g CO$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_La$</td>
<td>*</td>
<td></td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$k_1$</td>
<td>2221-10$^5$</td>
<td>10000</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>10$^4$-10$^{12}$</td>
<td>100000</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$pK_{a_1}$</td>
<td>6.36</td>
<td>6.36</td>
<td>-</td>
</tr>
<tr>
<td>$pK_{a_2}$</td>
<td>10.33</td>
<td>10.33</td>
<td>-</td>
</tr>
</tbody>
</table>

(*) Parameter considered for model calibration
4.3 Results and discussion

4.3.1. Data collection and derived information

Figure 4.2 (top) shows a typical result of a respirometric-titrmetric experiment. The results are repeatable and similar profiles were obtained when certain experimental conditions were modified (e.g. different spiked quantities of bicarbonate, different algal biomass concentration).

The dissolved oxygen profile is the result of the balance between (1) oxygen produced by the algae during the consumption of the pulse of bicarbonate which was added to the system and (2) oxygen removed from the system through transport to the atmosphere as described by Equation (2.3). Upon addition of an inorganic carbon source (in this experiment 100 g HCO$_3^-$ m$^{-3}$), the initial dynamic equilibrium is disturbed as more dissolved oxygen is produced than removed. This results in a rapid increase of the dissolved oxygen concentration. However, this increase is limited by the maximum growth rate of the algae (i.e. metabolic limitation) which leads to a new steady state (plateau in time interval 0.1-0.15 d). At some point the inorganic carbon source is depleted (approx. 0.16 d) and limits the DO production. This leads to a decrease in DO, eventually returning to the state the system was in prior to the addition of inorganic carbon source. Along with the consumption of bicarbonate, protons are removed from the system. Due to the fact that pH is controlled at a fixed set-point (here 7.5), proton addition is needed. According to the dashed line in Figure 4.2 (top), this happens at a constant rate (TPAR=11.80 g m$^{-3}$ d$^{-1}$) during the time interval between spiking and depletion of bicarbonate. After depletion, the proton addition reduces to the BSAR level, in this case 0.141 g m$^{-3}$ d$^{-1}$, or about 1% of the TPAR. This results in a HAR for the consumption of the pulse of HCO$_3^-$ of 11.70 g m$^{-3}$ d$^{-1}$. The specific HAR (expressed per unit biomass) at the beginning of the experiment ($p_{H}$) is determined to be 0.024 g H g$^{-1}$ DW. The total amount of protons added for the consumption of the added pulse of bicarbonate can be determined by integrating the titrimetric profile yielding 1.86 g H$^+$ m$^{-3}$. Equation (2.6) allows an exact calculation of the stoichiometrically required amount of acid (given the equations hold): a concentration of 100 g m$^{-3}$ HCO$_3^-$ yields 1.92 g H$^+$ m$^{-3}$ that needs to be added to maintain a fixed pH. Hence, the titrimetric method had in this case a recovery rate of 97%, proving to be accurate. The calculated OPR and OTR from the dissolved oxygen profile are shown in Figure 4.2 (bottom).
maximum OPR of the algae after spiking with the inorganic carbon has an average of about 250 g O₂ m⁻³ d⁻¹. Hence, the maximum rate of oxygen production per unit of DW of algae (pO₂, max) equals 0.523 g O₂ g DW⁻¹ d⁻¹. The total amount of oxygen produced is determined by integrating the OPR curve and equals 39.94 g O₂ m⁻³. This is significantly lower than the theoretical amount that can be produced according to Equation (2.6) from the amount of bicarbonate (100 g m⁻³) added to the system, being 68.30 g O₂ m⁻³. From these results the recovery rate only amounts to about 58.4%. This low recovery can be explained as follow. First, respiration is not taken into account when interpreting the data. Indeed, microalgae use oxygen for their maintenance metabolism, thus lowering the total amount of oxygen produced. This type of respiration is called dark respiration (Wolf et al., 2007). Also, with respect to the proton addition, dark respiration will have an influence on the proton addition rate. However, according to Kliphuis (2010), this is maximum 10% of the proton addition due to photosynthetic activity. In addition, photorespiration can occur at high oxygen to carbon dioxide ratio in the solution, and as such inhibition of the photosynthesis occurs (Nigel et al., 1977) Birmingham et al. (1981) stressed, that photorespiration is only inhibited at the CO₂ saturation level in the water. However, quantification of the photorespiration rate is difficult. This because this it depends on the ratio of concentration of O₂/CO₂ in the vicinity of the rubisco enzyme. According to Kliphuis (2010) it is very difficult to determine the latter. Ogren (1984) mentioned a formula to express the relative photorespiration for isolated Rubisco. However, in a whole cell, several transport processes play a role in the functioning of the Rubisco. Hence, the proposed formula was only an estimation. Kliphuis (2010) applied the expression and found that the maximum photorespiration rate is smaller than 4% of the photosynthetic activity. This was the case for experiments that were conducted under conditions similar to the ones presented in this chapter. Therefore, it can be concluded that photorespiration is negligible.
Figure 4.2: Example of a respirometric experiment (top figure). The full line illustrates the respirometric profile and the dashed line the proton addition. In the bottom figure, the resulting OPR (full line) and OTR (dashed line) in case of 100 g m\(^{-3}\) HCO\(_3^-\) and an algal concentration of 478 g DW m\(^{-3}\) and with a light intensity of 4875 lux. pH is controlled at 7.5. Temperature is set at 288 K.

Another possible explanation is that not the entire amount of inorganic carbon source is available for the algae to be consumed and as such for oxygen production. As mentioned before, next to oxygen stripping to the atmosphere, also stripping of carbon dioxide occurs. An initial concentration of 100 HCO\(_3^-\) g m\(^{-3}\) or 0.001639 mol l\(^{-1}\) total inorganic carbon corresponds, based on Equations (2.11) and (2.12), to 0.000149 mol l\(^{-1}\) H\(_2\)CO\(_3\), 0.001489 mol l\(^{-1}\) HCO\(_3^-\) and 9.39 x 10\(^{-7}\) mol l\(^{-1}\) CO\(_3^{2-}\) at pH 7.5. Consumption of 1 mol inorganic carbon leads to addition of 1 mol H\(^+\). As such the concentration of carbon dioxide in the respirometer can be calculated based on the addition of protons. Further, according to Equation (2.9), the carbon dioxide transfer rate can be calculated from concentration and is depicted in Figure 4.3, proving to be significant. Integrating this curve results in 22.82 g m\(^{-3}\) HCO\(_3^-\) transfer to the atmosphere. Accounting for this loss of inorganic carbon, a total recovery of 92.98 % is obtained. As such it can be concluded that there is a significant amount of inorganic carbon that is not available for the microalgae due to stripping resulting in rather low recovery when expressed in the amount of oxygen produced and that CO\(_2\) transfer should be incorporated in both data interpretation and modelling.
Figure 4.3: Visualisation of the CO\textsubscript{2} transfer rate (top) and HCO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{2} concentration (bottom) with 100 g m\textsuperscript{-3} and 478 g DW m\textsuperscript{-3} at pH 7.5 and 288 K. The K\textsubscript{L}a was set at 19 d\textsuperscript{-1}. In the bottom figure, the full line represents the calculated evolution of bicarbonate, and the dashed line the evolution of carbon dioxide.

### 4.3.2 Model calibration

The model was optimized by fitting its output to three different data sets of the respirometric experiments with 75 mg sodium bicarbonate (or 72.6 g HCO\textsubscript{3}\textsuperscript{-} m\textsuperscript{-3}) added to 267 g DW m\textsuperscript{-3} (Figure 4.4A), 75 mg sodium bicarbonate added to 252 g DW m\textsuperscript{-3} (Figure 4.4B) and 150 mg sodium bicarbonate (or 145.2 g HCO\textsubscript{3}\textsuperscript{-} m\textsuperscript{-3}) added to 459 g DW m\textsuperscript{-3} (Figure 4.4C). The model is able to describe the DO-profile acceptably well. The values of the optimized parameters are presented in Table 4.3.

As can been seen the maximum growth rates of the three different experiments are very similar and are comparable with values found in literature (Menaa et al., 2015). For these three experiments the K\textsubscript{L}a spans the range from 15.83 to 26.79 d\textsuperscript{-1}. The consumption rate of inorganic carbon is also plotted and is very similar in the tested cases.
Figure 4.4: Comparison of experimental data (dashed line) and model predictions (full line) for respirometric data: (A) 75 mg NaHCO$_3$ added to 267 g DW m$^{-3}$, (B) 75 mg NaHCO$_3$ added to 252 g DW m$^{-3}$ and (C) 150 mg NaHCO$_3$ added to 459 g DW m$^{-3}$. Further the inorganic carbon evolution is depicted.

Although the experimental conditions were very similar, differences in optimized $K_L \alpha$ values were observed. This could be explained by the fact that there was a difference in the level of mixing between the different experiments. Indeed, by calculating the experimental oxygen mass transfer coefficient, more specifically by the descending limb of the respirometric profile, it was observed that there was also some difference in the experimental values for $K_L \alpha$. These experimental calculated values were respectively 33 d$^{-1}$, 21 d$^{-1}$ and 13 d$^{-1}$.
Chapter 4

Good model performance, i.e. fit to the experimental data, and similar values for the calibrated parameters for the different datasets were obtained. Also, based upon the TIC criterium, good model performance was noted, with a TIC equal to 0.05, 0.04 and 0.05 respectively. However, a calibrated model is only able to predict respirometer behavior provided that the experimental conditions are similar. Hence, model predictions should be interpreted with care when predicting effects outside the range of values for which it was validated (e.g. larger spiked amounts of carbon source) or for degrees of freedom that were not yet validated (e.g. light intensity, temperature). However, the model results are promising and form a solid base for future research in exploring microalgae system behavior and its optimization.

Table 4.2: Values of the optimized parameters and corresponding Theil’s Inequality Coefficient for each separate test. The measured data are shown in Figure 4.4

<table>
<thead>
<tr>
<th>Biomass (g m$^{-3}$)</th>
<th>Bicarbonate pulse (g m$^{-3}$)</th>
<th>$K_a$ (d$^{-1}$)</th>
<th>$\mu_{\text{max}}$ (d$^{-1}$)</th>
<th>TIC (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>458</td>
<td>150</td>
<td>26.79 ± 0.02</td>
<td>0.52 ± 0.008</td>
<td>0.05</td>
</tr>
<tr>
<td>252</td>
<td>75</td>
<td>19.44 ± 0.04</td>
<td>0.48 ± 0.012</td>
<td>0.04</td>
</tr>
<tr>
<td>235</td>
<td>75</td>
<td>15.84 ± 0.09</td>
<td>0.52 ± 0.015</td>
<td>0.05</td>
</tr>
</tbody>
</table>

4.4 Conclusions

In this chapter a combined respirometric-titrmetric set-up and protocol for microalgae was developed that allows determination of microalgae kinetic parameters, inspired by classic respirometry in the activated sludge process. Chlorella vulgaris was used as test organism, since the occurrence of this microalgal species in wastewater treatment systems is reported. Further, a lot of research on the kinetics of this species has already been performed (Kim et al., 2010). The recovery of the respirometric data appeared to be lower than stoichiometrically (Stumm and Morgan, 1996) expected. This is shown to be attributed to carbon dioxide stripping during the experiment. Since the experiments that were presented in this chapter were conducted with non-limiting conditions of inorganic nitrogen, inorganic phosphorus, light intensity and temperature, it would be very interesting to assess whether this technique can be used to determine the kinetics when limitation of these factors are imposed.
Further a simple model based on solely inorganic carbon limitation was built to describe the observed respirometric and titrimetric system behavior. Model calibration was performed by using the sum of squared errors between experimental and calculated respirometric values as objective function. Two parameters were optimized, respectively the maximum specific growth rate and the oxygen mass transfer coefficient. This resulted in good correspondence of this optimized parameters in case of three different experiments. Further good visual correspondence between experimental and calculated profiles was observed. However it would be very interesting to extend the model so it could be validated when different environmental conditions are imposed.
Chapter 5

Assessing the effect of environmental conditions on the microalgal growth rate

Redrafted from


Abstract

Due to their complex nature a lot of research is performed on microalgal systems for wastewater treatment. In this work a previously developed combined respirometric-titrimetric approach was used for the determination of microalgal kinetics. A Plackett-Burman design with fold over was executed to investigate the influence of 8 different degrees of freedom regarding environmental conditions on 5 different aspects (or responses) related to the microalgal photosynthetic activity.

Results revealed a correlation between microalgal biomass concentration and both oxygen production rate and proton addition rate. When ammonium limiting conditions were implemented (molar N/P ratio = 7/1), a decrease of the titrimetric profile was observed. Although it was expected based on literature no limitation regarding light intensity and temperature could be observed. Furthermore, with respect to inorganic phosphorus no limitation was observed. Thus it could be concluded that these state conditions were not limiting in the ranges applied in this research. Overall it can be concluded that the combined respirometric-titrimetric approach is successful for determination of limiting and non-limiting components regarding wastewater nutrients and environmental conditions for microalgal growth.

5.1 Introduction

Knowledge of the kinetic process is crucial to understand the metabolic reactions taking place in an algal system. Such knowledge is the starting point of model development and hence prediction of reactor performance. The respirometric and titrimetric tools were successfully translated to a microalgal based wastewater treatment when only inorganic carbon was considered as limiting for the microalgal growth as described in Chapter 4. However in the scope of mimicking natural water systems, in this chapter the ability to assess different influencing factors with a combined respirometric-titrimetric setup is discussed. Chlorella vulgaris is used as reference species and was grown on a synthetic wastewater. It should be emphasized that all separate experiments were performed under continuous illumination. The maximum duration time of the experiments was similar to the time that algae are exposed to light under natural conditions taking into account the dial cycle. As such it was assumed that the microalgae were not stressed by the continuous illumination. Extensions of the kinetic
Assessing the effect of environmental conditions on the microalgal growth rate

model taking into account inorganic carbon limitation and carbonaceous equilibrium are needed and are proposed.

5.2 Materials and methods

5.2.1 Experimental design

The microalgal strain used for the experiments was *Chlorella vulgaris* obtained from the Laboratory of Protistology and Aquatic Ecology (Ghent University). The microalgal species was bred akin as described in Chapter 4 and each separate test was performed according to the developed protocol.

To explore the influence of different degrees of freedom on the microalgal growth, a Placket-Burman design with fold over (Box and Draper, 1987) was used. This is a two level experimental design that allows determining the main effect of degrees of freedom (or factors) on a certain response, assuming that interactions between the factors are negligible. When the latter is not the case, a fold over design is used to uncouple the main effect of factors from factor aliases. This means that the main effects can be distinguished from the 2-way interaction effects (Beres and Hawkins, 2001). The degrees of freedom used in the experimental design and their corresponding levels are summarized in Table 5.1. These values for nitrogen and phosphorus were taken from literature (Valderrama et al. 2002; Kozlowska et al., 2000). The values for temperature and light intensity were borrowed from Bougaran and Rémond (2012) where the combined influence and light intensity on microalgal growth rate was investigated.

As a 2 level Placket-Burman design imposes a fixed number of degrees of freedom, namely eleven, three dummy variables had to be included in the experimental setup. Eventually 24 experiments were executed, each at different conditions (see Appendix A, Table A.1). Limitation was implemented regarding P and N by diverging from the Redfield ratio of 16/1 (Sterner et al., 2008). The influence of all aforementioned degrees of freedom was examined for four different responses, $\text{OPR}_{max}$ (g O$_2$ m$^{-3}$d$^{-1}$), $\text{HAR}$ (g H$^+$ m$^{-3}$d$^{-1}$), total amount of oxygen produced (g O$_2$ m$^{-3}$) and total amount of protons added (g H$^+$ m$^{-3}$).
Chapter 5

Considering the titrimetric output, the HAR equals the slope of the proton addition curve (or titrigram) until the bicarbonate is depleted and the total amount of protons added is also determined when the bicarbonate is depleted. At this point the proton addition curve reaches a plateau (see Chapter 4). Further, the oxygen recovery and the expected total amount of protons added (proton recovery) are calculated based on the amount of added bicarbonate and the algal stoichiometry (Stumm and Morgan, 1996). The relative difference between the actual amount and the theoretical amount of oxygen produced or protons consumed was defined as the recovery.

Next to these responses, also the deviation of the proton addition curve from ideal, i.e. non-limiting behavior, was determined. In case of limiting conditions, the assimilation rate of bicarbonate will be limited, resulting in a deflection from linearity of the proton addition curve. Quantification of the deflection was done by measuring the difference between the actual amount of protons added and the maximal amount of protons added at the inclination point of the theoretical proton addition curve. This latter curve is obtained by assuming that the initial proton addition rate is maintained until all inorganic carbon is consumed.

Table 5.1: Degrees of freedom and different level values used in the experimental design

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low value</th>
<th>High value</th>
<th>Mean Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$ (g N m$^{-3}$)</td>
<td>3.39</td>
<td>33.87</td>
<td>18.63</td>
</tr>
<tr>
<td>NH$_4^+$ (g N m$^{-3}$)</td>
<td>7.78</td>
<td>77.8</td>
<td>42.79</td>
</tr>
<tr>
<td>PO$_4^{3-}$ (g P m$^{-3}$)</td>
<td>3.33</td>
<td>20</td>
<td>11.67</td>
</tr>
<tr>
<td>DW (g m$^{-3}$)</td>
<td>100</td>
<td>500</td>
<td>300</td>
</tr>
<tr>
<td>HCO$_3^-$ (g m$^{-3}$)</td>
<td>100</td>
<td>500</td>
<td>300</td>
</tr>
<tr>
<td>T (K)</td>
<td>288</td>
<td>299</td>
<td>294</td>
</tr>
<tr>
<td>I (lux)</td>
<td>4875</td>
<td>9750</td>
<td>7313</td>
</tr>
<tr>
<td>pH (-)</td>
<td>6</td>
<td>7.5</td>
<td>6.75</td>
</tr>
</tbody>
</table>
5.2.2 Analytical methods

Initial and final nutrients concentration were measured for each separate test with Hach-Lange, respectively LCK 303 (ammonium), LCK 348 (phosphate) and LCK 340 (nitrate) (www.Hach-Lange.com). Microalgal biomass was measured according to standard methods and expressed as g DW m$^{-3}$ (APHA, 2005).

5.3 Results and discussion

5.3.1 Exploration of the influence of biomass concentration

To explore the influence of biomass concentration on the oxygen production, two experiments were compared, mentioned as run number 4 and run number 6* in appendix A.1. Also, the initial settings and calculated responses are summarized in Table 5.2. In Figure 5.1, both oxygen profiles are shown, indicating a major difference. In case of low biomass concentration, a microalgal adaptation period can be observed of approximately 0.5 days. This could be explained by the fact that low biomass concentration results in initially low photosynthetic activity. The maximum oxygen production rate in case of high biomass concentration was 115.6 g O$_2$ m$^{-3}$d$^{-1}$ and for low biomass concentration 46.6 g O$_2$ m$^{-3}$d$^{-1}$. The photosynthetic activity per unit of biomass concentration was 0.26 g O$_2$ g$^{-1}$DW d$^{-1}$ for the experiment at high concentration and 0.48 g O$_2$ g$^{-1}$DW d$^{-1}$ for the experiment at low concentration. The lower photosynthetic activity per unit of biomass concentration at high biomass concentration could be explained by the self-shading effect that occurs. Considering the complete set of experimental runs an increasing maximum oxygen production rate could be observed (Figure 5.2). As such, the biomass concentration can be considered to be influential with respect to the maximum oxygen production rate. For the proton addition rate a similar trend was observed as is also indicated in Figure 5.2.
Table 5.2: Initial settings and derived responses for the experiments used to assess the influence of microalgal biomass concentration

<table>
<thead>
<tr>
<th>Exp.</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>PO$_4^{3-}$</th>
<th>DW</th>
<th>HCO$_3^-$</th>
<th>T</th>
<th>I</th>
<th>pO$_2$ max</th>
</tr>
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<tr>
<td>run</td>
<td>g N m$^{-3}$</td>
<td>g N m$^{-3}$</td>
<td>g P m$^{-3}$</td>
<td>g m$^3$</td>
<td>g m$^3$</td>
<td>K</td>
<td>lux</td>
<td>g O$_2$ g$^{-1}$ DW d$^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>2.89</td>
<td>6.04</td>
<td>4.67</td>
<td>442</td>
<td>100</td>
<td>299</td>
<td>9750</td>
<td>0.26</td>
</tr>
<tr>
<td>6*</td>
<td>3.3</td>
<td>78.63</td>
<td>5.02</td>
<td>78</td>
<td>100</td>
<td>299</td>
<td>9750</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Figure 5.1: Comparison of respirometric profiles for experimental run number 4 and run number 6*. Initial biomass concentration was 442 g DW m$^{-3}$ (run 4) and 78 g DW m$^{-3}$ (run 6).

Figure 5.2: Maximum oxygen production rate (left hand) and proton addition rate (right hand) in relation to the microalgal biomass concentration.
5.3.2 Assessment of the influence of pH on the availability of inorganic carbon

Since in Chapter 4 the assumption was made that a certain amount of inorganic carbon was lost due to stripping, it was investigated more thoroughly. This was done by first adding the bicarbonate, before the nutrients and algal biomass were added. At a rather acid pH (e.g. pH = 6) the dosed bicarbonate is converted into carbon dioxide. The latter then transfers to the atmosphere by the driving forces, respectively $K_La$ and the difference in concentration between the aqueous phase and gaseous phase. Such a stripping effect results in a pH rise and as such to a proton addition by the experimental set-up to maintain the pH at the initial set-point. The resulting titrimetric profile (Figure 5.3) has a first steep linear part due to the mass transfer of carbon dioxide to the atmosphere. After this stripping the algal biomass was added to the reactor. For this specific experiment that is shown in Figure 5.3, the biomass was added when the pH stabilized at the initial set-point, which was here at $t = 0.03 \text{d}^{-1}$. The ratio of the amount of protons added in the first period (5.12 g H⁺m⁻³) to the total amount of protons added (8.11 g H⁺m⁻³) is 0.63. As such 63 % or 31.5 g m⁻³ of the bicarbonate at pH = 6 is stripped before the microalgal activity starts. This is in accordance with the fact that at pH = 6 about 65 % of the inorganic carbon is present in the form of carbon dioxide (Judd and Stephenson, 2002). At pH 7.5 and 6.75 the bicarbonate concentration equals respectively 95 % and 75 % of the amount of inorganic carbon dosed. Because of this inorganic carbon equilibrium, the actual amount of bicarbonate available for photosynthetic activity was corrected by the above mentioned numbers (65% at pH = 6; 75 % at pH = 6.75 and 95 % at pH = 7.5). After this correction, a very good correlation ($R^2 = 0.9$) between the amount of protons added and the amount of initial bicarbonate (after correction) could be observed. When no correction factor is applied a much worse correlation is obtained ($R^2 = 0.47$) (Figure 5.4). For an even better description of the pH effect also the influence on pH of ammonium and nitrate consumption should be taken into account, although it is expected that the inorganic carbon equilibrium has the main influence on pH (Eriksen et al., 2007).

These findings confirm that taking into account the pH equilibrium during model development and experimentation is a prerequisite.
Chapter 5

Figure 5.3: Illustration of the stripping effect at initial pH = 6. Initial concentration bicarbonate dosed was 500 g m\(^{-3}\). The algal biomass was added to the reactor at \( t = 0.03 \) d.

Figure 5.4: Total amount of protons added in function of the initial bicarbonate concentration (corrected for initial stripping (\(\Delta\)) and not corrected for initial stripping (\(\Diamond\))).

5.3.3 Influence of inorganic nitrogen limitation on oxygen production and proton addition

In order to investigate the effect of inorganic nitrogen limitation on both experimental profiles, an experiment with both nitrate and ammonium limitation (run number 3) and an experiment with only ammonium limitation (run number 3*) were compared. In Table 5.3, the initial settings and different responses derived from the experimental profiles are summarized. In Figure 5.5 the corresponding respirometric and titrimetric profiles are illustrated.
Table 5.3: Initial settings and derived responses for the experiments used to assess the influence of nitrogen limitation

<table>
<thead>
<tr>
<th>Exp Run</th>
<th>NO$_3^-$ (g N m$^{-3}$)</th>
<th>NH$_4^+$ (g N m$^{-3}$)</th>
<th>PO$_4^{3-}$ (g P m$^{-3}$)</th>
<th>DW (g DW m$^{-3}$)</th>
<th>HCO$_3^-$ (g m$^{-3}$)</th>
<th>T (K)</th>
<th>I (lux)</th>
<th>pO$_2$ max (g O$_2$ g$^{-1}$ DW d$^{-1}$)</th>
<th>HAR (g H$^+$ m$^{-3}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>5.28</td>
<td>6.77</td>
<td>3.73</td>
<td>442</td>
<td>500</td>
<td>299</td>
<td>9750</td>
<td>0.17</td>
<td>2.91</td>
</tr>
<tr>
<td>3*</td>
<td>3.55</td>
<td>9.33</td>
<td>3.28</td>
<td>495</td>
<td>100</td>
<td>299</td>
<td>9750</td>
<td>0.41</td>
<td>11.68</td>
</tr>
</tbody>
</table>

In the first run, nitrogen limitation was evident as the initial total nitrogen concentration equals 12.1 g N m$^{-3}$ (5.3 g NO$_3^-$ -N m$^{-3}$ and 6.8 g NH$_4^+$-N m$^{-3}$) and the corresponding molar N/P ratio amounts 7/1. As a result, nitrate and ammonium are almost depleted at the end of the experiment (0.2 g NO$_3^-$ -N m$^{-3}$ and 0.3 g NH$_4^+$-N m$^{-3}$). Considering the second run (indicated with full line), no nitrogen limitation could be observed, because the nitrate removal is only 60% (initial concentration was 3.5 g NO$_3^-$ -N m$^{-3}$ and final concentration was 1.4 g NO$_3^-$ -N m$^{-3}$), although the ammonium was depleted at the end of the experiment (initial concentration was 9.3 g NH$_4^+$ -N m$^{-3}$ and final concentration was 0.02 g NH$_4^+$-N m$^{-3}$). The respirometric profiles illustrate a maximum oxygen concentration at approximately the same time (i.e. after 0.5 days). However, in case of both nitrate and ammonium limitation, the plateau in the profile is less extended and the dissolved oxygen concentration starts to decrease earlier. Also the OPRmax in case of only ammonium limitation is higher, respectively 207 g O$_2$ m$^{-3}$ d$^{-1}$ compared to 91 g O$_2$ m$^{-3}$ d$^{-1}$. The total amount of oxygen produced is also higher in case of only ammonium limitation (72.6 g O$_2$ m$^{-3}$ vs. 44.2 g O$_2$ m$^{-3}$).

With respect to the titrimetric profiles, a larger deviation from the theoretically expected curve in case both nitrogen species are limiting can be observed. For the experiment with only ammonium limitation, this deviation was 0.2 g H$^+$ m$^{-3}$ compared to 3.05 g H$^+$ m$^{-3}$. Concerning the proton addition rate, a difference could also be noticed, namely 2.2 g H$^+$ m$^{-3}$ d$^{-1}$ when both nitrate and ammonium are limiting compared to 11.7 g H$^+$ m$^{-3}$ d$^{-1}$ in case only ammonium is limiting. As due to this limitation, the proton addition caused by the consumption of inorganic carbon is limited.
5.3.4 Preferential uptake of inorganic nitrogen species

Analysis of inorganic nitrogen species, respectively nitrate and ammonium in the beginning and end of each experiment showed in general a preferential uptake of ammonium, which is also mentioned in literature (Schuler et al., 1952). Furthermore, an increased uptake of nitrate was observed, in case the initial ammonium concentration was low. This could be explained by the fact that when ammonium becomes limiting the microalgae start to use the other inorganic nitrogen species (Bienfang, 1975). Overall the ammonium uptake was 51% compared to 34% nitrate uptake.

In order to confirm the preferential uptake of ammonium compared to nitrate a paired samples t-test was performed with the available nitrogen uptake data (24 experiments). This resulted in a p-value of 0.006 indicating that the hypothesis that the ammonium removal is significantly higher than the nitrate removal is confirmed. As such an inhibition factor for growth on nitrate should be included in the respective kinetic equation that could be denoted as:

$$f_i^{NO_3^-} = \frac{K_i^{NH_4^+}}{S_{NH_4^+} + K_i^{NH_4^+}}$$

(5.1)

With $K_i^{NH_4^+}$ (g N m$^{-3}$) the inhibition parameter and $S_{NH_4^+}$ (g N m$^{-3}$) the dissolved ammonium concentration.
Assessing the effect of environmental conditions on the microalgal growth rate

The growth kinetics related to nitrate then becomes:

$$\rho_{Alg, NO_3^-} = \mu_{max} \left( \frac{S_{NO_3^-}}{K_{NO_3^-} + S_{NO_3^-}} \right) \left( \frac{K_i NH_4^+}{K_i NH_4^+ + S_{NH_4^+}} \right) X_{Alg}$$  (5.2)

With $S_{NO_3^-}$ (g N m$^{-3}$) the ambient nitrate concentration and $K_{NO_3^-}$ (g N m$^{-3}$) the half saturation coefficient for nitrate.

The growth kinetics related to ammonium can be denoted as:

$$\rho_{Alg, NH_4^+} = \mu_{max} \left( \frac{S_{NH_4^+}}{K_{NH_4^+} + S_{NH_4^+}} \right) X_{Alg}$$  (5.3)

With $S_{NH_4^+}$ (g N m$^{-3}$) the ambient ammonium concentration and $K_{NH_4^+}$ (g N m$^{-3}$) the half saturation coefficient for ammonium.

### 5.3.5 Influence of inorganic phosphorus on oxygen and proton addition

Regarding the effect of inorganic phosphorus, two experimental runs were compared. One experimental run was assumed to be phosphorus limited (run number 21, see Table A1 in the appendix), as the total initial nitrogen concentration equaled 66.9 g N m$^{-3}$ and initial phosphorus concentration was 6.17 g P m$^{-3}$. As such the molar N/P ratio equals approximately 25/1. For the second experimental run (number 22) the molar N/P ratio equaled 11/1. In Table 5.4, the initial settings and responses derived from the experimental profiles are tabulated. Concerning the respirometric results, the experiment with phosphorus limitation had an $OPR_{max}$ of 38 g O$_2$ m$^{-3}$ d$^{-1}$ whereas for the run without phosphorus limitation the $OPR_{max}$ equaled 74 g O$_2$ m$^{-3}$ d$^{-1}$. However, this difference cannot be explained by the effect of phosphorus limitation alone, because other parameters can play a role. In particular the algal biomass concentration is highly correlated to $OPR_{max}$. Values of respectively 0.14 g O$_2$ g$^{-1}$ DW d$^{-1}$ for the experiment without limitation and 0.09 g O$_2$ g$^{-1}$ DW d$^{-1}$ for the experiment with limitation were obtained when calculating the photosynthetic activity. In addition, it should be stressed that the difference in $OPR_{max}$ is not influenced by the difference in amount of bicarbonate that
was dosed. For both discussed experiments, the inorganic carbon is not limiting at the beginning of each separate experiment. So this cannot be the reason for the difference in $OPR_{max}$ that was observed. Hence, this strengthens the assumption that phosphorus limitation could potentially have an influence on the oxygen production. The titrimetric profiles showed a slight deviation in case of phosphorus limitation compared to when no limitation occurs. However with respect to the total amount of experiments executed no significant influence of phosphorus limitation was observed. Nevertheless for future modeling purposes a function describing phosphorus should be included. In order to describe this dependency on the ambient phosphorus concentration a Monod - equation can be used:

$$\rho_{Alg, PO_4^{3-}} = \mu_{max} \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) X_{Alg}$$  \hspace{1cm} (5.4)

With $S_{PO_4^{3-}}$ (g P m$^{-3}$) the ambient phosphate concentration and $K_{PO_4^{3-}}$ (g P m$^{-3}$) the half saturation coefficient for phosphate.

**Table 5.4: Initial settings and derived responses for the experiments used to assess the influence of phosphorus limitation**

<table>
<thead>
<tr>
<th>Exp</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>PO$_4^{3-}$</th>
<th>DW</th>
<th>HCO$_3^-$</th>
<th>T</th>
<th>I</th>
<th>pO$_2$ max</th>
<th>HAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run</td>
<td>(g N m$^{-3}$)</td>
<td>(g N m$^{-3}$)</td>
<td>(g P m$^{-3}$)</td>
<td>(g DW m$^{-3}$)</td>
<td>(g m$^{-3}$)</td>
<td>(K)</td>
<td>(lux)</td>
<td>(g O$_2$ g$^{-1}$ DW d$^{-1}$)</td>
<td>(g H$^+$ m$^{-3}$ d$^{-1}$)</td>
</tr>
<tr>
<td>21</td>
<td>3.59</td>
<td>63.31</td>
<td>6.17</td>
<td>486</td>
<td>500</td>
<td>299</td>
<td>4875</td>
<td>0.09</td>
<td>6.56</td>
</tr>
<tr>
<td>22</td>
<td>29.69</td>
<td>8.75</td>
<td>21.23</td>
<td>420</td>
<td>100</td>
<td>299</td>
<td>4875</td>
<td>0.14</td>
<td>5.85</td>
</tr>
</tbody>
</table>

5.3.6 Influence of temperature, light intensity on oxygen production and proton addition

To investigate the influence of temperature and light intensity on the oxygen production, two experimental runs were compared, respectively runs 23 and 5 (see Table A1 in the appendix). Initial nutrient concentrations, that are additionally mentioned in Table 5.5, were considered as non–limiting. Due to the fact that the microalgal breeding reactor was kept at ambient room temperature and adaptation to temperature changes occurs, the microalgae have a lower oxygen
Assessing the effect of environmental conditions on the microalgal growth rate

production rate at 288 K and I = 4875 lux compared to the experiment at T = 299 K. The photosynthetic activity however was very similar at 299 K (0.14 g O₂ g⁻¹ DW d⁻¹) and 288 K (0.15 g O₂ g⁻¹ DW d⁻¹). With respect to the oxygen recovery respective values of 41 % and 56 % were calculated. As such it can be concluded that no significant influence of temperature and light intensity on the oxygen production could be observed in the ranges applied, although it was expected based on literature (Bernard and Rémond, 2012). In Chapter 8, the influence of temperature and light intensity was investigated with a broader range of the light intensity and temperature. Also, experimental run number 5 has basically the same conditions than the run analyzed in Chapter 4 regarding the amount of bicarbonate dosed, biomass concentration, temperature, light and no limitation of nutrients. However, there are significant differences in the results calculated from the respirometric profile. More specifically, the maximum oxygen production rate is 250 g O₂ m⁻³ d⁻¹ in Chapter 4, compared to run 58 g O₂ m⁻³ d⁻¹ in experimental run number 5. This difference could potentially be explained by the fact that for these experiments different microalgal biomass was used. Moreover, there was a large time span between the experiments conducted in Chapter 4 and in this chapter. Possibly the microalgal biomass that was held in the exponential growth fase during the period in Chapter 4 was somewhat more active than the biomass used in this fase.

Table 5.5: Initial settings and derived responses for the experiments used to assess the influence of temperature and light intensity

<table>
<thead>
<tr>
<th>Exp Run</th>
<th>NO₃⁻ (g N m⁻³)</th>
<th>NH₄⁺ (g N m⁻³)</th>
<th>PO₄³⁻ (g P m⁻³)</th>
<th>DW (g DW m⁻³)</th>
<th>HCO₃⁻ (g m⁻³)</th>
<th>T (K)</th>
<th>I (lux)</th>
<th>pO₂ max (g O₂ g⁻¹ DW d⁻¹)</th>
<th>HAR (g H⁺ m⁻³ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.4</td>
<td>112</td>
<td>17.9</td>
<td>402</td>
<td>100</td>
<td>288</td>
<td>4875</td>
<td>0.15</td>
<td>3.00</td>
</tr>
<tr>
<td>12*</td>
<td>23.7</td>
<td>6.85</td>
<td>4.6</td>
<td>76</td>
<td>500</td>
<td>299</td>
<td>9750</td>
<td>0.79</td>
<td>5.34</td>
</tr>
<tr>
<td>18</td>
<td>28</td>
<td>10.5</td>
<td>22.8</td>
<td>378</td>
<td>500</td>
<td>288</td>
<td>4875</td>
<td>0.17</td>
<td>7.29</td>
</tr>
<tr>
<td>23</td>
<td>2.87</td>
<td>8.13</td>
<td>3.33</td>
<td>646</td>
<td>100</td>
<td>299</td>
<td>9750</td>
<td>0.17</td>
<td>NA</td>
</tr>
</tbody>
</table>

Concerning the influence of temperature and light intensity on the titrimetric profile, two other experiments were compared (run number 18 and 12* respectively, see Table A.1 in the appendix A and Table 5.5). No large difference in the titrimetric values was observed. The little difference
in total amount of protons added, respectively 3.73 g H⁺m⁻³ and 3.46 g H⁺m⁻³, is explained by
the small difference in initial pH when biomass and nutrients were dosed to the reactor.
Contrary to literature (Bernard and Rémond, 2012) no significant influence of temperature was
observed in the ranges applied, suggesting that in future other ranges should be investigated,
although again it was expected based on literature. Considering the total amount of experiments
performed, no significant effect of temperature and light intensity on the respirometric and
titrimetric profile could be observed. This was not really expected and could be explained by
the limits of the experimental design that was used. The fold over technique is specific for
determining the main effects on the microalgal growth rate. It does not take into account
possible interactions. However, by changing all the degrees of freedom, it was not possible to
observe the effect of only temperature or light intensity. Thus, at this stage of the research the
possible interactions were likely underestimated. In case an effect of temperature and light
intensity would have been noticed, then different models for describing this effect are available
in literature (Epply and Sloan, 1966; Bernard and Rémond, 2012).

### 5.3.7 Overall kinetic expression

Considering all above findings, an overall kinetic expression can now be proposed to describe
the microalgal growth depending on nutrient concentration, respectively inorganic carbon,
inorganic nitrogen and inorganic phosphorus. As mentioned before, a distinction should be
made between the growth on bicarbonate and carbon dioxide with respect to inorganic carbon.
Also a distinction should be made between nitrate and ammonium for inorganic nitrogen
species. As such following equations are proposed where Equation (5.5) and Equation (5.6)
describe the growth when carbon dioxide and one of the inorganic nitrogen species is used.
Equation (5.7) and Equation (5.8) describe the growth in case of bicarbonate assimilation with
one of the inorganic nitrogen species used.

\[
\rho_{\text{Alg}}(CO_2,NH_4^+,P,O_4^{3-}) = \mu_{\text{max}} \times \left( \frac{S_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{S_{NH_4^+}}{K_{NH_4^+} + S_{NH_4^+}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) X_{\text{Alg}}
\] (5.5)
Assessing the effect of environmental conditions on the microalgal growth rate

\[ \rho_{Alg}(CO_2, NO_3^-, PO_4^{3-}) = \mu_{max} \left( \frac{S_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{S_{NO_3^-}}{K_{NO_3^-} + S_{NO_3^-}} \right) \left( \frac{K_{NH_4^+}}{K_{NH_4^+} + S_{NH_4^+}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) X_{Alg} \]  

(5.6)

\[ \rho_{Alg}(HCO_3^-, NH_4^+, PO_4^{3-}) = \mu_{max} \left( \frac{S_{HCO_3^-}}{K_{HCO_3^-} + S_{HCO_3^-}} \right) \left( \frac{K_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{S_{NH_4^+}}{K_{NH_4^+} + S_{NH_4^+}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) X_{Alg} \]  

(5.7)

\[ \rho_{Alg}(HCO_3^-, NO_3^-, PO_4^{3-}) = \mu_{max} \left( \frac{S_{HCO_3^-}}{K_{HCO_3^-} + S_{HCO_3^-}} \right) \left( \frac{K_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{S_{NO_3^-}}{K_{NO_3^-} + S_{NO_3^-}} \right) \left( \frac{K_{NH_4^+}}{K_{NH_4^+} + S_{NH_4^+}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) X_{Alg} \]  

(5.8)

5.4 Conclusions

Combined respirometric-titrimetric responses of green microalgae were evaluated to assess the effect of inorganic carbon assimilation and the effect of the degrees of freedom influencing the microalgal growth on these responses. The results indicate that this is a successful methodology for microalgal systems, however it should be stressed that both profiles, respectively the respirometric and titrimetric profile should be investigated intensively for optimal information extraction. This is especially true in cases where limitation occurs which could not be observed by the respirometric profile, but clearly by the deflection of the titrimetric profile. Moreover, a preferential uptake of ammonium with respect to inorganic nitrogen was observed. No influence of light intensity, temperature and inorganic phosphorus could be observed in the ranges applied. This might suggest that further research is needed considering a lower concentration range of inorganic phosphorus. With respect to light intensity and temperature a stable growth rate could be concluded in the ranges applied. Considering the total amount of experiments performed, no significant effect of temperature and light intensity on the respirometric and titrimetric profile could be observed. This could be explained by limited experimental design that was used. The fold over technique is specific for determining the main effects on the microalgal growth rate. It does not take into account possible interactions. However, by changing all the degrees of freedom, it was not possible to observe the effect of only temperature or light intensity. Thus, at this stage of the research the possible interactions were
underestimated. In Chapter 8, the effect of light intensity and temperature was investigated more thoroughly.

Finally, an overall kinetic expression is proposed to describe the microalgal growth function of inorganic nitrogen, inorganic phosphorus and inorganic carbon. This kinetic expression will be used in Chapter 6
Chapter 6

Assessment of nutrient removal by microalgal biomass

Chapter 6

Abstract

Despite strict legislation, nowadays large amounts of nutrients are sometimes discharged in natural water systems by e.g. industry and agriculture. The impact of nutrients such as nitrogen and phosphorus can be harmful for the receiving water body. Hence, these nutrients need to be removed prior to discharge. As alternative for conventional wastewater treatment systems, microalgal systems have great potential for extensive nutrient removal. In this chapter a model-based approach was followed to describe nutrient removal by means of microalgae. The model was calibrated and validated with combined respirometric and titrimetric data. A global sensitivity analysis indicated the large influence of the maximum specific algal growth rate \( \mu_{\text{max}} \) and the oxygen mass transfer coefficient \( K_L a \). A parameter identifiability assessment illustrated that only these parameters could be used for accurate parameter calibration based on the available combined respirometric and titrimetric data. An optimisation using seven independent data sets resulted in \( \mu_{\text{max}} = 0.254 \pm 0.096 \text{ d}^{-1} \) and \( K_L a = 7.11 \pm 4.29 \text{ d}^{-1} \). Model validation with 2 additional data sets resulted in a Theil’s Inequality Coefficient (TIC) of 0.07 and 0.08 for the combined respirometric and titrimetric model output indicating good model predictive performance. Furthermore, good predictions of final concentration of dissolved inorganic nitrogen species, algal biomass and inorganic phosphorus were observed.

6.1 Introduction

Microalgal systems show great potential for extensive nutrient removal in wastewater treatment. Moreover in combination with bacterial biomass residing in activated sludge it can overcome the drawback of the high aeration cost of a conventional denitrifying and nitrifying system since the microalgae produce oxygen through photosynthetic activity (Van Den Hende et al., 2011). Microalgal systems are eco-friendly and offer the opportunity of cost-effective nutrient removal and biomass production. However algal separation is a concern in view of full scale application (Van Damme et al., 2013). Furthermore the produced microalgal biomass has different possibilities for valorisation such as energy source, fertilizer, biobased chemicals and feedstock for animals (Vilchez et al., 1997; Mulbry et al., 2008). However, microalgal systems
Assessment of nutrient removal by microalgal biomass

are in general more complex compared to conventional techniques given that its performance depends on various local environmental conditions such as light intensity, pH, temperature and availability of nutrients. As such it is of high interest to operate these wastewater treatment systems under optimal conditions in order to obtain optimal system performance.

With respect to the latter, mathematical models offer an efficient way to find the optimal settings for these conditions. However, accurate representation of algal growth is one of most difficult and poorly understood areas in water quality modelling. Algal growth is inherently complex, in general showing nonlinear responses to various environmental parameters such as temperature, light and several nutrients, as well as demonstrating poorly understood interactions among these separate factors (Bowie et al., 1985; Thomann and Mueller, 1987). Site-specificity also makes extrapolation from lab or other field studies inherently problematic. Furthermore, due to the fact that a diverse multi-species algal community is residing in natural systems it is difficult to assign a parameter value from literature which is mainly dedicated to a pure microalgal culture. As such accurately describing the microalgal kinetic growth kinetics remains a challenge. Several models for algal growth have been described in literature. Some of the models are focusing on a single environmental factor (Filali et al., 2011; Ogbanna et al., 1995). Other models combine an extensive set of causal hypotheses based on current understanding of how processes work, leading to more complex and detailed process descriptions (Coppens, 2016). However, more complex model structures are prone to identifiability problems given the scarce available experimental observations. This makes them difficult to calibrate and decreases their predictive power. Hence, for these models it is very important to assess the identifiability of the parameters, given a set of experimental data (Brun et al., 2001).

In this chapter an extended microalgae model is presented including nutrient and inorganic carbon removal, hereby balancing model prediction accuracy and complexity. The data used for model calibration and validation are combined respirometric-titrimetric data. Compared to Chapter 4, an extended approach is presented, since in the latter only the respirometric data were used for model calibration and furthermore the microalgae model is extended with nitrogen and phosphorus kinetics, calibrated and validated. A global sensitivity analysis is performed to identify the model parameters with the most influence on the model outputs. The identifiability of these parameters was assessed and subsequently the model was calibrated and validated.
Chapter 6

6.2 Materials and methods

6.2.1 Experimental data collection

Not all the different experimental runs mentioned in Chapter 5 were used in this topic. Two main criteria were implemented for selection of suitable data. First, the expected theoretical amount of protons added, based on the amount of initial bicarbonate and algal stoichiometry (Stumm and Morgan, 1996), was compared against the experimental amount of protons added. For this a cut–off value of 25 % was implemented. This means that experiments with relative deviations between expected and measured amount of protons of more than 25 % were not considered as suitable. Since the principal aim is to develop a microalgal growth model with a general approach for green microalgae the experimental N/P ratio (mol N/mol P) of the nutrient removal against the Redfield ratio was compared, assuming that all nitrogen and phosphorus is removed by assimilation. A threshold value N/P = 16 was used. Moreover in case of Chlorella vulgaris the optimal N/P is similar to the Redfield Ratio (Mandalam and Palsson, 1998).

6.2.2 Model development

Compared to the model presented in Chapter 4, four additional derived states were included, respectively dissolved nitrate, dissolved ammonium, dissolved phosphate and cumulative dissolved proton concentration. The proton addition rate is mainly effected by the inorganic carbon equilibrium and the stripping of carbon dioxide to the atmosphere. Although the uptake of nitrogen sources also influences the pH, preliminary model simulations taking into account the effect of nitrogen equilibria have shown that the effect of the latter is much smaller compared to the influence of the inorganic carbon equilibria. Hence, the mass balance equation for proton addition was limited to:

\[
\frac{dH^+}{dt} = -0.016 \rho_{dissoc} - 0.016 \rho_{hydrat}
\]  

(6.1)
Considering the microalgal growth rates on different inorganic carbon sources and nutrients, the proposed equations of Chapter 5, respectively Equations (5.5) to (5.8) will be implemented. As such the dynamic mass balance equations for nutrients can be denoted as:

\[
\frac{dNH_4^+}{dt} = -\frac{1}{Y_4} \rho_{Alg}(CO_2,NH_4^+,PO_4^{3-}) - \frac{1}{Y_4} \rho_{Alg}(HCO_3^-,NH_4^+,PO_4^{3-})
\]

\[
\frac{dNO_3^-}{dt} = -\frac{1}{Y_4} \rho_{Alg}(CO_2,NO_3^-,PO_4^{3-}) - \frac{1}{Y_4} \rho_{Alg}(HCO_3^-,NO_3^-,PO_4^{3-})
\]

\[
\frac{dPO_4^{3-}}{dt} = -\frac{1}{Y_5} \rho_{Alg}(CO_2,NH_4^+,PO_4^{3-}) - \frac{1}{Y_5} \rho_{Alg}(HCO_3^-,NH_4^+,PO_4^{3-}) - \frac{1}{Y_5} \rho_{Alg}(CO_2,NO_3^-,PO_4^{3-})
\]

With \(Y_4\) (g DW g\(^{-1}\) N) and \(Y_5\) (g DW g\(^{-1}\) P) the yield coefficients for growth on nitrogen (nitrate and ammonium) and phosphorus respectively.

The specific biokinetic processes and physical-chemical processes included in the model, which will be further on referred as the “Algnut” model, are provided in Table 6.1. This table is an extension of the Gujer matrix presented in Chapter 4, based on the experimental results that are discussed in Chapter 5.

### 6.2.3 Model parameter values

As can be deduced from Equations (2.5), (2.6), (6.5) and (6.6) the amount of oxygen produced by the photosynthetic activity of the microalgae depends on the inorganic nitrogen source that is used. As such the oxygen production yield in case of nitrate \((Y_7)\) was set at 1.26 g O\(_2\) g DW\(^{-1}\) and in case of ammonium \((Y_6)\) was set at 0.96 g O\(_2\) g DW\(^{-1}\).

\[
CO_2 + H_2O + 0.15 NH_4^+ \rightarrow CH_2O(NH_3)_{0.15} + O_2 + 0.15H^+
\]

\[
106HCO_3^- + 16H_2O + 16NH_4^+ + 92H^+ + HPO_4^{2-} \leftrightarrow C_{106}H_{263}O_{110}N_{16}P + 106O_2
\]
The yield coefficients for growth on inorganic nitrogen and inorganic phosphate were calculated based on the microalgal biomass composition (Stumm and Morgan, 1996) and were respectively 15.84 g DW g⁻¹ N and 110.93 g DW g⁻¹ P. For the half saturation coefficients for growth on inorganic nitrogen species, respectively ammonium and nitrate, and for growth on inorganic phosphorus values were adopted from literature, respectively $K_{NO_3^-} = 0.3$ g N m⁻³ (Tyrell, 1994), $K_{NH_4^+} = 0.3$ g N m⁻³ (Tyrell, 1994) and $K_{PO_4^{3-}} = 0.08$ g P m⁻³ (Moreno-Grau et al., 1996). The inhibition coefficient for growth on nitrate $K_i NH_4^+$ was set at 0.03 mg N m⁻³ (Dortch et al., 1990). Other parameter values were akin to those mentioned in Chapter 4. An overview of all parameters is given in Table 6.2.

---

**Chapter 6**

**Table 6.1: Gujer matrix of the “Algnut” model accounting with nutrient kinetics**

<table>
<thead>
<tr>
<th>Process</th>
<th>$S_{HCO_3^-}$</th>
<th>$S_{CO_2}$</th>
<th>$S_{CO_2^2-}$</th>
<th>$S_{O_2}$</th>
<th>$S_{NO_3^-}$</th>
<th>$S_{PO_4^{3-}}$</th>
<th>$S_{NH_4^+}$</th>
<th>$S_{H^+}$</th>
<th>$X_{Alg}$</th>
<th>Process rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on HCO₃⁻, NO₃⁻, PO₄³⁻</td>
<td>-1 $\frac{1}{Y_1}$</td>
<td>$Y_7$</td>
<td>-1 $\frac{1}{Y_4}$</td>
<td>-1 $\frac{1}{Y_5}$</td>
<td>1</td>
<td>$\rho_{Alg}(HCO_3^-,NO_3^-,PO_4^{3-})$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth on HCO₃⁻, NH₄⁺, PO₄³⁻</td>
<td>-1 $\frac{1}{Y_1}$</td>
<td>$Y_6$</td>
<td>-1 $\frac{1}{Y_5}$</td>
<td>-1 $\frac{1}{Y_4}$</td>
<td>1</td>
<td>$\rho_{Alg}(HCO_3^-,NH_4^+,PO_4^{3-})$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth on CO₂, NO₃⁻, PO₄³⁻</td>
<td>-1 $\frac{1}{Y_2}$</td>
<td>$Y_7$</td>
<td>-1 $\frac{1}{Y_4}$</td>
<td>-1 $\frac{1}{Y_5}$</td>
<td>1</td>
<td>$\rho_{Alg}(CO_2,NO_3^-,PO_4^{3-})$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth on CO₂, NH₄⁺, PO₄³⁻</td>
<td>-1 $\frac{1}{Y_2}$</td>
<td>$Y_6$</td>
<td>-1 $\frac{1}{Y_5}$</td>
<td>-1 $\frac{1}{Y_4}$</td>
<td>1</td>
<td>$\rho_{Alg}(CO_2,NH_4^+,PO_4^{3-})$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decay</td>
<td>-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{decay}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfer O₂</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{O_2,trans}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfer CO₂</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{CO_2,trans}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydration</td>
<td>1</td>
<td>-0.72</td>
<td></td>
<td>-0.016</td>
<td></td>
<td>$\rho_{hydrat}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissociation HCO₃⁻</td>
<td>-1</td>
<td>0.98</td>
<td></td>
<td>-0.016</td>
<td></td>
<td>$\rho_{dissoc}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiration</td>
<td>-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\rho_{resp}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6.2.4 Global sensitivity analysis

In order to assess the contribution of model parameters to the variation in the model output, a global sensitivity analysis was performed. Here all parameters are varied simultaneously over a predefined range (Table 6.3) in the parameter space (Neuman et al., 2009). For this, the Flexible Modelling Environment (FME) package of R was used. Since the probability density function (PDF) of each parameter was not known, a uniform distribution was assumed (Audenaert et al., 2013). Furthermore, a Latin Hypercube Sampling was used for the Monte Carlo simulations (Audenaert et al., 2013). At first several Monte Carlo runs were executed with respectively 500, 1000, 2000, 5000 and 10000 simulations in order to assess the amount of simulations needed for good data interpretation. Then a value for dissolved oxygen concentration and proton addition was taken at \( t = 0.2 \) d. At this point the dissolved oxygen concentration was still increasing due to algal activity, before depletion of the limiting nutrient. The resulting variability in the model output was then analyzed using a linear regression in SPSS (IBM, Armonck, NY USA) which resulted in regression coefficients that are an indication of the linear dependency between output variables and parameters.

Table 6.2: Parameter values used for the simulations with the Algnut model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Assigned value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{\text{max}} )</td>
<td>Max. specific growth rate</td>
<td>*</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>( b_{\text{max}} )</td>
<td>Max. decay rate</td>
<td>0.01</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>( r_{\text{max}} )</td>
<td>Max. respiration rate</td>
<td>0.01</td>
<td>d(^{-1})</td>
</tr>
<tr>
<td>( Y_1 )</td>
<td>Growth yield on HCO(_3^-)</td>
<td>0.549</td>
<td>g DW g HCO(_3^-) (^{-1})</td>
</tr>
<tr>
<td>( Y_2 )</td>
<td>Growth yield on CO(_2)</td>
<td>0.761</td>
<td>g DW g CO(_2) (^{-1})</td>
</tr>
</tbody>
</table>
### Table 6.2: Parameter values used for the simulations with Algnut model (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Assigned value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_4$</td>
<td>Growth yield on N</td>
<td>15.84</td>
<td>g DW g⁻¹ N</td>
</tr>
<tr>
<td>$Y_5$</td>
<td>Growth yield on P</td>
<td>110.93</td>
<td>g DW g⁻¹ P</td>
</tr>
<tr>
<td>$Y_6$</td>
<td>O₂ production on growth on NO₃⁻</td>
<td>1.24</td>
<td>g O₂ g⁻¹ DW</td>
</tr>
<tr>
<td>$Y_7$</td>
<td>O₂ production on growth on NH₄⁺</td>
<td>0.96</td>
<td>g O₂ g⁻¹ DW</td>
</tr>
<tr>
<td>$K_{HCO_3^-}$</td>
<td>Half saturation coefficient for HCO₃⁻</td>
<td>3</td>
<td>g HCO₃⁻ m⁻³</td>
</tr>
<tr>
<td>$K_{CO_2}$</td>
<td>Half saturation coefficient for CO₂</td>
<td>0.2</td>
<td>g CO₂ m⁻³</td>
</tr>
<tr>
<td>$k_1$</td>
<td>CO₂ hydratation rate</td>
<td>100000</td>
<td>d⁻¹</td>
</tr>
<tr>
<td>$k_2$</td>
<td>CO₂ dissociation rate</td>
<td>10000</td>
<td>d⁻¹</td>
</tr>
<tr>
<td>$pK_{a1}$</td>
<td>Acidity constant</td>
<td>6.36</td>
<td>-</td>
</tr>
<tr>
<td>$pK_{a2}$</td>
<td>Acidity constant</td>
<td>10.33</td>
<td>-</td>
</tr>
<tr>
<td>$K_{NH_4^+}$</td>
<td>Half saturation coefficient for NH₄⁺</td>
<td>0.02</td>
<td>g N m⁻³</td>
</tr>
</tbody>
</table>
Table 6.2: Parameter values used for the simulations with Algnut model (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Assigned value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_i NH_4^+$</td>
<td>Inhibition constant for growth on NO$_3^-$</td>
<td>0.02</td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$K_{NO_3^-}$</td>
<td>Half saturation coefficient for NO$_3^-$</td>
<td>0.02</td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$K_{PO_4^{3-}}$</td>
<td>Half saturation coefficient for PO$_4^{3-}$</td>
<td>0.0083</td>
<td>g P m$^{-3}$</td>
</tr>
<tr>
<td>$K_i CO_2$</td>
<td>Inhibition constant for growth on HCO$_3^-$</td>
<td>0.044</td>
<td>g CO$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_L a$</td>
<td>Oxygen mass transfer coefficient</td>
<td>*</td>
<td>d$^{-1}$</td>
</tr>
</tbody>
</table>

Table 6.3: Parameter ranges applied for the Monte Carlo simulation

<table>
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<tr>
<th>Parameter</th>
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<th>Upper bound</th>
<th>Unit</th>
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<tr>
<td>$\mu_{max}$</td>
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<td>2</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$K_L a$</td>
<td>5</td>
<td>25</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$K_{O_2}$</td>
<td>0.1</td>
<td>10</td>
<td>g O$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_{HCO_3^-}$</td>
<td>0.1</td>
<td>20</td>
<td>g HCO$_3^-$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_{CO_2}$</td>
<td>0.1</td>
<td>5</td>
<td>g CO$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_{NO_3^-}$</td>
<td>0.001</td>
<td>15</td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$K_{NH_4^+}$</td>
<td>0.001</td>
<td>15</td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$K_{PO_4^{3-}}$</td>
<td>0.0001</td>
<td>2</td>
<td>g P m$^{-3}$</td>
</tr>
<tr>
<td>$K_i CO_2$</td>
<td>0.001</td>
<td>5</td>
<td>g CO$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_i NH_4^+$</td>
<td>0.001</td>
<td>15</td>
<td>g N m$^{-3}$</td>
</tr>
</tbody>
</table>
6.3 Results and discussion

6.3.1 Data selection

In Table 6.4, seven experiments are summarized. In this table the initial conditions of each experiment, the difference between experimental and theoretical proton addition, and the molar N/P ratio of removed nutrients are mentioned. These experimental runs were used for model calibration. Further, the optimized parameter values and calculated TIC are summarized. All these values will be discussed below.

It should be stressed that considering the experimental run number 4 an extremely high N/P of removed nutrients was calculated. However, this experimental run was selected, because the profiles for dissolved oxygen and proton addition were as could be expected. This means that for example the point where the dissolved oxygen concentration starts to decrease due the depletion of inorganic carbon and the effect of $K_La$ corresponds to the point of declination in the titrimetric profile. Also the proton addition recovery was good. As such, it is assumed that nitrogen and phosphorous content were not analyzed correctly.

6.3.2 Global sensitivity analysis

With respect to the global sensitivity analysis (GSA), experimental run number seven was used for simulation. From the preliminary Monte Carlo runs with different number of simulations, it is inferred that from 2000 simulations on, the same conclusion could be made regarding the standardized regression coefficients. Consequently the results of the Monte Carlo run with $N = 2000$ will be discussed. Figure 6.2 represents the tornado plot summarizing sensitivities of all model parameters at $t = 0.2$ d with respect to dissolved oxygen concentration (left hand) and calculated proton addition (right hand). This clearly indicates the high sensitivity of the dissolved oxygen concentration towards the parameters $\mu_{max}$ (t-SRC= 11.87) and $K_La$ (t-SRC=-48.22). The negative value in case of $K_La$ indicates that an increase of this parameter will cause a lower dissolved oxygen level. Indeed when this parameter has higher values, more oxygen will be stripped out of the liquid phase and as such the dissolved oxygen concentration
Assessment of nutrient removal by microalgal biomass

will be lower. Considering $\mu_{\text{max}}$ (positive value), a higher value of this parameter causes higher dissolved oxygen concentration. Other parameters with some, although much lower influence on the dissolved oxygen concentration (t-SRC > 1.2) are $K_{i}CO_{2}$, $K_{NO_{3}^{-}}$ and $K_{PO_{4}^{3-}}$. With respect to the inhibition parameter for the growth on bicarbonate this could be explained by the fact that although the oxygen production yield is akin regardless the inorganic carbon source used, the kinetics for the assimilation of the two sources is different (Equations (4.1) and (4.2)). As such this parameter is influential. Considering the affinity constant for nitrate it could be explained by the fact that once the ammonium is depleted, the microalgae still can use the nitrate as inorganic nitrogen source. But if the nitrate is depleted, all activity stops. Also when nitrate is used, more oxygen is produced (stoichiometry) than in the case of ammonium as already mentioned (Stumm and Morgan, 1996; Eriksen, 2007). Regarding the proton addition similar observations were made for $K_{L}a$ and $\mu_{\text{max}}$. However it is noteworthy that for $K_{L}a$ a positive value was obtained (t-SRC = +31.32) and for $\mu_{\text{max}}$ a negative value was obtained (t-SRC = -38.9), indicating that the effect of these parameters compared to the dissolved oxygen concentration are opposite. A higher value of $K_{L}a$ in this case will cause more stripping of carbon dioxide and as such more protons will be added due to the shift of the chemical equilibrium if inorganic carbon, whilst a lower $\mu_{\text{max}}$ will result in higher impact of the shift in the chemical equilibrium and as such a higher proton addition. Furthermore, a high significance for the half saturation coefficients for growth on bicarbonate and carbon dioxide, respectively t-SRC = +45 and t-SRC = +7.52, was observed. This could be explained by the fact that the higher the half saturation coefficients, the lower the growth rate and as explained before this will cause more stripping and as such a higher proton addition. Also the inhibition constant for assimilation of bicarbonate was very significant (t-SRC = -117). Indeed, a higher value of this parameter indicates that the microalgae will switch to assimilate bicarbonate much swifter. According to Equations (2.5-2.6) more protons are consumed when bicarbonate is assimilated compared to carbon dioxide.
In addition, the identifiability of the most important parameters as determined in the SRC was evaluated. Figure 6.3 focusses on three parameters, respectively $\mu_{\text{max}}, K_La$ and $K_iCO_2$. The left hand side visualizes scatter plots of the SSE as function of the three parameters separately (in literature also referred as dotty plots). Clear minima in the scatter plot indicate a clear minimum in the SSE and as such, the potential to identify the optimal value of the parameter using the available experimental data.

As can be observed, the SSE has a clear minimum in case of the two first parameters, respectively for $K_La = 8.76$ d$^{-1}$ and $\mu_{\text{max}} = 0.28$ d$^{-1}$. In the case of the other parameter, no clear minimum could be indicated. This is also illustrated in the right hand side of the figures, where the marginal cumulative distributions of the 10 groups, coming out of the RSA are plotted. The marginal cumulative distributions of each parameter class illustrate no similarities in case of the maximum specific growth rate and the oxygen mass transfer coefficient. Such a course again indicates that the maximum specific growth rate and the oxygen mass transfer coefficient are identifiable parameters. However, in case of the inhibition coefficient for growth on bicarbonate due to carbon dioxide all marginal cumulative distributions cluster together indicating that this coefficient is not identifiable. From this it can be concluded that only two parameters are uniquely identifiable when using the combined respirometric - titrimetric data set. So only these parameters were considered in the model calibration. Other biokinetic parameter values were already discussed in the previous section.

Figure 6.2: Tornado plot of respirometric model output (left hand) and titrimetric model output (right hand). The parameters are ranged from most influential parameters on the top to non-influential parameters at the bottom.
Figure 6.3: Parameter dotty plot and regional sensitivity plot for maximum specific growth, oxygen mass transfer coefficient and inhibition coefficient for growth on carbon dioxide for the experiment used for the Monte Carlo simulation. In the regional sensitivity plot the lines are more dark grey with increasing value of objective function.
Table 6.4: Initial settings of the different experiments used for model calibration and corresponding optimized parameter values and TIC

<table>
<thead>
<tr>
<th>Run</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>PO$_4^{3-}$</th>
<th>DW</th>
<th>HCO$_3^-$</th>
<th>T</th>
<th>I</th>
<th>pH</th>
<th>∆S</th>
<th>N/P</th>
<th>µ$_{max}$</th>
<th>K$_a$</th>
<th>TIC</th>
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</thead>
<tbody>
<tr>
<td>No</td>
<td>(g N m$^{-3}$)</td>
<td>(g N m$^{-3}$)</td>
<td>(g P m$^{-3}$)</td>
<td>(g m$^{-3}$)</td>
<td>(g m$^{-3}$)</td>
<td>(K)</td>
<td>(lx)</td>
<td>(-)</td>
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<td>(d$^{-1}$)</td>
<td>(-)</td>
</tr>
<tr>
<td>1</td>
<td>5.6</td>
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<td>500</td>
<td>288</td>
<td>4875</td>
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<td>2</td>
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<td>1.75±0.011</td>
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<tr>
<td>2</td>
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<td>85.6</td>
<td>24.3</td>
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<td>500</td>
<td>299</td>
<td>9750</td>
<td>7.5</td>
<td>9</td>
<td>28</td>
<td>0.22±0.0006</td>
<td>6.97±0.024</td>
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<td>3.3</td>
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<td>5.0</td>
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<td>299</td>
<td>9750</td>
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<td>100</td>
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<td>12</td>
<td>0.32±0.0007</td>
<td>8.41±0.018</td>
<td>0.08</td>
</tr>
</tbody>
</table>

6.3.3 Model calibration

Seven combined datasets (Table 6.4) of dissolved oxygen concentration and proton addition with different initial environmental conditions were separately used for model calibration. As can be seen, all experimental runs have a TIC < 0.3 for the optimized parameter set, indicating good model performance. However, with respect to the second experimental run a TIC = 0.28 was observed, indicating that here less good correspondence between experimental data and model prediction was obtained. Furthermore, with respect to the optimized parameter, µ$_{max}$, similar values were found in literature (Menaa et al., 2015).

In order to illustrate the quality of a good model fit at optimized parameter settings, the experimental and simulated dissolved oxygen concentration (left hand) and proton addition (right hand) of experimental run 1 are depicted in Figure 6.4. Only a minor deviation between the experimental profiles and model prediction could be observed. Considering the dissolved oxygen profile, the model prediction and experimental values are the same until the descending part of the profile. Here the model prediction is underestimating the experimental data indicating that the K$_L$ value is somewhat too high as (after depletion of the substrate) the oxygen profile calculations are dominated by the K$_L$ value. In the increasing part the model prediction is very similar to the measured profile, which indicates a good estimation of the parameter µ$_{max}$, which dominates the oxygen profile calculation until the substrate is depleted. However looking at the proton addition profile, for the part of the curve that is determined by the µ$_{max}$, the model prediction is underestimating the experimental profile. This discrepancy could be explained by the fact that the sum of squared errors was used as objective function for the model calibration and since this value is higher with respect to the respirometric data than the value for the titrimetric data, the algorithm attaches more importance to the respirometric
Assessment of nutrient removal by microalgal biomass

data than the titrimetric data. To solve this, it was considered taking into account different weights in the objective function, respectively 1/10 and 1/50. However, the results of the recalibration did not indicate a better accordance between the predicted and measured values.

With respect to the titrimetric profile it was noted for all experiments, that the second part of the curve, this is at the level of the total amount of protons dosed, the model did not predict this accurately. This could be explained by the fact that it was assumed that the dosed bicarbonate concentration corresponds to the initial value of bicarbonate used for model simulation. Likely an error occurred in the preparation of the bicarbonate solution, as such, this is not the case. Additional measurement of the initial experimental concentration of bicarbonate could rule out this uncertainty.

Furthermore, it should be noted that the calculated and experimental proton addition profiles do not start at zero. This could be explained by initial carbon dioxide stripping (at pH = 6 for this experiment) as explained in Chapter 4.

![Figure 6.4: Comparison between experimental (dashed line) and predicted (full line) dissolved oxygen concentration (left) and proton addition (right) profile. Initial settings were 5.6 g NO₃⁻-N m⁻³, 70.9 g NH₄⁺-N m⁻³, 4.9 g PO₄³⁻-P m⁻³. Algal biomass was 82 g DW m⁻³. I = 4875 lux, pH = 6. TIC = 500 g HCO₃⁻ m⁻³.](image)

As an additional check, the predicted end values, in case of optimized parameter settings, for nitrogen (ammonium and nitrate), phosphate and algal biomass were compared to the experimental values at the end of each experiment for model calibration. This is illustrated in Figure 6.5. As can be seen, good correspondence between calculated and experimental values was obtained in case of phosphate, nitrate and algal biomass. With respect to ammonium some outliers were observed. Considering all experiments for model calibration, a divergence of 26.5 ± 27.5% for ammonium and 15.1 ± 14.7% for nitrate was noted.

105
Figure 6.5: Comparison of predicted end values of experimental end values for ammonium, nitrate, phosphate and algal biomass for the experimental runs used for model calibration with optimized parameter values for $K_L$, $a$ and $\mu_{max}$.

With respect to the outliers that were observed for ammonium, the predicted values were higher than the experimental values. Possible reasons could be stripping of ammonium as ammonia, heterotrophic activity or intracellular uptake of ammonium. These processes are not taken into account in the model structure. However, ammonia stripping is negligible at the pH values that are used in the experiments. Also the oxidation of ammonium into nitrate by bacterial activity is less likely, because this should increase the dissolved nitrate concentration. However, good correspondence between measured and calculated values for the latter were noted. As such the most plausible explanation is that intracellular ammonium storage occurred. Indeed, it is reported in literature that, when microalgae are stressed for a certain nutrient, they start to accumulate another nutrient. For the specific experiments with outliers, the experiments were run with limiting conditions for phosphorus. Hence, the microalgae could have accumulated ammonium intracellularly, resulting in a higher deviation between experimental and predicted end values for ammonium.
6.3.4 Model validation

Considering model validation, two remaining experimental runs were used that were not used in the calibration step. Initial settings of the first validation run were 2.87 g NO$_3^-$-N m$^{-3}$, 8.13 g NH$_4^+$-N m$^{-3}$, 3.33 g PO$_4^{3-}$-P m$^{-3}$ and 100 g HCO$_3^-$ m$^{-3}$. The initial biomass concentration was 646 g DW m$^{-3}$. For the second validation run, initial conditions of 4.03 g NO$_3^-$-N m$^{-3}$, 70.89 g NH$_4^+$-N m$^{-3}$, 26.57 g PO$_4^{3-}$-P m$^{-3}$ and 500 g HCO$_3^-$ m$^{-3}$. The initial biomass concentration was in this case 104 g DW m$^{-3}$. Both experiments were run at akin temperature, light intensity and pH, respectively T=293 K, I = 9750 lux and pH = 6.

It was decided to implement the mean value of experimental runs number 1, 3, 4, 5, 6 and 7, respectively 0.261 d$^{-1}$, for both validation experiments.

Furthermore, it was investigated by means of a multiple regression analysis whether a trend could be observed between model state variables and the optimized value of $K_La$ and $\mu_{max}$. For this however the poor calibration of data set 2 was not considered since the Theil’s Inequality Coefficient (TIC= 0.28) was almost equal to the threshold level of 0.3. However, only a significant relation between the microalgal biomass concentration and the mass transfer coefficient could be observed. Also, since this parameter depends on the temperature at which the experiments are run, the experiments at T= 299 K and T= 288 K were evaluated separately.

This resulted in a best fit relation which could be denoted as:

$$K_La = a X_{Alg}^b$$

(6.7)

At 288 K the following values were obtained $a = 0.013$ and $b = 1.537$ ($R^2 = 0.89$), while at 299 K, the following values were obtained: $a = 0.991$ and $b = 0.377$ ($R^2 = 0.67$).

It should be stressed that by introducing the use of equation (6.7) for model validation, that with respect to the model structure some improvement can be made. More specifically, by implementing a sub-model for the oxygen mass transfer coefficient as function of algal biomass and temperature. This will be discussed in Chapter 8, where more settings of temperature were used.

So, considering Equation (6.7), for the parameter $K_La$ a value of 11.36 d$^{-1}$ for the first validation experiment and 5.71 d$^{-1}$ for the second validation experiment was used.
Chapter 6

Furthermore, it should be emphasized that the proposed sub model for the oxygen mass transfer that is proposed in this chapter, was not used in further chapters. The reason for that is, that in Chapter 7, the reactor configuration was different and the medium was continuously sparged with carbon dioxide. As such the mass transfer coefficient will likely be completely different. Furthermore, in Chapter 8, the reactor configuration was different and the altitude at which the experiments were conducted in the latter chapter likely influenced the oxygen mass transfer coefficient.

In Figure 6.6 the calculated respirometric and titrimetric profiles in both experiments are compared to the experimental profiles. In case of the first validation experiment, very good correspondence between the experimental and calculated respirometric and titrimetric profiles could be observed. For the second validation experiment a slight deviation between the experimental and calculated profiles could be noted. However calculation of Theil’s Inequality Coefficient resulted in TIC = 0.05 for the first validation and TIC= 0.08 for the second validation, indicating good model performance in both cases.

Considering the total nitrogen deviation between experimental and calculated end value, a percentage value of 34 % for the first validation experiment and 33 % for the second validation experiment was noted. In the case of phosphorus this deviation was lower (17 % and 8 %). In addition, the deviation for biomass was 2 % and 25 %. Overall, these results were similar to those of the model calibrations. There also less good prediction of the total nitrogen values was noted.
Figure 6.6: Comparison between predicted (full line) and experimental (dashed line) dissolved oxygen concentration and proton addition profiles for the first (top) and second (bottom) validation experiments. Initial settings of the first validation run were 2.87 g NO\text{3}\text{-N} m^{-3}, 8.13 g NH\text{4}\text{-N} m^{-3}, 3.33 g PO\text{4}\text{-P} m^{-3} and 100 g HCO\text{3}\text{-} m^{-3}. The initial biomass concentration was 646 g DW m^{-3}. For the second validation run, initial conditions of 4.03 g NO\text{3}\text{-N} m^{-3}, 70.89 g NH\text{4}\text{-N} m^{-3}, 26.57 g PO\text{4}\text{-P} m^{-3} and 500 g HCO\text{3}\text{-} m^{-3}. The initial biomass concentration was in this case 104 g DW m^{-3}. Both experiments were run at akin temperature, light intensity and pH, respectively T=293 K, I = 9750 lux and pH = 6.

6.4 Conclusions

In this chapter, the model from Chapter 4 that describes microalgal growth as function of inorganic carbon was extended with growth functions for nitrogen and phosphorus in order to predict nutrient removal by microalgal biomass in wastewater.
Chapter 6

A global sensitivity analysis indicated two very significant parameters, namely the maximum specific growth rate ($\mu_{\text{max}}$) and oxygen mass transfer coefficient ($K_L a$) with respect to the dissolved oxygen concentration. Further considering the proton addition rate, it seemed that in addition the biokinetic parameters for microalgal growth on inorganic carbon became very significant. Mainly due to the fact that assimilation of inorganic carbon induces a shift on the equilibrium of these components and as such has an effect on the proton addition.

A parameter identifiability analysis further illustrated that only the maximum specific growth rate and the oxygen mass transfer coefficient were uniquely identifiable when using the combined dissolved oxygen concentration and proton addition data. Therefore, these two parameters where calibrated to seven different combined respirometric and titrimetric data sets resulting in a good model prediction with a calculated TIC-value lower than the threshold value of 0.3 for six of the seven separate calibrations. However, with the model proposed in this chapter, a less good fit than the model described in Chapter 4 was obtained, based on the TIC. This could be explained by the fact that in Chapter 4, the objective function consisted only of the respirometric values, whereas in this chapter, the combined respirometric and titrimetric data was used for model calibration. Further, more deviation between the predicted total amount of protons and the experimental total proton addition was observed. This could be explained by the uncertainty of the experimental dosed bicarbonate concentration. The latter could be overcome by analysis of the inorganic carbon concentration in the reactor, for example by means of an additional sensor.

With the optimized parameter settings two additional model validations were performed with calculated TIC values of 0.07 and 0.08, indicating good model performance. Overall it can be concluded that in this work the proposed (relative straightforward) model with a unique set of identifiable parameters to the specific data is able to describe the microalgal growth as function of environmental parameters such as pH and nutrients (inorganic carbon, inorganic nitrogen and inorganic phosphorus).
Chapter 7

Kinetic exploration of intracellular nitrate storage

Redrafted from


This Chapter involves joint work between Joeri Coppens and Bjorge Decostere. Joeri Coppens focused on the experimental work, whilst Bjorge Decostere focused on model development and model analysis.
Chapter 7

Abstract

Within sustainable resource management, the recovery of nitrogen and phosphorus nutrients from waste streams is becoming increasingly important. Although the use of microalgae has been described extensively in environmental biotechnology, the potential of nitrate accumulating microalgae for nutrient recovery has not been investigated yet.

In this chapter, a model accounting for intracellular nitrate storage kinetics was developed and thoroughly studied to understand and compare the storage capacity of different marine microalgae. The intracellular nitrate storage capacity was quantified for six marine microalgae in synthetic wastewater. Amphora coffeaeformis and Phaeodactylum tricornutum stored the highest amount of nitrate with respectively 3.15 g N l⁻¹ and 2.10 g l⁻¹ of cell volume, which accounted for 17.3 and 4.6 % respectively, of the total nitrogen content. Based upon further analyses these two species showed the highest potential for nutrient recovery. For this the experimental features of these two species were used for model analysis. In a first stage the identifiability of the biokinetic parameters was examined. Next, the kinetic model was calibrated for microalgal species based on experimental observations during batch growth experiments. Two kinetic constants were calibrated, namely the maximum specific growth rate \( \mu_{\text{max}} \) and the nitrate storage rate \( k_{\text{sto}} \). A significant difference was observed for the nitrate storage rate between both species. For P. tricornutum, the nitrate storage rate was much higher \( k_{\text{sto}} = 0.036 \text{ m}^3\text{ g}^{-1}\text{DW d}^{-1} \) compared to A. coffeaeformis \( k_{\text{sto}} = 0.0004 \text{ m}^3\text{ g}^{-1}\text{DW d}^{-1} \). Also this parameter did not differ from zero at 95 % confidence level. This suggest that P. tricornutum has a more efficient nitrate uptake ability and intracellular nitrate storage capacity and also indicates the need for determination of \( k_{\text{sto}} \) in order to quantify nitrate storage.

7.1 Introduction

Intensive agriculture has resulted in an increasing demand for nitrogen (N) and phosphorus (P) fertilizers, thereby pressuring the available nutrient sources. The recycling of nitrogen and phosphorus nutrients from waste streams is therefore becoming more and more imperative. In that aspect, studies have shown the feasibility to use microalgae to recover nutrients from different types of wastewater (Cai et al., 2013). Due to their low affinity constants, microalgae
are very promising for tertiary wastewater treatment with respect to nutrient removal. Also, the nitrate uptake kinetics of eukaryotic types of diatoms which reside in both benthic and pelagic zones of marine ecosystems have been studied to assess their applicability for phytoremediation of eutrophic coastal areas (Kwon et al., 2013; Naldi and Viaroli, 2002; Collos et al., 1992). In these marine ecosystems specific microalgae occur that have the capacity to store nitrate intracellularly in transitory cytoplasmic pools in concentrations up to several grams of nitrogen per liter (Bode et al., 1997; Dortch et al., 1984; Kamp et al., 2011; Lomas and Glibert, 2000; Needoba and Harrison, 2004). With nitrogen limited conditions, the intracellular nitrate is reduced and used as nitrogen source for growth. As such, the ability of these specific microalgae to store environmental nitrogen in their biomass is in a way remarkable and offers an attractive potential for biological nutrient recovery where conventional physical-chemical methods are not applicable. In general, a large quantity of the wastewater that is currently produced, such as domestic wastewater originates from freshwater resources. However, the treatment of saline wastewaters is nevertheless relevant. Due to the need for more sustainable fish production, intensive marine aquaculture has become a fast growing economic sector. In recirculating aquaculture systems, the seawater is recycled, which requires remediation of the nitrogen and phosphorus abundant in the wastewater stream. Besides aquaculture wastewater, also other wastewaters are characterized by a high salinity, such as landfill leachate (Di laconi et al., 2010), beverage production wastewater (Campos et al., 2010) and tannery wastewater (Cuartas-UrIBE et al., 2006). Due to the high salt concentrations, these streams are often difficult to treat by conventional wastewater treatment systems.

In this chapter the exploration of this potential of nitrate-storing diatoms for nutrient recovery is described. A screening was performed to determine the nitrate storage capacity of six diatom species. For the diatoms with the highest nitrate accumulation, the growth and nutrient uptake were analyzed. Based on these features a mathematical model was developed and used to compare the accumulation capacity of the best performing diatoms. The developed model describes inorganic carbon kinetics, nutrient removal and an extra cellular nitrogen uptake. Considering the modelling part, the most important kinetic parameters were identified by using Standardized Regression Coefficients in case of Monte Carlo sampling over the global parameter space during a global sensitivity analysis (Saltelli et al., 2005). In addition the identifiability of parameters to the combined output of dissolved nitrogen, dissolved phosphorus and microalgal biomass concentration was examined by using a Regional Sensitivity Analysis (Camacho and Gonzaléz, 2008). Further a collinearity study was
performed (Brun et al., 2001) to examine possible parameter subsets suitable for model calibration during a local sensitivity analysis. Based on this sensitivity analysis experimental data was used to infer the most important kinetic parameters for the two marine microalgae. The resulting parameter values were tested during a subsequent model validation with stationary data. Furthermore the parameter values were compared in terms of practical importance such as algal growth rate and nitrate storage capacity. Finally, a scenario analysis was performed to compare the microalgal system performance with a conventional denitrification system.

7.2 Material and methods

7.2.1 Cultivation of the strains

The benthic diatom strains *Amphora coffeaeformis* (CCMP127) and *Nitzschia punctata* (CCMP 561) and the pelagic strains *Skeletonema dohrnii* (CCMP 782), *Thalassiosira nordenskioeldii* (CCMP 995) and *Thalassiosira weissflogii* (CCMP 1336) were obtained from the Provasoli–Guillard National Center for Marine Algae and Microbiota (NCMA). The pelagic strain *Phaeodactylum tricornutum* (CCAP 1055/1) was obtained from the Flanders Institute for Biotechnology (VIB). In all experiments the diatoms were cultured axenic in sterile synthetic wastewater, modified from artificial seawater (ESAW) medium (Berges et al. 2001). Nitrate and phosphate concentrations were 100 mg N l\(^{-1}\) and 44 mg P l\(^{-1}\), respectively, i.e., a mass and molar N:P ratio of 2.3 and 5, respectively, to prevent phosphorus limitation in the medium. Cultures were aerated at 0.71 air l medium\(^{-1}\) with 0.22 μm filter sterilized 2 % carbon dioxide. The cultivation temperature was 293 K, the pH was maintained at 8.0–8.2 and continuous illumination was provided from the top by means of cool white lamps (Osram, Dulux L) at a light intensity of 8250 lux. Bacterial contamination of the cultures was checked throughout the experiments using phase contrast microscopy.
7.2.2 Quantification of intracellular nitrate

Diatoms were cultured in 0.8 L batch tests in synthetic wastewater. Tests were performed in quadruplicate and growth was monitored by optical density (OD) measurement at 450 and 670 nm. Intracellular nitrate storage was quantified under exponential and stationary growth phase. The cell disruption method was modified from Dortch (1982). 50 mL of algal suspension was filtered on a 0.45 μm Whatman glass fiber filter. Cells were washed three times with 50 mL of 3 % NaCl to remove extracellular nitrate. Filters were frozen at −80 °C for 15 min, after which cells were broken by adding three times 10 mL of hot (80 °C) distilled water to extract intracellular nitrate. Nitrate, nitrite, ammonium, and phosphate were determined after filtration as detailed below. Determination of the cell density was carried out using light microscopy with a cell counting chamber (KOVA® Glasstic, USA) at 100× magnification (Zeiss Axioskop 2, Germany). The width and length of cells (n=100) was determined at 1000× magnification using the image processing software tool ImageJ (Schneider et al., 2012). The cell volume was estimated by assuming a biconical cell shape for *P. tricornutum* and a cylindrical shape for the other five species (Kamp et al. 2011).

7.2.3 Kinetic experiments

Growth experiments were performed for *A. coffeaeformis* and *P. tricornutum* in 0.8 L batch tests. Cells in exponential growth were inoculated in synthetic wastewater at a cell density of 3 x 10^3 cells ml^-1. Tests were performed during 10 days and nitrate, nitrite, ammonium, and phosphate concentrations in the medium were measured daily. Growth was monitored daily by cell count (KOVA® Glasstic, USA), and cell concentrations were correlated to biomass concentrations by determining the dry weight content at different stages of the growth curve in parallel tests. Additional batch tests were performed for *P. tricornutum* collected at the late stationary phase (t = 14 days) after spiking with nitrate to reach the maximal cell density. Nutrient depleted cells at an initial cell density of 4 x 10^7 cells ml^-1 were spiked with nitrate and phosphate to a final concentration of 100 mg N l^-1 and 44 mg P l^-1. The cell density and nitrate, nitrite, ammonium, and phosphate in the medium were monitored during 3.5 days. All batch tests were performed in quadruplicate.
Chapter 7

7.2.4 Analytical techniques

Nitrate, nitrite and phosphate were analyzed after sample filtration using anion chromatography (Metrohm 761 Compact IC, Switzerland). Ammonium (Nessler method), total Kjeldahl nitrogen, and total phosphorus (molybdene--vanadate method) were determined according to standard methods (APHA, 2005). The total carbon and nitrogen contents of the biomass were determined using an elemental analyzer (ANCA-GSL PDZ Europe, UK).

7.2.5 Model development

To describe the microalgal growth and removal of nutrients in the aqueous phase, a kinetic model was set up based on the experimental observations. This model described in the previous chapter (Chapter 6) was extended with intracellular nitrate storage. As such the extended model contains 9 state variables: microalgal biomass concentration, concentration of different inorganic carbon species (CO$_3^{2-}$, HCO$_3^-$ and CO$_2$), concentration of nutrients (nitrate and phosphate), dissolved oxygen concentration and amount of nitrate stored. Since it was assumed that light intensity, temperature and pH, which were maintained at a constant level during the experiments, respectively 293 K, 9000 lux and pH = 7.75, were not limiting the microalgal growth, the specific growth rate can be denoted as function of total inorganic carbon, nitrogen, phosphorus and biomass concentration. Further only nitrate was used as inorganic nitrogen source. So, no inhibition factor for growth on nitrate was incorporated in the model structure. Also growth limitation due to self-shading effect was incorporated. This was described by an empirical function (Equation (7.1)) that was based on the experimental data. Indeed, a decrease in growth rate was observed when the biomass concentration was above 0.5 kg m$^{-3}$. In Figure 7.1, this empirical function is illustrated.

\[ f_X = \frac{k_X}{k_X + \frac{X_{Alg}}{1000} n_X} \]  

(7.1)

With $k_X$ (kg DW m$^{-3}$) and $n_X$ (-) parameters of this function.
Kinetic exploration of intracellular nitrate storage

As such the microalgal growth rate on nitrate, phosphate and inorganic carbon, respectively carbon dioxide (7.2) and bicarbonate (7.3) was denoted as:

$$
\rho_{Alg(CO_2,NO_3^-,PO_4^{3-})} = \mu_{max} \left( \frac{S_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{S_{NO_3^-}}{K_{NO_3^-} + S_{NO_3^-}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) \left( \frac{k_i x}{k_i x + \left( \frac{X_{Alg}}{1000} \right)^2} \right) X_{Alg}
$$

(7.2)

$$
\rho_{Alg(HCO_3^-,NO_3^-,PO_4^{3-})} = \\
\mu_{max} \left( \frac{S_{HCO_3^-}}{K_{HCO_3^-} + S_{HCO_3^-}} \right) \left( \frac{K_i CO_2}{K_i CO_2 + S_{CO_2}} \right) \left( \frac{S_{NO_3^-}}{K_{NO_3^-} + S_{NO_3^-}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) \left( \frac{k_i x}{k_i x + \left( \frac{X_{Alg}}{1000} \right)^2} \right) X_{Alg}
$$

(7.3)

The nitrate storage kinetics were expressed as a function of the algal biomass concentration, the ambient nitrate concentration $S_{NO_3^-}$ and the nitrate storage constant $k_{STO}$ ($m^3 g^{-1} DW d^{-1}$) (Equation (7.4)). A nitrate accumulation limitation factor was also included and was inspired by the P-uptake model by Henze et al. (2000) for enhanced biological P-removal (EBPR). This limitation factor is a function of the maximum nitrate uptake capacity $K_{cap}$ (g N g^{-1} DW), the microalgal biomass concentration, and the effective concentration of nitrate stored in the algal pools $X_{STN}$ (g N m^{-3}).

$$
\rho_{STO}^{NO_3^-} = k_{STO} S_{NO_3^-} \frac{K_{cap}}{K_{cap} + \left( \frac{X_{Alg}}{1000} \right)^2} X_{Alg}
$$

(7.4)

Furthermore, transfer of carbon dioxide and oxygen between the gaseous and liquid phase and the chemical equilibria of inorganic carbon were included in the model structure according to Equation (2.4) and Equation (2.9) mentioned in Chapter 2.
Chapter 7

In Table 7.1, an overview of the modeled processes and the reaction rates is given in the Gujer matrix of the model, which will be further one referred as the Algstor model.

**Tabel 7.1: Gujer matrix of the Algstor model**

<table>
<thead>
<tr>
<th>Proces</th>
<th>$X_{Alg}$</th>
<th>$S_{HCO_3}$</th>
<th>$S_{CO_2}$</th>
<th>$S_{H_2O}$</th>
<th>$S_{NO_3}$</th>
<th>$S_{PO_4^{3-}}$</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on nutrients and bicarbonate</td>
<td>1</td>
<td>$-\frac{1}{Y_1}$</td>
<td>$Y_3$</td>
<td>$-\frac{1}{Y_4}$</td>
<td>$\frac{1}{Y_5}$</td>
<td>$\frac{1}{Y_6}$</td>
<td>$\rho_1$</td>
</tr>
<tr>
<td>Growth on nutrients and carbon dioxide</td>
<td>1</td>
<td>$-\frac{1}{Y_2}$</td>
<td>$Y_3$</td>
<td>$-\frac{1}{Y_4}$</td>
<td>$\frac{1}{Y_5}$</td>
<td>$\frac{1}{Y_6}$</td>
<td>$\rho_2$</td>
</tr>
<tr>
<td>Growth on stored nitrate and bicarbonate</td>
<td>1</td>
<td>$-\frac{1}{Y_1}$</td>
<td>$Y_3$</td>
<td>$-\frac{1}{Y_4}$</td>
<td>$\frac{1}{Y_5}$</td>
<td>$\frac{1}{Y_6}$</td>
<td>$\rho_3$</td>
</tr>
<tr>
<td>Growth on stored nitrate and carbon dioxide</td>
<td>1</td>
<td>$-\frac{1}{Y_2}$</td>
<td>$Y_3$</td>
<td>$-\frac{1}{Y_4}$</td>
<td>$\frac{1}{Y_5}$</td>
<td>$\frac{1}{Y_6}$</td>
<td>$\rho_4$</td>
</tr>
<tr>
<td>Nitrate storage</td>
<td>1</td>
<td>$-1$</td>
<td>$1$</td>
<td>$\rho_5$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide transfer rate</td>
<td>1</td>
<td>$\rho_6$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen transfer rate</td>
<td>1</td>
<td>$\rho_7$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide hydration</td>
<td>$\rho_8$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicarbonate dissociation</td>
<td>$\rho_9$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decay</td>
<td>-1</td>
<td>$\rho_{10}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \rho_1 = \mu_{max} \left( \frac{S_{HCO_3}}{K_{HCO_3} + S_{HCO_3}} \right) \left( \frac{K_{1}CO_2}{K_{1}CO_2 + S_{CO_2}} \right) \left( \frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) \left( \frac{K_{1}x}{K_{x}X_{Alg}^{1000}} \right) X_{Alg} \]

\[ \rho_2 = \mu_{max} \left( \frac{S_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{S_{NO_3}}{K_{NO_3} + S_{NO_3}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) \left( \frac{K_{1}x}{K_{x}X_{Alg}^{1000}} \right) X_{Alg} \]

\[ \rho_3 = \mu_{max} \left( \frac{S_{HCO_3}}{K_{HCO_3} + S_{HCO_3}} \right) \left( \frac{K_{1}CO_2}{K_{1}CO_2 + S_{CO_2}} \right) \left( \frac{X_{STN}}{S_{NO_3} + X_{STN}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) \left( \frac{K_{1}x}{K_{x}X_{Alg}^{1000}} \right) X_{Alg} \]

\[ \rho_4 = \mu_{max} \left( \frac{S_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{X_{STN}}{S_{NO_3} + X_{STN}} \right) \left( \frac{S_{PO_4^{3-}}}{K_{PO_4^{3-}} + S_{PO_4^{3-}}} \right) \left( \frac{K_{1}x}{K_{x}X_{Alg}^{1000}} \right) X_{Alg} \]

\[ \rho_5 = k_{STO} \frac{S_{NO_3}}{K_{cap} + \frac{X_{STN}}{X_{Alg}}} X_{Alg} \]

\[ \rho_6 = K_{La red} (S_{CO_2}^{sat} - S_{CO_2}) \]

\[ \rho_7 = K_{La} (S_{O_2}^{sat} - S_{O_2}). \]

118
\[ \rho_8 = k_1 \left( \frac{S_{CO_2}}{44} - \frac{10^{-pH} S_{HCO_3}}{61 K_1} \right) \]

\[ \rho_9 = k_2 \left( \frac{S_{HCO_3}}{61} - \frac{10^{-pH} S_{CO_2}}{60 K_2} \right) \]

\[ \rho_{10} = b_{max} X_{Alg} \]

### 7.2.6 Model parameter values

The mass transfer coefficient between the water phase and gas phase was calculated based on the assumption that the mass of carbon dioxide in the sparging gas equals the mass transfer coefficient multiplied by the dissolved saturation of carbon dioxide concentration in the aqueous phase. As such the mass transfer coefficient could be calculated as:

\[ Q_{CO_2} C_{CO_2} = K_L a \text{ red}_{CO_2} S^{sat}_{CO_2} \quad (7.5) \]

Here \( Q_{CO_2} \) represents the sparging gas flow rate (m\(^3\) d\(^{-1}\)), \( C_{CO_2} \) the concentration of carbon dioxide in the sparging gas (g m\(^{-3}\)), \( K_L a \text{ red}_{CO_2} \), the carbon dioxide mass transfer coefficient (d\(^{-1}\)) and \( S^{sat}_{CO_2} \) the concentration of carbon dioxide at saturation level in the liquid phase (g m\(^{-3}\)). The latter can be calculated according to:

\[ S^{sat}_{CO_2} = K_H C_{CO_2} \quad (7.6) \]

In this equation, \( K_H \) represents the Henry coefficient, being 0.8317 (-) for this specific case.

Parameters related to the assimilation of inorganic carbon and chemical equilibria of inorganic carbon species were similar to previous chapters. The half saturation coefficients for growth on nitrate and phosphate were obtained from literature (Tyrell et al., 1999; Baldia et al., 1991). Further the values of the yield on nitrate and phosphate were calculated on the features of the elemental analysis of the biomass and will be discussed further in this chapter.
Chapter 7

7.2.7 Global sensitivity and parameter identifiability

In order to determine the contribution of each biokinetic parameter to changes in the model output a GSA was performed. As model output the dissolved nitrate concentration at a certain experimental time was evaluated. The biokinetic parameters were varied simultaneously over a predefined range (Table 7.3) in the parameter space. Since the PDF of each parameter was not known, a uniform distribution was assumed (Audenaert et al., 2013). Eventually 4 Monte Carlo runs were performed with a different number of simulations, respectively 200, 1000, 5000 and 10000. This was done to get an idea of the number of simulations that were sufficient to guarantee the quality of this Monte Carlo simulation. The Monte Carlo runs were evaluated by assessing the fit of the linear regression of the model. Then in case of the most suitable linear regression, the t-statistic value was evaluated. The results of this linear regression were depicted in a tornado plot, where the t-statistic values of each parameter are illustrated in decreasing order of the absolute t-statistic value.

Table 7.2: Kinetic parameter values used in the Algstor model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>P. tricornutum</th>
<th>A. Coffeaeformis</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>Max. specific growth rate</td>
<td>*</td>
<td>*</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$b_{\text{max}}$</td>
<td>Max. decay rate</td>
<td>0.001</td>
<td>0.001</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$Y_1$</td>
<td>Yield on HCO$_3^-$</td>
<td>0.549</td>
<td>0.549</td>
<td>g DW g$^{-1}$ HCO$_3^-$</td>
</tr>
<tr>
<td>$Y_2$</td>
<td>Yield on CO$_2$</td>
<td>0.761</td>
<td>0.761</td>
<td>g DW g$^{-1}$ CO$_2$</td>
</tr>
<tr>
<td>$Y_4$</td>
<td>Yield on NO$_3^-$</td>
<td>14.4</td>
<td>33</td>
<td>g DW g$^{-1}$ N</td>
</tr>
<tr>
<td>$Y_5$</td>
<td>Yield on PO$_4^{3-}$</td>
<td>66</td>
<td>125</td>
<td>g DW g$^{-1}$ P</td>
</tr>
<tr>
<td>$K_{\text{HCO}_3^-}$</td>
<td>Half saturation coefficient for HCO$_3^-$</td>
<td>3</td>
<td>3</td>
<td>g HCO$_3^-$ m$^{-3}$</td>
</tr>
</tbody>
</table>
Table 7.2: Kinetic parameter values used in the Algstor model (continued)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>P. tricornutum</th>
<th>A. Coffeaformis</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{CO2}$</td>
<td>Half saturation coefficient for CO$_2$</td>
<td>0.2</td>
<td>0.2</td>
<td>g CO$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$k_{L_a}$</td>
<td>CO$_2$ mass transfer rate</td>
<td>838</td>
<td>838</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$K_{NO3}^{-}$</td>
<td>Half saturation coefficient for NO$_3$-</td>
<td></td>
<td></td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$K_{PO4}^{3-}$</td>
<td>Half saturation coefficient for PO$_4$$^{3-}$</td>
<td>0.0083</td>
<td>0.0083</td>
<td>g PO$_4$ m$^{-3}$</td>
</tr>
<tr>
<td>$k_{i_x}$</td>
<td>Biomass inhibition constant***</td>
<td>1</td>
<td>1</td>
<td>g DW l$^{-1}$</td>
</tr>
<tr>
<td>$n_x$</td>
<td>Biomass inhibition exponent***</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>$k_{sto}$</td>
<td>Nitrate storage rate*</td>
<td>*</td>
<td>*</td>
<td>m$^{-3}$ g$^{-1}$DW d$^{-1}$</td>
</tr>
<tr>
<td>$k_{cap}$</td>
<td>Half saturation coefficient for nitrate storage</td>
<td>0.0004</td>
<td>0.0004</td>
<td>g NO$_3$-N g$^{-1}$ DW</td>
</tr>
</tbody>
</table>

7.3 Results and discussion

7.3.1 Intracellular nitrate storage of the different species

In Figure 7.1 the intracellular nitrate storage of the six different species during the exponential growth experiments is depicted. A large variation in nitrate storage was observed between the different species. *A. coffeaformis* and *P. tricornutum* stored the highest amount of nitrate. Further, the capacity to accumulate was not correlated to cell size or the habitat type (benthic or pelagic). Although phosphate was never limiting in the medium, intracellular phosphate
storage was not detected for any of the diatoms. Moreover, nitrite and ammonium were not observed to be stored intracellularly (Coppens, 2016).

Table 7.3: Ranges of the biokinetic parameters used for the Monte Carlo simulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Low level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h_{\text{max}}$</td>
<td>d$^{-1}$</td>
<td>0.525</td>
<td>1.575</td>
</tr>
<tr>
<td>$K_{\text{NO}_3}$</td>
<td>g N m$^{-3}$</td>
<td>0.065</td>
<td>0.195</td>
</tr>
<tr>
<td>$K_{\text{PO}_4}$</td>
<td>g P m$^{-3}$</td>
<td>0.004</td>
<td>0.012</td>
</tr>
<tr>
<td>$k_i x$</td>
<td>kg DW m$^{-3}$</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>$n_x$</td>
<td>(-)</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>$k_{\text{STO}}$</td>
<td>m$^3$ g$^{-1}$ DW d$^{-1}$</td>
<td>0.0145</td>
<td>0.0435</td>
</tr>
<tr>
<td>$K_{\text{cap}}$</td>
<td>g N g$^{-1}$ DW</td>
<td>0.0002</td>
<td>0.0006</td>
</tr>
<tr>
<td>$Y_4$</td>
<td>g DW g$^{-1}$ N</td>
<td>7.2</td>
<td>21.6</td>
</tr>
<tr>
<td>$Y_5$</td>
<td>g DW g$^{-1}$ P</td>
<td>33</td>
<td>99</td>
</tr>
</tbody>
</table>

Figure 7.1: Intracellular nitrate storage capacity of the six species during exponential growth.

In addition elementary analysis of the biomass composition for *A. coffeaeformis* and *P. tricornutum* during exponential growth resulted in a molar C/N/P ratio of 61/8/1 and 70/11/1. As such the biomass yield on nitrate ($Y_4$) for *P. tricornutum* was set at 14.4 g DW g$^{-1}$ N, which is similar to the yield proposed by Stumm and Morgan (1996). For the *A. coffeaeformis* biomass, the elementary analysis deviated from Stumm and Morgan and a value for of 33.0 g DW g$^{-1}$ N.
was calculated. The value of the yield on phosphate ($Y_5$) was set to 66 g DW g$^{-1}$ P, for the 
*P. tricornutum* 125 g DW g$^{-1}$ P for the *A. coffeaeformis* respectively.

### 7.3.2 Global sensitivity analysis and parameter identifiability

In Figure 7.2, the tornado plot of the corresponding t-values for the nitrate concentration at t=3d is depicted. As can be seen, four parameters are significant for the dissolved nitrate concentration, respectively $\mu_{\text{max}}$ (t-SRC = -151.26), $Y_4$(t-SRC = 44.00), $k_{STO}$ (t-SRC = -9.70) and $K_{\text{cap}}$ (t-SRC = -8.83).

![Tornado plot at t = 3 d for dissolved nitrate concentration. Parameters are ranged with highest value of t-SRC at the top of figure.](image)

Next, the identifiability of these four significant parameters was examined by means of the scatter plots related to the SSE and regional sensitivity analysis. In order to calculate the SSE the mean values of the fourfold performed experiments features for dissolved nitrate, the dissolved inorganic phosphorus and microalgal biomass in case of *P. tricornutum* were used. In Figure 7.3 the SSE as function of the four sensitive parameters ($\mu_{\text{max}}, k_{STO}, K_{\text{cap}}$ and $Y_4$) is presented in a scatter plot. Considering the maximum specific growth rate a clear minimum is observed. Next the Regional Sensitivity plot illustrates non-clustered lines in the case of the maximum specific growth rate. With respect to the other parameters that were indicated to be significant, no clear minimum in the scatter plots and only clustered lines in the Regional Sensitivity plots were observed. According to Camacho and Gonzales (2008), this is an
Chapter 7

indication that only the maximum specific growth rate is unique identifiable with respect to the combined model output.

Figure 7.3: Scatter plot (left hand) and Regional Sensitivity Analysis plot (right hand) for $\mu_{max}$, $k_{STO}$, $Y_4$, and $K_{cap}$. Dark shaded lines indicate a high value for the sum of errors.
7.3.3 Identifiability of parameter subsets: the collinearity index

The identifiability of the model parameters was further investigated according to Brun et al. (2001), presenting an appropriate method to tackle the problem of models with a lot of parameters that often lead to poorly identifiable or non–identifiable parameters. The identifiability of different parameter subsets to the different model outputs, respectively dissolved nitrate, dissolved inorganic phosphorus and microalgal biomass concentration was further evaluated by means of the collinearity index $Y_K$. It should be stressed that for this analysis, no experimental data was used. Only the parameter $\mu_{\text{max}}$ is uniquely identifiable (see above) to the combined model output, so only combinations with this parameter were considered. In Table 7.4 the different parameter subsets are summarized. To evaluate which parameter subsets were identifiable to the different model outputs, a threshold value of $Y_K = 20$ was used. (Brun et al., 2001). The results of this collinearity assessment, showed that, for parameter subsets consisting of 4 parameters and all combinations of three parameters, $Y_K$ was higher than 182 in all cases for the different model outputs. As such, these parameter subsets could not be considered for model calibration. Considering subsets with two parameters, the subset with parameters $Y_4$ and $\mu_{\text{max}}$ had a $Y_K = 35.92$, $Y_K = 24.22$ and $Y_K = 83.75$ for model outputs nitrate, algal biomass and phosphate respectively. This indicates the non–identifiability of these parameter subsets. Correlation between yield parameter and maximum specific growth rate, was also observed by parameters by Petersen et al. (2001).

Table 7.4: Different parameter subsets used to assess the collinearity

<table>
<thead>
<tr>
<th>Subset code</th>
<th>Number of parameters</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>$\mu_{\text{max}}, k_{\text{STO}}, K_{\text{cap}}, Y_4$</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>$\mu_{\text{max}}, k_{\text{STO}}, Y_4$</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>$\mu_{\text{max}}, K_{\text{cap}}, k_{\text{STO}}$</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>$\mu_{\text{max}}, K_{\text{cap}}, Y_4$</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>$\mu_{\text{max}}, Y_4$</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>$\mu_{\text{max}}, K_{\text{cap}}$</td>
</tr>
<tr>
<td>G</td>
<td>2</td>
<td>$\mu_{\text{max}}, k_{\text{STO}}$</td>
</tr>
</tbody>
</table>

Considering the parameter subset with $\mu_{\text{max}}$ and $k_{\text{STO}}$, a value of $Y_K < 1$ for all model outputs was noted, indicating that this parameter could be considered for model calibration. For the
Chapter 7

Parameter subset with parameters $\mu_{\text{max}}$ and $K_{\text{cap}}$ values for $Y_K < 1$ were observed for the model outputs phosphate and nitrate. With respect to the model output nitrate $Y_K$ was equal to 20.95. Although this exceeds the threshold value of 20, it was decided to consider this parameter subset for model calibration.

7.3.4 Model calibration

Concerning the combination of $\mu_{\text{max}}$ and $K_{\text{cap}}$ for model calibration, it was observed that no confidence interval could be calculated due to problems of singularity. As such, it could be also concluded that this combination of parameters is not suitable for model calibration to the experimental data available. This confirmed also, that even the $Y_K$ only slightly exceeds the threshold value of 20 (see previous section), it indicates parameter identifiability issues.

Eventually the maximum specific growth rate ($\mu_{\text{max}}$) and microalgal nitrate storage rate were considered for model calibration. This resulted in a value for $\mu_{\text{max}} = 1.05 \pm 0.002 \, \text{d}^{-1}$ and $\mu_{\text{max}} = 1.15 \pm 0.004 \, \text{d}^{-1}$ for $P. \text{tricornutum}$ and $A. \text{coffeaeformis}$ respectively. With respect to the nitrate storage rate, values were obtained of $k_{ST0} = 0.036 \pm 0.0015 \, \text{m}^3 \, \text{g}^{-1} \, \text{DW} \, \text{d}^{-1}$ and $k_{ST0} = 0.0004 \pm 0.0006 \, \text{m}^3 \, \text{g}^{-1} \, \text{DW} \, \text{d}^{-1}$ for $P. \text{tricornutum}$ and $A. \text{coffeaeformis}$, respectively. Furthermore, in case of $A. \text{coffeaeformis}$, $k_{ST0}$ does not differ significantly from zero (95% confidence interval), indicating the non-significance of this parameter for this species. Thus this microalgal species has very low capacity for additional intracellular nitrate storage. This could be expected based on the experimental observations. Despite the fact that in the case of $A. \text{coffeaeformis}$ more nitrate is incorporated in the microalgal biomass compared to $P. \text{tricornutum}$, less dissolved nitrate is removed from the liquid phase. This suggests that this microalgal species has a lower additional intracellular nitrate storage capacity.

In Figure 7.4, the different experimental values obtained from the growth experiments and model simulations with optimized parameter settings are depicted for $P. \text{tricornutum}$ (top) and $A. \text{coffeaeformis}$ (bottom). Regarding the first microalgal species, the model is able to describe the evolution of nitrate (TIC = 0.02) and biomass concentration (TIC = 0.02) rather accurate. For phosphate (TIC = 0.05) a slight deviation between the experimental data and model simulation was observed. This could be explained by the fact that several mechanisms such as adsorption, precipitation for P-removal could occur next to assimilation. At this stage of the
research, these phenomena were not included in the model due to the lack of experimental data in this regard. Furthermore, a less good fit for the *A. coffeaeformis* was noted, with TIC values between 0.06 and 0.10, however lower than the rejection threshold of 0.3. For this no plausible explanation could be found.

### 7.3.4 Nitrate storage evolution

With respect to the minor intracellular uptake rate of the *A. coffeaeformis* species, two scenarios for both microalgal species are illustrated in Figure 7.5. In the first scenario the optimized parameter value for the storage rate is used whereas in the second scenario the storage rate value was set at zero. This clearly indicates that the intracellular storage rate of *P. tricornutum* has a major influence on the dissolved nitrate concentration and as such offers advantages for additional nitrate removal and recuperation. Considering *A. coffeaeformis*, there is no difference in dissolved nitrate removal comparing the two scenarios and thus this species offers no advantage of additional nitrate removal.

Figure 7.4: Model simulation and experimental data of nitrate (Δ), phosphate (○) and biomass (◊) for *P. tricornutum* (top) and *A. coffeaeformis* (bottom) during the growth experiments. Initial settings were 100 g N m⁻³ and 50 g P m⁻³. Predicted values are represented by lines.
Figure 7.5: Evolution of dissolved nitrate concentration for *P. tricornutum* (left hand) and *A. coffeaeformis* (right hand) with two different nitrogen storage rates imposed, respectively 0.036 m$^3$ g$^{-1}$ DW d$^{-1}$ for *P. tricornutum* and 0.0004 m$^3$ g$^{-1}$ DW d$^{-1}$ for *A. coffeaeformis* (full lines). The dashed lines represent the nitrate evolution with $k_{STO} = 0$ m$^3$ g$^{-1}$ DW d$^{-1}$ for both species. Initial nitrate was 100 g N m$^{-3}$.

7.3.5 Model validation

Model validation was done by using the data obtained from a stationary growth experiment in which maximal cell density was reached. The reactor was spiked with nitrate and phosphate to obtain a final concentration of 100 g N m$^{-3}$ and 44 g P m$^{-3}$, respectively. For the simulations, optimized parameter settings of the growth experiments were used (as determined above). As can been deduced from Figure 7.6, each model is able to describe the measured data accurately. Furthermore regarding the goodness of fit, TIC values for nitrate and phosphate were calculated. In case of *P. tricornutum* values of 0.03 (nitrate) and 0.15 (phosphate) were noted. For the *A. coffeaeformis*, values of 0.10 (nitrate) and 0.09 (phosphate) were noted. In both cases, the TIC values for nitrate were in general higher than the values calculated for the growth experiments. More specifically, the higher TIC values were caused by the difference in dissolved nitrate concentration at the end of the stationary growth experiment. This concentration was always depleted by the experiments compared to the simulation results. A plausible explanation for this is that the biomass was composed of more organic nitrogen during the stationary growth experiments and this is not accounted for in the model. However the values are still lower than the acceptance threshold of 0.3 indicating good model performance.
Figure 7.6: Model simulation and experimental data of nitrate (Δ), phosphate (○) and biomass (◊) for *P. tricornutum* (top) and *A. coffeaeformis* (bottom) during the steady state experiments. Initial settings were 100 g N m$^{-3}$ and 44 g P m$^{-3}$. Predicted values are represented by lines.

### 7.3.6 Scenario analysis

The potential for nutrient recovery of *P. tricornutum* was further assessed by simulating a microalgal nutrient recovery system using the calibrated kinetic model. For this a scenario analysis was performed in WEST ®. The performance of the proposed nutrient recovery unit, which requires marine conditions, was compared to a conventional submerged moving bed biofilm reactor for seawater denitrification (Labelle et al. 2005). The algal biomass concentration in the system was controlled at 1 kg m$^{-3}$, while both influent characteristics and reactor volume were derived from Labelle et al. (2005). The initial nitrate and phosphate concentration was 53 g N m$^{-3}$ and 20 g P m$^{-3}$. The conventional denitrification system achieved a nitrate removal efficiency of 88% at a hydraulic retention time (HRT) of 0.04 d. The simulated microalgal nutrient recovery system resulted in a nitrate removal efficiency of 92 % at a HRT
of 1.5 d. Also a 55% phosphate removal efficiency was obtained. The increase of the HRT lowered the possible volumetric loading rate of the reactor from 1.3 to 0.04 kg N m⁻³ d⁻¹ compared to Labelle et al. (2005) (Figure 7.6). A maximal algal biomass production of 109 g DW d⁻¹ was obtained at an HRT of 1 d, which results in a nitrate and phosphate removal efficiency of 67 and 37%, respectively (Figure 7.6).

**Figure 7.6:** Simulation of the nitrate removal (Δ), phosphate removal (○) and algal biomass production (◊) in function of the hydraulic retention time in the reactor.

### 7.4 Conclusions

In this study, a model based kinetic exploration of two microalgal species for nutrient removal in synthetic wastewater was performed. These two microalgal species were selected after an experimental screening for intracellular nitrate storage, by featuring the highest potential amongst six microalgal species.

A parametric study was performed to assess the most significant parameters on the combined dissolved nitrate, dissolved inorganic phosphorus and microalgal biomass concentration during an exponential growth experiment. This resulted in an identifiable combination of the maximum specific growth rate (µ_max) and the intracellular nitrate storage rate (k_STO) which was used for model calibration.

Regarding the nitrate storage rate (k_STO), a value of 0.036 m³ g⁻¹ DW d⁻¹ for *P. tricornutum* and 0.0004 m³ g⁻¹ DW d⁻¹ for *A. coffeaeformis* was noted. Further, in case of *A. coffeaeformis*, the
Kinetic exploration of intracellular nitrate storage

nitrate storage rate \((k_{STO})\) does not differ significantly from zero (95% confidence interval), indicating the non-significance of this parameter for the species. As such the results suggest that \textit{P. tricornutum} is more effective for nitrate removal.

Although a less good fit for the model validation for both microalgal species was obtained, the threshold TIC value of 0.3 was never exceeded as such indicating good model performance.

To further assess the potential of \textit{P. tricornutum} for nutrient recovery and wastewater treatment, a microalgal nutrient recovery unit was simulated for the treatment of aquaculture wastewater and its performance was compared to a conventional denitrification system (Labelle et al. 2005). The simulation study showed that \textit{P. tricornutum} can obtain the same nitrate removal efficiency as the denitrification reactor by increasing the HRT of the system from 0.04 to 1.5 days, thereby reducing the loading rate of the system. The microalgal system on the other hand also results in simultaneous phosphate recovery. This valuable nutrient stays untreated in the denitrification reactor, which necessitates further treatment with iron or aluminum salts to remove it from the effluent before discharge. Also the dosage of an additional carbon source under the form of e.g. methanol is not required in the microalgal system. From a wastewater treatment perspective the algal unit is however unable to compete with the denitrification system due to the large reactor volume required.
Chapter 8

Kinetics of microalgae residing in a WSP

This research was conducted in the scope of an individual credit for scientific research, that was awarded to the author by the Foundation for Scientific Research (FWO) in Belgium
Abstract

Since the oxygen concentration is an important operational parameter of Waste Stabilization Ponds (WSPs), accurate insight in the kinetics of the microalgae living in such systems, is essential in view of system optimization. In this chapter the growth kinetics of two microalgal species, respectively *Chlorella vulgaris* and *Scenedesmus obliquus* was investigated when different conditions of light intensity and temperature were imposed. These microalgal species were isolated from the WSP situated in Ucubamba, Cuenca, Ecuador. This treatment plant is situated in the Andes at 2400 meters above sea level and is used for the treatment of the municipal wastewater of Cuenca. The combined respirometric and titrimetric set-up was used to assess the microalgal kinetics.

The experimental results illustrated the interdependent relationship of light intensity and temperature for both microalgal species, which had a significant influence on the microalgal growth rate. Consequently the growth kinetics, described in Chapter 6 were extended with a mathematical function that describes this relationship. Further additional combined respirometric and titrimetric data were used for model calibration and model validation. Based upon the Theil’s Inequality Criterium, the model described the features of dissolved oxygen and proton addition rather good for both microalgal species.

8.1 Introduction

Wastewater treatment by Waste Stabilization Ponds (WSP) is widely used in the world to treat different types of wastewater, ranging from domestic to industrial waste water. The most important advantage of this system is its simplicity in construction and operation. Moreover, WSP exhibits a high reliability because its operation depends mainly on biological processes and does not depend on equipment performance that can fail (Von Sperling, 2007). In the treatment of domestic wastewater by WSP, the aerobic stabilization of organic compounds by bacteria and the oxygen production and nutrient removal by algal (photosynthetic) activity are the main occurring natural processes. The oxygen demand by bacteria for the assimilation of organic substrate is met by the oxygen produced through algal photosynthetic activity. The photosynthetic activity of algae depends on several environmental conditions such as light,
Kinetics of microalgae residing in a WSP

temperature and availability of nutrients. The effect of nutrients has already been discussed elaborately in previous chapters. Therefore, this chapter focuses on the influence of light intensity and temperature. The oxygen production and consequently the amount of dissolved oxygen in the pond, is a fundamental operational parameter for both maintain a healthy aerobic biomass and to induce adverse conditions in the ponds for pathogen viability (Alvarado, 2013). Thus, it is essential to (accurately and frequently) quantify the influence of these conditions over the oxygen production as they have a major effect on the operation of the WSP. Further, in view of system optimization good insight in the kinetics of the microalgal biomass is a prerequisite. For this, the combined respirometric and titrimetric methodology was used in this chapter to determine the growth kinetics of two microalgal species that were isolated from the facultative pond of the full scale installation at Ucubamba, Ecuador. The two isolated microalgal species were *Chlorella vulgaris* and *Scenedesmus obliquus*. As far as known, no results of the kinetic behavior of the microalgal biomass in a WSP at high altitude has been reported in literature yet. In addition a kinetic growth model was developed based on the measurements and the experimental data were used for model calibration and validation.

8.2 Methods and materials

8.2.1 Cultivation of the microalgae

Both microalgae species were isolated from the biomass of the full scale WSP installation located at Ucubamba. Isolation was done by controlled growth on specific media. After isolation, the microalgae were bred axenic in continuous stirred 3.0 L reactors with ideal inorganic carbon, nutrients and light availability. Also the reactors were periodically sparged with air to prevent settlement of the microalgae on the walls of the breeding reactors. Further the microalgae were kept in exponential growth phase by bi-weekly refreshing of the breeding medium and harvesting of microalgae.
Chapter 8

8.2.2 Experimental protocol

The combined respirometric and titrimetric unit was similar to the set-up described in Chapter 4. The 1.6 L reactor vessel was heat-jacketed to allow temperature control (Alpha R8, www.lauda.de) enabling the exploration of system behaviour at different temperatures. The light cage enclosing the reactor entirely consisted of 36 fluorescent lamps (Voltech, T5 8 W). Light intensity was measured using a lux light meter (FC 840020, Sper Scientific).

Dissolved oxygen (DO) and pH were measured online with an oxygen (Inpro 6870i, Mettler Toledo) and pH electrode (Inpro R 4260 i/SG/120, Mettler Toledo) and the data logged using a PCI-MIO-16XE-50 data acquisition card using LabView (www.ni.com). The pH was controlled online at a user defined setpoint using a banded (+/- 0.1 pH) on-off feedback control algorithm implemented in LabView by dosing HCl or NaOH through two 3-way pinch solenoid valves (Cole Parmer). The rate and amount of 0.25 M HCl and 0.5 M NaOH dosed into the reactor vessel constitutes the titrimetric data.

For the respirometric tests microalgae from the breeding reactor were used, after centrifugation at 4000 rpm for 5 minutes (Heraeus Megafuge 8 Centrifuge, Thermo Scientific), the microalgae where then rinsed twice with demineralized water. Subsequently, the concentrated algae were diluted in 1.5 L of demineralized water. Demineralized water was used, because tap water can contain some alkalinity that can be used by the microalgae for growth. Next 100 ml of nutrient solution containing nitrogen and phosphorus was spiked into the solution. This nutrient solution was directly added after the microalgal biomass was diluted in the demineralized water to avoid osmotic shocks. Then, a sample (200 ml) was taken to analyse the initial microalgal biomass and nutrient concentrations. Finally, 100 ml of bicarbonate solution was spiked before the start of each test. As such, the amount of nutrients and algal biomass for each separate test remained constant, namely 15 g NH₄⁺-N m⁻³, 0.6 g NO₃⁻-N m⁻³, 1.5 g PO₄³⁻-P m⁻³ and 100 g HCO₃⁻ m⁻³. The microalgal biomass concentration was 100 g DW m⁻³. The pH was controlled at 7.5 ± 0.1 for each separate test.

In order to investigate the effect of light intensity and temperature and possible interaction between those environmental variables, a 2 level full factorial design (Box and Draper, 1987) was at first used. As such 7 experiments (Table 8.1) were performed for both microalgal species. The maximal photosynthetic activity, expressed as g O₂ g DW⁻¹d⁻¹ was considered as response.
variable (\(y\)). This value was derived from the oxygen production rate (OPR) curve, more specifically the maximum value of this curve. Next linear regression was used to determine the significant variables and possible interaction. The equation to describe this photosynthetic activity can be denoted as:

\[
y = b_0 + b_1X_1 + b_{12}X_1X_2 + b_2X_2
\]  
(8.1)

With \(b_0\), \(b_1\), \(b_{12}\) and \(b_2\) the different coefficients and \(X_1\), \(X_2\) the variables influencing the photosynthetic activity, respectively light intensity (lux) and temperature (K).

The interaction term in this equation is represented by \(b_{12}X_1X_2\). This means that when interaction is not significant, Equation (8.1) can be rewritten as:

\[
y = b_0 + b_1X_1 + b_2X_2
\]  
(8.2)

The statistical analysis was performed with SPSS.

In Table 8.1 the different settings of light intensity and temperature for both microalgal species are summarized.

### 8.2.3 Analytical methods

Nitrate, phosphate and ammonium were measured according to the standard methods (APHA, 2005). The microalgal dry weight concentration was determined by turbidity measurements, which was previously related to the microalgal dry weight by means of the linear equations shown below for \(Chlorella vulgaris\) and \(Scenedesmus obliquus\), respectively:

\[
Y = 3.06X - 8.54
\]  
(8.3)

\[
Y = 2.16X + 39.33
\]  
(8.4)

With \(Y\) (g DW m\(^{-3}\)) the microalgal dry weight concentration and \(X\) (FTU) the feature of turbidity measurement.
8.2.4 Modelling software

For the simulations described in this work, the Flexible Modelling Environment (FME) package (Soetaert and Herman, 2009) was used. Although this open access package only uses a textual interface, recently it has been more intensively used in view of ecological modelling (Haario et al., 2009; Mannina et al., 2012)

Parameter estimation was performed by the minimization of an objective function by using an optimization algorithm. The objective function was defined as SSE between model prediction and measurements. Minimization of the objective function was done by the Levenberg-Marquardt (Yu and Wilamowski, 2011) algorithm.

8.3 Results and discussion

8.3.1 Data interpretation and derived information

In Figure 8.1 dissolved oxygen profiles, proton addition profiles and OPR and OTR profiles (see Chapter 4) for Chlorella vulgaris and Scenedesmus obliquus are depicted. Both experiments were run at a light intensity of 10650 lux and a temperature of 308 K. Initial algal biomass concentration was 102 g DW m\(^{-3}\) for Chlorella vulgaris whereas for Scenedesmus obliquus it was 75 g DW m\(^{-3}\). No large difference could be observed between the dissolved oxygen profiles and proton addition profiles for both microalgal species. However when calculating the oxygen production profiles, very high values of OPR were observed until approximately 0.05 d. After this time, the OPR levels off to a certain value before it tends to zero. Whereas this trend was not observed for Scenedesmus obliquus. Here the OPR starts at a certain value and remains rather constant before it tends to zero. This trend was observed for all performed experiments.

In Table 8.2 the different calculated values are summarized. The higher \(OPR_{\max}\) for Chlorella vulgaris can be partially explained by the higher microalgal biomass concentration (see Chapter 4). However when taking into account the biomass, a \(pO_{2,\max}\) of 7.73 g O\(_2\) g\(^{-1}\) DW d\(^{-1}\) compared to \(pO_{2,\max} = 4.01\) g O\(_2\) g\(^{-1}\) DW d\(^{-1}\) for Scenedesmus obliquus.
Kinetics of microalgae residing in a WSP

was calculated. This higher photosynthetic activity might suggest a higher maximum specific growth rate in case of \textit{Chlorella vulgaris}. Considering the titrimetric values, also a higher proton rate for \textit{Chlorella vulgaris} was calculated. Moreover, the results of \textit{Chlorella vulgaris} were similar to the values obtained in previous chapters. As such it could be concluded that, despite the lower solubility of oxygen and carbon dioxide at higher altitude, it had no effect on the microalgal growth rate. In literature it is reported that microalgae can adapt to extreme environmental conditions (Mock and Kroon, 2003).

Table 8.1: Initial settings of light intensity and temperature and corresponding $p_{O_2,max}$ for \textit{S. obliquus} and \textit{C. vulgaris}

<table>
<thead>
<tr>
<th></th>
<th>\textit{S. obliquus}</th>
<th></th>
<th>\textit{C. vulgaris}</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (lux)</td>
<td>T (K)</td>
<td>Y (g O$_2$ g$^{-1}$ DW d$^{-1}$)</td>
<td>I (lux)</td>
</tr>
<tr>
<td>4810</td>
<td>298</td>
<td>0.29</td>
<td>4810</td>
</tr>
<tr>
<td>4810</td>
<td>283</td>
<td>0.37</td>
<td>4810</td>
</tr>
<tr>
<td>1000</td>
<td>283</td>
<td>0.16</td>
<td>4810</td>
</tr>
<tr>
<td>1000</td>
<td>306</td>
<td>0.29</td>
<td>10650</td>
</tr>
<tr>
<td>10650</td>
<td>306</td>
<td>1.00</td>
<td>1000</td>
</tr>
<tr>
<td>4810</td>
<td>306</td>
<td>0.64</td>
<td>10650</td>
</tr>
<tr>
<td>10650</td>
<td>283</td>
<td>0.40</td>
<td>10650</td>
</tr>
</tbody>
</table>

Table 8.2: Calculated values derived from the respirometric and titrimetric profile for the experiments illustrated in Figure 8.1

<table>
<thead>
<tr>
<th></th>
<th>\textit{C. vulgaris}</th>
<th>\textit{S. obliquus}</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(OPR_{\text{max}})</td>
<td>850.00</td>
<td>300.80</td>
<td>g O$_2$ m$^{-3}$ d$^{-1}$</td>
</tr>
<tr>
<td>(p_{O_2,\text{max}})</td>
<td>7.73</td>
<td>4.01</td>
<td>g O$_2$ g$^{-1}$ DW d$^{-1}$</td>
</tr>
<tr>
<td>O$_2$\text{produced}</td>
<td>0.87</td>
<td>0.74</td>
<td>g O$_2$ g$^{-1}$ DW</td>
</tr>
<tr>
<td>HAR</td>
<td>12.53</td>
<td>7.01</td>
<td>g H$^+$ m$^{-3}$ d$^{-1}$</td>
</tr>
<tr>
<td>H$^+$\text{added}</td>
<td>1.54</td>
<td>1.36</td>
<td>g H$^+$ m$^{-3}$</td>
</tr>
</tbody>
</table>
Figure 8.1: Experimental profiles of dissolved oxygen, proton addition, oxygen production rate and oxygen transfer rate for *Chlorella vulgaris* (red line) and *Scenedesmus obliquus* (black line). Both experiments were run at a light intensity of 10650 lux and a temperature of 308 K. Initial algal biomass concentration was 102 g DW m$^{-3}$ for *Chlorella vulgaris* whereas for *Scenedesmus obliquus* it was 75 g DW m$^{-3}$.

### 8.3.2 Determination of significance of factors

To determine the significance of light intensity and temperature, seven separate experiments were run for both microalgal species. The relative $p_{O_2,max}$ of each experiment was considered as respons variable for the linear regression. This was derived from the respirometric profiles. The settings of light intensity, temperature and the derived respons variable are mentioned in Table 8.1. Results of the linear regression (95 % confidence level) are given in Table 8.3. This results showed the significance of the interdependency of light intensity and temperature for both microalgal species. This could be expected by literature (Carvalho and Malcata., 2003) as already described in Chapter 2. As such this aspect should be taking into account for model
development. Further it seemed that the influence of light intensity is higher for *Chlorella vulgaris* compared to *Scenedesmus obliquus*.

### Table 8.3: Regression coefficients for *C. vulgaris* and *S. obliquus*

<table>
<thead>
<tr>
<th>coefficient</th>
<th><em>C. vulgaris</em></th>
<th><em>S. obliquus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>p value</td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td>$b_1$</td>
<td>$1.7 \times 10^{-3}$</td>
<td>$6.5 \times 10^{-4}$</td>
</tr>
<tr>
<td>$b_{12}$</td>
<td>$7.2 \times 10^{-4}$</td>
<td>$7.3 \times 10^{-4}$</td>
</tr>
<tr>
<td>$b_3$</td>
<td>$6 \times 10^{-6}$</td>
<td>$2.4 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

#### 8.3.3 Model development

In the previous section, experiments were performed to assess the effect of light intensity and temperature and the interdependent relationship between those variables. In this section, the model described in Chapter 6 is extended. This allows to describe several experiments conducted at different temperatures and light intensities by using one uniform combination of parameter values.

8.3.3.1 Microalgal growth

In the previous section the interrelationship between temperature and light intensity was observed. Here, the microalgal growth kinetics are extended compared to Chapter 6.

The interrelationship between temperature and light intensity can be explained by the fact that there is a direct relation between light intensity and activation energy. Furthermore, the light saturation level is influenced as mentioned before by the temperature, next to the prevailing light intensity (Carvalho and Malcata, 2003). To describe this relationship, Equation (2.28) was used. As such the different growth rates could be denoted as:

$$
\rho_{A_{lg}(CO_2,NH_4^+,PO_3^-)} = \mu_{max} \left( \frac{S_{CO_2}}{K_{CO_2} + S_{CO_2}} \right) \left( \frac{S_{NH_4^+}}{K_{NH_4^+} + S_{NH_4^+}} \right) \left( \frac{S_{PO_3^-}}{K_{PO_3^-} + S_{PO_3^-}} \right) \left( \frac{K_1 I}{K_2 T + I} \right) e^{\left(\frac{-\beta I}{T}\right)} X_{A_{lg}}
$$
\[ \rho_{\text{Alg}}(\text{CO}_2, \text{NO}_3^-, \text{PO}_4^{3-}) = \mu_{\text{max}} \left( \frac{S_{\text{CO}_2}}{K_{\text{CO}_2} + S_{\text{CO}_2}} \right) \left( \frac{S_{\text{NO}_3^-}}{K_{\text{NO}_3^-} + S_{\text{NO}_3^-}} \right) \left( \frac{K_i \text{NH}_4^+}{K_i \text{NH}_4^+ + S_{\text{NH}_4^+}} \right) \left( \frac{S_{\text{PO}_4^{3-}}}{K_{\text{PO}_4^{3-}} + S_{\text{PO}_4^{3-}}} \right) \left( \frac{K_1 I}{K_2 T + I} \right) e^{\left( \frac{-\beta I}{T} \right) X_{\text{Alg}}} \] (8.5)

\[ \rho_{\text{Alg}}(\text{HCO}_3^-, \text{NH}_4^+, \text{NO}_3^-, \text{PO}_4^{3-}) = \mu_{\text{max}} \left( \frac{S_{\text{HCO}_3^-}}{K_{\text{HCO}_3^-} + S_{\text{HCO}_3^-}} \right) \left( \frac{K_i \text{CO}_2}{K_i \text{CO}_2 + S_{\text{CO}_2}} \right) \left( \frac{S_{\text{NH}_4^+}}{K_i \text{NH}_4^+ + S_{\text{NH}_4^+}} \right) \left( \frac{S_{\text{PO}_4^{3-}}}{K_{\text{PO}_4^{3-}} + S_{\text{PO}_4^{3-}}} \right) \left( \frac{K_1 I}{K_2 T + I} \right) e^{\left( \frac{-\beta I}{T} \right) X_{\text{Alg}}} \] (8.6)

\[ \rho_{\text{Alg}}(\text{HCO}_3^-, \text{NO}_3^-, \text{PO}_4^{3-}) = \mu_{\text{max}} \left( \frac{S_{\text{HCO}_3^-}}{K_{\text{HCO}_3^-} + S_{\text{HCO}_3^-}} \right) \left( \frac{K_i \text{CO}_2}{K_i \text{CO}_2 + S_{\text{CO}_2}} \right) \left( \frac{K_i \text{NH}_4^+}{K_i \text{NH}_4^+ + S_{\text{NH}_4^+}} \right) \left( \frac{S_{\text{NO}_3^-}}{K_{\text{NO}_3^-} + S_{\text{NO}_3^-}} \right) \left( \frac{S_{\text{PO}_4^{3-}}}{K_{\text{PO}_4^{3-}} + S_{\text{PO}_4^{3-}}} \right) \left( \frac{K_1 I}{K_2 T + I} \right) e^{\left( \frac{-\beta I}{T} \right) X_{\text{Alg}}} \] (8.7)

8.3.3.2 Temperature dependent oxygen transfer rate

The influence of temperature on the \( K_L a \) was determined by performing separate experiments at different temperatures. For this only 1.5 l of synthetic wastewater was used. This wastewater was first sparged with carbon dioxide to remove the dissolved oxygen. Then the reactor vessel was stirred and the dissolved oxygen concentration was noted. The \( K_L a \) was calculated by:

\[ \frac{dO_2}{dt} = K_L a \left( S_{O_2}^{\text{sat}} - S_{O_2} \right) \] (8.9)

The experimentally determined values were then fitted to:

\[ K_L a \left( T \right) = K_L a_{\text{ref}} \theta^{(T-293)} \] (8.10)

With \( K_L a_{\text{ref}} \) (d\(^{-1}\)) the oxygen mass transfer coefficient at temperature of \( T = 293 \) K, and \( K_L a \left( T \right) \) (d\(^{-1}\)) the oxygen mass transfer coefficient at a certain temperature. Parameter \( \theta \) (-) was fitted using the experimental values by minimizing the SSE.
In Figure 8.2 the experimental oxygen mass transfer coefficient and calculated values are depicted with optimized parameter value $\theta$. In general good correspondence between the measured and calculated oxygen mass transfer coefficient was observed.

![Graph](image)

**Figure 8.2:** Calculated and experimental $K_La$ values when using $\theta = 1.045$.

### 8.3.4 Model parameter values

Considering the function of light intensity and temperature, parameters $K_1$, $K_2$ and $\beta$ were fitted to the relative photosynthetic activity ($pO_{2,max}$) values for the experiments described in section 8.3.2. In Figure 8.3 the experimental and calculated values of $pO_{2,max}$ for both microalgal species are illustrated after estimation of the three parameters by minimization of SSE. As can been seen rather good correspondence between calculated and experimental values was observed. However, some outliers were noted. The same results were used to perform the linear regression in order to assess the effect of temperature and light intensity on the relative maximum photosynthetic activity. The standard errors of the regression analysis were the highest for the experiments corresponding to the outliers in Fig 8.3. This was the case for both microalgal species. As such, it could be concluded that there were probably unexpected deviations on the experimental results. At that moment it was decided not to omit this experimental values, since this would reduce the points to determine this empirical function. The optimized parameter values are mentioned in Table 8.4.

Other biokinetic and physical-chemical parameters for *Chlorella vulgaris* were akin to the parameters mentioned in Chapter 6. With respect to the species *Scenedesmus obliquus* different
parameters for the yield coefficient on phosphorus and the half saturation coefficient for phosphorus, respectively \( Y_5 = 156.25 \, \text{g DW g}^{-1} \, \text{P} \) and \( K_{PO_4} = 0.037 \, \text{g P m}^{-3} \) (Sancho et al., 1997) were used. Sancho et al. (1997) determined this yield for phosphorus based on elementary analysis of the biomass. Also the half saturation coefficient was experimentally determined.

![Graph showing comparison of calculated and measured values of relative \( p_{O_2, max} \) with optimized parameters of \( K_1, K_2 \) and \( \beta \) for Scenedesmus obliquus (left hand figure) and Chlorella vulgaris (right hand figure).]

**8.3.5 Model calibration**

**8.3.5.1 Experiments with Chlorella vulgaris**

Two separate experiments were used for model calibration in case of both microalgal species. The maximum specific growth rate and the oxygen mass transfer coefficient were calibrated to the combined respirometric and titrimetric data. With Chlorella vulgaris the first experiment was performed at a light intensity of \( I = 10650 \, \text{lux} \). The temperature was controlled at 313 K and the initial microalgal biomass concentration equaled 91.75 g DW m\(^{-3}\). The second calibration experiment was run at a light intensity of 4810 lux and a temperature of 308 K. The initial biomass concentration was 104 g DW m\(^{-3}\). Like already mentioned before it was strived to use similar values of nitrate, phosphate and ammonium for each separate test, respectively 15 g NH\(_4\)\(^+\)-N m\(^{-3}\), 0.6 g NO\(_3\)-N m\(^{-3}\) and 0.2 g PO\(_4\)\(^3\)-P m\(^{-3}\). In Figure 8.4 the predicted and experimental dissolved oxygen profiles and proton addition profiles are illustrated.
Kinetics of microalgae residing in a WSP

For the first calibration experiment a TIC = 0.06 and TIC = 0.05 was calculated for the respirometric and titrimetric profile, indicating good model performance (Audenaert et al., 2010). Also good visual correspondence between experimental and predicted values was observed. Considering the second calibration experiment, a visual less good fit (respirometric profile) was observed. However still low values for TIC were calculated, namely TIC = 0.07 for the respirometric profile and TIC = 0.05 for the titrimetric profile. The optimized parameter values for the first experiment were $\mu_{\text{max}} = 0.56 \pm 0.0008 \, \text{d}^{-1}$ and $K_L a_{293} = 10.02 \pm 0.02 \, \text{d}^{-1}$. For the second calibration experiment $\mu_{\text{max}} = 0.62 \pm 0.0001 \, \text{d}^{-1}$ and $K_L a_{293} = 3.76 \pm 0.003 \, \text{d}^{-1}$ were obtained. The optimal values for the maximum specific growth rate were akin for the two experiments.

Figure 8.4: Experimental (dashed line) and predicted (full line) dissolved oxygen concentration (left hand) and proton addition (right hand) for the first (top) and second (bottom) calibration experiment with *Chlorella vulgaris*.

The optimal value of the oxygen mass transfer coefficient for the second calibration experiment was similar to the experimental determined value. For the first calibration experiment this
parameter was higher than experimentally determined. This could be explained by the fact that more intense mixing occurred during the experiments.

Table 8.4: Overview of the biokinetic parameter values used in the model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Assigned value</th>
<th>Assigned value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_{\text{max}}$</td>
<td>0.001</td>
<td>0.001</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$Y_1$</td>
<td>0.549</td>
<td>0.549</td>
<td>g DW g$^{-1}$ HCO$_3^-$</td>
</tr>
<tr>
<td>$Y_2$</td>
<td>0.761</td>
<td>0.761</td>
<td>g DW g$^{-1}$ CO$_2$</td>
</tr>
<tr>
<td>$Y_4$</td>
<td>15.84</td>
<td>15.84</td>
<td>g DW g$^{-1}$ N</td>
</tr>
<tr>
<td>$Y_5$</td>
<td>110.93</td>
<td>156.25</td>
<td>g DW g$^{-1}$ P</td>
</tr>
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<td>$Y_7$</td>
<td>1.24</td>
<td>1.24</td>
<td>g O$_2$ g$^{-1}$ DW</td>
</tr>
<tr>
<td>$Y_6$</td>
<td>0.96</td>
<td>0.96</td>
<td>g O$_2$ g$^{-1}$ DW</td>
</tr>
<tr>
<td>$K_{\text{HCO}_3^-}$</td>
<td>3</td>
<td>3</td>
<td>g HCO$_3^-$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_{\text{CO}_2}$</td>
<td>0.2</td>
<td>0.2</td>
<td>g CO$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_{\text{NH}_4^+}$</td>
<td>0.3</td>
<td>0.00018</td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$K_{\text{NO}_3^-}$</td>
<td>0.3</td>
<td>0.00018</td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$K_{\text{PO}_4^{3-}}$</td>
<td>0.08</td>
<td>0.037</td>
<td>g P m$^{-3}$</td>
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<tr>
<td>$k_1$</td>
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<td>d$^{-1}$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>100000</td>
<td>100000</td>
<td>d$^{-1}$</td>
</tr>
<tr>
<td>$K_{\text{i}CO}_2$</td>
<td>0.044</td>
<td>0.044</td>
<td>g CO$_2$ m$^{-3}$</td>
</tr>
<tr>
<td>$K_1$</td>
<td>12.23</td>
<td>122</td>
<td>(-)</td>
</tr>
<tr>
<td>$K_2$</td>
<td>21.75</td>
<td>200.5</td>
<td>(K$^+$)</td>
</tr>
<tr>
<td>$K_{\text{i}NH}_4^+$</td>
<td>0.02</td>
<td>0.02</td>
<td>g N m$^{-3}$</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.013</td>
<td>0.011</td>
<td>(-)</td>
</tr>
</tbody>
</table>
8.3.5.2 Experiments with *Scenedesmus obliquus*

The first calibration experiment with *Scenedesmus obliquus* was performed at a light intensity of $I = 1000$ lux and temperature $T = 306$ K. The initial microalgal biomass concentration was $92$ g DW m$^{-3}$. For the second calibration experiment, light intensity was set at $I = 10650$ lux and the temperature was controlled at $T = 313$ K.

![Figure 8.5: Predicted (full line) and experimental values (dashed line) of dissolved oxygen concentration (left hand figures) and proton addition (right hand figures) for the first (top) and second (bottom) calibration experiment with *Scenedesmus obliquus*.](image)

For the first calibration experiment a TIC = 0.06 and TIC = 0.03 for the dissolved oxygen concentration and proton addition was calculated, indicating good model performance. The second experiment had a TIC = 0.02 for both the dissolved oxygen concentration and proton addition. In general it can be concluded, that with optimized parameter settings, the correspondence between experimental values and predicted values is higher in case of *Scenedesmus obliquus* compared to *Chlorella vulgaris*. This might be expected, due to the fact that the parameters related to the light and temperature function, were first fitted to experimental...
Chapter 8

$p_{O_2,\text{max}}$ values (see section 8.3.4). For *Chlorella vulgaris* more outliers were observed. This might suggest optimization of this parameter values. However this was not done, because of possible identifiability issues and considering the overall good model performance. The optimized value of maximum specific growth rate and oxygen mass transfer coefficient were $\mu_{\text{max}} = 0.19 \pm 0.0004$, $K_La_{293} = 7.71 \pm 0.004 \text{ d}^{-1}$ and $\mu_{\text{max}} = 0.25 \pm 0.0004 \text{ d}^{-1}$, $K_La_{293} = 5.20 \pm 0.02 \text{ d}^{-1}$ for the first calibration and second calibration experiment respectively.

### 8.3 Model validation

#### 8.3.1 Experiments with *Chlorella vulgaris*

Two experimental runs were used as validation experiments. The mean value of the optimized values for the maximum specific growth rate was used for the simulations. As such a value of $\mu_{\text{max}} = 0.59 \text{ d}^{-1}$ was used. Considering the oxygen mass transfer coefficient, the mean of the four (i.e. for both microalgal species) calibration was used. So a value of $K_La_{293} = 6.43 \text{ d}^{-1}$ was used. The first validation experiment was run with a light intensity of $I = 10650$ lux and temperature $T = 298 \text{ K}$. The initial microalgal biomass concentration was $60.94 \text{ g DW m}^{-3}$. The second validation experiment was performed with a light intensity of $I = 10650$ lux and temperature $T = 293$. Initial biomass concentration was $58.55 \text{ g DW m}^{-3}$. In Figure 8.6 the predicted and experimental values of dissolved oxygen (left) and proton addition (right) are depicted. Good visual correspondence for both experiments in the first part of the respirometric profile was observed. In the descending part a slight deviation between experimental and predicted values could be noted. This could be explained by the difference of experimental and optimized values of oxygen mass transfer coefficient. Indeed, the experimental value was determined without microalgal biomass present in the reactor vessel and this can have influence on the parameter (Pittoors et al., 2014). Considering the titrimetric profile also good correspondence in the first part was observed and minor deviation between experimental values and predicted values after the declination point. This can be explained by the fact that the amount of bicarbonate dosed to the reactor vessel slightly deviated from the foreseen amount. To overcome this drawback, an additional sensor to online measure the inorganic concentration in the liquid phase might be suggested. Overall good model performance could be concluded.
since the calculated TIC values were 0.19 for the respirometric profile and 0.04 for the titrimetric profile in case of the first validation experiment. For the second validation experiment TIC = 0.06 and TIC = 0.05 for the titrimetric and respirometric profile respectively.

Figure 8.6: Experimental (dashed line) and predicted (full line) profiles for the first (top) and second (bottom) validation experiments with *Chlorella vulgaris*. The first experiment was run with $I = 10650$ lux and $T = 298$. Initial biomass concentration was 60.94 g DW m$^{-3}$. The second experiment was run with $I = 1650$ lux and $T = 273$ K. Initial biomass concentration was 58.55 g DW m$^{-3}$.

8.3.2 Experiments with *Scenedesmus obliquus*

For this species, also two experimental runs were used as model validation. The first experiment was performed with a light intensity $I = 10650$ lux and $T = 306$K. Initial biomass concentration was 91.17 g DW m$^{-3}$. For the second experiment, $I = 4810$ lux and $T = 293$ K. Here the initial biomass concentration was 103. 34 g DW m$^{-3}$. A value of $\mu_{max} = 0.217$ d$^{-1}$ and $K_L a_{20} = 6.43$ d$^{-1}$ was used for both simulations. In Figure 8.7 the predicted and experimental values of
dissolved oxygen (left hand figure) and proton addition (right hand figure) are depicted. Similar observations and conclusions as for *Chlorella vulgaris* could be made. For the first experiment, a TIC = 0.07 and TIC = 0.06 for the respirometric data and titrimetric data was calculated. For the second experiment, the TIC = 0.09 for both data series.

![Figure 8.7: Experimental (dashed line) and predicted (full line) profiles for the first (top) and second (bottom) validation experiments with *Senedesmus obliquus*. The first experiment was run with I= 10650 lux and T= 306 K. Initial biomass concentration was 91.17 g DW m\(^{-3}\). The second experiment was run with I = 4810 lux and T = 293 K. Initial biomass concentration was 103.34 g DW m\(^{-3}\).](image)

8.3.3 Validation of the extended model with additional experimental data

In addition, the extended model performance was assessed by the validation of this model using experimental data of *Chlorella vulgaris*, described in Chapter 6. The parameter values and optimized maximum specific growth rate and oxygen mass transfer coefficient were taken from this chapter. Initial settings of the were 2.87 g NO\(_3\)\(^{-}\)N m\(^{-3}\), 8.13 g NH\(_4^+\)-N m\(^{-3}\), 3.33 g PO\(_4^{3-}\)-P m\(^{-3}\) and 100 g HCO\(_3^-\) m\(^{-3}\). The initial biomass concentration was 646 g DW m\(^{-3}\). In Figure 8.8
the experimental and predicted values for the proton addition and dissolved oxygen concentration are depicted. The TIC was 0.07 for the respirometric profile and 0.05 for the titrimetric profile, indicating good model performance. However, the TIC was somehow higher than the TIC calculated with the Algnut model. Also visually a difference could be observed. Both profiles had an overestimation of the maximum specific growth rate. Further it seemed that the oxygen mass transfer coefficient was slightly underestimated. This might suggest that the parameters with respect to the interdependent light and temperature function should be assessed for identifiability or should be determined more accurately by additional experiments in order to define the empirical relation more accurately.

![Figure 8.8: Experimental (dashed line) and predicted (full line) profiles for the additional validation experiment. Initial settings were 2.87 g NO3⁻-N m⁻³, 8.13 g NH₄⁺-N m⁻³, 3.33 g PO₄³⁻-P m⁻³ and 100 g HCO₃⁻ m⁻³. The initial biomass concentration was 646 g DW m⁻³.](image)

**8.4 Conclusions**

In this chapter the combined respirometric and titrimetric methodology was used to compare the microalgal growth kinetics of two microalgal species that were prior isolated from the biomass of a WSP. Results showed the interdependent effect of light intensity and temperature on the growth rate for both microalgal species. As such the model developed in Chapter 6 was extended with a mathematical function which describes this interdependent relationship. Good
Chapter 8

model performance with optimized parameter values of maximum specific growth rate and oxygen mass transfer coefficient was obtained. Further model validation with two additional experiments illustrated good model performance for both microalgal species. Next the optimized values for the maximum specific growth rates were $\mu_{\text{max}} = 0.590 \text{ d}^{-1}$ for *Chlorella vulgaris* and $\mu_{\text{max}} = 0.217 \text{ d}^{-1}$ for *Scenedesmus obliquus*. This agrees with the fact that the first microalgal species is the most dominant species present in the WSP. Also when calculating the $\text{OPR}_{\text{max}}$, a higher value for *Chlorella vulgaris* was noted. According to Menaa et al. (2015), the maximum specific growth rate for the *Chlorella vulgaris* is also higher than for *Scenedesmus obliquus*. Possible explanation could be that the metabolism for the latter species is different. Probably other pigments are more synthesized (for example carotenoids) instead of chlorophyll. This can then induce cell shading and as such less photosynthetic activity.

The fact that a difference in growth rate between the two species was observed could be interesting in view of valorisation of the microalgal biomass.

A next step in the research would be to assess the ability of the model to describe the behavior of the full scale waste stabilization pond. This can be achieved by integrating the model developed in this chapter with existing activated sludge model (ASM1). Based on the results of these simulations one could then conclude that either the model is able to describe the full scale installation well or the parameter set regarding the microalgal kinetics should be reconsidered. For this purpose additional calibration could be needed.
Kinetics of microalgae residing in a WSP
Chapter 9

General conclusions and future perspectives
9.1 Introduction

In this dissertation, the autotrophic growth kinetics of microalgae for wastewater treatment applications was examined by the combination of practical experiments and mathematical modelling. Throughout the work, several mathematical models were developed, calibrated and validated to experimental data. The experiments for the determination of the growth kinetics were performed with two microalgal species, namely *Chlorella vulgaris* and *Scenedesmus obliquus*. These microalgal species were chosen because a lot of information about the growth kinetics of these species is reported in literature. Also these two species are in general abundant in systems for wastewater treatment such as for example WSPs. (Pham et al., 2014). The approach was to start from a very simplistic model that was extended in different stages in order to lead to a mechanistic model including several processes that influence the microalgal growth rate and as such the removal of nutrients from wastewater. This in view of system performance optimization. In Table 9.1 the aspects that are included in the research chapters are summarized and the link between the different chapters is emphasized.

<table>
<thead>
<tr>
<th>Table 9.1: Overview of the different aspects mentioned in the different chapters</th>
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<tbody>
<tr>
<td><strong>Chapter</strong></td>
</tr>
<tr>
<td>Chapter 4</td>
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<tr>
<td>Chapter 5</td>
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<tr>
<td>Chapter 6</td>
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<tr>
<td>Chapter 7</td>
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<td>Chapter 8</td>
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</tbody>
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Throughout this dissertation, the knowledge concerning the microalgal growth kinetics and mathematical modelling increased significantly. In the first research chapter, namely Chapter 4, a simple model was built taking into account solely inorganic carbon limitation. Also, similarly to the respirometry with activated sludge, only the respirometric data was considered for model calibration. Two parameters, respectively the maximum specific growth rate ($\mu_{\text{max}}$) and the oxygen mass transfer coefficient ($K_L a$), were chosen for calibration by trial and error. In Chapter 5, it was observed that the titrimetric profile offers additional valuable information concerning the microalgal growth kinetics. More specifically, taking into account the values that were derived from these profiles, allowed to close the inorganic carbon balance.
Consequently, the cumulative proton concentration was included in the model structure of Chapter 6 as steady state variable. Also, only effect of inorganic nitrogen and microalgal biomass concentration on the dissolved oxygen profiles and proton addition profiles was observed. Likely, the reason for this was the limitation of the used statistic experimental design. For this it was decided to implement an alternative experimental design in Chapter 8. A parametric identifiability study (Chapter 6) illustrated that only the maximum specific growth rate ($\mu_{max}$) and oxygen mass transfer coefficient ($K_La$) were uniquely identifiable from the combined respirometric and titrimetric data. This might suggest that the choice of these two parameters for model calibration, made in Chapter 4, was a good choice.

The final model that is proposed in this dissertation (Chapter 8), is able to describe the specific experimental features very good when considering the Theil’s Inequality Coefficient as criterium for model performance, for the two different microalgal species. This when using one set of parameter combinations for each microalgal species. Further the proposed model accounts for several factors that influence the microalgal growth rate. Such models are also reported in literature, but are in general very mechanistic. Also the combined respirometric and titrimetric methodology, that was used as tool for the experimental test, has proven to be a solid and trustworthy technique to measure the microalgal kinetics. This method is by this a promising and easy to perform technique as alternative for more complex methods such as pulse amplitude modulated (PAM)-fluorometry or methods where the features can only be related to the microalgal kinetics after a certain adaptation period.

However, the proposed final model also has some drawback. A major one is the fact that this model can only be validated in case of autotrophic growth conditions. Also, the parameter settings that are proposed can only be used for the specific microalgal species that are mentioned. When using microalgal biomass for wastewater treatment, the latter is very difficult or even impossible to control, when using cost-effective systems. Validation of the developed growth model with data from a full scale installation would be a next logical step. In case of insufficient model performance, certain parameter setting should be reconsidered. This implements that probably for different type of wastewaters or different systems, the model needs to be recalibrated with new respirometric and titrimetric features. Nevertheless the methodology and proposed model offers a solid base.

In the following text, the main conclusions of each chapter and opportunities and perspectives for future research are highlighted.
Chapter 9

9.2 Development of a new technique to measure algal growth kinetics

In Chapter 4 a new method to measure the algal kinetics was described. This approach is similar to a method used to measure the growth kinetics of activated sludge, namely combined respirometry and titrimetry. However, the microalgal metabolism differs from the activated sludge metabolism. Microalgae produce oxygen with abundant light intensity and inorganic carbon and nutrients whereas activated sludge consumes oxygen due to consumption of organic carbon. Here tests were performed with only inorganic carbon limitation. Other factors such as nitrogen, phosphorus and light intensity were chosen not to be limiting the microalgal growth. The results revealed that, in order to have good insight in the microalgal kinetics both profiles should be evaluated. Indeed, the inorganic carbon equilibrium in the liquid phase has an important role in which form the inorganic carbon is present and can be consequently assimilated by the microalgae. Further inorganic carbon present as carbon dioxide can be stripped and not be available for microalgal assimilation. As such this will have a large influence on the proton addition. These phenomena cannot be observed by evaluating the dissolved oxygen profile. Also this explains that the experimental amount of oxygen produced is much lower than theoretically expected according to Stumm and Morgan (1998). This in contrary to the experimental amount of protons added by the photosynthetic activity corresponds to the theoretical amount (Stumm and Morgan, 1998) very well. This is because the titrimetric profile also takes into account the proton addition due to stripping and inducing shifts in the chemical equilibrium of inorganic carbon in the liquid phase.

Further, a model was developed accounting for inorganic carbon kinetics and the chemical equilibrium of inorganic carbon. Two parameters, respectively the maximum specific growth rate and the oxygen mass transfer coefficient between liquid and gas phase were considered for model calibration, with the experimental data of dissolved oxygen as objective function. Good visual correspondence between experimental and predicted values was obtained. Also the optimized values for the maximum specific growth rate were akin to values found in literature.

In Chapter 4, the combined respirometric and titrimetric methodology was proven to be a solid method to determine the microalgal growth kinetics when only inorganic carbon limitation was implemented. Also the developed model was able to describe the experimental dissolved oxygen data accurately. Assessing this methodology when different environmental factors were used was a logical next step in this research. This was performed in Chapter 5.
9.3 Optimization of nutrient removal in wastewater by microalgal biomass based on combined respirometric and titrimetric data

In Chapter 5, the assessment of the combined respirometric and titrimetric methodology was described when different environmental conditions were imposed. This was done mimicking the natural environment. For this a statistical experimental design was used. Experimental results revealed that in the ranges applied only nitrogen limitation could be observed by the combined data. Although it was expected from literature, no significant influence of light intensity, phosphorus or temperature on the respirometric and titrimetric profiles could be observed. Regarding phosphorus, possible reason might be that the concentrations that were implemented in the separate tests, were too high, so no limiting effect could be observed. Further the preference of ammonium as nitrogen source was observed. Based on the experimental observations, kinetic expressions for growth on inorganic carbon and nutrients were proposed.

These equations were incorporated in the model developed in Chapter 6. Because next to a good model structure, also accurate experimental data is needed in view of model calibration and validation, a selection was made of the data generated in Chapter 5. Two selection criteria were used. A first selection criterion was the comparison of the experimental proton addition and the theoretical proton addition. The second selection criterion was the correspondence between the nitrogen to phosphorus ratio and the theoretical Redfield ratio.

A global sensitivity analysis and parameter identifiability study revealed that only two parameters were uniquely identifiable to the combined respirometric and titrimetric data. Consequently these two parameters, namely the maximum specific growth rate and the oxygen mass transfer coefficient were calibrated to the selected experimental data resulting in good correspondence between experimental features and predicted values.

Moreover, model validation with two additional experimental runs illustrated that the experimental features were akin to the values predicted by the model.
9.4 Kinetic exploration of intracellular nitrate storage

The model structure developed in Chapter 6 was extended with an intracellular nitrate storage process to assess this storage capacity for marine microalgae. Modelling of this intracellular nitrate storage ability has not been reported in literature before. A preliminary experimental screening of six different marine microalgal species indicated two species with highest storage capacity, which were considered for further model based analysis. After a parameter identifiability assessment, the maximum specific growth rate and the intracellular nitrate storage rate constant were calibrated to the experimental features. The results revealed that \textit{P. tricornutum} had a higher intracellular nitrate storage rate. Further the latter did not differ significantly from zero for \textit{A. coffeaformis}.

9.5 Growth kinetics of microalgae residing in WSP

In Chapter 8 the combined respirometric and titrimetric methodology was used to compare the microalgal growth kinetics of two microalgal species that were isolated from the biomass of a WSP situated at 2400 m above sea level. Results showed the interdependent effect of light intensity and temperature on the growth rate for both microalgal species. As such the model developed in Chapter 6 was extended with a mathematical function which describes this interdependent relationship. Good model performance was obtained with optimized parameter values for maximum specific growth rate and oxygen mass transfer coefficient. Further model validation with two additional experiments illustrated good model performance for both microalgal species. This resulted in optimized values of $\mu_{\text{max}} = 0.590 \, \text{d}^{-1}$ for \textit{Chlorella vulgaris} and $\mu_{\text{max}} = 0.217 \, \text{d}^{-1}$ for \textit{Scenedesmus obliquus}. This agrees with the fact that the first microalgal species is the most dominant species present in the WSP.
9.6 Perspectives and opportunities for future research

In this dissertation, two major issues were handled, namely the development of a methodology to measure the microalgal kinetics, which is rather easy to perform and which can be used to determine the kinetics accurately under different environmental conditions. Next a model was developed which was successfully calibrated and validated to the specific experimental data. In view of system optimisation mathematical modelling offers a promising and useful tool. Although results are very promising, some aspects should be considered for future research.

First, in this dissertation, only autotrophic microalgal growth was considered and assessed by lab scale experiments. However, in full scale or pilot scale systems additional growth conditions, such as for example mixotrophic or heterotrophic, may occur depending on the environmental conditions such as light intensity and temperature and the physical – chemical condition of the treated waste water. This could also change the microalgal diversity present in the system.

For this it would be interesting to test in a first stage the different alternative microalgal growth conditions on lab scale with different microalgal species. These different growth conditions can be induced by adding different organic carbon sources with different conditions of temperature and light intensity imposed. In a next step, samples of biomass of the microalgal system can be taken to perform different separate tests. In a final step experimental runs with the microalgal biomass and specific types of wastewater could be performed. Consequently model structure changes should be made. Kinetics and stoichiometry describing mixotrophic and/or heterotrophic microalgal growth should be incorporated. Further considering the fact that a real type of wastewater is used, additional physic-chemical phenomena, such as for example precipitation, should be included.

Another aspect that would be interesting for future research, is the assessment of the combined respirometric and titrimetric methodology for wastewater treatment systems with microalgal biomass in combination with activated sludge. In general these combinations occur in microalgal bacteria flocs systems and in WSPs. Both of these systems offer advantages in operation costs such as aeration and also harvesting of the biomass. Kinetics of activated sludge are already rigorously described in literature. In this dissertation results of microalgal kinetics are mentioned. Consequently it would be very interesting to run experiments under autotrophic,
mixotrophic and heterotrophic growth conditions with the biomass of WSP and microalgal bacteria flocs to assess the growth kinetics. The features of this experiments could then be used for model calibration and validation in view of system optimization. The model structure could be a merging of the known ASM(1) model and the model developed in this dissertation extended with the alternative microalgal growth conditions. This takes the assumption that for example in case of microalgal bacteria flocs, the bacteria and microalgae are completely mixed and no stratification (layers) occur in the Microalgal Bacteria flocs.

Finally considering the experimental set-up, it would be interesting to use additional on line measurement to improve the model calibration and validation. More specific a sensor to measure the inorganic carbon concentration in the liquid phase. This could overcome the drawback of inaccuracies of model prediction, for example in the final proton addition concentration. Next to model structure, adequate experimental data and correct initial simulation settings are prerequisite. Also considering the combination of microalgal species and activated sludge this additional data could have added value for the determination of the growth kinetics. Hence carbon dioxide is formed by bacterial assimilation of organic carbon, which is then used by the microalgal biomass.
Table A.1: Initial settings, analysis results and derived variables for the performed experimental runs

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(*) Repeated experiment

(**) Not measured or calculated
Table A.1: Initial settings, analysis results and derived variables for the performed experimental runs (continued)

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Table A.1: Initial settings, analysis results and derived variables for the performed experimental runs (continued)

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<th>Run n°</th>
<th>O₂prod (mg O₂ l⁻¹)</th>
<th>OPRmax (mg O₂ l⁻¹ d⁻¹)</th>
<th>HAR (mg H⁺ l⁻¹ d⁻¹)</th>
<th>H_added (mg H⁺ l⁻¹)</th>
<th>P(O₂) (mg O₂ g⁻¹ DW d⁻¹)</th>
<th>O₂ th (mg O₂ l⁻¹)</th>
<th>O₂ recov (%)</th>
<th>H⁺ recov (%)</th>
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</table>

(*) Repeated experiment  (**) Not measured or calculated
SAMENVATTING

Algen worden beschouwd als een van de oudste levensvormen op onze planeet. Ze komen voor in zowel zoet water, zout water of brak water. Met microalgen worden algen bedoeld die te klein zijn om met het blote oog waar te nemen. In hoofdzaak kunnen twee grote groepen onderscheiden worden, namelijk de eukaryotische (bv. groene microalgen) en de prokaryotische (cyanobacteriën). De belangrijkste gemeenschappelijke eigenschap tussen die twee groepen is, dat de groei hoofdzakelijk wordt veroorzaakt door fotosynthese reacties, waarbij invallend licht intracellulair wordt omgezet in energie voor groei. Bij deze fotosynthese worden anorganische koolstof en nutriënten (zoals stikstof en fosfor) vastgelegd in de biomassa van de microalgen.

Hierdoor wordt het gebruik van systemen voor afvalwaterzuivering met microalgen biomassa is een veelbelovende techniek, dat een aantal voordelen biedt ten opzichte van conventionele systemen. De nutriënten worden enerzijds verwijderd uit het afvalwater en kunnen ook gerecupereerd worden uit de biomassa. Voorbeelden waarin de biomassa kan gevaloriseerd worden zijn bijvoorbeeld biopolymeren, biokleurstoffen, als bestanddeel voor biobrandstof of meer down – stream procestechnologie zoals anaërobe vergisting. Bovendien hebben microalgen de eigenschap dat ze goed blijven groeien bij lage nutriëntgehaltes in het afvalwater, wat voordeel biedt dat ze kunnen gebruikt worden voor tertiaire afvalwaterzuivering. Dit wordt steeds belangrijker door de strengere lozingsnormen die worden opgelegd.

Echter, de groei van microalgen is een complexer proces in vergelijking met actief slib. Verschillende omgevingsomstandigheden zoals lichtintensiteit, temperatuur en de fysico-chemische samenstelling van het afvalwater kunnen een belangrijke invloed hebben op de groei. Goed inzicht in de groeikinetiek is daarom van groot belang om de performantie van het systeem te kunnen begrijpen, controleren en optimaliseren. Hierbij kunnen wiskundige modellen van groot belang zijn omdat ze in staat zijn de proces效率tie te voorspellen bij variërende operationele instellingen en watersamenstelling. Aan de hand van deze modellen kunnen talrijke scenario’s worden doorgerekend alvorens het proces op volle schaal wordt toegepast.
Samenvatting

Ondanks intensief wetenschappelijk onderzoek hieromtrent, is het aantal biokinetische groeimodellen voor microalgen die rekening houden met alle invloedsfactoren relatief beperkt. Naast modelontwikkeling, is een goede bepaling van de groeikinetiek belangrijk. De methoden die in de literatuur beschreven zijn, zijn meestal gebaseerd op de zogenaamde “indirecte “ metingen, zoals bijvoorbeeld organisch materiaal, chlorofyl gehalte en carotenoidgehalte. Groot nadeel van deze methoden, is dat deze analysemethoden tijdrovend kunnen zijn en vrij duur analysemateriaal behoeven. Bovendien zijn de resultaten van die methoden sterk afhankelijk van de omgevingsomstandigheden waarbij het experiment wordt uitgevoerd en zijn moeilijk te vertalen naar andere omgevingsomstandigheden.

In dit doctoraatsonderzoek werd enerzijds een methode ontwikkeld om op een eenvoudige, snelle, directe doch accurate manier de kinetiek van microalgen onder autotrofe groeiomstandigheden te bepalen. Hierbij werd gesteund op een methode die reeds gebruikt wordt om de kinetiek van actief slib te bepalen, namelijk gecombineerde respirometrie en titrimetrie. Bij deze methode, worden twee variabelen on-line gemeten, namelijk het opgeloste zuurstof gehalte en de protonadditie (om de pH constant te houden). Respirometrie met microalgen zal leiden tot zuurstofproductie door assimilatie van anorganische koolstof en nutriënten onder invloed van licht. Bovendien zal door de fotosynthese de oplossing alkalischer worden. De protonen die moeten toegevoegd worden om de zuurtegraad op een bepaald instelpunt te houden is de protonadditie. Uit deze bekomen data kan de kinetiek van de microalgen afgeleid worden.

In een eerste fase werd de experimentele set-up ontwikkeld en gebruikt voor het bepalen van de kinetiek bij experimenten waarbij enkel anorganische koolstof als beperkend substraat werd beschouwd. De resultaten toonden aan dat, naast het gebruik van respirometrie, het titrimetrisch profiel een noodzakelijke aanvulling is voor een correcte interpretatie van de resultaten. Dit omdat de totale zuurstofproductie, die werd berekend uit het zuurstofprofiel, niet in overeenstemming was met de theoretisch verwachte hoeveelheid na toevoeging van een bepaalde hoeveelheid anorganische koolstof. Immers, uit het zuurstofprofiel kan niet afgeleid worden of de anorganische koolstof wordt gebruikt voor groei of het uit de vloeistof gestript wordt door de menging van de reactor. Dit in tegenstelling tot het totaal aantal protonen dat wel in overeenstemming is met de theoretisch verwachte hoeveelheid. Dit kan verklaard worden door het feit dat veranderingen in het chemisch evenwicht van anorganische koolstof in water wel degelijk de protonadditie beïnvloedt. Hieruit kon afgeleid worden dat de verandering in dit
evenwicht en het strippen van koolstofdioxide belangrijke aspecten zijn die zeker dienden in rekening gebracht te worden bij data-interpretatie en gebruik van de data voor modelontwikkeling. Inderdaad, er werd een eerste simpel model ontwikkeld waarbij via trial en error twee parameters, namelijk de maximale specifieke groeisnelheid ($\mu_{\text{max}}$) en de zuurstof overdracht coëfficiënt ($K_L\alpha$) gekalibreerd werden aan het zuurstofprofiel van 3 afzonderlijke experimenten. Hierbij werd telkens goede overeenstemming tussen gemeten en voorspelde waarden gevonden.

De uitbreiding van het gebruik van de methodologie voor de identificatie van de groeikinetiek bij verschillende instellingen van nutriënten, licht, temperatuur en biomassaconcentratie was de daaropvolgende logische stap. Hierbij werd aan de hand van een experimentele statistische proefopzet nagegaan of de hoofdeffecten van deze factoren op de respirometrische en titrimetrische data konden bepaald worden. Echter bleek enkel een duidelijk effect van stikstof en biomassaconcentratie op de experimentele data merkbaar. Dit was enigszins te wijten aan het feit dat de experimentele proefopzet erop gericht was om enkel de hoofdeffecten na te gaan en waarbij de interactie tussen bepaalde vrijheidsgraden kon verwaarloosd worden. Dit bleek niet het geval te zijn. Ook bleek dat het gebied waarbinnen licht en temperatuur werden gevarieerd bij toekomstige experimenten dient worden uitgebreid om een duidelijk hoofdeffect waar te nemen. Op basis van deze resultaten werd beslist om het simpel model uit te breiden met kinetiek voor stikstof ($\text{NO}_3^-$ en $\text{NH}_4^+$) en fosfor ($\text{PO}_4^{3-}$). Deze laatste werd ook in het uitgebreide model meegenomen, omdat fosfor een essentieel element is voor de groei van de microalgen.

In het daaropvolgend onderzoek werd het uitgebreide model onderworpen aan een parameterstudie, waarbij de identificeerbaarheid van de parameters ten opzichte van de gecombineerde respirometrische en titrimetrische data werd nagegaan. Daaruit bleek dat de parameters die in het simpele model via trial en error werden gekozen voor modelkalibratie, ook de enige parameters zijn die uniek identificeerbaar zijn voor deze experimentele data. Bijgevolg werden ook hier de maximale specifieke groeisnelheid ($\mu_{\text{max}}$) en de zuurstof overdracht coëfficiënt ($K_L\alpha$) gebruikt voor de kalibratie. Ook hier werd er een goede overeenkomst tussen de experimentele en voorspelde waarden waargenomen. Bijkomstig werd de performantie van het model getoetst aan de hand van het Theil’s Inequality Criterium (TIC), waarbij telkens de randvoorwaarde van 0.3 niet werd overschreden. Hieruit kon besloten worden dat het uitgebreide model de experimentele waargenomen waarden goed kon
beschrijven met optimale parameterwaarden van de maximale specifieke groeisnelheid ($\mu_{\text{max}}$) en zuurstof overdracht coëfficiënt ($K_L a$). Bijkomstig werd het model gevalideerd aan twee bijkomende experimenten. Voor de simulaties werd hierbij als $\mu_{\text{max}}$ het gemiddelde van de verschillende geoptimaliseerde waarden genomen, namelijk $\mu_{\text{max}} = 0.261$ d$^{-1}$. De waarde van $K_L a$ werd bepaald via een empirische vergelijking als functie van de biomassa. Hierdoor werd voor het eerste validatie experiment $K_L a = 11.31$ d$^{-1}$ en voor het tweede validatie experiment $K_L a = 5.71$ d$^{-1}$. Ook bij de validatie werd goede model performantie waargenomen op basis van het TIC, namelijk TIC = 0.05 en TIC = 0.08.

In een laatste fase werd de experimentele methode gebruikt voor de identificatie van de groeikinetiek van microalgen die vooraf werden geïsoleerd uit een Waste Stabilization Pond (WSP) gesitueerd in het Andesgebergte in Ecuador. Dit is een open pondsysteem waarin microalen en bacteriën samen voorkomen voor het behandelen van het huishoudelijk afvalwater van een nabijgelegen stad (Cuenca). Het hoofddoel hiervan was nagaan of er enig verschil in groeisnelheid merkbaar was tussen twee soorten microalgen die van nature voorkomen in het WSP. Hiervoor werd ter plaatse een experimentele set-up gebouwd. Bovendien werd in deze fase ook opnieuw nagegaan of er invloed van temperatuur en licht kon afgeleid worden, omdat dit in voorgaand onderzoek niet kon bepaald worden, ondanks dat dit volgens de literatuur werd verwacht. Hiervoor werd er een bredere range voor licht en temperatuur gebruikt. De experimentele resultaten toonden aan dat het effect van temperatuur en lichtintensiteit wel op de fotosyntheseactiviteit wel significant is in het gebied dat werd toegepast. Bovendien kon de interactie tussen licht en temperatuur ook waargenomen worden. Bijgevolg werd het model ook uitgebreid met een wiskundige functie die deze interactie tussen licht en temperatuur beschrijft. Dit model werd dan gekalibreerd en gevalideerd aan bijkomstige experimenten met beide soorten microalgen. De resultaten hiervan toonden opnieuw een goede overeenkomst tussen de experimentele data en de voorspelde data. Dit zowel visueel als op basis van het Theil’s Inequality Criterium dat steeds onder de grenswaarde van 0.3 bleef. Ook kon een verschil vastgesteld worden tussen de groeisnelheid van beide soorten microalgen. Dit verschil werd ook eerder in de literatuur vermeld en kan hoogstwaarschijnlijk verklaard worden door het verschil in pigmenten dat intracellulair wordt gesynthetiseerd. Invloed van de hoogte en bijgevolg een verlaagde atmosferische druk kon niet waargenomen worden. Dit kon waarschijnlijk verklaard worden door het feit dat de microalgen aangepast zijn aan deze omstandigheid.
Doorheen dit proefschrift werd de specifieke experimentele methodologie ontwikkeld ter identificatie van de autotrofe groeikinetiek van microalgen onder variërende omstandigheden van nutriënten, licht en temperatuur. Hierbij werd een wiskundig model stelselmatig uitgebreid om tot een model die deze factoren in rekening brengt. Met de ontwikkelde methodologie en wiskundig model werd een solide basis gelegd voor verder onderzoek voor het gebruik van microalgen systemen voor afvalwaterzuivering. De methodologie zou in toekomstig onderzoek kunnen gebruikt worden voor de identificatie van de groeikinetiek van microalgen onder heterotrofe of mixotrofe groeimstandigheden. Ook de combinatie van microalgen met bacteriën zou kunnen onderzocht worden. Zulke systemen zijn meer waarschijnlijk bij het behandelen van afvalwater in bijvoorbeeld open pond systemen. Deze systemen zijn immers minder complex in uitvoering en vergen minder operationele en onderhoudskosten.
Algae are considered as one of the oldest life forms on Earth. They are able to reside in different aqueous environments, including fresh water, saline or brackish water. Microalgae are the algae that are only visible with the use of a microscope. In general, two major groups can be distinguished, respectively eukaryotic microalgae (chlorophyte) or prokaryotic (cyanobacteria). For both groups, their growth is based on photosynthetic reactions, where light intensity is converted intracellularly in energy that consequently can be used for growth. Inorganic carbon such as carbon dioxide or bicarbonate are in presence of nutrients (nitrogen and phosphorus) assimilated in the microalgal biomass.

The use of microalgal systems for wastewater treatment is a promising technique that has several advantages compared to conventional wastewater treatment systems. Besides removal of nitrogen and phosphorus from wastewater streams, nutrients are converted into valuable compounds that can be valorized as feedstock for biofuels, biopolymers or as feedstock for more down-streams process technology such as anaerobic digestion. Moreover microalgae have the capacity to grow on low environmental concentrations of nutrients, which makes the use of this systems for effluent polishing possible. This becomes more important with the more stringent environmental legislation.

However, the microalgal metabolism is somehow more complex compared to the metabolism of activated sludge. Different environmental conditions such as light intensity, temperature and physical-chemical composition of wastewater can have significant influence on the microalgal growth rate. Good insight in the microalgal growth kinetics is therefore essential in view of system performance, control and optimization. Hereby the development of mathematical models can be of great use, because with this technique the performance for different operational settings and water composition can be predicted. Based upon these models, different scenarios can be calculated, prior to the implementation of these systems in the real world.

Despite intensive scientific research, microalgal growth models balancing complexity with accuracy are rarely reported in literature. Next to model development, accurate determination of the microalgal growth kinetics is a prerequisite. In general, the methods that are reported in literature are based on proxy measurements such as for example organic matter or chlorophyll content. The major drawback however of such measurements is the fact that the features of such measurements can only be correlated to the microalgal growth under stable environmental
conditions. With altering environmental conditions, a certain adaption period is needed before the features can be correlated to the microalgal growth rate.

In this dissertation, a methodology to determine the microalgal autotrophic growth rate on a simple, fast but accurate way was developed. This methodology is based upon a the combined respirometric and titrimetric technique that is very well known in the scientific field to determine the growth kinetics of activated sludge. With this method, two variables are measured on-line, namely the dissolved oxygen concentration in the liquid phase and the proton addition (in order to keep the pH constant). The metabolism of microalgae differs however from the metabolism of activated sludge. Where bacteria consume oxygen by the assimilation of organic carbon, microalgae produce oxygen with abundant light intensity, inorganic carbon and nutrients. Besides, the photosynthetic activity will induce an increase of the pH. As such protons will be dosed to maintain the pH at a user defined set-point. The resulting respirometric and titrimetric profiles can be used for the determination of the microalgal growth kinetics.

At first, the combined respirometric and titrimetric methodology was developed and used to assess the microalgal growth kinetics with only one limiting factor, namely the amount of inorganic carbon. The results revealed that, next to the interpretation respirometric results, interpretation of the titrimetric profile is essential. This because the fact that the total amount of oxygen produced, calculated experimentally, did not correspond to the theoretical amount of oxygen produced, expected by the addition of inorganic carbon. The reason for this was that the amount of inorganic carbon that is not available for the assimilation due to possible stripping to the atmosphere cannot be deduced from the respirometric profile. This in contrast to the titrimetric profile, where changes in the chemical equilibrium of inorganic carbon and possible stripping of carbon dioxide to the atmosphere will influence the proton addition. As such it could be concluded that the phenomena of changes in the chemical equilibrium and possible stripping should be taken into account when interpreting the experimental results and using the data for model development. Indeed, a simple model was developed taking into account inorganic carbon limitation. By trial and error, it was then decided to consider the microalgal maximum specific growth rate ($\mu_{\text{max}}$) and the oxygen mass transfer coefficient ($K_L a$) for model calibration. For this three separate respirometric profiles were used. Good correspondence between simulated and experimental values were noted.
Summary

Subsequently the methodology was extended to different settings of environmental factors, respectively light intensity, temperature, nitrogen, phosphorus and microalgal biomass concentration. For this an experimental statistic design was implemented to assess the main effects of these degrees of freedom on several responses that are related to the photosynthetic activity. Only significant influence of nitrogen and biomass concentration could be observed. This could be explained by the fact that the experimental statistic design focusses on the main effect of degrees of freedom. As such no interaction is taking into account. Apparently this is a reason why no main effect of temperature and light intensity could be observed. Also the range of these degrees of freedom should be broadened in future experiments. Based upon the experimental results, it was decided at this stage of the research to expand the earlier developed simple model with kinetics for nitrogen species (NH$_4^+$ and NO$_3^-$) and phosphorus (PO$_4^{3-}$). The latter because phosphorus is an essential element for the microalgal growth.

In a next stage, the parameter included in this expanded model were assessed for identifiability. The results of this illustrated that the two parameters that where at first chosen by trial and error for model calibration, were the only parameters that were uniquely identifiable to the combined respirometric and titrimetric data. Consequently these two parameters, respectively the maximum specific growth rate ($\mu_{\text{max}}$) and the oxygen mass transfer coefficient ($K_La$) were calibrated to seven separate experiments. With optimized parameter settings, good visual correspondence between experimental and predicted profiles was noted. Further the model performance was evaluated by using the Theil’s Inequality Criterium (TIC). For all calibration experiments, the threshold value of 0.3 was not exceeded. Additional model validation with two other experiments also illustrated good correspondence between model prediction and measured profiles. For the simulations, the $\mu_{\text{max}}$ was taken as the mean value of the different separate optimized values, namely $\mu_{\text{max}} = 0.261$ d$^{-1}$. For the $K_La$ an empirical relation was defined as function of the microalgal biomass. As such a value of $K_La = 11.31$ d$^{-1}$ for the first validation experiment and $K_La = 5.71$ d$^{-1}$ for the second validation experiment was used. Also TIC did not exceed the threshold value for these experiments, respectively TIC = 0.05 and TIC = 0.08.

In the final part of the research, the experimental methodology was used to assess the growth kinetics of microalgal species that were isolated from a waste stabilization pond (WSP) situated in the Andes in Ecuador. This is an open pond system, where the combination of bacteria and microalgae is used to treat the domestic wastewater of a nearby city (Cuenca). The main goal
of the research was to investigate whether there is a difference in growth kinetics between both microalgal species. For this an experimental set-up analogues to the set-up developed earlier was built. Further in this specific stage, it was decided to investigate if the influence of light intensity and temperature is significant for the microalgal growth rate. This was done, because although expected, it was not observed in the preceding research. As such a broader range of light intensity and temperature was used. It was observed by the experimental results, that the effect of light intensity and temperature is significant in the ranges that were applied. Also the interaction between light intensity and temperature was significant. Consequently the microalgal growth kinetics were expanded with a function that describes this interdependent relation. Further the model was calibrated with two additional separate experiments for both microalgal species. Also the maximum specific growth rate ($\mu_{\text{max}}$) and the oxygen mass transfer coefficient ($K_{L\alpha}$) were used for model calibration. The results showed again good correspondence between simulated and experimental dissolved oxygen evolution and proton addition for both microalgal species. This for the visual aspect as based on the TIC. Also a difference between the optimized maximum specific growth rate between species was noted. This could be explained by the fact that different pigments are synthesized between species. Influence of height and as such by decreased atmospheric pressure was not noted. This could possibly be explained by the fact that microalgae are able to adapt to this elevated situation.

Throughout this dissertation a specific experimental methodology was developed and used to assess the identification of the autotrophic microalgal growth kinetics, even under varying conditions of environmental factors, such as nutrients, light intensity, biomass concentration and temperature. Also a mathematical model was expanded from a very simple model to a more mechanistic model taking into account all these environmental factors. This experimental methodology and the developed model are a solid base for future research involving wastewater treatment systems with microalgal biomass. It could be used for the identification of the growth kinetics with heterotrophic and mixotrophic conditions. Also the combination of microalgae and bacteria would be interesting to investigate. Such combination would occur in the treatment
Summary

of wastewater in open pond systems, which aim for less complex compared and less costly waste water treatment.


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Personal data

Name: Bjorge Decostere
Adres: Kleine Kerkstraat 61
        9050 Gent
Nationality: Belgium
Date of birth: 25th February 1974
Place of birth: Oostende
Civil status: divorced
Email: Bjorge.Decostere@Ugent.be
Secondary Email: bdcostere@gmail.com

Education

1995 – 1997: Graduate Chemistry, Université Technique de Lille, France

2004 – 2007: Master of Science in Industrial Engineering: Environmental Science, College University of West – Flanders, Belgium

Professional activities

1997 – 1999: Lab technician at the compagnie Européene de Development Techniques Industrières, Gravelinnes, France

1999 – 2001: Quality Control at Berry Yarns, Cominnes, Belgium
Curriculum Vitae

2007 – 2008: Project Engineer within the research project “The use of electrospun nanofiber membrane for water filter applications “, partly funded by the Institute for Encouragement of Innovation by means of Science and Technology in Flanders (IWT), Department of Industrial Engineering and Technology (ENBICHEM) of the College University West – Flanders, Belgium

2008 – 2012: Teaching assistant Environmental Sciences, College University West –Flanders, Belgium

2012 – 2016: Teaching assistant Environmental Sciences, Department of Industrial Biological Sciences, University of Ghent, Belgium

Teaching activities

Practical courses and exercises:

- Soil and groundwater remediation
- Air purification technology
- Solid waste treatment
- Waste water treatment
- Soil sciences
- Water technology
- Environmental sanitation and legislation

MSc. Theses supervised

2007 – 2008: B. Thienpondt. De constructie van een membraanbioreactor als mogelijk toepassingsgebied voor een elektrogesponnen nanovezel membraan. Department of Industrial Sciences, College University West- Flanders, Belgium

2008 – 2009: B. Cardoen. Aanpak van de problematiek van minerale olie in compost. Department of Industrial Sciences, College University West- Flanders, Belgium

2008 – 2009: K. Lenoir. Evaluatie van het elektrogesponnen nanovezel membraan toegepast bij membraanbioreactoren. Department of Industrial Sciences, College University West- Flanders, Belgium
Curriculum Vitae

2009 – 2010: K. Schouteten. Toepasbaarheid van effectieve micro-organismen in een biologische waterzuivering. Department of Industrial Sciences, College University West-Flanders, Belgium

2010 – 2011: L. Simoens. Modelmatig ontwerp van een individuele afvalwater behandeling voor een dierenasiel. Department of Industrial Sciences, College University West-Flanders, Belgium

2010 – 2011: S. El Hilali. Implementatie van autotrofe stikstofverwijdering op stortplaatspercolaat. Department of Industrial Sciences, College University West-Flanders, Belgium

2011 – 2012: G. Vermeeren. Haalbaarheidsstudie naar in-situ remediatietechnieken voor chroom 6. Department of Industrial Sciences, College University West-Flanders, Belgium

2011 – 2012: M. Bulckaert. Evaluatie van geactiveerd persulfaat als ISCO voor een met chloororbenzenen verontreinigde bodem. Department of Industrial Sciences, College University West-Flanders, Belgium

2012 – 2013: K. Vromant. Oriënterend onderzoek naar low-cost sensoren voor het meten van ammoniakconcentraties in stallucht. Department of Industrial Sciences, College University West-Flanders, Belgium

2012 – 2013: J. Verhulst. Optimaliseren van bestaande aerobe degradatietests. Department of Industrial Sciences, College University West-Flanders, Belgium

2012 – 2013: M. Duyck. Modelmatige analyse van de kinetische parameters van microalgen. Department of Industrial Sciences, College University West-Flanders, Belgium

2013 – 2015: J. De Craene. Optimalisatie van de verwijdering van nutrienten uit afvalwater met microalgen biomassa: een modelgebaseerde aanpak. Department of Industrial Biological Sciences, Ghent University, Belgium

2014 – 2015: W. Malfait. Bodemsanering van gechloreerde ethanen. Department of Industrial Biological Sciences, Ghent University, Belgium
Curriculum Vitae

2015 – 2016: P. Knockaert. Bepalen van de groeikinetiek van MaB-vlokken met de gecombineerde respirometrische en titrimetrische methodologie. Department of Industrial Biological Sciences, Ghent University, Belgium

Erasmus (Exchange students) projects supervised

2011 – 2012: (June – September): Y. Ding. The use of a combined respirometric and titrimetric unit for microalgal kinetics determination, Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Belgium

2012 – 2013: (September – December): PM. Porter. Factors influencing the microalgal growth: overview and modelling. Department of Industrial Sciences, College University West – Flanders, Belgium

Publications

Peer-Reviewed (in the scope of dissertation)


Curriculum Vitae


- Other Peer-Reviewed

  
  
  
  
  
  
  

201
Curriculum Vitae


Not listed in the ISI Web of Knowledge


Conference proceedings (in the scope of dissertation)

- Decostere B, Duyck M, Vervaeren H, Maere T, Van Hulle SWH, Nopens I (2013) Determination of degrees of freedom influencing the microalgal kinetic growth rate with a respirometric-titrmetric methodology. ICA conference on instrumation and automation, Narbonne France
Other Conference proceedings