Growth and Modeling of Freshwater Algae as a Function of Media Inorganic Carbon Content

Mary Watson
*Clemson University, mkwatso@clemson.edu*

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GROWTH AND MODELING OF FRESHWATER ALGAE AS A FUNCTION OF MEDIA INORGANIC CARBON CONTENT

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Biosystems Engineering

by
Mary Katherine Watson
May 2009

Accepted by:
Dr. Caye M. Drapcho, Committee Chair
Dr. Terry H. Walker
Dr. Charles E. Turick
ABSTRACT

In response to global climate change, the Department of Energy (DOE) has specified advanced biological processes, such as cultivation of algal biomass in alkaline ponds, as part of a carbon management plan. The goal of this thesis was to investigate use of a mixed freshwater algal culture for biological carbon mitigation. Extensive review of carbonate dynamics, laboratory investigations to characterize algal growth, and development of a dynamic algal growth model were completed.

The presented literature review summarizes carbonate equilibria and kinetics needed for development of carbon mitigation technologies, especially in freshwaters. Reaction mechanisms, equilibrium relationships, kinetic rate constants, and kinetic rate laws are used to develop mass balance equations (MBEs) for species concentrations in closed systems. Several strategies for quantifying reaction-enhanced CO₂ transport are presented to develop carbonate species MBEs for open systems.

Batch algal growth was analyzed in closed and open batch reactors. Specific growth rates, biomass production, and peak pH generally increase with increasing initial TIC concentration in closed and open reactors. Closed algal cultures kinetically responded to CO₂, HCO₃⁻, and CO₃²⁻ concentrations ($\mu_{\text{max}} = 0.0726 \text{ hr}^{-1}$, $K_{\text{CO}_2} = 4.47 \times 10^{-8}$, $K_{\text{HCO}_3} = 5.70 \times 10^{-4}$, $K_{\text{CO}_3} = 8.70 \times 10^{-4}$), which suggests employment of carbon concentrating mechanisms (CCMs). Analysis of batch growth in open reactors revealed that carbon sequestered per supplied TIC exponentially ($R^2 = 0.9717$) decreased with increasing initial TIC.
Dynamic mathematical models aimed at predicting algal biomass and carbonate species concentrations in closed and open batch reactors were developed. The \( \text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-} \) substitutable substrates Monod equation for predicting TIC-limited algal specific growth rates best estimated biomass concentrations in closed and open batch reactors. However, inaccuracies were observed for some water chemistry parameters. The closed batch reactor model was calibrated based on photosynthetic oxygen production and verified using data from laboratory investigations. A sensitivity analysis for the open batch reactor model suggests that photosynthetic oxygen production and biomass light attenuation coefficients should be further investigated to improve open algal growth model simulations.
DEDICATION

I dedicate this work to my parents, David and Ann Marie Watson, who have always challenged me to pursue my goals. Their unconditional love and support motivates me to strive for success. I would also like to thank Joshua Pelkey for his encouragement, and I look forward to continuing our graduate careers together.
ACKNOWLEDGMENTS

I am indebted to each of my committee members for their review of my Masters project. Their guidance and input greatly contributed to this research. Specifically, I would like to thank my committee chair, Dr. Caye M. Drapcho, for her support throughout my undergraduate and graduate careers. She is a gifted educator and researcher, and I have valued the opportunity to learn from her. I look forward to our continued friendship and collaboration.

Several groups and individuals have aided me in my graduate studies. For financial support, I thank donors of the Wade Stackhouse Fellowship, the Calhoun Honors College, and SC-Life. For technical support, I thank Scott Davis of the Clemson Aquaculture Facility and Kathy Moore of the Clemson Agricultural Service Laboratory. Finally, I appreciate Joshua Pelkey’s Matlab support.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter/Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Background Information</td>
<td>1</td>
</tr>
<tr>
<td>Scope of Completed Research</td>
<td>5</td>
</tr>
<tr>
<td>II. CARBONATE SYSTEM EQUILIBRIUM AND KINETICS IN CLOSED AND OPEN ENVIRONMENTS: A LITERATURE REVIEW</td>
<td>8</td>
</tr>
<tr>
<td>Abstract</td>
<td>8</td>
</tr>
<tr>
<td>Introduction</td>
<td>8</td>
</tr>
<tr>
<td>Model Development</td>
<td>10</td>
</tr>
<tr>
<td>Summary</td>
<td>33</td>
</tr>
<tr>
<td>Conclusions</td>
<td>34</td>
</tr>
<tr>
<td>References</td>
<td>34</td>
</tr>
<tr>
<td>III. GROWTH OF FRESHWATER ALGAE AS A FUNCTION OF MEDIA INORGANIC CARBON CONTENT</td>
<td>38</td>
</tr>
<tr>
<td>Abstract</td>
<td>38</td>
</tr>
<tr>
<td>Introduction</td>
<td>38</td>
</tr>
<tr>
<td>Literature Review</td>
<td>40</td>
</tr>
<tr>
<td>Experimental Methods</td>
<td>49</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>55</td>
</tr>
<tr>
<td>Summary</td>
<td>84</td>
</tr>
</tbody>
</table>
### Table of Contents (Continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conclusions</td>
<td>84</td>
</tr>
<tr>
<td>References</td>
<td>85</td>
</tr>
<tr>
<td><strong>IV. MODELING OF FRESHWATER ALGAL GROWTH</strong></td>
<td></td>
</tr>
<tr>
<td>as a function of media inorganic carbon content</td>
<td>89</td>
</tr>
<tr>
<td>Abstract</td>
<td>89</td>
</tr>
<tr>
<td>Introduction</td>
<td>90</td>
</tr>
<tr>
<td>Model Development</td>
<td>91</td>
</tr>
<tr>
<td>Closed Batch Reactor Model</td>
<td>112</td>
</tr>
<tr>
<td>Open Batch Reactor Model</td>
<td>127</td>
</tr>
<tr>
<td>Summary</td>
<td>141</td>
</tr>
<tr>
<td>Conclusions</td>
<td>142</td>
</tr>
<tr>
<td>References</td>
<td>142</td>
</tr>
<tr>
<td><strong>VI. REMARKS AND RECOMMENDATIONS</strong></td>
<td>147</td>
</tr>
<tr>
<td><strong>APPENDICES</strong></td>
<td>149</td>
</tr>
<tr>
<td>A: Sampling Schedule</td>
<td>150</td>
</tr>
<tr>
<td>B: Biomass Quantification</td>
<td>153</td>
</tr>
<tr>
<td>C: Additional Kinetic Analysis Data</td>
<td>159</td>
</tr>
<tr>
<td>D: Bath Growth Curves</td>
<td>163</td>
</tr>
<tr>
<td>E: Preliminary Data for Open Batch Reactors</td>
<td>172</td>
</tr>
<tr>
<td>F: Heterotrophic Plate Counts</td>
<td>175</td>
</tr>
<tr>
<td>G: Impact of Carbon Mitigation by Freshwater Algae</td>
<td>184</td>
</tr>
<tr>
<td>H: Matlab® Code for Algal Growth Models</td>
<td>186</td>
</tr>
<tr>
<td>I: Calibration and Verification of Closed Algal Growth Model using Data from Unadjusted Initial pH Reactors</td>
<td>200</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Summary of equilibrium constants and values at 25°C for carbonate system reactions.</td>
<td>14</td>
</tr>
<tr>
<td>2.2</td>
<td>Dependence of carbonate system equilibrium constants on absolute temperature (Kelvin).</td>
<td>15</td>
</tr>
<tr>
<td>2.3</td>
<td>Temperature dependence of $K_{H_2CO_3}$ and $K_h$ in freshwater systems.</td>
<td>15</td>
</tr>
<tr>
<td>2.4</td>
<td>Summary of $k_{CO_2}$ measurements at 25°C.</td>
<td>18</td>
</tr>
<tr>
<td>2.5</td>
<td>Summary of $k_{H_2CO_3}$ measurements at 25°C.</td>
<td>19</td>
</tr>
<tr>
<td>2.6</td>
<td>Additional constants describing carbonate reactions at 25°C in freshwater.</td>
<td>21</td>
</tr>
<tr>
<td>3.1</td>
<td>Summary of equilibrium constants and values at 25°C for carbonate system reactions.</td>
<td>42</td>
</tr>
<tr>
<td>3.2</td>
<td>Summary of completed experiments.</td>
<td>49</td>
</tr>
<tr>
<td>3.3</td>
<td>Modified BG11 media used to cultivate freshwater algae.</td>
<td>51</td>
</tr>
<tr>
<td>3.4</td>
<td>Specific growth rates of closed freshwater algal cultures supplied with various initial amounts of inorganic carbon.</td>
<td>57</td>
</tr>
<tr>
<td>3.5</td>
<td>Stoichiometry (C:N:P) of closed freshwater algal cultures.</td>
<td>58</td>
</tr>
<tr>
<td>3.6</td>
<td>Estimates of kinetic constants calculated from Lineweaver-Burk plots for freshwater algal growth assuming $CO_2$, $HCO_3^-$, $CO_3^{2-}$, or TIC as sole inorganic carbon sources.</td>
<td>65</td>
</tr>
<tr>
<td>3.7</td>
<td>Kinetic parameters for single substrate models obtained using SAS proc nlin.</td>
<td>66</td>
</tr>
</tbody>
</table>
List of Tables (Continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8</td>
<td>Comparison of specific growth rates on each carbonate species to measured specific growth rate using data between 75 and 100 hr in 87.5% C reactor</td>
<td>66</td>
</tr>
<tr>
<td>3.9</td>
<td>Calculation of $%BP_{CO_3}$ using data from 50% C reactor</td>
<td>68</td>
</tr>
<tr>
<td>3.10</td>
<td>Stoichiometry (C:N:P) of open freshwater algal cultures</td>
<td>76</td>
</tr>
<tr>
<td>3.11</td>
<td>Estimation of atmospheric carbon sequestered in open batch algal reactors after 1100 hr growth</td>
<td>82</td>
</tr>
<tr>
<td>3.12</td>
<td>Estimation of pond volume required to abate 2008 Clemson University carbon emissions</td>
<td>83</td>
</tr>
<tr>
<td>4.1</td>
<td>Summary of kinetic constants for carbonate system reactions at 25°C</td>
<td>95</td>
</tr>
<tr>
<td>4.2</td>
<td>Summary of algal biomass and water extinction coefficients</td>
<td>103</td>
</tr>
<tr>
<td>4.3</td>
<td>Mass balance equations for TIC and carbonate species assuming $CO_2$ and $HCO_3^-$ as single substrates</td>
<td>104</td>
</tr>
<tr>
<td>4.4</td>
<td>Mass balance equations for TIC and carbonate species assuming $CO_2$, $HCO_3^-$, and $CO_3^{2-}$ as substitutable substrates</td>
<td>105</td>
</tr>
<tr>
<td>4.5</td>
<td>Mass balance equations for $CO_2$ in open systems for $CO_2$, $HCO_3^-$, and $CO_3^{2-}$ as single and substitutable substrates</td>
<td>106</td>
</tr>
<tr>
<td>4.6</td>
<td>Rates of species utilization by algae using $CO_2$, $HCO_3^-$, and $CO_3^{2-}$ as single and substitutable substrates</td>
<td>107</td>
</tr>
<tr>
<td>4.7</td>
<td>Specific growth rates of algae for $CO_2$, $HCO_3^-$, and $CO_3^{2-}$ as single and substitutable substrates</td>
<td>108</td>
</tr>
</tbody>
</table>
List of Tables (Continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>Summary of completed experiments to provide data for verification of dynamic algal growth model</td>
<td>110</td>
</tr>
<tr>
<td>4.9</td>
<td>Kinetic parameters describing inorganic-carbon-limited freshwater algal growth</td>
<td>110</td>
</tr>
<tr>
<td>4.10</td>
<td>Stoichiometric parameters describing TIC-limited freshwater algal growth</td>
<td>111</td>
</tr>
<tr>
<td>4.11</td>
<td>Kinetic and physical parameters describing light-limited freshwater algal growth</td>
<td>111</td>
</tr>
<tr>
<td>4.12</td>
<td>Initial values used for comparison, calibration, and/or verification of closed and open growth models</td>
<td>112</td>
</tr>
<tr>
<td>A.1-5</td>
<td>Sampling schedules for closed and open batch reactors</td>
<td>150</td>
</tr>
<tr>
<td>C.1</td>
<td>ANOVA tables for single-substrate Monod models with CO$_2$, HCO$_3^-$, CO$_3^{2-}$, or TIC as inorganic carbon source</td>
<td>160</td>
</tr>
<tr>
<td>C.2</td>
<td>Calculation of percent biomass production (%BP) attributed to assimilation of carbonate species for adjusted initial pH Runs 2C and 3C</td>
<td>162</td>
</tr>
<tr>
<td>F.1</td>
<td>Summary of method for reporting CFU from heterotrophic plate counts</td>
<td>175</td>
</tr>
<tr>
<td>I.1</td>
<td>Initial values for comparison, calibration, and verification of closed and open algal growth models</td>
<td>200</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.1</td>
<td>World marketed energy use by fuel type from 1980 to 2030</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>World energy-related CO(_2) emissions by fuel type from 1990 to 2030</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>Effect of annual CO(_2) emissions on atmospheric CO(_2) concentration</td>
<td>3</td>
</tr>
<tr>
<td>2.1</td>
<td>Reaction scheme for CO(_2) hydration</td>
<td>10</td>
</tr>
<tr>
<td>2.2</td>
<td>Aqueous reactions of bicarbonate and carbonate</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Idealized depiction of unenhanced versus chemically-enhanced CO(_2) flux</td>
<td>25</td>
</tr>
<tr>
<td>2.4</td>
<td>Simulation of pH and temperature effects on a(_k)</td>
<td>29</td>
</tr>
<tr>
<td>2.5</td>
<td>Simulation of boundary layer thickness (L) effect on EF at 25(^\circ)C</td>
<td>31</td>
</tr>
<tr>
<td>3.1</td>
<td>Model for <em>Scenedesmus</em> CCMs</td>
<td>46</td>
</tr>
<tr>
<td>3.2</td>
<td>Open and closed algal batch reactors at 138 hr.</td>
<td>50</td>
</tr>
<tr>
<td>3.3</td>
<td>Cell densities within closed batch reactors supplied with various initial</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>amounts of inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>Elemental composition of freshwater algal cultures supplied with various</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>initial TIC concentrations</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>pH within closed algal cultures supplied with various initial TIC</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>concentrations</td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>TIC within closed algal cultures supplied with various initial TIC</td>
<td>60</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>CO$_2$ within closed algal cultures supplied with various initial TIC concentrations</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>HCO$_3^-$ within closed algal cultures supplied with various initial TIC concentrations</td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>CO$_3^{2-}$ within closed algal cultures supplied with various initial TIC concentrations</td>
<td></td>
</tr>
<tr>
<td>3.10</td>
<td>Relationship between biomass, TIC, and carbonate species concentrations in 27.5% C reactor</td>
<td></td>
</tr>
<tr>
<td>3.11</td>
<td>Relationship between biomass, TIC, and carbonate species concentrations in 87.5% C reactor</td>
<td></td>
</tr>
<tr>
<td>3.12</td>
<td>Lineweaver-Burk plots for determination of $\mu_{max}$ and $K_S$ for single substrate models</td>
<td></td>
</tr>
<tr>
<td>3.13</td>
<td>Observed biomass yields of algal cultures in closed batch reactors supplied with various initial amounts of inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>3.14</td>
<td>Relationship between initial TIC concentration and observed biomass yield of mixed freshwater algal cultures</td>
<td></td>
</tr>
<tr>
<td>3.15</td>
<td>Percent biomass production (%BP) for individual carbonate species for Runs 2C and 3C</td>
<td></td>
</tr>
<tr>
<td>3.16</td>
<td>Determination of decay constant for mixed freshwater algal culture supplied with various initial amounts of inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>3.17</td>
<td>Determination of oxygen mass transfer velocity for open batch reactor containing modified BG11 media with different TIC concentrations</td>
<td></td>
</tr>
<tr>
<td>3.18</td>
<td>Photographs of algal cells from open batch reactors</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>3.19</td>
<td>Total suspended solids concentrations within closed batch reactors supplied with various initial amounts inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>3.20</td>
<td>Plot of natural log of biomass concentration versus time used to determine specific growth rates of open algal cultures supplied with various amounts of TIC</td>
<td></td>
</tr>
<tr>
<td>3.21</td>
<td>Elemental composition of open freshwater algal cultures supplied with various initial TIC concentrations</td>
<td></td>
</tr>
<tr>
<td>3.22</td>
<td>pH of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>3.23</td>
<td>pH and TSS of algal culture in 50% C reactor</td>
<td></td>
</tr>
<tr>
<td>3.24</td>
<td>Alkalinity of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>3.25</td>
<td>TIC concentrations within open batch algal cultures supplied with high (100%) and low (25%) inorganic carbon</td>
<td></td>
</tr>
<tr>
<td>3.26</td>
<td>CO$_2$ concentrations within open batch algal cultures</td>
<td></td>
</tr>
<tr>
<td>3.27</td>
<td>HCO$_3^-$ concentrations within open batch algal cultures</td>
<td></td>
</tr>
<tr>
<td>3.28</td>
<td>CO$_3^{2-}$ concentrations within open batch algal cultures</td>
<td></td>
</tr>
<tr>
<td>3.29</td>
<td>Percent carbon sequestered (%TIC$_{seq}$) by open freshwater algal cultures as a function of initial TIC</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Reaction scheme for CO$_2$ hydration</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>Aqueous acid-base reactions of bicarbonate and carbonate</td>
<td></td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3-16</td>
<td>Comparison of closed algal growth model predictions when various Monod models are used to quantify TIC-limited algal specific growth rates (75% C reactor; Run 2C)</td>
<td>115</td>
</tr>
<tr>
<td>4.17-24</td>
<td>Effect of p on closed algal growth model predictions (25% Reactor; Run 2C)</td>
<td>120</td>
</tr>
<tr>
<td>4.25-38</td>
<td>Model predictions for calibrated closed algal growth model (50% Reactor; Run 2C)</td>
<td>123</td>
</tr>
<tr>
<td>4.39-52</td>
<td>Comparison of open algal growth model predictions when various Monod models are used to quantify TIC-limited algal specific growth rates (50% C reactor; Run 1O)</td>
<td>128</td>
</tr>
<tr>
<td>4.53-66</td>
<td>Effect of p on open algal growth model predictions (50% C reactor; Run 1O)</td>
<td>133</td>
</tr>
<tr>
<td>4.67-80</td>
<td>Effect of $K_B$ on open algal growth model predictions (50% C reactor; Run 1O)</td>
<td>137</td>
</tr>
<tr>
<td>B.1</td>
<td>Dry weights of closed batch algal reactors supplied with varying initial amounts of inorganic carbon</td>
<td>153</td>
</tr>
<tr>
<td>B.2</td>
<td>Optical densities at 750 nm of algal cultures within closed batch reactors supplied with varying initial amounts of inorganic carbon ≤</td>
<td>154</td>
</tr>
<tr>
<td>B.3</td>
<td>Calibration curve relating TSS to OD at 750 nm</td>
<td>155</td>
</tr>
<tr>
<td>B.4</td>
<td>Relationship between cell density and TSS of closed batch algal cultures cultivated with varying initial TIC concentrations (Runs 2C and 3C)</td>
<td>156</td>
</tr>
<tr>
<td>B.5</td>
<td>Calibration curves relating cell density to OD at 750 nm for closed algal cultures supplied with various initial amounts of inorganic carbon</td>
<td>156</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.6</td>
<td>Dry weights of open batch algal reactors supplied with varying initial amounts of inorganic carbon</td>
</tr>
<tr>
<td>B.7</td>
<td>Optical densities at 750 nm of open batch algal reactors supplied with varying initial amounts of inorganic carbon</td>
</tr>
<tr>
<td>B.8</td>
<td>Calibration curve relating OD to biomass for open batch algal reactors supplied with varying initial amounts of inorganic carbon</td>
</tr>
<tr>
<td>C.1</td>
<td>Lineweaver-Burk plots for determination of $\mu_{\text{max}}$ and $K_S$ for single substrate models (Run 1C)</td>
</tr>
<tr>
<td>C.2</td>
<td>Monod plots generated using kinetic data estimated by SAS and experimental data from Runs 1C, 2C, and 3C</td>
</tr>
<tr>
<td>C.3</td>
<td>Sample calculations for $%BP_{\text{CO}_3}$ using data from 50% C reactor of Run 2C</td>
</tr>
<tr>
<td>D.1-4</td>
<td>Relationship between biomass, TIC, and carbonate species concentrations in reactors from Run 1C</td>
</tr>
<tr>
<td>D.5-8</td>
<td>Relationship between biomass, TIC, and carbonate species concentrations in reactors from Run 2C</td>
</tr>
<tr>
<td>D.9-12</td>
<td>Relationship between biomass, TIC, and carbonate species concentrations in reactors from Run 3C</td>
</tr>
<tr>
<td>D.13-16</td>
<td>Relationship between biomass and pH in reactors from Run 1O</td>
</tr>
<tr>
<td>E.1</td>
<td>Dry weights of open batch algal reactors supplied with varying initial amounts of inorganic carbon (Prelim. 1O)</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.2</td>
<td>Natural log of biomass concentration versus time used to determine specific growth rates of open algal cultures supplied with various amounts TIC (Prelim. 1O)</td>
<td>173</td>
</tr>
<tr>
<td>E.3</td>
<td>Alkalinity of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon (Prelim. 1O)</td>
<td>173</td>
</tr>
<tr>
<td>E.4</td>
<td>pH of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon (Prelim. 1O)</td>
<td>174</td>
</tr>
<tr>
<td>E.5</td>
<td>Total inorganic carbon concentrations within open batch algal cultures supplied with varying initial amounts of inorganic carbon (Prelim. 1O)</td>
<td>174</td>
</tr>
<tr>
<td>F.1-5</td>
<td>Concentrations of heterotrophic bacteria in open batch reactors initially supplied with various TIC concentrations at 734 hr</td>
<td>176</td>
</tr>
<tr>
<td>F.6</td>
<td>Photographs of heterotrophic plates containing samples from open batch reactors at 836 hr.</td>
<td>180</td>
</tr>
<tr>
<td>F.7-11</td>
<td>Concentrations of heterotrophic bacteria in open batch reactors initially supplied with various TIC concentrations at 836 hr</td>
<td>181</td>
</tr>
<tr>
<td>I.1-8</td>
<td>Comparison of closed algal growth model predictions when various Monod models are used to quantify TIC-limited algal specific growth rates (25% Reactor; Run 1C)</td>
<td>201</td>
</tr>
<tr>
<td>I.9-22</td>
<td>Model predictions for calibrated closed algal growth model (50% Reactor; Run 1C)</td>
<td>203</td>
</tr>
</tbody>
</table>
BACKGROUND INFORMATION

Energy Consumption Trends

World energy consumption is predicted to increase through 2030, with majority of energy being derived from coal, natural gas, and liquid fuels (Figure 1.1). Consumption of renewable energy and coal will likely increase the most, at annual rates of 2.1 and 2.0 percent, respectively. Renewable energy sources are favored due to heightened public awareness about adverse environmental impacts of fossil fuel combustion, while coal offers competitive economic advantages [1].

Figure 1.1: World marketed energy use by fuel type from 1980 to 2030 [1].
Carbon Dioxide Emissions

Combustion of fossil fuels is problematic because it releases CO$_2$, the most abundant anthropogenic greenhouse gas (GHG), into the atmosphere. Energy-related CO$_2$ emissions are expected to increase an average of 1.7 percent per year from 2005 to 2030. Contribution of CO$_2$ emissions from coal, the most carbon-intensive fuel, is expected to increase from 41 to 44 percent in 2030 (Figure 1.2) [1].

![Figure 1.2: World energy-related CO$_2$ emissions by fuel type from 1990 to 2030 [1].](image)

Atmospheric CO$_2$ concentrations are predicted to increase as emissions increase, although natural and anthropogenic carbon sinks will reduce this effect (Figure 1.3). Currently, the concentration of atmospheric CO$_2$ is 380 ppm. Even with measures to abate emissions, this concentration is expected to rise to 450 ppm by 2030 [1]. Carbon
capture and storage strategies, not included in EIA/DOE predictions, have the potential to mitigate 1 billion metric tons of CO₂ annually [1].

![Figure 1.3: Effect of annual CO₂ emissions on atmospheric CO₂ concentrations [1].](image)

**Carbon Management Plan**

To combat increasing CO₂ emissions and atmospheric concentrations, the Department of Energy (DOE) has outlined a three-tired plan for carbon management [2,3]:

1. increase efficiency of primary energy conversion so that fewer units of primary fossil energy are required to provide same energy service,

2. substitute lower-carbon or carbon-free energy sources for current sources, and

3. implement carbon sequestration technologies.
Carbon sequestration refers to the capture and storage of carbon that would otherwise add to atmospheric concentrations [4]. This strategy has received considerably less attention than the first two tiers of the DOE carbon management plan; however, it has potential to be a “major tool” for reducing the effects of fossil fuels on global climate change [3].

Six focus areas have been proposed by DOE as promising carbon sequestration strategies [3]: (1) separation and capture of CO₂, (2) ocean sequestration, (3) carbon sequestration in terrestrial ecosystems, (4) sequestration of CO₂ in geological formations, (5) advanced biological processes for sequestration, and (6) advanced chemical approaches to sequestration.

**Biological Carbon Sequestration**

As part of the U.S. DOE carbon management plan, advanced biological processes will be implemented by 2025 to sequester carbon from concentrated combustion gases and dispersed point sources. These processes are designed to enhance natural biological processes that sequester atmospheric carbon into terrestrial plants, aquatic photosynthetic species, and microbial communities [3].

One process of interest is sequestration of CO₂ in reduced carbon compounds. Expansion of forests to mitigate rising CO₂ concentrations has been shown to be feasible. DOE suggests cultivation of algal biomass as an alternative approach for carbon sequestration [3]. However, since biomass decay releases CO₂ into the atmosphere, biomass must be strategically stored or utilized to ensure sequestration or abatement. For instance, biomass could be harvested, converted to biofuels, and used to reduce fossil
fuel use [5]. Marine cultures are advantageous because production is not limited by water availability. However, the ability of some algae and cyanobacteria to survive in high alkalinity ponds may enhance sequestration. In this case, the amount of inorganic carbon dissolved in solution increases, due to increasing chemical hydration rates with increasing pH. Ultimately, this maximizes the availability of inorganic carbon to aquatic organisms for biofixation [3].

**SCOPE OF COMPLETED RESEARCH**

**Project Objectives**

The goal of this project was to investigate use of a mixed freshwater algal culture for biological abatement of atmospheric carbon. The objectives of the research were as follows:

1. to complete an extensive literature review on carbonate equilibrium and kinetics in closed and open environments, with specific focus on compiling kinetic rate constants for freshwater,
2. to characterize algal growth in closed and open batch reactors to assess carbon mitigation potential, and
3. to develop a dynamic algal growth model to predict algal biomass and carbonate species concentrations as a function of media inorganic carbon concentration.
Project Summary

Dissemination of this research to explore the use of algal cultures for carbon mitigation will be completed in five chapters.

1. Chapter One. Introduction: Projections for world energy demand and CO₂ emissions, compiled by the Energy Information Administration (EIA), are examined. The U.S. DOE carbon management plan is presented, with focus on biological mitigation.

2. Chapter Two. Carbonate System Equilibrium and Kinetics in Open and Closed Environments: A Literature Review: A review of literature on carbonate dynamics is presented. Reaction mechanisms, equilibrium relationships, and kinetic rate laws are used to develop mass balance equations (MBEs) for carbonate species concentrations in closed systems. MBEs are expanded to include reaction-enhanced diffusion of CO₂ into open systems.

3. Chapter Three. Growth of Freshwater Algae as a Function of Media Inorganic Carbon Content: The analytical results of laboratory investigations are presented and discussed. Effects of reactor environment (closed or open) on freshwater algal growth and culture chemistry parameters are investigated. Quantification and impact of carbon utilization by open algal cultures is completed.

4. Chapter Four. Modeling of Freshwater Algal Growth as a Function of Media Inorganic Carbon Content: Dynamic mathematical models intended to predict algal biomass and carbonate species concentrations in closed and open batch reactors are presented. The systems of MBEs developed in Chapter Two are expanded to include
the effects of algal growth on carbonate species concentrations, and are solved using Matlab®. Model calibration and verification are completed.

5. **Chapter Five. Remarks and Recommendations**: Based on results of this project, areas of continued research are suggested.

**REFERENCES**


CHAPTER TWO

CARBONATE SYSTEM EQUILIBRIUM AND KINETICS IN CLOSED AND OPEN ENVIRONMENTS: A LITERATURE REVIEW

ABSTRACT

Use of carbon-based energy sources is increasing atmospheric CO$_2$ concentrations and leading to global climate change [1]. As part of a carbon management plan the U.S. Department of Energy (DOE) has outlined several research focus areas to develop carbon sequestration technologies.

The goal of this literature review is to summarize carbonate equilibria and kinetics needed for development of carbon sequestration strategies, especially in freshwaters. Specifically, reaction mechanisms, equilibrium relationships, and kinetic rate laws are used to develop mass balance equations (MBEs) for species concentrations in closed systems. Several strategies for quantifying reaction-enhanced CO$_2$ transport are presented to develop carbonate species MBEs for open systems. These general models can be applied to a variety of carbon mitigation strategies.

INTRODUCTION

World energy consumption is predicted to increase through 2030, with majority of energy being derived from coal, natural gas, and liquid fuels. Combustion of these fossil fuels releases CO$_2$, which is currently the most abundant anthropogenic greenhouse gas
(GHG) in the atmosphere. Energy-related CO\textsubscript{2} emissions are expected to increase an average of 1.7 percent per year from 2005 to 2030. Atmospheric CO\textsubscript{2} concentrations are predicted to increase as emissions from fossil fuel combustion increase, although natural and anthropogenic carbon sinks will reduce this effect. Currently, the concentration of atmospheric CO\textsubscript{2} is 380 ppm. Even with measures to abate emissions, this concentration is expected to rise to 450 ppm by 2030 [1].

To combat increasing CO\textsubscript{2} emissions and atmospheric concentrations, the Department of Energy (DOE) has outlined a plan for carbon management, which includes development of carbon sequestration technologies [2,3]. Carbon sequestration refers to the capture and storage of carbon that would otherwise add to atmospheric concentrations [4]. Six research focus areas have been proposed by DOE [3] to develop carbon sequestration technologies: (1) separation and capture of CO\textsubscript{2}, (2) ocean sequestration, (3) carbon sequestration in terrestrial ecosystems, (4) sequestration of CO\textsubscript{2} in geological formations, (5) advanced biological processes for sequestration, and (6) advanced chemical approaches to sequestration.

The goal of this literature review is to provide a comprehensive examination of carbonate chemistry in closed and open aqueous environments. The objectives are to: (1) present reaction mechanisms for carbonate reactions, (2) compile early reports of kinetic rate constants for freshwater, and (3) develop systems of differential equations to describe carbonate species concentrations in closed and open systems.
MODEL DEVELOPMENT

Parameters used to Characterize Carbonate Systems

A carbonate system is characterized by measuring two of the following parameters: pH, total inorganic carbon (TIC), alkalinity (ALK), or fugacity ($f_{CO_2}$) [5].

The pH is a measure of hydrogen ions in solution (equation 1).

$$\text{pH} = -\log[H^+]. \tag{1}$$

The TIC concentration (equation 2) is the sum of all inorganic carbon species, while alkalinity describes the acid-neutralizing capacity of a solution (equation 3) [6].

$$\text{TIC} = [CO_2(\text{aq})] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]. \tag{2}$$

$$\text{ALK} = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]. \tag{3}$$

Reaction Mechanisms

The reversible reactions responsible for carbonate species conversion include CO$_2$ hydration and hydroxylation, as well as HCO$_3^-$ protolysis and hydrolysis.

Hydration of Carbon Dioxide

Hydration of CO$_2$ in aqueous systems occurs by two distinct pathways (Figure 2.1) [6-11].

![Figure 2.1: Reaction scheme for CO$_2$ hydration.](image)
Path I leads to the direct formation of HCO$_3^-$ and H$^+$ (equation 4), while paths II and III lead to formation of H$_2$CO$_3$ (equation 5) followed by HCO$_3^-$ and H$^+$ (equation 6). This reaction scheme is different than the classical reaction scheme presented by Kern [12], which only considers equations 5 and 6. Eigen [7] and Ho and Sturtevant [9] assert that there is no experimental evidence to support the classical reaction scheme.

The equilibrium for equation 5 lies very far to the left; thus, most unionized CO$_2$ exists as CO$_2$ (aq) [6]. As a result, H$_2$CO$_3$ may be neglected, or considered as part of H$_2$CO$_3^*$, which represents the combined concentration of CO$_2$ and H$_2$CO$_3$ (equation 7).

*Hydroxylation of Carbon Dioxide*

Hydroxylation of CO$_2$ (equation 8) is especially important in high pH systems because it contributes significantly to CO$_2$ disappearance at pH $\geq$ 7.5 and dominates at pH $\geq$ 10 [6,10,12,13].
Protolysis and Hydrolysis of Bicarbonate

Acid-base equilibria between bicarbonate and carbonate are described using the universal reaction scheme proposed by Eigen [14] (Figure 2.2).

\[
\begin{align*}
\text{Protolysis} & \quad (I) \\
\text{Hydrolysis} & \quad (II) \\
HCO_3^- + H_2O & \rightleftharpoons H^+ + OH^- + HCO_3^- \\
H^+ + CO_3^{2-} + H_2O & \rightleftharpoons HCO_3^- + H_2O
\end{align*}
\]

Figure 2.2. Aqueous reactions of bicarbonate and carbonate [15].

Protolysis (path I) describes bicarbonate dissociation (equation 9), while hydrolysis (path II) occurs when \( HCO_3^- \) combines with \( OH^- \) (equation 10). Dissociation of water (path III) connects these two pathways (equation 11). Not all authors mentioned here considered equation 10, although it was used by Eigen [14], Kern [12], Patel et.al. [16], Zeebe and Wolf-Gladrow [15], and Cents et.al. [17].

\[
\begin{align*}
HCO_3^- & \overset{k_{5}}{\underset{k_{-5}}{\rightleftharpoons}} H^+ + CO_3^{2-} \\
HCO_3^- + OH^- & \overset{k_{6}}{\underset{k_{-6}}{\rightleftharpoons}} CO_3^{2-} + H_2O \\
H_2O & \overset{k_{7}}{\underset{k_{-7}}{\rightleftharpoons}} H^+ + OH^-
\end{align*}
\]  

Equilibrium Considerations

Equilibrium constants for the carbonate system, defined as molar ratios of products to reactants, at 25°C are summarized in Table 2.1.
Relationships describing the effect of temperature on carbonate system equilibrium constants have been reported by several authors (Table 2.2). Equations relating $K_{H2CO3}$ and $K_b$ to temperature have not been found in the literature; however, Wissburn et.al. [18] measured $K_{H2CO3}$ in aqueous solution between 5 and 45ºC using a high field conductance technique (Table 2.3). Similarly, Edsall and Wyman [19] tabulated values of K for various temperatures (Table 2.3).
Table 2.1: Summary of equilibrium constants and values at 25°C for carbonate system reactions in freshwater systems.

<table>
<thead>
<tr>
<th>Equilibrium Reactions</th>
<th>Definitions and Relationships</th>
<th>Eq. No.</th>
<th>pK at 25°C</th>
<th>Source</th>
</tr>
</thead>
</table>
| \( \text{CO}_2(\text{aq}) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \) | \[ K_h = \frac{[\text{H}_2\text{CO}_3]}{[\text{CO}_2(\text{aq})]} \] | (12) | 2.59 | Edsall [10]
| \( \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \) | \[ K_{\text{H}_2\text{CO}_3} = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \] | (13a) | 3.76 | Wissbrun et.al. [18]
| \( \text{CO}_2(\text{aq}) \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \) | \[ K_1 = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2]} = \frac{K_{\text{H}_2\text{CO}_3}}{1 + K_h} \] | (14) | 6.352 | Harned and Davis [20]
| \( \text{HCO}_3^- + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-} \) | \[ K_2 = \frac{[\text{H}^+][\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} \] | (15) | 10.329 | Harned and Davis [20]
| \( \text{HCO}_3^- + \text{OH}^- \rightleftharpoons \text{CO}_3^{2-} + \text{H}_2\text{O} \) | \[ K_3 = \frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-][\text{OH}^-]} = \frac{K_2}{K_w} \] | (16) | -3.667 | Hikita et.al. [21]
| \( \text{CO}_2(\text{aq}) + \text{OH}^- \rightleftharpoons \text{HCO}_3^- \) | \[ K_4 = \frac{[\text{HCO}_3^-]}{[\text{CO}_2(\text{aq})][\text{OH}^-]} = \frac{K_1}{K_w} \] | (17) | -7.645 | calculated
| \( \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^- \) | \[ K_w = \frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]} \] | (18) | 13.997 | Edsall [10]

1 Calculated by Edsall and Wyman [19] using equation 14 with \( K_{\text{H}_2\text{CO}_3} \) from Wissbrun et.al. [18] and \( K_1 \) from Harned and Davis [20].
2 This relationship is derived by expressing \( K_3 \) as the molar ratio of products to reactants for reaction 10. The concentration of hydroxide ions, \([\text{OH}^-]\), is then replaced with \( K_w/[\text{H}^+] \) from equation 18.
3 Calculated from equation 21.
4 This relationship is derived by expressing \( K_4 \) as the molar ratio of products to reactants for reaction 8. The concentration of hydroxide ions, \([\text{OH}^-]\), is then replaced with \( K_w/[\text{H}^+] \) from equation 18.
5 calculated using equation 18 and appropriate values from Table 2.1.
Table 2.2: Dependence of carbonate system equilibrium constants on absolute temperature (Kelvin) in freshwater systems.

<table>
<thead>
<tr>
<th>Equilibrium Constant</th>
<th>Temperature Dependence</th>
<th>Eq. No.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>$\ln K_1 = 290.9097 - \frac{14554.21}{T} - 45.0575 \ln (T)$</td>
<td>(19)</td>
<td>[22]</td>
</tr>
<tr>
<td>$K_2$</td>
<td>$\ln K_2 = 207.6548 - \frac{11843.79}{T} - 33.6485 \ln (T)$</td>
<td>(20)</td>
<td>[22]</td>
</tr>
<tr>
<td>$K_3$</td>
<td>$\log (K_3) = \frac{1568.94}{T} + 0.4134 - 0.006737T$</td>
<td>(21)</td>
<td>[21]</td>
</tr>
<tr>
<td>$K_W$</td>
<td>$\log (K_W) = -\frac{4470.99}{T} + 6.0875 - 0.01706T$</td>
<td>(22)</td>
<td>[6,23]</td>
</tr>
</tbody>
</table>

1Similar information applicable to marine systems can be obtained from Millero [5], Zeebe and Wolf-Gladrow [15], or DOE [24].

Table 2.3: Temperature dependence of $K_{H_2CO_3}$ and $K_h$ in freshwater systems [18,19].

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>$K_{H_2CO_3} (\times 10^{-4})$</th>
<th>$K_h (\times 10^{-3})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.56</td>
<td>2.00</td>
</tr>
<tr>
<td>15</td>
<td>1.76</td>
<td>2.12</td>
</tr>
<tr>
<td>25</td>
<td>1.72</td>
<td>2.58</td>
</tr>
<tr>
<td>35</td>
<td>1.67</td>
<td>2.96</td>
</tr>
</tbody>
</table>

$K_h$ values calculated by Edsall and Wyman [19] using equation 14 with $K_{H_2CO_3}$ from Wissburn et.al. [18] and $K_1$ from Harned and Davis [20].

Kinetic Considerations

Kinetic modeling of a carbonate system requires formulation of rate laws and determination of rate constants, with special consideration of CO$_2$ hydration kinetics.

Rate Law for Carbon Dioxide Hydration

The rate law for CO$_2$ hydration (equation 23) is developed based on equations 4 and 5.

$$\left( \frac{d[CO_2 (aq)]}{dt} \right)_{hydration} = k_{-1}[H^+][HCO_3^-] - k_{+1}[CO_2 (aq)] + k_{-2}[H_2CO_3] - k_{+2}[CO_2 (aq)].$$ (23)
Assuming equilibrium of equation 6, this rate law is simplified by substituting equation 13b into equation 23 to yield equation 24a. This is a valid assumption since values for \( k_{+3} \) and \( k_{-3} \) are much larger than any of the other rate constants for reactions appearing in Figure 2.1 [6].

\[
K_{H_{2}CO_{3}}\left[H_{2}CO_{3}\right] = \left[H^{+}\right]\left[HCO_{3}^{-}\right]. \quad (13b)
\]

\[
\left(\frac{d\left[CO_{2}\right]_{aq}}{dt}\right)_{hydration, modified} = (k_{-1} \cdot K_{H_{2}CO_{3}} + k_{-2})\left[H_{2}CO_{3}\right] - (k_{+1} + k_{+2})\left[CO_{2}\right]. \quad (24a)
\]

Since the overall rate of formation/disappearance of CO\(_2\) or HCO\(_3^-\) is measured, several authors define composite kinetic constants based on equation 24a [6-11]. Composite kinetic constants \( k_{CO_{2}} \) (equation 25) and \( k_{H_{2}CO_{3}} \) (equation 26) are related to the equilibrium constant \( K_{h} \) (equation 27); in addition, they may be used in formulation of the rate law for CO\(_2\) hydration (equation 24b) [19].

\[
k_{CO_{2}} = k_{+1} + k_{+2}. \quad (25)
\]

\[
k_{H_{2}CO_{3}} = k_{-1} \cdot K_{H_{2}CO_{3}} + k_{-2}. \quad (26)
\]

\[
K_{h} = \frac{k_{CO_{2}}}{k_{H_{2}CO_{3}}}. \quad (27)
\]

\[
\left(\frac{d\left[CO_{2}\right]_{aq}}{dt}\right)_{hydration, modified} = k_{H_{2}CO_{3}}\left[H_{2}CO_{3}\right] - k_{CO_{2}}\left[CO_{2}\right]. \quad (24b)
\]

Again assuming equilibrium of reaction 6, kinetics of CO\(_2\) hydration can be expressed in terms of HCO\(_3^-\) and H\(^+\), rather than H\(_2\)CO\(_3\). The true first acidity constant for H\(_2\)CO\(_3\) is rearranged as equation 13c and substituted into equation 23c to yield equation 24c.
\[
[H_2CO_3] = \frac{[H^+] [HCO_3^-]}{K_{HCO_3}}. \tag{13c}
\]

\[
\left( \frac{d[CO_2(aq)]}{dt} \right)_{	ext{hydration, modified}} = \frac{k_{HCO_3}}{K_{HCO_3}} [HCO_3^-][H^+] - k_{CO_2} [CO_2]. \tag{24c}
\]

According to Stumm and Morgan [6], the modified rate law for CO$_2$ hydration (equations 24a through c) corresponds to a simplified reaction scheme (equation 28).

\[
CO_2(aq) + H_2O \xrightleftharpoons[k_{H2CO_3}]{k_{CO_2}} H_2CO_3 \xrightleftharpoons[\text{very fast}]{k_{K_1}} H^+ + HCO_3^- \tag{28}
\]

Several authors [13,25-28] report composite kinetic constants for a similar summary reaction (equation 29).

\[
CO_2(aq) + H_2O \xrightleftharpoons[k_+]{k_-} H^+ + HCO_3^- \tag{29}
\]

According to Zeebe and Wolf-Galdrow [15] and Johnson [11], the equilibrium constant for equation 29 is equal to $K_1$, and the rate law is the same as shown in equation 24c. As a result, kinetic constants for CO$_2$ hydration and $K_1$ are interrelated (equations 30 through 32).

\[
k_+ = k_{CO_2}. \tag{30}
\]

\[
k_- = \frac{k_{HCO_3}}{K_{HCO_3}} = \frac{k_-}{K_1} = \frac{k_{CO_2}}{K_1}. \tag{31}
\]

\[
K_1 = \frac{k_{CO_2} \cdot K_{HCO_3}}{k_{HCO_3}} = \frac{k_+}{k_-}. \tag{32}
\]
Determination of Kinetic Constants

CO₂ hydration (equation 28) and hydroxylation (equation 8) reactions are considerably slower than subsequent acid-base reactions (equations 6 and 9); therefore, the rate constants $k_{\text{CO₂}}$ and $k_{\text{H}_2\text{CO}_3}$ have been measured more often than other rate constants.

Rate Constants for Carbon Dioxide Hydration

Edsall [10] summarized measured values of $k_{\text{CO₂}}$ (Table 2.4) as 0.0021 s⁻¹ at 0°C; however, more variation was observed at 25°C (Table 2.4). Stumm and Morgan [6] report $k_{\text{CO₂}}$ to be between 0.025 and 0.04 s⁻¹ at 25°C. Kern [12] reports this value as 0.030 s⁻¹.

<table>
<thead>
<tr>
<th>$k_{\text{CO₂}}$ (s⁻¹)</th>
<th>Authors</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0275</td>
<td>Mills and Urey [29]</td>
<td>isotope exchange</td>
</tr>
<tr>
<td>0.0257</td>
<td>Pinsent et.al. [30]</td>
<td>thermal</td>
</tr>
<tr>
<td>0.0358</td>
<td>Ho and Stutevant [9]</td>
<td>pH; stopped flow</td>
</tr>
<tr>
<td>0.0375</td>
<td>Gibbons and Edsall [8]</td>
<td>pH; stopped flow</td>
</tr>
<tr>
<td>0.043</td>
<td>Eigen et.al. [7]</td>
<td>pressure and temp. jump</td>
</tr>
<tr>
<td>0.0339</td>
<td>Mean</td>
<td></td>
</tr>
</tbody>
</table>

Portielje and Lijklema [31] compiled data on the relationship between $k_{\text{CO₂}}$ and temperature (T, Kelvin), and formulated equation 33. A value of 0.035 s⁻¹ is calculated at 25°C, which is close to the average from Table 2.4.

$$\log(k_{\text{CO₂}}) = 10.685 - \frac{3618}{T}. \quad (33)$$
Determination of $k_{H_2CO_3}$ can be completed at low (<5) or neutral (6-8) pH, and requires a value for $K_{H_2CO_3}$. However, the effect of uncertainty in $K_{H_2CO_3}$ at experimental conditions is more pronounced in neutral pH systems (Table 2.5) [10]. Stumm and Morgan [6] and Kern [12] report $k_{H_2CO_3}$ as 20 s$^{-1}$.

### Table 2.5: Summary of $k_{H_2CO_3}$ measurements at 25°C [10].

<table>
<thead>
<tr>
<th>$k_{H_2CO_3}$ (s$^{-1}$)</th>
<th>Authors</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.5</td>
<td>Rossi-Bernardi and Berger [32]</td>
<td>electrode; continuous flow</td>
</tr>
<tr>
<td>20.0</td>
<td>Edsall [10]</td>
<td>optical; stopped flow</td>
</tr>
<tr>
<td>15.1$^a$</td>
<td>Eigen et al. [7]</td>
<td>temperature jump</td>
</tr>
<tr>
<td>17.5$^a$</td>
<td>Ho and Sturtevant [9]</td>
<td>optical; stopped flow</td>
</tr>
<tr>
<td>13.7$^a$</td>
<td>Gibbons and Edsall [8]</td>
<td>optical; stopped flow</td>
</tr>
<tr>
<td>22.1</td>
<td>Patel et al. [16]</td>
<td>concentration jump; spectrophotometric</td>
</tr>
<tr>
<td>19.0</td>
<td>Mean</td>
<td></td>
</tr>
</tbody>
</table>

$^a$measurements made in buffers ranging from pH 6 to 8.

Sirs [33] developed a quantitative relationship between temperature (T, Kelvin) and $k_{H_2CO_3}$ (equation 34). At 25°C, this equation yields a value of 23.1 s$^{-1}$, which is very close to the average of values displayed in Table 2.5.

$$\log(k_{H_{2}CO_{3}}) = 13.770 - \frac{3699}{T}. \quad (34)$$

**Kinetic Constants for Carbon Dioxide Hydroxylation**

Unlike kinetic constants for CO$_2$ hydration, investigations completed to determine $k_+4$ (and subsequently $k_4$) have yielded conclusive results [6,10,12]. Edsall [10] reports that the most accurate measurements of $k_+4$ are those of Pinsent et al. [30] and Sirs [33]. Pinsent et al. [30] reports a value of 8500 M$^{-1}$·s$^{-1}$ for $k_+4$ at 25°C. The reverse kinetic
constant is then calculated as $2 \times 10^{-4} \, s^{-1}$, using equation 17 with values of $K_1$ and $K_W$ from Table 2.3.

Equations describing the effect of temperature ($T$, Kelvin) on $k_{+4}$ (equation 35) [33] and $k_{-4}$ (equation 36) [34] predict values of $8053 \, M^{-1} \cdot s^{-1}$ and $2.25 \times 10^{-4} \, s^{-1}$ at $25^\circ C$, respectively.

$$
\log (k_{+4}) = 13.589 - \frac{2887}{T}.
$$

$$
\log (k_{-4}) = 14.88 - \frac{5524}{T}.
$$

**Additional Carbonate Kinetic Constants**

Kinetic constants for the first and second dissociations of $\text{H}_2\text{CO}_3$ (equations 6 and 9) and water ionization (equation 11) have not been examined in detail. In fact, authors using kinetic constants for the first dissociation of $\text{H}_2\text{CO}_3$ in both freshwater and seawater cite only work by Eigen and Hames [35], in which $k_3$ was determined to be $4.7 \times 10^{10} \, M^{-1} \cdot s^{-1}$ at zero ionic strength (Table 2.6). The value for $k_{+3}$ is calculated by using the value for $K_{\text{H}_2\text{CO}_3}$ given in Table 2.3.

Measurements of $k_{+5}$ and $k_{-5}$ describing the second dissociation of $\text{H}_2\text{CO}_3$ have not been presented. Zeebe and Wolf-Gladrow [15] suggest that the value of $k_{-5}$ is likely close in magnitude to $k_3$ (Table 2.6); thus, $k_{+5}$ is calculated using $K_2$. For any assumption of $k_{+5}$ and $k_{-5}$ to be valid, the ratio of these constants must be equal to $K_2$, and they must be chosen so that equilibrium is established almost instantaneously. The assumption of Zeebe and Wolf-Gladrow [15] is therefore very appropriate.
Kinetic constants describing bicarbonate hydrolysis ($k_{+6}$ and $k_{-6}$) were determined at 25°C and an ionic strength of 1.0 M (Table 2.6) [14]. For reference, the ionic strength of seawater is approximately 0.7 M [15]. Values for these constants in freshwater have not been found in the literature.

Finally, $k_{-7}$ characterizing water ionization has been determined by Eigen [14] to be $1.4 \times 10^{11} \text{ M}^{-1} \cdot \text{s}^{-1}$ (Table 2.6). The value for $k_{+7}$ is determined using the value for $K_W$ presented in Table 2.3.

<table>
<thead>
<tr>
<th>Kinetic Rate Constant</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{+3}$</td>
<td>$8 \times 10^6$</td>
<td>s$^{-1}$</td>
<td>calculated$^4$</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$4.7 \times 10^{10}$</td>
<td>M$^{-1} \cdot$ s$^{-1}$</td>
<td>Eigen and Hames [35]</td>
</tr>
<tr>
<td>$k_{+4}$</td>
<td>8500</td>
<td>M$^{-1} \cdot$ s$^{-1}$</td>
<td>Sirs [33]</td>
</tr>
<tr>
<td>$k_4$</td>
<td>$1.9 \times 10^{-4}$</td>
<td>s$^{-1}$</td>
<td>calculated$^3$</td>
</tr>
<tr>
<td>$k_5$</td>
<td>2.34</td>
<td>s$^{-1}$</td>
<td>calculated$^4$</td>
</tr>
<tr>
<td>$k_{+5}$</td>
<td>$5 \times 10^{10}$</td>
<td>M$^{-1} \cdot$ s$^{-1}$</td>
<td>Zeebe and Wolf-Gladrow [15]$^5$</td>
</tr>
<tr>
<td>$k_{+6}$</td>
<td>$6 \times 10^9$</td>
<td>M$^{-1} \cdot$ s$^{-1}$</td>
<td>Eigen [14]$^6$</td>
</tr>
<tr>
<td>$k_6$</td>
<td>$1.292 \times 10^6$</td>
<td>s$^{-1}$</td>
<td>calculated$^6$</td>
</tr>
<tr>
<td>$k_{+7}$</td>
<td>$1.411 \times 10^{-3}$</td>
<td>M$\cdot$ s$^{-1}$</td>
<td>calculated$^8$</td>
</tr>
<tr>
<td>$k_7$</td>
<td>$1.4 \times 10^{11}$</td>
<td>M$^{-1} \cdot$ s$^{-1}$</td>
<td>Eigen [14]</td>
</tr>
</tbody>
</table>

$^1$Similar information applicable to seawater can be obtained Zeebe and Wolf-Gladrow [15] or Johnson [11].

$^2$Calculated using $K_{H_2CO_3} = k_{+3}/k_{-3}$ ($K_{H_2CO_3}$ from Table 2.3).

$^3$Calculated using $K_1 = k_4/k_{-5}$ ($K_1$ and $K_W$ from Table 2.3).

$^4$Calculated using $K_2 = k_5/k_5$ ($K_2$ from Table 2.3).

$^5$k_{-5} assumed to be approximately equal to $k_3$, since no experimental data available.

$^6$Calculated using $K_3 = k_{+4}/k_{+5}$ (from Table 2.3).

$^7$Calculated using $K_4 = k_{+5}/k_{+6}$ ($K_1$ from Table 2.3).

$^8$Calculated using $K_W = k_{+7}/k_{-7}$ ($K_W$ from Table 2.3).
Modeling Carbonate Kinetics in a Closed System

Kinetic rate laws for reactions describing a closed carbonate system are used to express mass balance equations (MBEs) for each carbonate species. Two approaches may be used to develop these MBEs, depending on whether $\text{H}_2\text{CO}_3$ (CA) is considered to contribute significantly to the total $\text{CO}_2$ (or $\text{H}_2\text{CO}_3^*$) pool.

MBEs Assuming $\text{CO}_2$ and $\text{H}_2\text{CO}_3$ to be Separate Species

If $\text{H}_2\text{CO}_3$ is not considered negligible, the Stumm and Morgan [6] $\text{CO}_2$ hydration summary reaction (equation 28) is considered with remaining carbonate reactions (equations 8 through 11). Using this approach, the “fast” reaction in equation 28 is modeled using kinetic constants defined by equation 6. MBEs for carbonate species are formulated by combining chemical rate laws (equations 37 through 43).

\[
\frac{d[\text{CO}_2]}{dt}_{\text{closed,CA}} = k_{\text{H}_2\text{CO}_3}[\text{H}_2\text{CO}_3] - k_{\text{CO}_2}[\text{CO}_2] + k_{-4}[\text{HCO}_3^-] - k_{-4}[\text{CO}_3^-][\text{OH}^-]. \quad (37)
\]

\[
\frac{d[\text{H}_2\text{CO}_3]}{dt}_{\text{closed,CA}} = k_{\text{CO}_2}[\text{CO}_2] - k_{\text{H}_2\text{CO}_3}[\text{H}_2\text{CO}_3] + k_{-3}[\text{HCO}_3^-][\text{H}^+] - k_{-3}[\text{H}_2\text{CO}_3]. \quad (38)
\]

\[
\frac{d[\text{HCO}_3^-]}{dt}_{\text{closed,CA}} = k_{-5}[\text{H}_2\text{CO}_3] - k_{-5}[\text{H}^+][\text{HCO}_3^-] + k_{-4}[\text{CO}_2][\text{OH}^-] - k_{-4}[\text{HCO}_3^-] + k_{-5}[\text{HCO}_3^-][\text{OH}^-] + k_{-3}[\text{CO}_3^-]. \quad (39)
\]

\[
\frac{d[\text{CO}_3^-]}{dt}_{\text{closed,CA}} = k_{-5}[\text{HCO}_3^-] - k_{-5}[\text{H}^+][\text{CO}_3^-] + k_{-6}[\text{HCO}_3^-][\text{OH}^-] - k_{-6}[\text{CO}_3^-]. \quad (40)
\]

\[
\frac{d[\text{H}^+]}{dt}_{\text{closed,CA}} = k_{-5}[\text{H}_2\text{CO}_3] - k_{-5}[\text{H}^+][\text{HCO}_3^-] + k_{-5}[\text{HCO}_3^-] - k_{-5}[\text{H}^+][\text{CO}_3^-] + k_{-6}[\text{HCO}_3^-][\text{OH}^-]. \quad (41)
\]

\[
\frac{d[\text{OH}^-]}{dt}_{\text{closed,CA}} = k_{-4}[\text{HCO}_3^-] - k_{-4}[\text{CO}_2][\text{OH}^-] - k_{-6}[\text{HCO}_3^-][\text{OH}^-] + k_{-6}[\text{CO}_3^-] + k_{-7}[\text{H}^+][\text{OH}^-]. \quad (42)
\]

\[
\frac{d[\text{H}_2\text{CO}_3^*]}{dt}_{\text{closed,CA}} = \left(\frac{d[\text{CO}_2]}{dt}_{\text{closed,CA}} + \frac{d[\text{H}_2\text{CO}_3]}{dt}_{\text{closed,CA}}\right). \quad (43)
\]
**MBEs Assuming \( H_2CO_3 \) to be Negligible**

If \( H_2CO_3 \) is considered negligible, the \( CO_2 \) hydration summary reaction shown as equation 29 should be considered with remaining carbonate reactions (equations 8 through 11). By considering the kinetic rate laws for each of these reactions, MBEs for carbonate species are formulated (equations 44 through 46). MBEs for \( CO_3^{2-} \) and \( OH^- \) are not affected by choice of \( CO_2 \) summary reaction (equations 40 and 42).

\[
\frac{d[CO_2]}{dt}_{\text{closed}} = k_1[H^+][HCO_3^-] - k_8[CO_2] + k_{14}[HCO_3^-] - k_{14}[CO_2][OH^-]. \quad (44)
\]

\[
\frac{d[HCO_3^-]}{dt}_{\text{closed}} = \frac{k_1[CO_2] - k_{14}[HCO_3^-] + k_{12}[CO_2][OH^-] - k_{14}[HCO_3^-] + k_{15}[CO_3^{2-}] - k_{15}[HCO_3^-] - k_{26}[HCO_3^-][OH^-] + k_{26}[CO_3^{2-}]. \quad (45)
\]

\[
\frac{d[H^+]}{dt}_{\text{closed}} = \frac{k_1[CO_2] - k_{14}[HCO_3^-] + k_{15}[HCO_3^-] - k_{25}[HCO_3^-] - k_{26}[HCO_3^-][OH^-]} + k_{27} - k_{27}[H^+][OH^-]. \quad (46)
\]

**Modeling Inorganic Carbon System in Open Systems**

**Carbon Dioxide Absorption**

In an open carbonate system, diffusion of atmospheric \( CO_2 \) across the system boundary occurs (equation 47).

\[
CO_2(g) + \frac{1}{2}H_2O \rightleftharpoons CO_2(aq). \quad (47)
\]

Henry’s Law (equation 48) quantitatively describes the equilibrium absorption.

At 25°C, \( K_H \) is 3434.92 Pa/M and \( p_{CO_2} \) is 32.02 Pa [6].

\[
K_H = \frac{[CO_2(aq)]_{\text{sat}}}{p_{CO_2}}. \quad (48)
\]

Where, \( K_H = \) Henry’s Law constant (Pa/M), \( [CO_2(aq)]_{\text{sat}} = \) equilibrium \( CO_2 \) concentration (mol/L), and \( p_{CO_2} = CO_2 \) partial pressure (Pa).
Film Model

The rate of gas transfer into an open carbonate system is described using film theory [36]. For CO$_2$, the resistance to mass transfer occurs in the liquid, where a quiescent boundary layer of finite thickness (L) extends from the gas-liquid interface to the bulk solution. At the interface, CO$_2$ concentration is equal to (CO$_2$)$_{sat}$. At position L, CO$_2$ concentration is equal to that in the bulk solution, (CO$_2$)$_{bulk}$.

Based on the film theory [36], three cases exist for absorption of a gas into liquid [26,27,37,38]: unenhanced diffusion, kinetically-enhanced diffusion, and equilibrium-enhanced diffusion.

**Case 1: Unenhanced Diffusion**

Case 1 describes mass transfer by diffusion, without reaction of CO$_2$ in the boundary layer, as calculated by Fick’s first law (equation 49) [37,38].

$$\frac{dC_{O_2}}{dt} = \frac{D_{CO_2}}{L} \cdot a \cdot \left[ (CO_2)_{sat} - (CO_2)_{bulk} \right]$$  \hspace{1cm} (49)

Where, $D_{CO_2}$ = liquid diffusivity of CO$_2$ (m$^2$/s), $L$ = boundary layer thickness (m), and $a$ = reactor interfacial area (m$^{-1}$).

The dependence of $D_{CO_2}$ on temperature (equation 50) has been described using an Arrhenius relationship, which yields a value of $1.916 \times 10^{-9}$ m$^2$/s at 25ºC [39,40].

$$D_{CO_2} = A_{CO_2} e^{\frac{-E_a}{RT}}.$$  \hspace{1cm} (50)

Where, $A$ = Arrhenius constant for CO$_2$ ($5019 \times 10^{-9}$ m$^2$/s), $E_a$ = activation energy ($19,510$ J/mol), $R$ = gas constant (8.3142 J/K·mol), and $T$ = absolute temperature (K).
**Case 2: Kinetically-Enhanced Diffusion**

**Diffusion-Reaction Equation**

If CO$_2$ remains in the boundary layer long enough for hydration and hydroxylation reactions to occur, then the CO$_2$ reaction rate must be considered in Fick’s Law (equation 51) [27]. Reactions in the boundary layer cause the CO$_2$ concentration gradient to vary with depth in the boundary layer (Figure 2.3), with the profile being steepest just below the interface [31].

\[
\frac{d[\text{CO}_2]}{dt} = D_{\text{CO}_2} \frac{\partial^2[\text{CO}_2]}{\partial z^2} + \left( \frac{\partial[\text{CO}_2]}{\partial t} \right)_{\text{reaction}}.
\]  

(51)

**Figure 2.3:** Idealized depiction of unenhanced (dashed line) versus chemically-enhanced (solid line) CO$_2$ flux through a stagnant boundary layer [27,31].

**Enhanced TIC Transport Model**

In the enhanced TIC transport model, CO$_2$ conversion reactions occur in the boundary layer, with pH varying with distance [28,38,38]. Conversion of CO$_2$ in the
boundary layer creates gradients of CO\textsubscript{2}, HCO\textsubscript{3}\textsuperscript{−}, and CO\textsubscript{3}\textsuperscript{2−}. Thus, the TIC flux (N\textsubscript{TIC}) is the sum of individual species fluxes (equation 52) [28].

\[
N_{TIC} = -D_{CO_2} \frac{d[CO_2]}{dx} - D_{HCO_3^-} \frac{d[HCO_3^-]}{dx} - D_{CO_3^{2-}} \frac{d[CO_3^{2-}]}{dx}.
\] (52)

**Modified Enhanced TIC Transport Models**

Other authors have simplified this model. Bolin [41], Hoover and Berkshire [42], and Smith [27] assume pH to be constant in the boundary layer, and equal to the value in the bulk solution. Hoover and Berkshire [42] defend that pH can be considered constant in the boundary layer since the mobility of H\textsuperscript{+} is nearly eight times that of HCO\textsubscript{3}\textsuperscript{−}. However, Quinn and Otto [28] argue that a constant pH assumption violates electroneutrality in the boundary layer. Alternatively, an analytical solution can be developed (based on r, a\textsubscript{k}, Da, or EF) by considering flux at the air-water interface to eliminate ionic transport terms in equation 52 [15,27].

1. Enhanced flux in terms of r

In developing an analytical solution for enhanced TIC flux, Smith [27] considers both CO\textsubscript{2} hydration and hydroxylation in the boundary layer (equation 44b).

\[
- \left\{ \frac{\partial [CO_2]}{\partial t} \right\}_{reaction} = -[CO_2] \left\{ \frac{k_r [H^+] + k_s K_w}{[H^+] K} \right\} + [HCO_3^-] \left\{ \frac{k_r [H^+] + k_s K_w}{K} \right\}.
\] (44b)

Assuming steady-state conditions, equation 44b is substituted into equation 51 to yield equation 51b.
\[
\frac{\partial^2 [CO_2]}{\partial z^2} = \frac{1}{D_{CO_2}} \left( [CO_2] \left\{ \frac{k_+ [H^+] + k_{i_4} K_W}{[H^+]} \right\} + \left\{ HCO_3^- \right\} \left\{ \frac{k_+ [H^+] + k_{i_4} K_W}{K_l} \right\} \right).
\]

(51b)

Equation 51b is further modified by assuming that the concentration of HCO_3^- is constant throughout the boundary layer. Furthermore, this concentration is estimated by solving equation 14 for [HCO_3^-], and substituting this expression into equation 51b to yield equation 51c. Smith [27] has also considered the pH in the boundary layer to be equal to that in the bulk solution; thus, the value for [H^+] in equation 14 is determined using the pH of the bulk solution.

\[
-\frac{\partial^2 [CO_2]}{\partial z^2} = \frac{1}{D_{CO_2}} \left\{ [CO_2] \left\{ \frac{k_+ [H^+] + k_{i_4} K_W}{[H^+]} \right\} \cdot \left\{ [CO_2] - [CO_2]_{bulk} \right\} \right\}.
\]

(51c)

The CO_2 gradient at the air-water interface is determined by Smith [27] for z equal zero (equations 53, 54a, 54b).

\[
\left. \frac{d[CO_2]}{dz} \right|_{z=0} = r \cdot \frac{\cosh(r \cdot L)}{\sinh(r \cdot L)} \cdot \left\{ [CO_2]_{sat} - [CO_2]_{bulk} \right\}.
\]

(53)

Where,

\[
r = \sqrt{\frac{k_+ [H^+] + k_{i_4} K_W}{D_{CO_2} [H^+]}}.
\]

(54a)

Finally, the chemically-enhanced flux of TIC (F_e) is expressed by multiplying equation 54 by D_{CO_2} to yield equation 55 [27].
2. Enhanced flux in terms of reacto-diffusive length \( (a_k) \)

Using the concept of reacto-diffusive length \( (a_k) \) rather than \( r \), Zeebe and Wolf-Gladrow [15] also develop an enhanced TIC flux equation. The reacto-diffusive length \( (a_k) \) is a measure of the relative importance of diffusion and reaction, based on the reaction rate constant \( (k) \) and diffusivity \( (D) \) (equation 56). An expression of \( a_k \) for a carbonate system is developed in a similar manner as \( r \); however, comparison of equations 54b and 57 reveal that an inverse relationship exists between the parameters (equation 58).

\[
a_k = \frac{2}{\sqrt{k}}. \tag{56}
\]

For a carbonate system,

\[
a_k = \sqrt{\frac{D_{CO_2}}{k_a + k_{s,ad} [OH^-]}}. \tag{57}
\]

Thus,

\[
r = \frac{1}{a_k}. \tag{58}
\]

The parameter \( a_k \) (and consequently \( r \)) is used to characterize a diffusion-reaction system. Generally, when \( a_k \) is high, reaction in the boundary layer can be neglected. Values for \( a_k \) or \( r \) cannot be used alone to conclusively determine whether diffusion or reaction controls \( CO_2 \) absorption into an open carbonate system. Rather, these parameters must be compared to the boundary layer thickness.

In addition, temperature and pH can significantly affect \( a_k \), due to the impact on reaction rates. Generally, increases in temperature decrease the value of \( a_k \) because
reactions in the boundary layer occur more rapidly. Increases in pH also decrease $a_k$ due to an increase in hydroxyl ion concentration, which encourages hydroxylation in the boundary layer. Thus, increases in temperature and pH increase the significance of reactions in the boundary layer. At pH below approximately 8.5, temperature controls the value of $a_k$. The effects of temperature become negligible above pH 11, as $a_k$ approaches zero (Figure 2.4).

![Figure 2.4: Simulation of pH and temperature effects on $a_k$. Figure development completed using equation 57 to quantify $a_k$ for various temperatures and pH. Equations 22, 33, 35, 50 were used to describe the effect of temperature on $K_w$, $k_+$, $k_{+4}$, and $D_{CO_2}$.)](image-url)
3. Enhanced flux in terms of Damkohler number (Da) and enhancement factor (EF)

Enhanced TIC flux (equation 55a or 55b) can also be described using dimensionless mass transfer parameters, such as the Damkohler number (Da) or enhancement factor (EF). In an open carbonate system, the Damkohler number (equation 59) represents the ratio of the diffusion time scale \((L^2/D)\) to the reaction time scale \((1/k)\) [15].

\[
Da = \left( \frac{L}{a_k} \right)^2.
\]

(59)

Like \(a_k\) and \(r\), the Da number is used to characterize the relative importance of diffusion and reaction. Specifically, diffusion dominates at small Da numbers \((Da \ll 1)\), while reaction dominates at large Da numbers \((Da >> 1)\) [15]. Unlike \(a_k\) and \(r\), the Da number directly incorporates the effects of boundary layer thickness on the relative importance of diffusion and reaction.

The enhancement factor (EF) is analogous to the Da number and describes the ratio of enhanced TIC flux \((F_e)\) to unenhanced TIC flux \((F)\) (equation 60) [15,27]. EF can be expressed in terms of \(r\), \(a_k\), or Da (equations 61a 61b, and 61c, respectively). As this parameter increases, the relative importance of reactions in the boundary layer increases (Figure 2.5).

\[
EF = \frac{F_e}{F}.
\]

(60)

Where,

\[
EF = r \cdot L \cdot \coth(r \cdot L),
\]

(61a)
EF = \frac{L}{a_k} \coth \left( \frac{L}{a_k} \right), \text{ or} \quad (61b)

EF = \sqrt{Da} \coth \left( \sqrt{Da} \right). \quad (61c)

Figure 2.5: Simulation of boundary layer thickness (L) effect on EF at 25°C. Note that zero on the y-axis corresponds to unenhanced diffusion (Case 1). This figure was developed by calculating EF for a range of pH using equation 61.

Case 3: Equilibrium-Enhanced Diffusion

The upper limit for chemically-assisted diffusion occurs when reaction rates are essentially infinite so that equilibrium exists in the boundary layer [27,28]. Bolin [41] and Smith [27] assume pH to be constant in the boundary layer and assert that the
maximum EF is equal to the ratio of free CO\textsubscript{2} to TIC in the boundary layer. Using the definition of TIC (equation 2) and the definitions K\textsubscript{1} and K\textsubscript{2} (equations 14 and 15, respectively), the maximum EF is expressed in terms of only [H\textsuperscript{+}], K\textsubscript{1}, and K\textsubscript{2} (equation 62) [27]. Without assuming constant pH in the boundary layer, Quinn and Otto [28] derive a more stringent model for equilibrium-enhanced diffusion.

\[
(\text{EF})_{\text{max}} = \frac{[\text{H}^+]^2 + K_1[H^+]K_2}{[H^+]^2}. \tag{62}
\]

*Mass Balance Equations in Open Carbonate Systems*

Mass balance equations for an open carbonate system can be developed by considering MBEs for each carbonate species with enhanced TIC flux. Since development of enhanced flux equations by Smith [27] and Zeebe and Wolf-Gladrow [15] consider flux at the air-water interface to eliminate ionic flux, inorganic carbon transported into the system is in the form of CO\textsubscript{2}. Thus, MBEs for HCO\textsubscript{3}\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-}, H\textsuperscript{+}, and OH\textsuperscript{-} are expressed as equations 45, 40, 46, and 42, respectively. The MBE for CO\textsubscript{2} in an open system (equation 63) is developed by combining equations 44 and 55c.

\[
\left(\frac{d[\text{CO}_2(\text{aq})]}{dt}\right)_{\text{open}} = \left(\frac{d[\text{CO}_2]}{dt}\right)_{\text{closed}} + \left\{\text{EF} \cdot \frac{D_{\text{CO}_2} \cdot a}{L} \cdot \left([\text{CO}_2]_{\text{sat}} - [\text{CO}_2]_{\text{bulk}}\right)\right\}. \tag{63}
\]

Where, a = interfacial area (m\textsuperscript{-1}).
SUMMARY

Models were presented to describe carbonate kinetics in closed and open systems, and a summary of kinetic constants is given in Tables 2.4 through 2.6. The following major concepts were discussed.

1. Characterization of a carbonate system requires measurement of two of the following parameters: pH, TIC, ALK, or $f_{CO2}$.

2. The carbonate system of reactions includes $CO_2$ hydration and hydroxylation, $HCO_3^-$ protolysis and hydrolysis, and water ionization.

3. The elementary rate law describing the three $CO_2$ hydration reactions is simplified by assuming the first $H_2CO_3$ dissociation reaction to be at equilibrium and defining composite kinetic constants.

4. Measurements of kinetic constants describing $CO_2$ hydration ($k_{CO2}$ and $k_{H2CO3}$) have been reported, although variation exists in these values. Kinetic constants for $CO_2$ hydroxylation ($k_{+4}$ and $k_{-4}$) have been reported with more certainty. Values for remaining kinetic constants are sparse, and in some cases unavailable.

5. To model an open carbonate environment, diffusion of atmospheric $CO_2$ across the system boundary must be considered. Three cases exist for absorption of a gas into a liquid: (A) unenhanced diffusion, (B) kinetically-enhanced diffusion, and (C) equilibrium-enhanced diffusion.
6. The rate of kinetically-enhanced CO\textsubscript{2} diffusion is quantified by multiplying Fick’s First Law by an enhancement factor (EF). This parameter can be determined based on the Smith [27] “r”, reacto-diffusive length (\(a_k\)), or Damkohler number (Da).

7. Mass balance equations (MBEs) for carbonate species in a closed system are formulated by combining kinetic rate laws for carbonate reactions. To describe an open system, only the CO\textsubscript{2} MBE is altered to include transport of atmospheric CO\textsubscript{2}.

**CONCLUSIONS**

As atmospheric CO\textsubscript{2} concentrations and global temperatures continue to escalate, researchers must develop creative methods to offset these trends. Proposed solutions are expansive, and range from sequestration by photosynthetic organisms to oceanic sequestration [4]. The systems of differential equations presented for carbonate systems can be used to model and evaluate carbon sequestration strategies in aqueous systems.

**REFERENCES**


[20] HS Harned, RJ Davis. The ionization constant of carbonic acid in water and the solubility of carbon dioxide in water and aqueous salt solutions from 0 to 50°, J. Am. Chem. Soc. 65 (1943) 2030-2037.


CHAPTER THREE

GROWTH OF FRESHWATER ALGAE AS A FUNCTION OF MEDIA INORGANIC CARBON CONTENT

ABSTRACT

To investigate use of a freshwater algal culture for carbon mitigation, batch growth was analyzed in closed and open batch reactors. Trials were completed with unadjusted and adjusted initial pH at four initial TIC concentrations. Results show that specific growth rates, biomass production, and peak pH generally increase with increasing initial TIC in closed and open reactors. Batch growth curves indicate uptake of all carbonate species, which suggests employment of carbon concentrating mechanisms (CCMs) by algal cultures. Estimation of Monod constants ($\mu_{\text{max}} = 0.0726$ hr$^{-1}$, $K_{\text{CO}_2} = 4.47 \times 10^{-8}$, $K_{\text{HCO}_3} = 5.70 \times 10^{-4}$, $K_{\text{CO}_3} = 8.70 \times 10^{-4}$) were significant at the 5% level, indicating that algal growth responded to all TIC forms. Analysis of observed biomass yields (5.1 to 7.1 mg X/mg C) suggests that nearly 100% of biomass was produced using $\text{HCO}_3^-$ and $\text{CO}_3^{2-}$, while less than 1% was produced using $\text{CO}_2$. Analysis of batch growth in open reactors shows that carbon sequestered per supplied TIC exponentially ($R^2 = 0.9717$) decreased with increasing initial TIC.
INTRODUCTION

Atmospheric CO$_2$ concentrations are predicted to increase as emissions from fossil fuel combustion increase, although natural and anthropogenic carbon sinks will reduce this effect. Currently, the concentration of atmospheric CO$_2$ is 380 ppm. Even with measures to abate emissions, this concentration is expected to rise to 450 ppm by 2030 [1].

To combat increasing CO$_2$ emissions and atmospheric concentrations, the Department of Energy (DOE) has outlined a carbon management, which includes development of carbon sequestration technologies. Carbon sequestration refers to the capture and storage of carbon that would otherwise add to atmospheric concentrations [2]. Cultivation of algal biomass as an approach for carbon sequestration has been proposed [3]. However, since biomass decay releases CO$_2$ into the atmosphere, biomass must be strategically stored or utilized to ensure sequestration or abatement. For instance, biomass could be harvested, converted to biofuels, and used to reduce fossil fuel use [4]. The ability of some algae and cyanobacteria to survive in high alkalinity ponds may enhance mitigation. In this case, the amount of total inorganic carbon (TIC) dissolved in solution increases, due to increasing chemical hydration rates with increasing pH. Ultimately, this maximizes the availability of inorganic carbon to aquatic organisms for biofixation [3].
The goal of this project was to investigate use of a mixed freshwater algal culture for atmospheric carbon mitigation. The objectives of the research were as follows:

1) to compare effects of initial TIC concentration on algal growth and culture chemistry in closed and open batch reactors,
2) to quantify kinetic parameters ($\mu_{\text{max}}$, $K_S$, $Y_{X/S}$, and $b$) to characterize algal growth and aid in determination of carbonate species used by algal cultures, and
3) to determine the relationship between concentration of carbon sequestered by open algal cultures and initial TIC.

LITERATURE REVIEW

Closed Carbonate Systems

*Equilibrium Reactions*

Equilibrium reactions occurring in carbonate systems include the hydration of CO$_2$ (equation 1), the first dissociation reaction of H$_2$CO$_3$ (equation 3), the second dissociation reaction of H$_2$CO$_3$ (equation 7), and the ionization of water (equation 13) [5]. Since the equilibrium for reaction 1 lies far to the left, most unionized CO$_2$ in solution exists as CO$_2$(aq). Thus, the hydration of CO$_2$ and first dissociation reaction of H$_2$CO$_3$ can be combined (equation 5) [5,6].

Additional carbonate reactions may occur. Some authors also consider HCO$_3^-$ hydrolysis (equation 9) [6-10]. In addition, hydroxylation of CO$_2$ (equation 11) is
important in high pH systems because it contributes significantly to CO$_2$ disappearance at pH above 7.5, while it dominates at pH above 10 [5,8,11,12].

The equilibrium constants describing the carbonate system can be defined as the molar ratios of products to reactants (Table 3.1) [5,6].
Table 3.1: Summary of equilibrium constants and values at 25°C for carbonate system reactions.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CO}_2$ (aq) + $\text{H}_2\text{O}$ $\rightleftharpoons$ $\text{H}_2\text{CO}_3$</td>
<td>(1)</td>
<td></td>
<td>(2)</td>
<td>2.59</td>
<td>Edsall [11]</td>
</tr>
<tr>
<td>$\text{H}_2\text{CO}_3$ $\rightleftharpoons$ $\text{H}^+$ + $\text{HCO}_3^-$</td>
<td>(3)</td>
<td>$K_{\text{H}_2\text{CO}_3} = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$</td>
<td>(4)</td>
<td>3.76</td>
<td>Wissbrun et.al. [13]</td>
</tr>
<tr>
<td>$\text{CO}_2$ (aq) $\rightleftharpoons$ $\text{H}^+$ + $\text{HCO}_3^-$</td>
<td>(5)</td>
<td>$K_1 = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}<em>2]} = \frac{K</em>{\text{H}_2\text{CO}_3}}{1 + K_h}$</td>
<td>(6)</td>
<td>6.352</td>
<td>Harned and Davis [14]</td>
</tr>
<tr>
<td>$\text{HCO}_3^- + \text{H}_2\text{O}$ $\rightleftharpoons$ $\text{H}^+$ + $\text{CO}_3^{2-}$</td>
<td>(7)</td>
<td>$K_2 = \frac{[\text{H}^+][\text{CO}_3^{2-}]}{[\text{HCO}_3^-]}$</td>
<td>(8)</td>
<td>10.329</td>
<td>Harned and Davis [14]</td>
</tr>
<tr>
<td>$\text{HCO}_3^- + \text{OH}^- \rightleftharpoons$ $\text{CO}_3^{2-} + \text{H}_2\text{O}$</td>
<td>(9)</td>
<td>$K_3 = \frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-][\text{OH}^-]} = \frac{K_2}{K_w}$</td>
<td>(10)</td>
<td>-3.667</td>
<td>Hikita et.al. [15]</td>
</tr>
<tr>
<td>$\text{CO}_2$ (aq) + $\text{OH}^-$ $\rightleftharpoons$ $\text{HCO}_3^-$</td>
<td>(11)</td>
<td>$K_4 = \frac{[\text{HCO}_3^-]}{[\text{CO}_2 (aq)][\text{OH}^-]} = \frac{K_1}{K_w}$</td>
<td>(12)</td>
<td>-7.645</td>
<td>calculated$^1$</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}$ $\rightleftharpoons$ $\text{H}^+$ + $\text{OH}^-$</td>
<td>(13)</td>
<td>$K_w = \frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]}$</td>
<td>(14)</td>
<td>13.997</td>
<td>Edsall [11]</td>
</tr>
</tbody>
</table>

$^1$Calculated using $K_1$ and $K_w$. 
Total Inorganic Carbon and Alkalinity

Total inorganic carbon (equation 15) is defined as the sum of all inorganic carbon species, while alkalinity (equation 16) describes the acid-neutralizing capacity of a solution. Ionization fractions are calculated to quantify relative amounts of CO$_2$ (aq), HCO$_3^-$, and CO$_3^{2-}$ (equations 17 through 22) [5].

\[
\text{TIC} = \left[ \text{H}_2\text{CO}_3^- \right] + \left[ \text{HCO}_3^- \right] + \left[ \text{CO}_3^{2-} \right], \quad \text{and} \quad (15)
\]

\[
\text{ALK} = \left[ \text{HCO}_3^- \right] + 2\left[ \text{CO}_3^{2-} \right] + \left[ \text{OH}^- \right] - \left[ \text{H}^+ \right]. \quad (16)
\]

\[
\left[ \text{CO}_2 (\text{aq}) \right] = \text{TIC} \cdot \alpha_0 \quad (17) \quad \alpha_0 = \left\{ 1 + \left( \frac{\text{K}_1}{\left[ \text{H}^+ \right]} \right) + \left( \frac{\text{K}_1 \text{K}_2}{\left[ \text{H}^+ \right]^2} \right) \right\}^{-1} \quad (18)
\]

\[
\left[ \text{HCO}_3^- \right] = \text{TIC} \cdot \alpha_1 \quad (19) \quad \alpha_1 = \left\{ \frac{\left[ \text{H}^+ \right]}{\text{K}_1 + \text{K}_2/\left[ \text{H}^+ \right]^2} \right\}^{-1} \quad (20)
\]

\[
\left[ \text{CO}_3^{2-} \right] = \text{TIC} \cdot \alpha_2 \quad (21) \quad \alpha_2 = \left\{ \frac{\left[ \text{H}^+ \right]^2}{(\text{K}_1 \text{K}_2) + \left[ \text{H}^+ \right]/\text{K}_2 + 1} \right\}^{-1} \quad (22)
\]

Open Carbonate Systems

Equilibrium Considerations

In an open carbonate system, the TIC concentration is increased due to diffusion of atmospheric CO$_2$ across the system boundary (equation 23).

\[
\text{CO}_2 (g) \overset{\text{K}_\text{H}}{\rightleftharpoons} \left[ \text{CO}_2 (\text{aq}) \right] \quad (23)
\]

Henry’s Law (equation 24) quantitatively describes the equilibrium for reaction 23 (Stumm and Morgan, 1981). At 25°C, K$_H$ is 3434.92 Pa/M and p$_{\text{CO}_2}$ is 32.02 Pa. [5].

\[
\text{K}_H = \frac{\left[ \text{CO}_2 (\text{aq}) \right]}{\text{p}_{\text{CO}_2}} \quad (24)
\]
Where, $K_H = \text{Henry’s Law constant (Pa/M)}$, $[\text{CO}_2 \ (\text{aq})]_{\text{sat}} = \text{equilibrium CO}_2$ concentration (mol/L), and $p_{\text{CO}_2} = \text{CO}_2$ partial pressure (Pa).

**Kinetic Considerations**

**Fick’s Law**

For the case where chemical enhancement does not occur in the boundary layer, gas transfer into an open system is described using Fick’s Law (equation 25) [16].

$$\frac{dA}{dt} = (k_La)_A \cdot [(A)_{\text{sat}} - (A)_{\text{bulk}}].$$  (25)

Where, $(k_L)A = \text{mass transfer velocity of gas A (m/s)}$, $a = \text{interfacial area (m}^{-1})$, $(A)_{\text{sat}} = \text{equilibrium concentration of A (mol/L)}$, and $(A)_{\text{bulk}} = \text{concentration of A in bulk medium (mol/L)}$.

**Determination of Mass Transfer Velocity and Interfacial Area Product ($k_La$)**

Quantification of the rate of CO$_2$ transfer into an open carbonate system requires determination of $(k_La)_{\text{CO}_2}$, which can be estimated based on the $k_La$ for dissolved oxygen (DO) [17]. Oxygen is suggested as a reference compound because, like CO$_2$, resistance to mass transfer occurs in the liquid boundary layer [17].

An estimate of $(k_La)_{\text{DO}}$ is determined from experimental measurements and equation 26, which is obtained by integrating equation 25 between $t_1$ (initial) and $t_2$ [18].

$$-(k_La)_{\text{DO}} \cdot t = \ln \left( \frac{DO_{\text{sat}} - DO_{\text{bulk}}}{DO_{\text{sat}} - DO_{\text{initial}}} \right).$$  (26)

The $k_La$ for CO$_2$ is then determined using $k_La$ for DO in the same reactor under the same environmental conditions (equation 27). The values of $D_{\text{CO}_2}$ and $D_{\text{O}_2}$ at 25°C are of $1.916 \times 10^{-9}$ [19,20] and $2.306 \times 10^{-9}$ m$^2$/s [17], respectively.
Inorganic-Carbon-Limited Algal Growth

Although phosphorous is usually the rate-limiting nutrient in freshwater systems, inorganic carbon is often limiting in “artificial and highly enriched” systems [21]. King [22] and Novak and Brune [21] show a Monod response between CO$_2$ and specific growth rate of several green algae, although Goldman et.al. [23] defend a similar relationship for TIC. Recent information on carbon concentrating mechanisms (CCMs) has expanded this discussion.

CCMs refer to strategies or processes that organisms employ in CO$_2$-deficient environments to achieve intracellular concentrations higher than would exist by passive diffusion alone [24]. Price and Badger [24] cite low CO$_2$ availability in natural waters occurs due to slow diffusion of CO$_2$, incomplete equilibrium of waters with the atmosphere, and decreasing equilibrium CO$_2$ concentrations with increasing pH. However, dual carboxylase and oxygenase activities of Rubisco, the enzyme that catalyzes the first reaction in CO$_2$ fixation, necessitates that this substrate be present in high concentrations to prevent photorespiration [25]. As a result, organisms rely on numerous types of CCMs to enhance carbon fixation.

It is suspected that all cyanobacteria, most eukaryotic algae, and some aquatic plants employ some type of CCM [25], although these mechanisms vary between species. Cyanobacteria generally acquire CO$_2$ and HCO$_3^-$ from the bulk medium by diffusive and active transport, respectively. Some studies also suggest utilization of CO$_3^{2-}$ by

$$\frac{(k_l a)_{CO_2}}{(k_l a)_{DO}} = \left(\frac{D_{CO_2}}{D_{O_2}}\right)^\frac{1}{3}. \quad (27)$$
cyanobacteria [26,27]. Many green algae can actively transport both \( \text{CO}_2 \) and \( \text{HCO}_3^- \) across the plasma membrane (Figure 3.1).

![Figure 3.1: Model for Scenedesmus CCMs [28].](image)

**Stoichiometry of Algal Growth**

Redfield [29] observed that C:N:P ratios of zooplankton and phytoplankton in various oceanic regions were relatively constant at 106:16:1. This has been verified for organisms in the ocean by large data sets and precise measurement techniques [30].

A stoichiometric equation describing algal growth (equation 28) is developed using Redfield [29] proportions [5,30]. Algal biomass is assumed to have a molecular formula of \( \text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} \), alternatively represented as \( \text{(CH}_2\text{O})_{106}\text{(NH}_3\text{)}_{16}\text{(H}_3\text{PO}_4\text{)} \), with a molecular weight of 3552 g/mol. The Redfield biomass yield \( (\text{Y}_{\text{X/S}}) \) for inorganic-carbon-limited algal growth is 2.79 mg X/mg C. No similar equations have been reported for \( \text{HCO}_3^- \) or \( \text{CO}_3^{2-} \) as inorganic carbon sources.

\[
106\text{CO}_2 + 16\text{NO}_3^- + \text{HPO}_4^{2-} \xrightarrow{\text{respir.}} 122\text{H}_2\text{O} + 18\text{H}^+ + \frac{\text{X}}{2} \xrightarrow{\text{photos.}} \text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} + 138\text{O}_2
\]  

(28)
Sterner and Elser [30] note that, depending on severity of nutrient limitation and ratio of nutrient supply, considerable deviations from Redfield [29] ratios are observed in laboratory cultures. Rhee [31] studied *Scenedesmus* at a constant growth rate in chemostat cultures, and found that cellular N:P ratios nearly matched N:P ratios provided in media. Goldman et al. [32] observed C:P and N:P of *Dunaliella tertiolecta* to be almost independent of growth rate when N and P were supplied at or below Redfield [29] ratios. However, when N:P was supplied above the Redfield [29] ratio, biomass nutrient content (C:P and N:P) decreased significantly with increasing growth rate.

Studies on effects of inorganic carbon limitation on algal C:N:P ratios are sparse [30]. Some evidence exists to support decreasing C:N and C:chl *a* with decreasing inorganic-carbon-limited growth rate in marine diatoms [33]. However, biomass nutrient ratios were independent of CO$_2$ concentration in the marine diatom *Skeletonema costatum* when cultivated under P-limited conditions [34]. Although observations on the effects of inorganic carbon on nutrient ratios of cyanobacteria and eukaryotic algae have been reported by several authors, no universal trend has been identified.

*Inorganic-Carbon-Limited Growth Rate*

CCMs may allow cyanobacteria and algae to utilize multiple carbonate species. The single-substrate Monod model (equation 29) may be used to model inorganic-carbon limited algal growth with CO$_2$, HCO$_3$-, CO$_3^{2-}$ or TIC as substrate [35].

$$
\mu_C = \frac{\mu_{\text{max}} [C]}{K_C + [C]},
$$

(29)
Where, $\mu_C =$ inorganic-carbon-limited specific growth rate ($hr^{-1}$), $\mu_{max} =$ maximum specific growth rate ($hr^{-1}$), $C = CO_2$, $HCO_3^-$, $CO_3^{2-}$, or TIC (mol/L C), and $K_C =$ half-saturation constant (mol/L C).

Simultaneous use of multiple carbonate species may be modeled using the Monod equation for substitutable substrates [36]. A preferred substrate ($C_{pfd}$) is used when available; however, as $C_{pfd}$ becomes depleted, cells use an alternative substrate ($C_{alt}$). Growth rate on $C_{pfd}$ is modeled by equation 29, while growth rate on $C_{alt}$ ($\mu_{C,SS}$) is inhibited by presence of $C_{pfd}$ (equation 30). Possible combinations include CO$_2$ as the preferred substrate, with either $HCO_3^-$ or $CO_3^{2-}$ as the alternative substrate.

$$\mu_{C,SS} = \mu_{max} \left( \frac{[C_{alt}]}{K_{C,alt} + [C_{alt}]} \right) \left( \frac{K_{C,pfd}}{[C_{pfd}] + K_{C,pfd}} \right). \quad (30)$$

**Scenedesmus Cultivation**

A mixed freshwater algal culture, containing predominately *Scenedesmus*, was used for experimental trials. These green algae are usually elliptical, contain spines, and grow in rows of 4, 8, or 16 cells [37]. Several growth mediums suggest an optimum pH of 7.0 to 8.0 for *Scenedesmus* [37]. Novak and Brune [21] suggest an optimum temperature of 21 to 27°C with light intensity above 90 µE/m$^2$-s.
EXPERIMENTAL METHODS

Algal growth in closed and open batch systems was compared. Three experiments were conducted in closed reactors at four TIC levels. Two experiments were completed in open reactors at four TIC levels, with initial pH unadjusted (Table 3.2). Sampling schedules for each experiment are included in Appendix A.

Table 3.2: Summary of completed experiments.

<table>
<thead>
<tr>
<th>Initial pH</th>
<th>Initial TIC Concentrations (g/L Na₂CO₃)</th>
<th>Initial TIC¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Open Experiments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelim. 1O</td>
<td>unadjusted 0.05, 0.10, 0.15, 0.20</td>
<td>25, 50, 75, 100%</td>
</tr>
<tr>
<td>Run 1O</td>
<td>unadjusted 0.05, 0.10, 0.15, 0.20</td>
<td>25, 50, 75, 100%</td>
</tr>
<tr>
<td><strong>Closed Experiments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Run 1C</td>
<td>unadjusted 0.05, 0.10, 0.15, 0.20</td>
<td>25, 50, 75, 100%</td>
</tr>
<tr>
<td>Run 2C</td>
<td>adjusted to 10.3 0.05, 0.10, 0.15, 0.20</td>
<td>25, 50, 75, 100%</td>
</tr>
<tr>
<td>Run 3C</td>
<td>adjusted to 10.3 0.025, 0.075, 0.125, 0.175</td>
<td>12.5, 37.5, 62.5, 87.5%</td>
</tr>
</tbody>
</table>

¹Percentage of Na₂CO₃ concentration, as recommended by modified BG11.

Bioreactor Configuration

Four liter glass vessels (0.0762 m diameter and 0.232 m height) were used to culture mixed algal cultures in open and closed systems (Figure 3.2). Closed reactors were fitted with No. 8 stoppers, which were fabricated to provide a sampling port and connection for tubing containing 12 g AscariteII®. This chemical was used to allow the headspace pressure to equilibrate with the atmosphere, without permitting carbon dioxide to enter reactors.
Culture Methods

Culture Media

Freshwater algal inoculum was obtained from Lake Hartwell, SC and cultured using a modified BG11 media (Table 3.3), with 12.5 to 100% of recommended Na$_2$CO$_3$ supplied as inorganic carbon source.
Table 3.3: Modified BG11 media used to cultivate freshwater algae [38].

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>1.5 g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.04 g</td>
</tr>
<tr>
<td>MgSO₄ 7H₂O</td>
<td>0.075 g</td>
</tr>
<tr>
<td>CaCl₂ 2H₂O</td>
<td>0.036 g</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>6.0 mg</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>6.0 mg</td>
</tr>
<tr>
<td>EDTA</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Na₂CO₃¹</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Trace Metal Mix A5</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>1.0 L</td>
</tr>
<tr>
<td>Trace Metal Mix A5</td>
<td></td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>2.86 g</td>
</tr>
<tr>
<td>MnCl₂ 4H₂O</td>
<td>1.81 g</td>
</tr>
<tr>
<td>ZnSO₄ 7H₂O</td>
<td>0.222 g</td>
</tr>
<tr>
<td>Na₂MoO₄ 2H₂O</td>
<td>0.39 g</td>
</tr>
<tr>
<td>CuSO₄ 5H₂O</td>
<td>0.079 g</td>
</tr>
<tr>
<td>Co(NO₃)₂ 6H₂O</td>
<td>49.4 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

¹Amount listed is the recommended mass designated as 100% C.

Precultures and Inoculum Preparation

Precultures for closed and open batch reactors, containing predominately *Scenedesmus*, were prepared in 4L reactors under the same environmental conditions as test reactors. Precultures for experimental trials were inoculated into test reactors while in exponential phase. The same mass of algal cells was inoculated into each test reactor, by centrifugation and dilution to equal OD.

Environmental Conditions

Cultures were grown in a controlled-environment room maintained at 25°C (Climate Technologies, Inc.; Model G3). Reactors were placed on shelves with four 40 W cool white fluorescent bulbs (ACE F40 Universal DLX) positioned 20.3 cm above
initial liquid level in reactors. Average illuminance in the controlled-environmental chamber was measured using an environmental quality meter (Sper Scientific Ltd.; Model 850070). Average photosynthetically active radiation (PAR) was calculated to be 121 µE/m²-s, as per Sager and McFarlane [39]. All cultures were mixed using stir plates set at 300 rpm and large stir bars.

**Culture Maintenance and Sample Handling**

Distilled water was added to account for evaporation, and samples were collected in 100 mL reagent bottles with modified screw-cap tops to minimize exchange of CO₂ during filtration and titration. Two holes were drilled in caps to allow for addition of algal sample, pH determination, and addition of titrant.

**Algal Biomass Quantification and Identification**

Algal identification, cell density (CD), total suspended solids (TSS), and optical density (OD) were monitored. Cultures were visually identified by Scott Davis of the Clemson Aquaculture Facility. Cell densities were determined using a Meiji phase contrast microscope (Martin Microscope Co.) and a 0.1 mm deep, two-chamber hemacytometer. TSS concentrations were determined using the Membrane Filter Method [40] with 0.2 µm filters. OD at 750 nm was determined, as per Method 8111 G [40]. Calibration curves were prepared to relate these parameters.

**pH, Alkalinity, and Total Inorganic Carbon**

pH was monitored in all reactors using a VWR® sympHony® Gel 3-in-1 pH Electrode and VWR® sympHony® pH meter (model SP70P). The pH electrode was calibrated using 4.01, 7.41, and 10.40 buffers before sampling.
Alkalinity (ALK) was monitored, as per Method 2320 B [40], with 0.02 or 0.1 N H$_2$SO$_4$ as titrant.

Total inorganic carbon concentrations were calculated using pH and alkalinity data and equation 16. Concentrations of carbonate species were calculated using equations 17 through 22 [5].

**Characterization of Mass Transfer into Open Reactors**

(k$_{L,a}$)$_{DO}$ was determined for open reactors containing media with 0 or 100% C and equation 26, as per ASCE [41]. These values were used to estimate (k$_{L,a}$)$_{CO2}$ using equation 27.

**Determination of Algal Growth Kinetic Parameters and Stoichiometry**

*Specific Growth Rate and Decay Constant*

Specific growth rate (µ) was determined by fitting a linear regression to natural log of biomass versus time data for the exponential growth phase. Decay constant (b) was estimated by fitting a linear equation to decay data. Regression slopes were identified as specific growth and decay rates.

*Kinetic Constants*

Monod kinetic parameter values (µ$_{\text{max}}$ and K$_S$), considering CO$_2$, HCO$_3^-$, CO$_3^{2-}$, or TIC as substrate, were determined for closed batch reactor data using Lineweaver-Burk plots [36] and Statistical Application Software (SAS). In SAS, initial values of µ$_{\text{max}}$ and K$_S$ were those determined from Lineweaver-Burk plots.
**Biomass Yield**

Observed $Y_{X/S}$ for each closed culture was determined as the slope of a linear regression relating biomass to TIC during exponential growth [18]. Analysis of $Y_{X/S}$ was completed based on TIC, rather than individual carbonate species, because pH was not controlled throughout experiments.

Biomass yields were used to compare the contribution of each carbonate species to total biomass production. First, experimental biomass production was calculated over the growth period (time $t_1$ until time $t_2$) (equation 31).

$$
\Delta X_{\text{exp.}} = X_{t_2} - X_{t_1}.
$$

(31)

The concentration of each carbonate species utilized by algal cultures ($S_{\text{utilization}}$) was also determined (equation 32) over the growth period ($S_{t_1}$ and $S_{t_2}$). Changes in species concentrations due to shifts in pH ($\Delta S_{\text{pH}}$) were quantified as the difference between final measured TIC and final TIC that would have existed if pH had been maintained at 10.3 during Runs 2 and 3 (equation 33).

$$
S_{t_2} = S_{t_1} \pm \Delta S_{\text{pH}} - S_{\text{utilization}}.
$$

(32)

$$
\Delta S_{\text{pH}} = TIC_{t_2} \left( \alpha_{x_{t_2}} - \alpha_{x_{t_1}} \right).
$$

(33)

Where, $TIC_{t_2} =$ TIC concentration at time 2 (mol/L C), $\alpha_{x_{t_2}} =$ ionization fraction at t2 ($\alpha_{t_2}$ for CO$_2$, $\alpha_{1_{t_2}}$ for HCO$_3^-$, $\alpha_{2_{t_2}}$ for CO$_3^{2-}$), and $\alpha_{x_{t_1}} =$ ionization fraction t1 ($\alpha_{t_1}$ for CO$_2$, $\alpha_{1_{t_1}}$ for HCO$_3^-$, $\alpha_{2_{t_1}}$ for CO$_3^{2-}$).

Using observed $Y_{X/S}$, the concentration of biomass produced using each carbonate species ($\Delta X_{\text{theoretical}}$) was determined (equation 34).
\[ \Delta X_{\text{theoretical}} = S_{\text{utilization}} \cdot (Y_{X/S})_{\text{observed}} \cdot \]  

(34)

Finally, the percentage of biomass production attributed to carbonate species \( S \) \((\%BP_S)\) was calculated using equation 35.

\[ \%BP_S = \frac{\Delta X_{\text{theoretical}}}{\Delta X_{\text{actual}}} \cdot 100 \text{, and} \]  

(35)

**Stoichiometry of Algal Growth**

Algal cultures were prepared using treatments from Runs 2C and 1O to provide algal biomass for quantification of C:N:P ratios. Samples were analyzed by Kathy Moore of the Agricultural Service Laboratory at Clemson University. Carbon and nitrogen were analyzed using an Elementar Vario Macro (Mt. Laurel, NJ), while phosphorous was quantified using a Spectro ARCOS ICP (Mahwah, NJ).

**RESULTS AND DISCUSSION**

**Effects of TIC Concentration on Algal Growth in Closed Batch Reactors**

*Species Identification*

Algal cultures contained predominantly *Scenedesmus*, with low inorganic carbon (12.5 and 25% C) reactors containing mostly single cells and high inorganic carbon (87.5 and 100% C) reactors containing four-cell clusters.
Biomass Quantification and Analysis

TSS, OD, and CD

Typical batch growth responses were observed for closed reactors (Figure 3.3). Within each run, peak biomass concentrations were found to increase with increasing initial TIC concentration. Furthermore, peak biomass concentrations were achieved later in high carbon reactors (100% C and 87.5% C) than in other reactors.

![Graph showing cell densities within closed batch reactors supplied with various initial amounts of inorganic carbon (Runs 2C, 3C: adjusted initial pH).]

Calibration curves were prepared to relate CD, OD and TSS. No substantial differences in OD:TSS calibration slopes were observed for concentrated algal samples from Run 1C (avg. OD:TSS = 9.07 × 10^-4 L/mg, avg. R^2 = 0.984) (Figure B.3). Linear
relationships were observed between CD and OD (Figure B.5), with an average OD/CD of $2.31 \times 10^{-8}$ mL/cell (avg. $R^2 = 0.741$).

**Determination of Specific Growth Rates**

In general, specific growth rates of algal cultures within closed batch reactors increased with increasing initial TIC concentration (Table 3.4). However, in two runs, the specific growth rate of cells from the 100% C reactor was lower than in the 75% C reactor. It is possible that higher pH in 100% C reactors caused adverse physiological effects on algal cells.

**Table 3.4:** Specific growth rates of closed freshwater algal cultures supplied with various initial amounts of inorganic carbon (Run 1C: unadjusted initial pH; Runs 2C, 3C: adjusted initial pH).

<table>
<thead>
<tr>
<th></th>
<th>Run 1C</th>
<th></th>
<th>Run 2C</th>
<th></th>
<th>Run 3C</th>
</tr>
</thead>
<tbody>
<tr>
<td>% C</td>
<td>$\mu$ (hr$^{-1}$)</td>
<td>$R^2$</td>
<td>% C</td>
<td>$\mu$ (hr$^{-1}$)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>25</td>
<td>0.0191</td>
<td>0.9902</td>
<td>25</td>
<td>0.0326</td>
<td>0.9428</td>
</tr>
<tr>
<td>50</td>
<td>0.0216</td>
<td>0.9977</td>
<td>50</td>
<td>0.0394</td>
<td>0.9631</td>
</tr>
<tr>
<td>75</td>
<td>0.0305</td>
<td>0.9997</td>
<td>75</td>
<td>0.0458</td>
<td>0.9848</td>
</tr>
<tr>
<td>100</td>
<td>0.0273</td>
<td>0.9133</td>
<td>100</td>
<td>0.0387</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

**Stoichiometry**

Carbon composition of algal biomass cultivated in closed batch reactors linearly increased with increasing initial TIC (Figure 3.4), while no relationship was observed between cellular N and P content and initial TIC. The average particulate nitrogen and phosphorous concentrations were 7.83 and 4.16%, respectively, with standard deviations of 0.863 and 0.386%, respectively. Due to high P content of algal biomass, molar C:P, N:P, and MW were significantly lower than predicted by Redfield [29] ratios (Table 3.5).
Figure 3.4: Elemental composition of closed freshwater algal cultures supplied with various initial TIC concentrations.

Table 3.5: Stoichiometry (C:N:P) of closed freshwater algal cultures.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Carbon (% C)</th>
<th>Nitrogen (% C)</th>
<th>Phosphorus (% C)</th>
<th>C:N</th>
<th>MW (g/mol)</th>
<th>Yx/s (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>19.12 (mol¹)</td>
<td>6.16 (mol¹)</td>
<td>3.67 (mol¹)</td>
<td>8.02</td>
<td>300.2</td>
<td>4.06</td>
</tr>
<tr>
<td>50</td>
<td>21.43 (mol¹)</td>
<td>6.18 (mol¹)</td>
<td>3.83 (mol¹)</td>
<td>8.96</td>
<td>299.7</td>
<td>4.04</td>
</tr>
<tr>
<td>75</td>
<td>23.63 (mol¹)</td>
<td>7.67 (mol¹)</td>
<td>4.40 (mol¹)</td>
<td>7.81</td>
<td>353.8</td>
<td>3.77</td>
</tr>
<tr>
<td>100</td>
<td>25.71 (mol¹)</td>
<td>10.16 (mol¹)</td>
<td>4.49 (mol¹)</td>
<td>6.54</td>
<td>428.8</td>
<td>3.52</td>
</tr>
<tr>
<td>Redfield Ratios</td>
<td>35.83 (mol¹)</td>
<td>106 (mol¹)</td>
<td>6.31 (mol¹)</td>
<td>0.872</td>
<td>3353.2</td>
<td>2.79</td>
</tr>
</tbody>
</table>

¹Calculated as mol element per mol P.
²Algal molecular formula assumed to be (CH₂O)ₓ(NH₃)ᵧ(H₃PO₄)ₐ; x = mol C/mol P and y = mol N/mol P.
**Alkalinity and Total Inorganic Carbon Speciation**

Despite utilization of NO$_3$-N by algae, alkalinity within closed reactors remained relatively constant over time, due to low net biomass.

Due to uptake of hydrogen ions by algal cells, pH increased over time in all closed reactors (Figures 3.5). The final pH in each reactor increased with increasing initial TIC concentration within each run. In Run 1C, where initial pH was not adjusted, the initial pH for 25% C reactor was nearly a full pH unit lower than higher TIC reactors, likely due to other media components.

---

**Figure 3.5:** pH within closed algal cultures supplied with various initial TIC concentrations (Run 1C: unadjusted initial pH; Runs 2C, 3C: adjusted initial pH).
TIC declined in reactors, as carbon was consumed to form new algal cell mass (Figure 3.6). Although initial TIC varied from 0.001 to 0.0028 mol/L C for adjusted initial pH runs, final TIC concentrations reached approximately 0.0075 mol/L C.

CO$_2$ (Figure 3.7) and HCO$_3^-$ (Figure 3.8) decreased in closed reactors, indicating that these species were likely used as inorganic carbon sources. The response of carbonate varied between reactors (Figure 3.9). CO$_3^{2-}$ remained relatively constant in lower C reactors, while decreasing in higher C reactors after 50 to 75 hr. The decrease in carbonate concentration, coupled with significant growth after 75 hr in 87.5 and 100% C reactors, suggests that carbonate may have been used as an inorganic carbon source.
Figure 3.7: CO$_2$ within closed algal cultures supplied with various initial TIC concentrations (Run 1C: unadjusted initial pH; Runs 2C, 3C: adjusted initial pH).

Figure 3.8: HCO$_3^-$ within closed algal cultures supplied with various initial TIC concentrations (Run 1C: unadjusted initial pH; Runs 2C, 3C: adjusted initial pH).
Figure 3.9: $\text{CO}_3^{2-}$ within closed algal cultures supplied with various initial TIC concentrations (Run 1C: unadjusted initial pH; Runs 2C, 3C: adjusted initial pH).

Characterization of Inorganic-Carbon-Limited Algal Growth

Batch Growth Curves

Plots of biomass and inorganic carbon species versus time indicate decline of only $\text{CO}_2$ and $\text{HCO}_3^-$ in low TIC reactors (Figure 3.10), and decrease in all carbonate species in high carbon reactors (Figure 3.11). Similar charts were prepared for other reactors (Appendix D).
Figure 3.10: Relationship between biomass, TIC, and carbonate species concentrations in 37.5% C reactor (Run 3C: initial pH adjusted to 10.3).

Figure 3.11: Relationship between biomass, TIC, and carbonate species concentrations in 87.5% C reactor (Run 3C: initial pH adjusted to 10.3).
Maximum Specific Growth Rate and Half Saturation Constants

Determination of µ-max and K_S were completed using data from 25, 50, and 75% C reactors from Runs 1C and 2C, since an increase in specific growth rate was not observed when initial TIC was increased from 75 to 100% C. Data from all reactors in Run 3C were used.

1. Linear Technique: Lineweaver-Burk Plots

For adjusted initial pH Runs 2C and 3C, Lineweaver-Burk plots (Figure 3.12) depict that algal specific growth rates increased with increasing carbonate species concentrations. For unadjusted initial pH Run 1C (Figure 3.12), the Lineweaver-Burk plot does not show increasing specific growth rate with CO_2. This suggests that CO_2 did not serve as the only inorganic carbon source.

Kinetic parameters resulting from Run 1C (Figure C.1) were less reliable than those obtained from adjusted initial pH Runs 2C and 3C, due to lower coefficients of determination (R^2) and variation in calculated µ_max values. R^2 for kinetic parameters from adjusted pH runs were significantly higher (Table 3.6). Also, estimates of µ-max from the three single-substrate models are close in magnitude for both Trials 2C and 3C, although estimates vary some between the two runs. Fundamentally, µ-max is a constant that should not change with substrate.
Figure 3.12: Lineweaver-Burk plots for determination of $\mu$-max and $K_S$ for single substrate models.

Table 3.6: Estimates of kinetic constants calculated from Lineweaver-Burk plots for freshwater algal growth assuming $CO_2$, $HCO_3^-$, $CO_3^{2-}$, or TIC as sole inorganic carbon source (Runs 2C and 3C).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$\mu$-max (hr$^{-1}$)</th>
<th>$K_S$ (mol/L C)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CO_2$</td>
<td>0.0737</td>
<td>2.811 $\times$ 10$^{-8}$</td>
<td>0.9954</td>
</tr>
<tr>
<td></td>
<td>0.0967</td>
<td>1.020 $\times$ 10$^{-7}$</td>
<td>0.8750</td>
</tr>
<tr>
<td>$HCO_3^-$</td>
<td>0.0738</td>
<td>0.000368</td>
<td>0.9956</td>
</tr>
<tr>
<td></td>
<td>0.0950</td>
<td>0.00116</td>
<td>0.9380</td>
</tr>
<tr>
<td>$CO_3^{2-}$</td>
<td>0.0704</td>
<td>0.000688</td>
<td>0.9895</td>
</tr>
<tr>
<td></td>
<td>0.0689</td>
<td>0.00105</td>
<td>0.9401</td>
</tr>
<tr>
<td>TIC</td>
<td>0.0715</td>
<td>0.00101</td>
<td>0.9918</td>
</tr>
<tr>
<td></td>
<td>0.0782</td>
<td>0.00217</td>
<td>0.9505</td>
</tr>
</tbody>
</table>

2. Nonlinear Technique: Statistical Application Software (SAS)

Estimations of kinetic parameters by SAS (Table 3.7) were similar to those obtained from Lineweaver-Burk plots (Table 3.6). However, $\mu_{\text{max}}$ values determined using SAS were closer in magnitude between Runs 2C and 3C than those estimated by
linearization. Comparison of p values from ANOVA tables (Table C.1) show that all estimations were significant at the 5% level or lower.

Table 3.7: Kinetic parameters for single substrate models obtained using SAS proc nlin.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( \mu_{\text{max}} ) (hr(^{-1}))</th>
<th>( K_S ) (mol/L C)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 2C</td>
<td>Run 3C</td>
<td>Avg.</td>
<td>Run 2C</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>0.0756</td>
<td>0.0700</td>
<td><strong>0.0728</strong></td>
</tr>
<tr>
<td>HCO(_3)</td>
<td>0.0756</td>
<td>0.0730</td>
<td><strong>0.0743</strong></td>
</tr>
<tr>
<td>CO(_3)(^2-)</td>
<td>0.0728</td>
<td>0.0691</td>
<td><strong>0.0710</strong></td>
</tr>
<tr>
<td>TIC</td>
<td>0.0738</td>
<td>0.0714</td>
<td><strong>0.0726</strong></td>
</tr>
</tbody>
</table>

Based on estimations for \( \mu_{\text{max}} \) and \( K_S \) for carbonate species (Table 3.7), specific growth rates using each carbonate species between 75 and 100 hr for 87.5% C reactor (Figure 3.11) further suggest that CO\(_2\) did not serve as the sole inorganic carbon source (Table 3.8).

Table 3.8: Comparison of specific growth rates on each carbonate species to measured specific growth rate using data between 75 and 100 hr in 87.5% C reactor.

<table>
<thead>
<tr>
<th>Avg. Species Concentrations (mol/L C)</th>
<th>Specific Growth Rate (hr(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(_2) ( 2.405 \times 10^{-9} )</td>
<td>0.00371</td>
</tr>
<tr>
<td>HCO(_3) ( 1.417 \times 10^{-4} )</td>
<td>0.0145</td>
</tr>
<tr>
<td>CO(_3)(^2-) ( 1.034 \times 10^{-3} )</td>
<td>0.0394</td>
</tr>
<tr>
<td>Biomass(^1) N/A</td>
<td>0.0209</td>
</tr>
</tbody>
</table>

\(^1\)Specific growth rate calculated using measured biomass data.
Biomass Yield

Ranging from 3.529 to 9.806 mg X/mgC, observed $Y_{X/S}$ (Figure 3.13) were considerably higher than theoretical values (Table 3.5) and the Redfield [29] $Y_{X/S}$ (2.79 mg X/mg C).

Due to discrepancies between observed $Y_{X/S}$ from 12.5 and 25% C reactors, no relationship between $Y_{X/S}$ and initial TIC was found (Figure 3.14). Omission of the 25% C reactor may suggest a decreasing logarithmic relationship; however, more experimentation is required at low initial TIC concentrations to verify this trend.
Figure 3.14: Relationship between initial TIC concentration and observed biomass yield of mixed freshwater algal cultures.

Analysis of individual species used for algal growth revealed that biomass formed using HCO$_3^-$ and CO$_3^{2-}$ each represented between 40 and 50% of the total biomass production, while biomass produced using CO$_2$ contributed very little to total biomass production (Figure 3.15). Sample calculations for %BP$_{CO_3}$ using data from 50% C reactor of Run 2C are provided in Table 3.9, and Table C.2 includes all calculations.

Table 3.9: Calculation of %BP$_{CO_3}$ using data from 50% C reactor (Run 2C).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Calculations</th>
<th>Variable</th>
<th>Eq. No.</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>t$_1$ (0 hr)</td>
<td>t$_2$ (71 hr)</td>
<td>ΔX$_{exp}$</td>
<td>31</td>
</tr>
<tr>
<td>X</td>
<td>4.961</td>
<td>68.36</td>
<td>(CO$<em>3^{2-}$)$</em>{pH}$</td>
<td>33</td>
</tr>
<tr>
<td>CO$_3^{2-}$</td>
<td>$8.954 \times 10^{-4}$</td>
<td>$8.095 \times 10^{-4}$</td>
<td>(CO$<em>3^{2-}$)$</em>{utilization}$</td>
<td>32</td>
</tr>
<tr>
<td>TIC</td>
<td>N/A</td>
<td>9.40807 $\times 10^{-3}$</td>
<td>ΔX$_{theoretical}$</td>
<td>34</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>0.5287</td>
<td>0.8604</td>
<td>%BP$_{CO_3}$</td>
<td>35</td>
</tr>
<tr>
<td>Y$_{XS}$</td>
<td>6.392</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.15: Percent biomass production (%BP) from individual carbonate species for Runs 2C and 3C (note: %BP for CO$_2$ much smaller than for other species).

Decay Constant

Decay constants were independent of initial TIC (Figure 3.16). The average decay rate constant was 0.00285 hr$^{-1}$ with a standard deviation of 0.000503 hr$^{-1}$. Lower final pH in 25% and 50% C reactors (10.84 and 11.20, respectively) than in 75% and 100% C reactors (11.40 and 11.51) may account for decay phase beginning later in low C reactors.
Figure 3.16: Determination of decay constant for mixed freshwater algal culture supplied with various initial amounts of inorganic carbon.

**Open Batch Reactors**

*Characterization of Gas Transfer*

Linearization of reaeration curves for open reactors containing modified BG11 media and either 0 or 100% C suggest that gas transfer did not significantly vary based on initial TIC (Figure 3.17). Average \((k_{La})_{CO2}\) was calculated as 0.00473 min\(^{-1}\), using the average \((k_{La})_{DO}\) (0.00520 min\(^{-1}\)) and equation 27.
Species Identification

Like closed reactors, open algal cultures contained predominantly *Scenedesmus*, with low inorganic carbon (12.5 and 25% C) reactors containing mostly single cells and high inorganic carbon (87.5 and 100% C) reactors containing four-cell clusters (Figure 3.18).
Figure 3.18: Algal cells from 25% C (left) and 100% C (right) open batch reactors.

Biomass Quantification and Analysis

TSS and OD

Typical growth phases were not observed for open algal cultures (Figure 3.19). Due to transfer of atmospheric CO$_2$ into reactors, decay phase was not observed. Net biomass concentrations were approximately ten times higher in open reactors (730 to 990 mg/L) than in closed reactors (42 to 110 mg/L)
Calibration curves relating TSS to OD (Figure B.8) indicate slight differences in algal composition within open reactors based on initial TIC. The average OD:TSS for 50-75% C reactors was $1.31 \times 10^{-3}$ L/mg, with a standard deviation of $2.08 \times 10^{-5}$ L/mg (avg $R^2 = 0.980$), while the OD:TSS for the 25% reactor was $1.54 \times 10^{-3}$ L/mg ($R^2 = 0.944$). Samples from the 25% C reactor exhibited higher OD for a given biomass concentration than those from higher inorganic carbon reactors. This corresponds to the observation that low inorganic carbon reactors contained more single-cells than high inorganic carbon reactors.
Determination of Specific Growth Rates

Two growth phases were observed for each open reactor (Figure 3.20). Exponential growth phase (EP1) occurred between 0 and 91 hours, while a second growth phase (GP2) occurred after 280 hr. Both EP1 and GP2 growth rates generally increased with increasing initial TIC concentration. EP1 growth rates (0.0253 to 0.0368 hr\(^{-1}\)) were of the same magnitude as those in closed reactors (0.0191 to 0.0458 hr\(^{-1}\)), while GP2 growth rates were considerably lower (0.00184 to 0.00267 hr\(^{-1}\)).

Figure 3.20: Plot of natural log of biomass concentration versus time used to determine specific growth rates of open algal cultures supplied with various amounts of TIC (EP1: exponential growth phase; GP2: second growth phase).
Stoichiometry

No relationship was observed between nitrogen and phosphorous content of open algal biomass and TIC treatment; however, particulate carbon composition may linearly decrease with increasing initial TIC (Figure 3.21). The average nitrogen and phosphorous contents of open algal biomass were 6.78 and 2.91% with standard deviations of 0.509 and 0.522%, respectively. Molar C:P, N:P, and MW for open algal biomass were lower than those predicted by Redfield [29] ratios, although they were higher than those for closed algal biomass (Table 3.5). Theoretical $Y_{X/S}$ (2.85 to 2.90 mg X/mg C) were comparable to the Redfield [29] $Y_{X/S}$ of 2.79 mg X/mg C (Table 3.10).

![Graph showing elemental composition of open freshwater algal cultures supplied with various initial TIC concentrations.](image)

$Y_{SC} = -0.291x + 52.273$

$R^2 = 0.8386$

Figure 3.21: Elemental composition of open freshwater algal cultures supplied with various initial TIC concentrations.
Table 3.10: Stoichiometry (C:N:P) of open freshwater algal cultures.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Carbon (%)</th>
<th>Nitrogen (%)</th>
<th>Phosphorous (%)</th>
<th>C:N</th>
<th>MW(^2)</th>
<th>Y(_{X/S})</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>50.10</td>
<td>35.81</td>
<td>7.42</td>
<td>4.53</td>
<td>3.63</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>45.39</td>
<td>45.09</td>
<td>6.18</td>
<td>5.26</td>
<td>2.60</td>
<td>1</td>
</tr>
<tr>
<td>75</td>
<td>45.35</td>
<td>39.60</td>
<td>6.82</td>
<td>5.10</td>
<td>2.96</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>44.65</td>
<td>46.87</td>
<td>6.71</td>
<td>6.04</td>
<td>2.46</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Calculated as mol element per mol P.
2 Algal molecular formula assumed to be (CH\(_2\)O\(_x\))(NH\(_3\))\(_y\)(H\(_3\)PO\(_4\)); x = mol C/mol P and y = mol N/mol P.

**pH, Alkalinity and Total Inorganic Carbon Speciation**

Culture pH was affected by uptake of H\(^+\) by algal cells, as well as entry of atmospheric CO\(_2\) into reactors. During EP1, pH increased considerably in all reactors (Figure 3.22), as cells grew at relatively high growth rates. Although biomass concentrations increased considerably during GP2, pH appeared to reach a maximum near 11.5 (Figure 3.22).

Lack of decay phases in open reactors (Figure 3.19) may be attributed to the relationship between biomass concentration and pH (Figure 3.23) (Appendix D). Algal cultures grew until inhibited by external pH during EP1. As growth rates slowed during GP2, diffusion of atmospheric CO\(_2\) into reactors lowered pH, and a suitable environment was restored for algal growth. This cycle would likely continue until cultures became limited by another nutrient or light availability.
Figure 3.22: pH of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon.

Figure 3.23: pH and TSS of algal cultures in 50% C reactor (Run 1O).
Due to utilization of NO$_3$-N by dense algal cultures, alkalinity (Figure 3.24) increased by a factor of four in the low C reactor (50 to 200 mg/L CaCO$_3$), and by a factor of two in the high C reactor (200 to 400 mg/L CaCO$_3$). This contrasts with closed reactors in which alkalinity remained constant due to low net biomass.

![Graph showing alkalinity of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon.](image)

**Figure 3.24:** Alkalinity of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon.

Total inorganic carbon concentrations fluctuated over time due to changes in culture pH and alkalinity (Figure 3.25). As growth rates slowed and TIC increased after 600 – 800 hr, carbon became sequestered in the media.
Carbon dioxide (Figure 3.26) and bicarbonate (Figure 3.27) concentrations generally decreased over time due to uptake by algal cultures and increases in culture pH. Carbonate concentrations (Figure 3.28) mimic the same fluctuations as TIC, with concentrations increasing after 600-800 hr.
Figure 3.26: Carbon dioxide concentrations within open batch algal cultures supplied with high (100%) and low (25%) inorganic carbon.

Figure 3.27: Bicarbonate concentrations within open batch algal cultures supplied with high (100%) and low (25%) inorganic carbon.
Quantification of Carbon Mitigation by Open Algal Cultures

Total sequestered carbon ($\text{TIC}_{\text{seq}}$), composed of carbon sequestered in media ($\text{TIC}_{\text{seq, media}}$) and in algal biomass ($\text{TIC}_{\text{seq, X}}$), generally increased with increasing initial TIC (Table 3.11). Interestingly, $\text{TIC}_{\text{seq}}$ per initial TIC (% $\text{TIC}_{\text{seq}}$) exponentially decreased with increasing TIC (Figure 3.29). Future experimentation is required to verify this trend.
Table 3.11: Estimation of atmospheric carbon sequestered in open batch algal reactors after 1100 hr of growth.

<table>
<thead>
<tr>
<th>Treatment (% C)</th>
<th>TIC_{seq, media} (mg/L C)</th>
<th>TIC_{seq, X} (mg/L C)</th>
<th>TIC_{seq} (^1) (mg/L C)</th>
<th>%TIC_{seq} (^2) (mg/L C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>6.80</td>
<td>362.23</td>
<td>369.03</td>
<td>36.96</td>
</tr>
<tr>
<td>50</td>
<td>6.60</td>
<td>421.79</td>
<td>428.38</td>
<td>21.76</td>
</tr>
<tr>
<td>75</td>
<td>16.85</td>
<td>393.67</td>
<td>410.53</td>
<td>18.65</td>
</tr>
<tr>
<td>100</td>
<td>10.64</td>
<td>433.65</td>
<td>444.28</td>
<td>15.06</td>
</tr>
</tbody>
</table>

\(^1\)TIC_{seq} = TIC_{seq, media} + TIC_{seq, X} - TIC_{t=0}, where, TIC_{seq, media} = TIC_{t=1100}, and TIC_{seq, X} = (X_{t=1100} - X_{t=0}) * (%C/100).

\(^2\)%TIC_{seq} = TIC_{seq} / TIC_{initial}.

Figure 3.29: Percent carbon sequestered (%TIC\(_{seq}\)) by open freshwater algal cultures as a function of initial TIC.
**Impact of Algal Carbon Mitigation**

To illustrate the impact of carbon mitigation by freshwater algae, the pond volume required to offset emissions from Clemson University was estimated. In 2008, Clemson fossil fuel usage totaled 600,000 MMBTU [42]. Using carbon contents and oxidation efficiencies listed in Table 3.12, approximately $3.96 \times 10^7$ kg CO$_2$ was emitted. Based on Table 3.11, the studied freshwater algal culture can utilize about 400 mg C/L over 1100 hr in light ($4.87 \times 10^3$ g CO$_2$/m$^3$-yr assuming 10 hr light/day) when supplied with 25 to 100% C and mixing. Thus, an outdoor algal pond totaling 8.14 $\times$ 10$^6$ m$^3$ would be required to abate Clemson University carbon emissions. Calculations are included in Appendix G.

<p>| Table 3.12: Estimation of pond volume required to abate 2008 Clemson University carbon emissions. |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Units</th>
<th>Usage (MMBTU)$^1$</th>
<th>Coal</th>
<th>Natural Gas</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200,000</td>
<td>400,000</td>
<td>600,000</td>
<td></td>
</tr>
<tr>
<td>Carbon Content$^2$</td>
<td>(g C/1000 BTU)</td>
<td>25.49</td>
<td>14.47</td>
<td>N/A</td>
</tr>
<tr>
<td>Fraction Oxidized$^2$</td>
<td>(%)</td>
<td>99</td>
<td>99.5</td>
<td>N/A</td>
</tr>
<tr>
<td>Annual CO$_2$ Emissions</td>
<td>(kg CO$_2$/yr)</td>
<td>$1.85 \times 10^7$</td>
<td>$2.11 \times 10^7$</td>
<td>$3.96 \times 10^7$</td>
</tr>
<tr>
<td>Required Pond Volume$^3$</td>
<td>(m$^3$)</td>
<td>$3.80 \times 10^3$</td>
<td>$4.34 \times 10^3$</td>
<td>$8.14 \times 10^3$</td>
</tr>
</tbody>
</table>

$^1$million BTU.

$^2$[43].

$^3$Total pond volume corresponds to a $8.9 \times 10^6$ m$^2$ (2200 acre) by 0.9144 m (3 ft) deep pond.
SUMMARY

Experiments were conducted to investigate carbon mitigation by a mixed freshwater algal culture. The following statements summarize the results.

1. Specific growth rates and net biomass concentrations increased with increasing TIC. Biomass carbon content increased based on initial TIC, while a contradictory trend was observed in open reactors. TIC increased and ALK remained constant in closed batch reactors, while net increases were observed in open reactors.

2. Algal cultures likely utilized all carbonate species, with $\mu_{\text{max}} = 0.0726 \text{ hr}^{-1}$, $K_{\text{CO}_2} = 4.47 \times 10^{-8}$, and $K_{\text{HCO}_3} = 5.70 \times 10^{-4}$, and $K_{\text{CO}_3} = 8.70 \times 10^{-4}$ describing TIC-limited algal growth. Due to discrepancies in experimental data, no relationship was found between $Y_{X/S}$ and initial TIC. Decay constant was independent of TIC.

3. Total carbon sequestered by open algal cultures increased based on initial TIC, while carbon sequestered per supplied TIC (%TIC$_{seq}$) exponentially decreased ($R^2 = 0.9717$) with increasing initial TIC.

CONCLUSIONS

Evidence of global warming necessitates that scientists and engineers develop innovative strategies to abate rising CO$_2$ emissions. Assimilation of CO$_2$ by freshwater algae is an appealing strategy because biomass can be harvested and converted to biofuels and other bioproducts. Furthermore, preliminary calculations show that an
outdoor algal pond \[8.9 \times 10^6 \text{ m}^2 \text{ (2200 acre)}\] by 0.9144 m (3 ft)\] could sequester the 3.96 \times 10^7 \text{ kg CO}_2 emitted in 2008 from use of coal and natural gas at Clemson University. Thus, cultivation of algae in freshwater systems can serve as a feasible component of a carbon management plan.

REFERENCES


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CHAPTER FOUR
MODELING OF FRESHWATER ALGAL GROWTH AS A FUNCTION OF
MEDIA INORGANIC CARBON CONTENT

ABSTRACT

The U.S. Department of Energy (DOE) has identified advanced biological processes as a research focus area for development of carbon management technologies. One such process is cultivation of algae in alkaline ponds, where accelerated rates of CO₂ hydration at high pH enhance inorganic carbon availability.

Dynamic mathematical models aimed at predicting algal biomass and carbonate species concentrations in closed and open batch reactors were developed. Modeling carbon-limited algal specific growth rate with Monod kinetics, considering CO₂, HCO₃⁻, and CO₃²⁻ as substitutable substrates, provided the best estimates for both length of exponential growth and peak biomass concentration in closed batch reactors. After calibrating the closed algal growth model for photosynthetic oxygen production, biomass, CO₂, and HCO₃⁻ concentrations were well-predicted, while CO₃²⁻ concentrations were under-predicted after 50 hr. Consideration of all carbonate species as substitutable substrates also best approximated peak biomass concentrations in open batch reactors; however, other model predictions were flawed. A sensitivity analysis suggests that photosynthetic oxygen production and biomass light attenuation coefficients should be further investigated to improve open algal growth model simulations.
INTRODUCTION

World energy consumption is predicted to increase through 2030, with majority of energy being derived from coal, natural gas, and liquid fuels. Combustion of these fossil fuels releases \( \text{CO}_2 \) into the atmosphere and contributes to global climate change. Energy-related \( \text{CO}_2 \) emissions are expected to increase an average of 1.7 percent per year from 2005 to 2030, increasing the atmospheric concentration from 380 to 450 ppm [1].

To combat increasing \( \text{CO}_2 \) emissions and atmospheric concentrations, the Department of Energy (DOE) has outlined a carbon management plan, which includes development of carbon sequestration technologies [2,3]. Carbon sequestration refers to the capture and storage of carbon that would otherwise add to atmospheric concentrations [4]. One possible strategy is cultivation of algal biomass in alkaline ponds, where increased \( \text{CO}_2 \) hydration rates at high pH may maximize availability of inorganic carbon to cultures for biofixation [3]. However, since biomass decay releases \( \text{CO}_2 \) into the atmosphere, biomass must be strategically stored or utilized to ensure carbon mitigation. For instance, biomass could be harvested, converted to biofuels, and used to reduce fossil fuel use [5].

The goal of this paper is to present a dynamic algal growth model intended to predict biomass and carbonate species concentrations in closed and open systems, to aid in design of carbon mitigation biosystems. The objectives of the research were as follows:
1. to compare the ability of single and substitutable substrate Monod models for predicting inorganic-carbon-limited algal growth in closed and open batch reactors,

2. to evaluate the effectiveness of calibrating model predictions based on the stoichiometric coefficient for photosynthetic oxygen production (p) and/or biomass light attenuation coefficient (K_B), and

3. to verify the ability of algal growth models to predict algal biomass and carbonate species concentrations.

MODEL DEVELOPMENT

Modeling Closed Carbonate Systems

To model a closed carbonate system, the mechanisms and kinetics of carbonate system reactions must be considered.

Reaction Mechanisms

The reversible reactions responsible for carbonate species conversion include CO_2 hydration and hydroxylation, as well as HCO_3^− protolysis and hydrolysis.

Hydration of Carbon Dioxide

Hydration of CO_2 in aqueous systems occurs by two distinct pathways (Figure 4.1) [6-11].
Path I leads to the direct formation of $\text{HCO}_3^-$ and $\text{H}^+$ (equation 1), while paths II and III lead to formation of $\text{H}_2\text{CO}_3$ (equation 2) followed by $\text{HCO}_3^-$ and $\text{H}^+$ (equation 3). The equilibrium for equation 2 lies very far to the left; thus, most unionized CO$_2$ exists as CO$_2$ (aq) [10]. As a result, the concentration of H$_2$CO$_3$ may be considered negligible in many cases.

$$\text{CO}_2(\text{aq}) + \text{H}_2\text{O} \xrightleftharpoons[k_{-1}]{k_1} \text{H}^+ + \text{HCO}_3^-$$  \hspace{1cm} (1)

$$\text{CO}_2(\text{aq}) + \text{H}_2\text{O} \xrightleftharpoons[k_{-2}]{k_2} \text{H}_2\text{CO}_3$$  \hspace{1cm} (2)

$$\text{H}_2\text{CO}_3 \xrightleftharpoons[k_{-3}]{k_3} \text{H}^+ + \text{HCO}_3^-$$  \hspace{1cm} (3)

Several authors [12-16] present a summary reaction (equation 4) and composite kinetic constants ($k_+$ and $k_-$) for the scheme shown in Figure 4.1. Development of equation 4 is presented by [17].

$$\text{CO}_2(\text{aq}) + \text{H}_2\text{O} \xrightleftharpoons[k_-]{k_+} \text{H}^+ + \text{HCO}_3^-$$  \hspace{1cm} (4)
Hydroxylation of Carbon Dioxide

Hydroxylation of CO$_2$ (equation 5) is especially important in high pH systems because it contributes significantly to CO$_2$ disappearance at pH $\geq 7.5$ and dominates at pH $\geq 10$ [9,10,12,18].

$$\text{CO}_2 (aq) + \text{OH}^- \overset{k_4}{\underset{k_{4a}}{\rightleftharpoons}} \text{HCO}_3^-$$  \hspace{1cm} (5)

Protolysis and Hydrolysis of Bicarbonate

Acid-base equilibria between bicarbonate and carbonate is described using the universal reaction scheme proposed by Eigen [19], as shown in Figure 4.2.

Protolysis (path I) describes bicarbonate dissociation (equation 6), while hydrolysis (path II) occurs when HCO$_3^-$ combines with OH$^-$ (equation 7). Dissociation of water (path III) connects these two pathways (equation 8). Not all reviewed literature considered equation 7, although it was used by Eigen [19], Kern [18], Patel et.al. [21], Zeebe and Wolf-Gladrow [20], and Cents et.al. [22]. This equation was not used in algal growth models because unreliable results were obtained.

Figure 4.2: Aqueous acid-base reactions of bicarbonate and carbonate [20].
\[
\text{HCO}_3^- \overset{k_{s5}}{\underset{k_{-5}}{\rightleftharpoons}} \text{H}^+ + \text{CO}_3^{2-} \quad (6)
\]

\[
\text{HCO}_3^- + \text{OH}^- \overset{k_{s6}}{\underset{k_{-6}}{\rightleftharpoons}} \text{CO}_3^{2-} + \text{H}_2\text{O} \quad (7)
\]

\[
\text{H}_2\text{O} \overset{k_{s7}}{\underset{k_{-7}}{\rightleftharpoons}} \text{H}^+ + \text{OH}^- \quad (8)
\]

**Kinetic Rate Constants**

Quantification of reaction rates in carbonate systems requires values for kinetic rate constants (Table 4.1). A detailed summary of kinetic rate constants is provided by Watson and Drapcho [17].
Table 4.1: Summary of kinetic constants for carbonate system reactions at 25°C in freshwater [17].

<table>
<thead>
<tr>
<th>Kinetic Rate Constant</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_+$</td>
<td>$3.550 \times 10^{-2}$</td>
<td>s$^{-1}$</td>
<td>[23]$^1$</td>
</tr>
<tr>
<td>$k_-$</td>
<td>$7.983 \times 10^4$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>calculated$^2$</td>
</tr>
<tr>
<td>$k_{+3}$</td>
<td>$9.164 \times 10^6$</td>
<td>s$^{-1}$</td>
<td>calculated$^3$</td>
</tr>
<tr>
<td>$k_{3}$</td>
<td>$4.7 \times 10^{10}$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Eigen and Hames [24]</td>
</tr>
<tr>
<td>$k_{+4}$</td>
<td>$8.053 \times 10^3$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Sirs [25]$^4$</td>
</tr>
<tr>
<td>$k_{4}$</td>
<td>$1.824 \times 10^{-4}$</td>
<td>s$^{-1}$</td>
<td>calculated$^5$</td>
</tr>
<tr>
<td>$k_{+5}$</td>
<td>$2.344$</td>
<td>s$^{-1}$</td>
<td>calculated$^6$</td>
</tr>
<tr>
<td>$k_{5}$</td>
<td>$5 \times 10^{10}$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Zeebe and Wolf-Gladrow [20]$^7$</td>
</tr>
<tr>
<td>$k_{+6}$</td>
<td>$6 \times 10^9$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Eigen [19]$^8$</td>
</tr>
<tr>
<td>$k_{6}$</td>
<td>$1.292 \times 10^6$</td>
<td>s$^{-1}$</td>
<td>calculated$^9$</td>
</tr>
<tr>
<td>$k_{+7}$</td>
<td>$1.410 \times 10^{-3}$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>calculated$^{10}$</td>
</tr>
<tr>
<td>$k_{7}$</td>
<td>$1.4 \times 10^{11}$</td>
<td>M$^{-1}$s$^{-1}$</td>
<td>Eigen [19]</td>
</tr>
</tbody>
</table>

$^1$Calculated using $k_+ = 10^{(0.685 - 3618/T)}$, where T is absolute temperature (K).
$^2$Calculated using $K_1 = k_+/k_-$, where $K_1$ is the equilibrium constant for equation 4 and p$K_1 = 6.352$ [26].
$^3$Calculated using $K_{H2CO3} = k_{+3}/k_{-3}$, where $K_{H2CO3}$ is the equilibrium constant for equation 2 and p$K_{H2CO3} = 3.71$ [27].
$^4$Calculated using $k_{+4} = 10^{(13.589 - 2887/T)}$, where T is absolute temperature (K).
$^5$Calculated using $K_4 = k_{+4}/K_w/K_1$, where $K_w$ is the equilibrium constant for equation 8 and p$K_w = 13.997$ [9].
$^6$Value for $k_{+5}$ assumed to be approximately equal to $k_{+3}$ since no experimental data available.
$^7$Value measured by Eigen [19] at ionic strength of 1.0M. No value for freshwater found in literature.
$^8$Calculated using $K_5 = k_{+6}/k_{-6}$, where $K_5$ is the equilibrium constant for equation 7, and p$K_5 = -3.667$ [28].
$^9$Calculated using $K_7 = k_{+7}/k_{-7}$. 

---

95
Mass Balance Equations

To model a closed carbonate system in which the concentration of H$_2$CO$_3$ is assumed negligible, the CO$_2$ hydration summary reaction shown as equation 4 should be considered with remaining carbonate reactions (equations 5 through 8). Using kinetic rate laws for each of these reactions, MBEs for carbonate species are formulated (equations 9 through 13).

\[
\frac{d[CO_2]}{dt}_{\text{closed}} = k_5[H^+][HCO_3^-] - k_4[CO_2] + k_{-4}[HCO_3^-] - k_{-4}[CO_2][OH^-]. 
\] (9)

\[
\frac{d[HCO_3^-]}{dt}_{\text{closed}} = k_4[CO_2] - k_5[H^+][HCO_3^-] + k_{-4}[CO_2][OH^-] - k_{-4}[HCO_3^-] + k_{-3}[H^+][CO_3^{2-}] - k_{-5}[HCO_3^-] - k_{-6}[HCO_3^-][OH^-] + k_{-4}[CO_3^{2-}]. 
\] (10)

\[
\frac{d[CO_3^{2-}]}{dt}_{\text{closed}} = k_{-5}[HCO_3^-] - k_{-5}[H^+][CO_3^{2-}] + k_{-6}[HCO_3^-][OH^-] - k_{-6}[CO_3^{2-}]. 
\] (11)

\[
\frac{d[H^+]}{dt}_{\text{closed}} = k_5[CO_2] - k_4[H^+][HCO_3^-] + k_{-5}[HCO_3^-] - k_{-5}[H^+][CO_3^{2-}] + k_{-7}[-k_{-5}[H^+][OH^-]]. 
\] (12)

\[
\frac{d[OH^-]}{dt}_{\text{closed}} = k_{-4}[HCO_3^-] - k_{-4}[CO_2][OH^-] - k_{-6}[HCO_3^-][OH^-] + k_{-6}[CO_3^{2-}] + k_{-7}[-k_{-5}[H^+][OH^-]]. 
\] (13)

Modeling Open Carbonate Systems

Carbon Dioxide Absorption

In an open carbonate system, diffusion of atmospheric CO$_2$ across the system boundary occurs (equation 14). Henry’s Law (equation 15) quantitatively describes the equilibrium for CO$_2$ absorption. At 25°C, $K_H$ is 3434.92 Pa/M and $p_{CO_2}$ is 32.02 Pa [10].

\[
CO_2(g) \rightleftharpoons K_H \times CO_2(aq). 
\] (14)

\[
K_H = \frac{[CO_2(aq)]_{\text{sat}}}{p_{CO_2}}. 
\] (15)
Where, $K_H =$ Henry’s Law constant (Pa/M), $[\text{CO}_2 \text{(aq)}]_{\text{sat}} =$ equilibrium CO$_2$ concentration (mol/L), and $p_{\text{CO}_2} =$ CO$_2$ partial pressure (Pa).

**Film Model**

Transfer of CO$_2$ into an open carbonate system is described using film theory [29], where resistance to mass transfer occurs in the liquid boundary layer. Diffusion of CO$_2$ into an aqueous system is kinetically-enhanced, due to conversion of CO$_2$ in this boundary layer. The enhanced rate of CO$_2$ transfer (equation 16) is quantified using an enhancement factor (EF) (equation 17), which is defined as the ratio of enhanced flux ($F_e$) to unenhanced flux ($F$). Calculation of EF can be completed using a reacto-diffusive length ($a_k$), which is defined as equation 18 for a carbonate system [20]. A review of methods for quantifying enhanced CO$_2$ transport is provided by Watson and Drapcho [17].

\[
\left( \frac{d[\text{CO}_2]}{dt} \right)_{\text{transfer}} = \text{EF} \cdot \left( \frac{D_{\text{CO}_2}}{L} \cdot a \cdot \left[ [\text{CO}_2]_{\text{sat}} - [\text{CO}_2]_{\text{bulk}} \right] \right).
\]  

\[
\text{EF} = \frac{L}{a_k} \cdot \coth \left( \frac{L}{a_k} \right).
\]  

\[
a_k = \sqrt{\frac{D_{\text{CO}_2}}{k_+ + k_{+\text{OH}^-}}}.
\]

Where, $D_{\text{CO}_2} =$ liquid diffusivity of CO$_2$ (m$^2$/s), $L =$ boundary layer thickness (m), $a =$ interfacial area (m$^{-1}$), and $[\text{CO}_2]_{\text{bulk}} =$ CO$_2$ concentration in bulk medium (mol/L C).
Mass Balance Equations

Differential equations describing closed and open carbonate systems differ only in the MBE for CO$_2$. In an open system, the CO$_2$ MBE is formulated by considering reaction rates and enhanced CO$_2$ transport (equation 19).

$$\left( \frac{d\left[\text{CO}_2\,\text{(aq)}\right]}{dt}\right)_{\text{open}} = \left( \frac{d\left[\text{CO}_2\,\text{(aq)}\right]}{dt}\right)_{\text{closed}} + \left( \frac{d\left[\text{CO}_2\,\text{(aq)}\right]}{dt}\right)_{\text{transfer}}.$$  \hspace{1cm} (19)

Modeling Algal Growth

Inorganic-Carbon-Limited Algal Growth

Although phosphorous is usually the rate-limiting nutrient in freshwater systems, inorganic carbon is often limiting in “artificial and highly enriched” systems [30]. King [31] and Novak and Brune [30] show a Monod response between CO$_2$ and specific growth rate of several green algae, although Goldman et.al. [32] defend a similar relationship for TIC. Recent information on carbon concentrating mechanisms (CCMs) has expanded this discussion.

It is suspected that all cyanobacteria, most eukaryotic algae, and some aquatic plants employ CCMs [33], which are strategies or processes that organisms employ in CO$_2$-deficient environments to achieve intracellular CO$_2$ concentrations higher than would exist by passive diffusion alone [34]. Most authors defend that CCMs facilitate uptake of CO$_2$ and/or HCO$_3^-$ from bulk medium [35,36]; however, CO$_3^{2-}$ transport may also occur [37,38].
**Growth Rates**

CCMs may allow cyanobacteria and algae to utilize multiple carbonate species. The single-substrate Monod model (equation 20) can be used to model inorganic-carbon limited algal growth with CO$_2$, HCO$_3^-$, CO$_3^{2-}$ or TIC as substrate [39].

$$\mu_C = \frac{\mu_{\text{max}} [C]}{K_C + [C]},$$  \hspace{1cm} \text{(20)}

Where, $\mu_C = \text{inorganic-carbon-limited specific growth rate (hr}^{-1})$, $\mu_{\text{max}} = \text{maximum specific growth rate (hr}^{-1})$, $C = \text{CO}_2$, HCO$_3^-$, CO$_3^{2-}$, or TIC (mol/L C), and $K_C = \text{half-saturation constant for inorganic-carbon-limited growth (mol/L C)}$.

Simultaneous use of multiple carbonate species may be modeled using the Monod equation for substitutable substrates [40]. A preferred substrate ($C_{\text{pfd}}$) is used when available; however, as $C_{\text{pfd}}$ becomes depleted, cells use an alternative substrate ($C_{\text{alt}}$). Growth rate on $C_{\text{pfd}}$ is modeled by equation 20, while growth rate on $C_{\text{alt}}$ ($\mu_{C,\text{SS}}$) is inhibited by presence of $C_{\text{pfd}}$ (equation 21). Possible combinations include CO$_2$ as the preferred substrate, with either HCO$_3^-$ or CO$_3^{2-}$ as the alternative substrate (Table 4.7).

$$\mu_{C,\text{SS}} = \mu_{\text{max}} \left( \frac{[C_{\text{alt}}]}{K_{C,\text{alt}} + [C_{\text{alt}}]} \right) \left( \frac{K_{C,\text{pfd}}}{[C_{\text{pfd}}]} + K_{C,\text{pfd}} \right).$$  \hspace{1cm} \text{(21)}

**Biomass Production/Decay Rates**

The rate of biomass formation ($r_X$) is formulated by considering an appropriate equation for $\mu$ (equation 22), while the rate of biomass decay ($r_D$) is quantified using a decay constant, $b$ (equation 23).

$$r_X = \mu \cdot X, \text{ and}$$  \hspace{1cm} \text{(22)}
Nutrient Utilization Rates

A stoichiometric equation describing algal growth (equation 24) with CO$_2$ as inorganic carbon source is developed using Redfield [41] proportions [10,42]. Redfield [41] observed that C:N:P ratios of zooplankton and phytoplankton in various oceanic regions were relatively constant at 106:16:1. Algal biomass is assumed to have a molecular formula of C$_{106}$H$_{263}$O$_{110}$N$_{16}$P, alternatively represented as (CH$_2$O)$_{106}$(NH$_3$)$_{16}$(H$_3$PO$_4$).

\[
106\text{CO}_2 + 16\text{NO}_3^- + \text{HPO}_4^{2-} + 122\text{H}_2\text{O} + 18\text{H}^+ \overset{\text{photos.}}{\rightarrow} \overset{\text{resp.}}{\text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P} + 138\text{O}_2}
\] (24)

This stoichiometric equation can be generalized for algal cultures with C:N:P ratios (x:y:1) that vary from the Redfield [41] proportions.

\[
x \cdot \text{CO}_2 + y \cdot \text{NO}_3^- + \text{HPO}_4^{2-} + \left(-x - 3y + 2p\right) \cdot \text{H}_2\text{O} + \left(4x + 9y - 4p + 2\right) \cdot \text{H}^+ \overset{\text{photos.}}{\rightarrow} \overset{\text{resp.}}{\left\{(\text{CH}_2\text{O})_x (\text{NH}_3)_y (\text{H}_3\text{PO}_4)\right\} + p \cdot \text{O}_2}
\] (25)

Stoichiometric equations describing algal growth on HCO$_3^-$ or CO$_3^{2-}$ have not been previously found in the literature. However, equations were developed by re-balancing equation 25 with HCO$_3^-$ (equation 26) and CO$_3^{2-}$ (equation 27) as inorganic carbon sources.

\[
x \cdot \text{HCO}_3^- + y \cdot \text{NO}_3^- + \text{HPO}_4^{2-} + \left(-2x - 3y + 2p\right) \cdot \text{H}_2\text{O} + \left(5x + 9y - 4p + 2\right) \cdot \text{H}^+ \overset{\text{photos.}}{\rightarrow} \overset{\text{resp.}}{\left\{(\text{CH}_2\text{O})_x (\text{NH}_3)_y (\text{H}_3\text{PO}_4)\right\} + p \cdot \text{O}_2}
\] (26)

\[
x \cdot \text{CO}_3^- + y \cdot \text{NO}_3^- + \text{HPO}_4^{2-} + \left(-2x - 3y + 2p\right) \cdot \text{H}_2\text{O} + \left(6x + 9y - 4p + 2\right) \cdot \text{H}^+ \overset{\text{photos.}}{\rightarrow} \overset{\text{resp.}}{\left\{(\text{CH}_2\text{O})_x (\text{NH}_3)_y (\text{H}_3\text{PO}_4)\right\} + p \cdot \text{O}_2}
\] (27)
The stoichiometric coefficient for photosynthetic oxygen production \((p)\) can be experimentally determined or estimated. Redfield [41] reports that 2 moles of oxygen are liberated during biomass synthesis per carbon atom, while an additional four oxygen atoms are produced for oxidation of each nitrogen atom. Thus, the Redfield [41] prediction for photosynthetic oxygen production \((p_r)\) is given by equation 28.

\[
p_r = \frac{1}{2}(2x + 4y). \tag{28}
\]

Rates of species utilization (equation 29) are expressed based on inorganic carbon source and an appropriate stoichiometric algal growth equation (equation 25, 26, or 27). In this expression, a “factor” is used to represent the molar ratio of species utilized per mol of biomass formed. Table 4.6 summarizes rates of species utilization and production for various inorganic carbon sources.

\[
r_{S,C-source} = \text{factor} \cdot \mu \cdot X. \tag{29}
\]

Where, \(r_{S,C-source}\) = rate of species (S) utilization for an inorganic carbon source (C-source), \(S = \text{CO}_2, \text{HCO}_3^-, \text{CO}_3^{2-}, \text{or H}^+\), and \(C_{source} = \text{CO}_2, \text{HCO}_3^-, \text{or CO}_3^{2-}\).

**Light-Limited Algal Growth**

**Beer-Lambert Law**

Algal growth is significantly impacted by light availability, which is quantified using the Beer-Lambert Law. This law is commonly applied to estimate light attenuation in natural and engineered systems (equation 30), and is applicable for relatively low total suspended solids concentrations, monochromatic light, and unidirectional path [43].

\[
I_z = I_0 e^{-Kz}. \tag{30}
\]
Where, \( I_Z \) = scalar irradiance at depth \( z \) (\( \mu \text{mol/m}^2\text{-s} \)), \( I_0 \) = incident irradiance at the surface (\( \mu \text{mol/m}^2\text{-s} \)), \( K \) = extinction coefficient (m\(^{-1}\)), and \( z \) = depth (m).

The average scalar irradiance (\( I_{\text{avg}} \)) in a reactor is determined by integrating equation 30 over the reactor depth (\( d \)), which yields equation 31 [43].

\[
I_{\text{avg}} = \frac{I_0 \left(1 - e^{-Kd}\right)}{K \cdot d}.
\] (31)

The extinction coefficient (\( K \)) in the Beer-Lambert law accounts for factors that cause light attenuation. In a bioreactor, the overall attenuation coefficient (\( K \)) is composed of similar factors for the media (\( K_M \)) and biomass (\( K_B \)) (equation 32) [44].

The biomass extinction coefficient is multiplied by some measure of biomass concentration (\( X \)), such as total suspended solids, secchi disk visibility, or chlorophyll a [44].

\[
K = K_M + K_B \cdot X
\] (32)

Several researchers determined a linear relationship between TSS and the extinction coefficient (Table 4.2). However, some results suggest that a hyperbolic model is more appropriate for high biomass concentrations above 1300 mg/L [45].
Table 4.2: Summary of algal biomass and water extinction coefficients.

<table>
<thead>
<tr>
<th>K_M (m⁻¹)</th>
<th>K_B (m²/g)</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.97</td>
<td>0.0575</td>
<td><em>Selenastrum capricornutum</em></td>
<td>[43]</td>
</tr>
<tr>
<td>1.4</td>
<td>0.0592</td>
<td>--</td>
<td>[46]</td>
</tr>
<tr>
<td>--</td>
<td>0.038 – 0.041</td>
<td><em>Porphyridium cruentum</em></td>
<td>[47]</td>
</tr>
<tr>
<td>--</td>
<td>0.035</td>
<td><em>Tetraselmis.</em></td>
<td>[48]</td>
</tr>
<tr>
<td>--</td>
<td>0.0382 – 0.1169¹</td>
<td><em>Isochrysis galbana</em></td>
<td>[49]</td>
</tr>
</tbody>
</table>

¹K_B calculated for various dilution rates and incident irradiances.

Specific Growth Rate

The effect of light limitation on algal growth rate is modeled using Monod kinetics, based on I_avg and a light half-saturation constant (K_S,I) (equation 33). For the case where nutrient and light limitations simultaneously occur, Monod models for nutrient and light-limited specific growth rates may be expanded by multiplication.

\[
\mu_l = \mu_{max} \left( \frac{I_{avg}}{K_{S,I} + I_{avg}} \right).
\]  

(33)

Modeling Algal Growth in Carbonate Systems

The effects of algal growth on carbonate systems are quantified by constructing MBEs to reflect chemical and biological kinetics. Specifically, MBEs for carbonate species, specific growth rates, and rates of substrate utilization are affected by the source of inorganic utilized by algal cells (Tables 4.3 through 4.7). Algal growth in an open environment is also affected by diffusion of CO₂ into the system (Table 4.5).
Table 4.3: Mass balance equations for TIC and carbonate species assuming CO$_2$ and HCO$_3^-$ as single substrates.

<table>
<thead>
<tr>
<th></th>
<th>CO$_2$ as Single Substrate</th>
<th>HCO$_3^-$ as Single Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d[CO_2]/dt$</td>
<td>$\left(\frac{d\left<a href="aq">CO_2\right</a>}{dt}\right)<em>{\text{closed}} - r</em>{\text{C,CO}_2}$</td>
<td>$\left(\frac{d\left<a href="aq">CO_2\right</a>}{dt}\right)_{\text{closed}}$</td>
</tr>
<tr>
<td>$d[HCO_3^-]/dt$</td>
<td>$\left(\frac{d\left[HCO_3^-\right]}{dt}\right)_{\text{closed}}$</td>
<td>$\left(\frac{d\left[HCO_3^-\right]}{dt}\right)<em>{\text{closed}} - r</em>{\text{C,HCO}_3}$</td>
</tr>
<tr>
<td>$d[CO_3^{2-}]/dt$</td>
<td>$\left(\frac{d\left[CO_3^{2-}\right]}{dt}\right)_{\text{closed}}$</td>
<td>$\left(\frac{d\left[CO_3^{2-}\right]}{dt}\right)_{\text{closed}}$</td>
</tr>
<tr>
<td>$d[H^+]/dt$</td>
<td>$\left(\frac{d\left[H^+\right]}{dt}\right)<em>{\text{closed}} - r</em>{\text{H,CO}_2}$</td>
<td>$\left(\frac{d\left[H^+\right]}{dt}\right)<em>{\text{closed}} - r</em>{\text{H,HCO}_3}$</td>
</tr>
<tr>
<td>$d[OH^-]/dt$</td>
<td>$\left(\frac{d\left[OH^-\right]}{dt}\right)_{\text{closed}}$</td>
<td>$\left(\frac{d\left[OH^-\right]}{dt}\right)_{\text{closed}}$</td>
</tr>
<tr>
<td>$d[\text{TIC}]/dt$</td>
<td>$\frac{d\left[CO_2\right]}{dt} + \frac{d\left[HCO_3^-\right]}{dt} + \frac{d\left[CO_3^{2-}\right]}{dt}$</td>
<td>$\frac{d\left[CO_2\right]}{dt} + \frac{d\left[HCO_3^-\right]}{dt} + \frac{d\left[CO_3^{2-}\right]}{dt}$</td>
</tr>
<tr>
<td>$d[X]/dt$</td>
<td>$(\mu_{\text{CO}_2} \cdot X) - (b \cdot X)$</td>
<td>$(\mu_{\text{HCO}_3} \cdot X) - (b \cdot X)$</td>
</tr>
</tbody>
</table>


Table 4.4: Mass balance equations for TIC and carbonate species assuming CO$_2$, HCO$_3^-$, and CO$_3^{2-}$ as substitutable substrates.

<table>
<thead>
<tr>
<th>Equation</th>
<th>CO$_2$ and HCO$_3^-$ as Substitutable Substrates</th>
<th>CO$_2$, HCO$_3^-$, and CO$_3^{2-}$ as Substitutable Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{d[CO_2]}{dt}$</td>
<td>$\left( \frac{d[CO_2 (aq)]}{dt} \right)<em>{\text{closed}} - r</em>{C,CO_2}$</td>
<td>$\left( \frac{d[CO_2 (aq)]}{dt} \right)<em>{\text{closed}} - r</em>{C,CO_2}$</td>
</tr>
<tr>
<td>$\frac{d[HCO_3^-]}{dt}$</td>
<td>$\left( \frac{d[HCO_3^-]}{dt} \right)<em>{\text{closed}} - r</em>{C,HCO_3}$</td>
<td>$\left( \frac{d[HCO_3^-]}{dt} \right)<em>{\text{closed}} - r</em>{C,HCO_3}$</td>
</tr>
<tr>
<td>$\frac{d[CO_3^{2-}]}{dt}$</td>
<td>$\left( \frac{d[CO_3^{2-}]}{dt} \right)_{\text{closed}}$</td>
<td>$\left( \frac{d[CO_3^{2-}]}{dt} \right)<em>{\text{closed}} - r</em>{C,CO_3}$</td>
</tr>
<tr>
<td>$\frac{d[H^+]}{dt}$</td>
<td>$\left( \frac{d[H^+]}{dt} \right)<em>{\text{closed}} - r</em>{H,CO_2} - r_{H,HCO_3}$</td>
<td>$\left( \frac{d[H^+]}{dt} \right)<em>{\text{closed}} - r</em>{H,CO_2} - r_{H,HCO_3} - r_{H,CO_3}$</td>
</tr>
<tr>
<td>$\frac{d[OH^-]}{dt}$</td>
<td>$\left( \frac{d[OH^-]}{dt} \right)_{\text{closed}}$</td>
<td>$\left( \frac{d[OH^-]}{dt} \right)_{\text{closed}}$</td>
</tr>
<tr>
<td>$\frac{d[TIC]}{dt}$</td>
<td>$\frac{d[CO_2]}{dt} + \frac{d[HCO_3^-]}{dt} + \frac{d[CO_3^{2-}]}{dt}$</td>
<td>$\frac{d[CO_2]}{dt} + \frac{d[HCO_3^-]}{dt} + \frac{d[CO_3^{2-}]}{dt}$</td>
</tr>
<tr>
<td>$\frac{d[X]}{dt}$</td>
<td>$\left( \mu_{CO_2} \cdot X \right) + \left( \mu_{HCO_3,SS} \cdot X \right) - \left( b \cdot X \right)$</td>
<td>$\left( \mu_{CO_2} \cdot X \right) + \left( \mu_{HCO_3,SS} \cdot X \right) + \left( \mu_{CO_3,SS} \cdot X \right) - \left( b \cdot X \right)$</td>
</tr>
</tbody>
</table>
Table 4.5: Mass balance equations for CO\(_2\) in open systems\(^1\) for CO\(_2\), HCO\(_3^-\), and CO\(_3^{2-}\) as single and substitutable substrates.

<table>
<thead>
<tr>
<th>Single Substrate Models</th>
<th>Substitutable Substrates Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO(_2) as Single Substrate</td>
<td>CO(_2) and HCO(_3^-) as Substitutable Substrates</td>
</tr>
</tbody>
</table>
| \[
\frac{d}{dt} \left[ \text{CO}_2 \,(aq) \right]_{\text{closed}} + \left\{ \text{EF} \cdot \frac{D_{\text{CO}_2}}{L} \left( [\text{CO}_2]_{\text{sat}} - [\text{CO}_2]_{\text{bulk}} \right) \right\} = -r_{c,\text{CO}_2}
\] | \[
\frac{d}{dt} \left[ \text{CO}_2 \,(aq) \right]_{\text{closed}} + \left\{ \text{EF} \cdot \frac{D_{\text{CO}_2}}{L} \left( [\text{CO}_2]_{\text{sat}} - [\text{CO}_2]_{\text{bulk}} \right) \right\} = -r_{c,\text{CO}_2}
\] |
| HCO\(_3^-\) as Single Substrate | CO\(_2\), HCO\(_3^-\), and CO\(_3^{2-}\) as Substitutable Substrates |
| \[
\frac{d}{dt} \left[ \text{CO}_2 \,(aq) \right]_{\text{closed}} + \left\{ \text{EF} \cdot \frac{D_{\text{CO}_2}}{L} \left( [\text{CO}_2]_{\text{sat}} - [\text{CO}_2]_{\text{bulk}} \right) \right\} = -r_{c,\text{CO}_2}
\] | \[
\frac{d}{dt} \left[ \text{CO}_2 \,(aq) \right]_{\text{closed}} + \left\{ \text{EF} \cdot \frac{D_{\text{CO}_2}}{L} \left( [\text{CO}_2]_{\text{sat}} - [\text{CO}_2]_{\text{bulk}} \right) \right\} = -r_{c,\text{CO}_2}
\] |

\(^1\)Model for algal growth in open batch reactors differs from model for closed systems only in expression of \(d[\text{CO}_2]/dt\). Thus, remaining equations in Tables 4.3, 4.4, 4.7, and 4.6 are also applicable for open algal growth model.
Table 4.6: Rates of species utilization by algae using CO$_2$, HCO$_3^-$, and CO$_3^{2-}$ as single and substitutable substrates$^1$.

<table>
<thead>
<tr>
<th>CO$_2$ as Single Substrate</th>
<th>CO$_2$ and HCO$_3^-$ as Substitutable Substrates</th>
<th>CO$_2$ and CO$_3^{2-}$ as Substitutable Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_{c,CO_2} = x \cdot \mu_{CO_2} \cdot X$</td>
<td>$r_{c,CO_2} = x \cdot \mu_{CO_2} \cdot X$</td>
<td>$r_{c,CO_2} = x \cdot \mu_{CO_2} \cdot X$</td>
</tr>
<tr>
<td>$r_{H,CO_2} = (4x + 9y - 4p + 2) \cdot \mu_{CO_2} \cdot X$</td>
<td>$r_{H,CO_2} = (4x + 9y - 4p + 2) \cdot \mu_{CO_2} \cdot X$</td>
<td>$r_{H,CO_2} = (4x + 9y - 4p + 2) \cdot \mu_{CO_2} \cdot X$</td>
</tr>
<tr>
<td>$r_{c,HCO_3} = x \cdot \mu_{HCO_3} \cdot X$</td>
<td>$r_{c,HCO_3} = x \cdot \mu_{HCO_3} \cdot X$</td>
<td>$r_{c,HCO_3} = x \cdot \mu_{HCO_3} \cdot X$</td>
</tr>
<tr>
<td>$r_{H,HCO_3} = (5x + 9y - 4p + 2) \cdot \mu_{HCO_3} \cdot X$</td>
<td>$r_{H,HCO_3} = (5x + 9y - 4p + 2) \cdot \mu_{HCO_3} \cdot X$</td>
<td>$r_{H,HCO_3} = (5x + 9y - 4p + 2) \cdot \mu_{HCO_3} \cdot X$</td>
</tr>
</tbody>
</table>

$^1$x and y correspond to algal C:N:P ratio of x:y:1. Rates of substrate utilization determined using equations 25, 26, and 27.
Table 4.7: Specific growth rates of algae for CO$_2$, HCO$_3^-$, and CO$_3^{2-}$ as single and substitutable substrates.

<table>
<thead>
<tr>
<th>Single Substrate Models</th>
<th>Substitutable Substrates Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{CO2}$</td>
<td>$\mu_{HCO3}$</td>
</tr>
</tbody>
</table>
| \[
\frac{\mu_{max}\left[CO_2\text{ (aq)}\right]}{K_{CO2} + \left[CO_2\text{ (aq)}\right]} \left\{ \frac{I_{avg}}{K_{S,1} + I_{avg}} \right\}
\] | \[
\frac{\mu_{max}\left[HCO_3^-\right]}{K_{HCO_3} + \left[HCO_3^-\right]} \left\{ \frac{I_{avg}}{K_{S,1} + I_{avg}} \right\}
\] |
| $\mu_{HCO3}$           | $\mu_{CO3, SS}$                |
| \[
\frac{\mu_{max}\left[HCO_3^-\right]}{K_{HCO_1} + \left[HCO_3^-\right]} \left\{ \frac{K_{CO_2}}{K_{CO_2} + \left[CO_2\text{ (aq)}\right]} \right\} \left\{ \frac{I_{avg}}{K_{S,1} + I_{avg}} \right\}
\] | \[
\frac{\mu_{max}\left[CO_3^{2-}\right]}{K_{CO_1} + \left[CO_3^{2-}\right]} \left\{ \frac{K_{CO_2}}{K_{CO_2} + \left[CO_2\text{ (aq)}\right]} \right\} \left\{ \frac{I_{avg}}{K_{S,1} + I_{avg}} \right\}
\] |
Modeling Software

Algal growth models were developed using Matlab® R2007B software with MBEs displayed in Tables 4.3 and 4.4 for closed batch reactors and Table 4.5 for open batch reactors. The systems of ordinary differential equations (ODEs) were solved for user-defined initial conditions using ODE15s and ODE23tb solvers provided by Matlab®. These solvers are used for “stiff” models which contain rapidly and slowly changing components [50]. The developed algal growth model considers both rapid carbonate kinetics and relatively slow algal growth kinetics. Matlab® code for algal growth models is included in Appendix H.

Model Inputs

A series of investigations was completed by Watson and Drapcho [51] to characterize freshwater algal growth as a function of media inorganic carbon content in closed and open batch reactors. Experiments were conducted by inoculating a freshwater algal inoculum into 4L reactors containing a modified BG11 media with various concentrations of Na₂CO₃ (Table 4.8). All reactors were exposed to 121 µE/m²-s at 25°C in a controlled-environment room.
Table 4.8: Summary of completed experiments to provide data for verification of dynamic algal growth model.

<table>
<thead>
<tr>
<th>Open Experiments</th>
<th>Closed Experiments$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prelim. 1O</strong></td>
<td>Run 1C unadjusted</td>
</tr>
<tr>
<td>Initial pH</td>
<td>Initial TIC Concentrations (g/L Na$_2$CO$_3$)</td>
</tr>
<tr>
<td>0.05, 0.10, 0.15, 0.20</td>
<td>0.05, 0.10, 0.15, 0.20</td>
</tr>
<tr>
<td><strong>Run 1O</strong></td>
<td>Run 2C adjusted to 10.3</td>
</tr>
<tr>
<td>unadjusted</td>
<td>0.05, 0.10, 0.15, 0.20</td>
</tr>
<tr>
<td><strong>Run 1C</strong></td>
<td>Run 3C adjusted to 10.3</td>
</tr>
<tr>
<td>unadjusted</td>
<td>0.025, 0.075, 0.125, 0.175</td>
</tr>
</tbody>
</table>

$^1$Percentage of Na$_2$CO$_3$ concentration, as recommended by modified BG11.
$^2$Closed batch reactors fitted with AscariteII® vents to allow headspace pressure to equilibrate with the atmosphere, without permitting CO$_2$ entry.

Inorganic-Carbon-Limited Algal Growth

Average kinetic parameters for single substrate Monod models were determined using data from closed batch reactors in which initial pH were set to 10.3 (Table 4.9). In addition, C:N:P ratios were measured to quantify algal growth stoichiometry (Table 4.10). Kinetic parameters are assumed to describe inorganic-carbon-limited algal growth in closed and open batch reactors, while stoichiometric data varied with reactor environment and initial TIC concentration.

Table 4.9. Kinetic parameters describing inorganic-carbon-limited freshwater algal growth.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>hr$^{-1}$</td>
<td>0.0726</td>
</tr>
<tr>
<td>$K_{\text{CO}_2}$</td>
<td>mol/L C</td>
<td>$4.47 \times 10^{-8}$</td>
</tr>
<tr>
<td>$K_{\text{HCO}_3}$</td>
<td>mol/L C</td>
<td>$5.70 \times 10^{-4}$</td>
</tr>
<tr>
<td>$K_{\text{CO}_3}$</td>
<td>mol/L C</td>
<td>$8.70 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

$^1\mu_{\text{max}}$ independent of inorganic carbon source.
Table 4.10. Stoichiometric parameters describing TIC-limited freshwater algal growth.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Closed Batch Reactors</th>
<th>Open Batch Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25% C</td>
<td>50% C</td>
<td>75% C</td>
</tr>
<tr>
<td>x</td>
<td>mol C/mol X</td>
<td>6.16</td>
<td>6.18</td>
</tr>
<tr>
<td>y</td>
<td>mol N/mol X</td>
<td>1.01</td>
<td>0.947</td>
</tr>
<tr>
<td>z</td>
<td>mol P/mol X</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MW</td>
<td>g/mol</td>
<td>300.2</td>
<td>299.7</td>
</tr>
</tbody>
</table>

Mass Transfer Characteristics

Quantification of enhanced TIC flux (equation 19) requires an estimate for boundary layer thickness. Using open reactors mixed at 300 rpm and containing modified BG11 media with either 0 or 100% Na$_2$CO$_3$, the average ($k_{LA}$)CO$_2$ and boundary layer thickness were determined to be 0.00446 min$^{-1}$ and 117 µm, respectively.

Light Limited Algal Growth

Constants required for quantification of the effects of light on algal growth were determined from the literature (Table 4.11). Incident light intensity ($I_0$) and reactor depth (d) for the experiments of Watson and Drapcho [51] are also displayed in Table 4.11.

Table 4.11. Kinetic and physical parameters describing light-limited freshwater algal growth.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_0$</td>
<td>µE/m$^2$-s</td>
<td>121</td>
</tr>
<tr>
<td>$K_{S,I}$</td>
<td>µE/m$^2$-s</td>
<td>45.9$^1$</td>
</tr>
<tr>
<td>$K_M$</td>
<td>m$^{-1}$</td>
<td>1.97$^2$</td>
</tr>
<tr>
<td>$K_B$</td>
<td>m$^2$/g</td>
<td>0.0575$^2$</td>
</tr>
<tr>
<td>d</td>
<td>m</td>
<td>0.232</td>
</tr>
</tbody>
</table>

$^1$From Conwell and Drapcho [52] for a similar mixed freshwater algal culture.
$^2$From Benson and Rush [43] for Selenastrum capricornutum. Authors cite similar values for other types of algal biomass.
Initial Values

Algal growth model simulations require specification of the initial values for carbonate species, TIC, and biomass (Table 4.12).

Table 4.12. Initial values used for comparison, calibration, and/or verification of closed and open algal growth models.

<table>
<thead>
<tr>
<th>Species</th>
<th>Units</th>
<th>Closed Reactors</th>
<th>Open Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25% C</td>
<td>50% C</td>
</tr>
<tr>
<td>CO₂</td>
<td>(mol/L C)</td>
<td>5.571E-08</td>
<td>7.980E-08</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>(mol/L C)</td>
<td>5.571E-04</td>
<td>7.980E-04</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>(mol/L C)</td>
<td>6.251E-04</td>
<td>8.954E-04</td>
</tr>
<tr>
<td>H⁺</td>
<td>(mol/L C)</td>
<td>5.012E-11</td>
<td>5.012E-11</td>
</tr>
<tr>
<td>OH⁻</td>
<td>(mol/L C)</td>
<td>1.995E-04</td>
<td>1.995E-04</td>
</tr>
<tr>
<td>TIC</td>
<td>(mol/L C)</td>
<td>1.182E-03</td>
<td>1.694E-03</td>
</tr>
<tr>
<td>X¹</td>
<td>(mol/L X)</td>
<td>1.836E-05</td>
<td>1.655E-05</td>
</tr>
</tbody>
</table>

1Initial mass-based biomass concentrations were converted to a molar basis using experimentally determined MW values.

CLOSED BATCH REACTOR MODEL

Model Comparisons

Four models for TIC-limited specific growth rate were evaluated in the algal growth model: (1) CO₂ as single substrate, (2) HCO₃⁻ as single substrate, (3) CO₂ and HCO₃⁻ as substitutable substrates, and (4) CO₂, HCO₃⁻ and CO₃²⁻ as substitutable substrates. Each Monod expression for specific growth rate yielded similar trends. Simulations and residual plots for the 75% C reactor from Run 2C are provided as example (Figures 4.3 through 4.16).
Models incorporating only use of CO$_2$ and HCO$_3^-$ over-predicted the length of exponential growth, while under-predicting peak biomass concentration (Figure 4.3). Consideration of all carbonate species as substitutable substrates generally predicted both the time period of exponential growth and peak biomass concentration (Figure 4.3). The latter model accurately estimates CO$_2$ (Figure 4.11) and HCO$_3^-$ (Figure 4.13) concentrations throughout the growth period; however, CO$_3^{2-}$ (Figure 4.15) concentrations after 50 hr are under-predicted. This decrease in CO$_3^{2-}$ occurs due to the increase in biomass concentration correctly predicted between 75 and 100 hr. Similarly, this model very closely estimates TIC concentrations before 50 hr (Figure 4.5); however, future values are low due to under-predicted CO$_3^{2-}$ concentrations. Carbonate and TIC concentrations are not as severely under-predicted for other models, because they do not consider depletion of CO$_3^{2-}$ by algal growth.

Residuals for pH (Figure 4.8) and alkalinity (Figure 4.10) are highest for the CO$_2$/HCO$_3^-$/CO$_3^{2-}$ substitutable substrates model. Estimates of pH by HCO$_3^-$ single substrate and CO$_2$/HCO$_3^-$ substitutable substrates models are close to experimental data because biomass concentrations are under-predicted by these models. It is also noted that alkalinity predictions by the CO$_2$/HCO$_3^-$/CO$_3^{2-}$ substitutable substrates model are high, even though carbonate predictions are very low after 50 hr. This results due to high pH predictions, which causes an over-contribution of OH$^-$ to alkalinity.

Overall, the substitutable substrates model incorporating all carbonate species best represents data from test closed batch reactors, although low CO$_3^{2-}$ predictions near the end of exponential growth causes similar under-predictions for TIC. Accurate
predictions for culture chemistry parameters by $\text{HCO}_3^-$ single substrate and $\text{CO}_2/\text{HCO}_3^-$ substitutable substrates models are only artificial because of incorrect predictions in both the shape and peak concentration of the biomass batch growth curve. Thus, the model considering use of all carbonate species by algal cells was selected for model calibration and verification.
Figures 4.3 - 4.4 (left): Biomass (top) and residuals (bottom) for closed algal growth models (75% C reactor; Run 2C). Figures 4.5 - 4.6 (right): TIC (top) and residuals (bottom) for closed algal growth models (75% C reactor; Run 2C).
Figures 4.7 - 4.8 (left): pH (top) and residuals (bottom) for closed algal growth models (75% C reactor; Run 2C).
Figures 4.9 - 4.10 (right): Alk (top) and residuals (bottom) for closed algal growth models (75% C reactor; Run 2C).
Figures 4.11 - 4.12 (left): CO$_2$ (top) and residual plots (bottom) for closed algal growth models (75% reactor; Run 2C). Figures 4.13 - 4.14 (right): HCO$_3^-$ (top) and residuals (bottom) for closed algal growth models (75% reactor; Run 2C).
Figures 4.15 - 4.16: $\text{CO}_3^{2-}$ (left) and residual plots (right) for closed algal growth models (75% C reactor; Run 2C).
**Model Calibration**

The algal growth model using the \( \text{CO}_2/\text{HCO}_3/\text{CO}_3^{2-} \) substitutable substrates model was calibrated for the coefficient for photosynthetic oxygen production (p) (equations 24 through 27) using data from 25% and 75% C reactors from Run 2C (Figures 4.17 through 4.24). This coefficient was found to greatly impact model predictions of pH, and consequently alkalinity. After varying this stoichiometric variable from the predicted Redfield [41] value (p) to 1.30*p, it was observed that approximately 1.25*p provided the best predictions of pH. As a result of more accurate pH predictions, alkalinity simulations clearly reflect the under-prediction of CO\(_3^{2-}\) by this model. A similar procedure was completed using data from unadjusted initial pH reactors (Appendix I).
Figures 4.17 - 4.18 (left): Effect of $p$ on pH (top) and residuals (bottom) (25% reactor; Run 2C; closed model).
Figures 4.19 - 4.20 (right): Effect of $p$ on alkalinity (top) and residuals (bottom) (25% reactor; Run 2C; closed model).
Figures 4.21 - 4.22 (left): Effect of $p$ on pH (top) and residuals (bottom) (75% reactor; Run 2C; closed model).
Figures 4.23 - 4.24 (right): Effect of $p$ on alkalinity (top) and residuals (top) (75% reactor; Run 2C; closed model).
Model Verification

The algal growth model with an adjusted p was verified using data from the 50% C reactor of Run 2C (Figures 4.25 through 4.38). Increasing $p_t$ by a factor of 1.25 improved pH predictions (Figure 4.29), while further bettering CO$_2$ (Figure 4.33) and HCO$_3^-$ (Figure 4.35) estimates. However, estimates of CO$_3^{2-}$ (Figure 4.37) are still too low after 50 hr, leading to errors in TIC and alkalinity predictions. In general, the calibrated closed algal growth model provides better predictions for the adjusted initial pH reactors. A similar procedure was completed using data from unadjusted initial pH reactors (Appendix I).
Figures 4.25 - 4.26 (left): Biomass (top) and residuals (bottom) for calibrated closed model (50% reactor; Run 2C).
Figures 4.27 - 4.28 (right): TIC (top) and residuals (bottom) for calibrated closed model (50% reactor; Run 2C).
Figures 4.29 - 4.30 (left): pH (top) and residuals (bottom) for calibrated closed model (50% reactor; Run 2C).
Figures 4.31 - 4.32 (right): Alk (top) and residuals (bottom) for calibrated closed model (50% reactor; Run 2C).
Figures 4.33 - 4.34 (left): CO$_2$ (top) and residuals (bottom) for calibrated closed model (50% reactor; Run 2C).
Figures 4.35 - 4.36 (right): HCO$_3^-$ (top) and residuals (bottom) for calibrated closed model (50% reactor; Run 2C).
Figures 4.37 - 4.38: \( \text{CO}_3^{2-} \) (left) and residuals (right) for calibrated closed model (50% reactor; Run 2C).
OPEN BATCH REACTOR MODEL

Model Comparisons

Four equations for TIC-limited specific growth rate were evaluated in the open algal growth model, assuming carbonate species as single and substitutable substrates. Simulations and residual plots for the 50% C reactor from Run 1O are provided to compare model predictions (Figures 4.39 through 4.52).

Several trends in model predictions were observed for the four Monod expressions. All models, except CO$_2$ as single substrate, well-predicted CO$_2$ and HCO$_3^-$ concentrations throughout the growth period. Consideration of HCO$_3^-$ as a single substrate and as a substitutable substrate for CO$_2$ generally over-predicted CO$_3^{2-}$, while consideration of all carbonate species as substitutable substrates under-predicted CO$_3^{2-}$ concentrations (Figure 4.51). As a result, similar faults were observed in TIC simulations (Figure 4.41). Furthermore, these two models over-predicted pH (Figure 4.43), although residuals (Figure 4.44) were higher for the CO$_2$/HCO$_3^-$/CO$_3^{2-}$ substitutable substrates model. Due to high pH estimations, alkalinity (Figure 4.45) was over-estimated for the latter model, even though CO$_3^{2-}$ was under-predicted. Finally, biomass concentrations (Figure 4.39) in open reactors before 700 hr were best predicted when HCO$_3^-$ was considered as a single substrate and as a substitutable substrate for CO$_2$; however, later concentrations were most accurately approximated by the CO$_2$/HCO$_3^-$/CO$_3^{2-}$ substitutable substrates model. Overall, errors in model predictions differed between the four Monod expressions for TIC-limited specific growth rate.
Figures 4.39 - 4.40 (left): Biomass (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.41 - 4.42 (right): TIC (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.43 - 4.44 (left): pH (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.45 - 4.46 (right): Alk (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.47 - 4.48 (left): $\text{CO}_2$ (top) and residuals (bottom) for open algal growth models (50% C reactor). Figures 4.49 - 4.50 (right): $\text{HCO}_3^-$ (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.51 - 4.52: $\text{CO}_3^{2-}$ (left) and residual plots (right) for open algal growth models (50% C reactor).
Sensitivity Analysis

Since deficiencies existed in the open algal growth model when all four Monod expressions were compared, sensitivity analyses were completed to identify parameters that should be further investigated to improve model predictions. TIC-limited specific growth rate was modeled assuming all carbonate species as substitutable substrates.

Small adjustments to $p$ significantly affected model predictions (Figures 4.53 through 4.66). Specifically, when this parameter was increased above $p_r$, biomass, pH, and alkalinity predictions were observed to decrease. A value of $1.04*p_r$ appears to best represent pH and alkalinity data; however, this modification causes low biomass predictions.

The biomass light attenuation coefficient ($K_B$) was found to impact all model predictions (Figures 4.67 through 4.80). Increasing this value from the Benson and Rush [43] value ($K_{B-BR}$) to $20*K_{B-BR}$, decreases biomass, pH, and alkalinity predictions, while increasing TIC and carbonate species estimations. A value of $5*K_{B-BR}$ improves predictions to reflect increases in $CO_3^{2-}$ and TIC later in the growth period; however, this increase does not adequately reduce pH and alkalinity estimates.

Examination of the effects of $p$ and $K_B$ on model predictions suggests that manipulation of neither parameter alone adequately calibrates the open algal growth model. Additional laboratory investigations are required to quantify one or both variables.
Figures 4.53 - 4.54 (left): Effect of p on biomass (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.55 - 4.56 (right): Effect of p on TIC (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.57 - 4.58 (left): Effect of p on pH (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.59 - 4.60 (right): Effect of p on alk (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.61 - 4.62 (left): Effect of p on CO₂ (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.63 - 4.64 (right): Effect of p on HCO₃⁻ (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.65 - 4.66: Effect of p on $\text{CO}_3^{2-}$ (left) and residuals (right) for open algal growth models (50% C reactor).
Figures 4.67 - 4.68 (left): Effect of $K_B$ on biomass (top) and residuals (bottom) for open algal growth models (50% C reactor).

Figures 4.69 - 4.70 (right): Effect of $K_B$ on TIC (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.71 - 4.72 (left): Effect of $K_B$ on pH (top) and residuals (bottom) for open algal growth models (50% C reactor).

Figures 4.73 - 4.74 (right): Effect of $K_B$ on alk (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.75 - 4.76 (left): Effect of $K_B$ on $CO_2$ (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.77 - 4.78 (right): Effect of $K_B$ on $HCO_3^-$ (top) and residuals (bottom) for open algal growth models (50% C reactor).
Figures 4.79 - 4.80: Effect of $K_B$ on CO$_2$ (left) and residuals (right) for open algal growth models (50% C reactor).
SUMMARY

Dynamic algal growth models intended to predict algal biomass and carbonate species concentrations in closed and open batch reactors were developed and evaluated. The following statements summarize the results.

1. The CO$_2$/HCO$_3^-$/CO$_3^{2-}$ substitutable substrates model best predicted both the length of exponential growth and peak biomass concentration in closed batch algal reactors. Models considering only use of CO$_2$ and/or HCO$_3^-$ significantly over-estimated the length of exponential growth, while under-predicting peak biomass concentrations.

2. Calibration of the closed algal growth model suggests that the stoichiometric coefficient for photosynthetic oxygen production (p) is 1.25 times the predicted Redfield [41] value ($p_r$).

3. Verification of the closed algal growth model shows it to accurately estimate biomass, CO$_2$, and HCO$_3^-$ concentrations. However, under-prediction of carbonate concentrations adversely impacts TIC and alkalinity estimations after 50 hr.

4. In open batch reactors, HCO$_3^-$ single substrate and CO$_2$/HCO$_3^-$ substitutable substrates models best predicted biomass concentrations before 700 hr, while the CO$_2$/HCO$_3^-$/CO$_3^{2-}$ substitutable substrates model best estimated later biomass concentrations.

5. A sensitivity analysis for the open batch reactor model showed p and $K_B$ to significantly impact model predictions; however, neither parameter alone could be used for model calibration.
CONCLUSIONS

As atmospheric CO$_2$ concentrations and global temperatures continue to escalate, researchers must develop creative methods to offset these trends. Cultivation of algal biomass in large outdoor ponds is an appealing strategy because biomass can be harvested and converted to biofuels to reduce use of traditional carbon-intensive fuels. Once further work is completed to improve the presented algal growth model, it can be used to aid in design and optimization of systems to produce algae for carbon mitigation and other bioproducts.

REFERENCES


[26] HS Harned, RJ Davis. The ionization constant of carbonic acid in water and the solubility of carbon dioxide in water and aqueous salt solutions from 0 to 50°, J. Am. Chem. Soc. 65 (1943) 2030-2037.


[40] CPL Grady, GT Daigger, HC Lim, Biological Wastewater Treatment: Principles and Practice, CRC Press 1999.


CHAPTER FIVE

REMARKS AND RECOMMENDATIONS

Continued research is needed to further explore use of mixed freshwater algae for biological carbon mitigation. Several experiments in closed and open algal reactors are suggested.

1) Additional trials in closed batch reactors should be completed with lower initial TIC (less than 25%). This would clarify the discrepancy between $Y_{X/S}$ data between 12.5% and 25% C reactors (Figure 3.14), and possibly reveal a trend between initial TIC and observed $Y_{X/S}$. Measurement of C:N:P ratios from these reactors may also strengthen the linear relationship (Figure 3.4) between initial TIC and carbon content of closed algal biomass.

2) Completion of open batch reactor experiments at lower initial TIC should be completed to verify the exponential trend between initial TIC and $\%\text{TIC}_{eq}$ (Figure 3.29). Specifically, algal growth in a 0% C reactor should be characterized to verify the intercept shown in Figure 3.29, which suggests that this reactor would demonstrate a $\%\text{TIC}_{eq}$ of 56%. Algal C:N:P data from these reactors could be used to strengthen the linear relationship between initial TIC and carbon content of open algal biomass (Figure 3.21).

3) Analysis of open batch reactors supplied with equal initial TIC and different initial pH could be investigated to determine optimum conditions for carbon mitigation.
Lower pH may lead to increased specific growth rates; however, growth periods may be shortened due to decreased CO₂ hydration rates.

4) Determination of photosynthetic oxygen production and biomass light attenuation coefficients in closed and open reactors could improve algal growth model simulations.

Global climate change, resulting partly from increased anthropogenic CO₂ emissions, has severe consequences. Atmospheric concentrations between 450 to 600 ppmv will likely lead to dry-season rainfall reductions as severe as the dust bowl era, while concentrations in excess of this range may cause an irreversible 0.4 to 1.0 m rise in sea level [1]. Thus, it is imperative that scientists and engineers research and develop effective mitigation technologies to protect terrestrial and aquatic ecosystems. Although abatement by cultivation of algal biomass in alkaline ponds may not be the sole solution, it has potential to serve as part of a diverse carbon management plan.

REFERENCE

APPENDICES
Sampling schedules for all experiments are outlined in Appendix A, with the “Total” column indicating the total volume removed from each reactor. Tables A.1, A.2, and A.3 outline sampling schedules for Runs 1C, 2C, and 3C, respectively. Tables A.4 and A.5 outline sampling schedules for Prelim. 1O and Run 1O.

Table A.1: Sampling schedule for closed batch reactors with unadjusted initial pH (Run 1C).

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Table A.2: Sampling schedule for closed batch reactors with adjusted initial pH (Run 2C).

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Table A.3: Sampling schedule for closed batch reactors with adjusted initial pH (Run 3C).

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Table A.4: Sampling schedule for open batch reactors (Prelim. 1O)\(^1\).

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\(^1\)Evaporation of water from open reactors was monitored, and DI water was added on each sampling day to account for evaporation. However, the amount of water added was not recorded.
Table A.5: Sampling schedule for open batch reactors (Run 1O).

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APPENDIX B
BIOMASS QUANTIFICATION

CLOSED BATCH REACTORS

Raw Data

Total suspended solids data is included as Figure B.1, while OD data is included as Figure B.2. Cell density data is presented in Chapter Three.

Figure B.1: Dry weights of closed batch algal reactors supplied with varying initial amounts of inorganic carbon.
Calibration Curves

Calibration curves relating OD to TSS were constructed using concentrated algal samples from Run 1C (Figure B.3).
Calibration curves relating cell density to TSS and OD are included as Figures B.4 and B.5, respectively. Poor correlations were observed between CD and TSS, likely due to variation in TSS data caused by low biomass concentrations in closed reactors. Coefficients of determination ($R^2$) were higher when intercepts were not forced to be zero; however, TSS should be near zero in the absence of algal cells. Linear relationships were generally observed between CD and OD.
Figure B.4: Relationship between cell density and TSS of closed batch algal cultures cultivated with varying initial TIC concentrations (Runs 2C and 3C).

Figure B.5: Calibration curves relating cell density to OD at 750 nm for closed algal cultures supplied with various initial amounts of inorganic carbon (Runs 2C and 3C).
OPEN BATCH REACTORS

Total suspended solids and OD data are shown in Figures B.6 and B.7, respectively. Linear correlations were observed between OD and TSS (Figure B.8).

Figure B.6: Dry weights of open batch algal reactors supplied with varying initial amounts of inorganic carbon.
Figure B.7: Optical densities at 750 nm of open batch algal reactors supplied with varying initial amounts of inorganic carbon.

Figure B.8: Calibration curve relating OD to biomass concentration for open batch algal reactors supplied with varying initial amounts of inorganic carbon.
APPENDIX C

ADDITIONAL KINETIC ANALYSIS DATA

LINEWEAVER-BURK PLOTS

Lineweaver-Burk plots for unadjusted initial pH Run 1C (Figure C.1) provided less reliable estimates of kinetic parameters $\mu_{\text{max}}$ and $K_S$ than those from Runs 2C and 3C (Chapter Three).

![Figure C.1. Lineweaver-Burk plots for determination of $\mu$-max and $K_S$ for single substrate models (Run 1C).](image-url)
STATISTICAL APPLICATION SOFTWARE (SAS)

Estimations of $\mu_{\text{max}}$ and $K_s$ determined using SAS and data from Runs 2C and 3C were presented in Chapter Three. ANOVA tables are displayed in Table C.1, and Monod plots are displayed in Figure C.2.

Table C.1: ANOVA tables for single-substrate Monod models with CO$_2$, HCO$_3^-$, CO$_3^{2-}$, or TIC as inorganic carbon source.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Approx. Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CO$_2$ single-substrate model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (Run 2C)</td>
<td>2</td>
<td>0.00471</td>
<td>0.00236</td>
<td>5020.28</td>
<td>0.0100</td>
</tr>
<tr>
<td>Error</td>
<td>1</td>
<td>4.693E-7</td>
<td>4.693E-7</td>
<td>5020.28</td>
<td>0.0100</td>
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<tr>
<td>Uncorrected Total</td>
<td>3</td>
<td>0.00471</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (Run 3C)</td>
<td>2</td>
<td>0.00390</td>
<td>0.00195</td>
<td>152.23</td>
<td>0.0065</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>0.000026</td>
<td>0.000013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncorrected Total</td>
<td>4</td>
<td>0.00393</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HCO$_3^-$ single-substrate model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (Run 2C)</td>
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<td>0.00236</td>
<td>5352.33</td>
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<tr>
<td>Error</td>
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<td>4.402E-7</td>
<td>5352.33</td>
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<td>Uncorrected Total</td>
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<td></td>
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<tr>
<td>Model (Run 3C)</td>
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<td>0.00391</td>
<td>0.00195</td>
<td>207.83</td>
<td>0.0048</td>
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<tr>
<td>Error</td>
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<td>0.000019</td>
<td>9.403E-6</td>
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<tr>
<td>Uncorrected Total</td>
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<td>0.00393</td>
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</tr>
<tr>
<td><strong>CO$_3^{2-}$ single-substrate model</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (Run 2C)</td>
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<td>0.00471</td>
<td>0.00236</td>
<td>2142.36</td>
<td>0.0153</td>
</tr>
<tr>
<td>Error</td>
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<td>1.1E-6</td>
<td>1.1E-7</td>
<td>2142.36</td>
<td>0.0153</td>
</tr>
<tr>
<td>Uncorrected Total</td>
<td>3</td>
<td>0.00471</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (Run 3C)</td>
<td>2</td>
<td>0.00391</td>
<td>0.00196</td>
<td>262.62</td>
<td>0.0038</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>0.000015</td>
<td>7.449E-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncorrected Total</td>
<td>4</td>
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<td></td>
</tr>
<tr>
<td><strong>TIC single-substrate model</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (Run 2C)</td>
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<td>0.00471</td>
<td>0.00236</td>
<td>2778.37</td>
<td>0.0134</td>
</tr>
<tr>
<td>Error</td>
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<td>8.48E-7</td>
<td>8.48E-7</td>
<td>2778.37</td>
<td>0.0134</td>
</tr>
<tr>
<td>Uncorrected Total</td>
<td>3</td>
<td>0.00471</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model (Run 3C)</td>
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<td>260.68</td>
<td>0.0038</td>
</tr>
<tr>
<td>Error</td>
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<td>0.000015</td>
<td>7.504E-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncorrected Total</td>
<td>4</td>
<td>0.00393</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure C.2. Monod plots generated using kinetic data estimated by SAS and experimental data from Runs 1C, 2C, and 3C.

PERCENT BIOMASS PRODUCTION (PBP)

Estimates of %BP for all carbonate species from Runs 2C and 3C were presented in Chapter Three. Sample calculations for %BP_{CO_3} using data from the 50% C reactor of Run 2C are presented in Figure C.3. Results of all calculations are summarized in Table C.2.
\[
\Delta X_{\text{exp}} = 68.36 - 4.961 = 63.40 \text{ mg X/L}
\]
\[
\Delta \left( \text{CO}_3^{2-} \right)_{\text{pH}} = 9.408 \times 10^{-4} \left( 0.8604 - 0.5287 \right) = 3.121 \times 10^{-4} \text{ mol C/L}
\]
\[
8.095 \times 10^{-4} = 8.954 \times 10^{-4} + 3.121 \times 10^{-4} - \left( \text{CO}_3^{2-} \right)_{\text{utilization}}
\]
\[
\Rightarrow \left( \text{CO}_3^{2-} \right)_{\text{utilization}} = 3.980 \times 10^{-4} \text{ mol L/C}
\]
\[
\Delta X_{\text{theoretical}} = \left( 3.980 \times 10^{-4} \right) \left( 12,000 \right) \left( 6.392 \right) = 30.5 \text{ mg X/L}
\]
\[
\% \text{BP} = \frac{30.5}{63.40} \times 100 = 48.2 \%
\]

Figure C.3. Sample calculations for %BP\text{CO}_3 using data from 50\% \text{C} reactor of Run 2C.

Table C.2: Calculation\(^1\) of percent of biomass production (%BP) attributed to assimilation of carbonate species for adjusted initial pH Runs 2C and 3C.

<table>
<thead>
<tr>
<th>TIC</th>
<th>Run 2C (%C)</th>
<th>Run 3C (%C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>37.5</td>
<td>62.5</td>
</tr>
<tr>
<td>2 \times (TIC)u \times 10^{-4}</td>
<td>5.407</td>
<td>7.527</td>
</tr>
<tr>
<td>\Delta X_{\text{TIC}}</td>
<td>33.3</td>
<td>57.7</td>
</tr>
<tr>
<td>%BP_{\text{TIC}}</td>
<td>79.5</td>
<td>91.1</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>5.407</td>
<td>7.527</td>
</tr>
<tr>
<td>\Delta X_{\text{CO}_2} \times 10^{-3}</td>
<td>1.569</td>
<td>3.602</td>
</tr>
<tr>
<td>%BP_{\text{CO}_2}</td>
<td>0.0037</td>
<td>0.0046</td>
</tr>
<tr>
<td>5 \times (HCO_3^-)u \times 10^{-4}</td>
<td>2.548</td>
<td>3.547</td>
</tr>
<tr>
<td>\Delta X_{\text{HCO}_3}</td>
<td>15.7</td>
<td>27.2</td>
</tr>
<tr>
<td>%BP_{\text{HCO}_3}</td>
<td>37.4</td>
<td>42.9</td>
</tr>
<tr>
<td>5 \times (CO_3^{2-})u \times 10^{-4}</td>
<td>2.859</td>
<td>3.980</td>
</tr>
<tr>
<td>\Delta X_{\text{CO}_3}</td>
<td>17.6</td>
<td>30.5</td>
</tr>
<tr>
<td>%BP_{\text{CO}_3}</td>
<td>42.0</td>
<td>48.2</td>
</tr>
<tr>
<td>11 \times (X)</td>
<td>38.0</td>
<td>63.4</td>
</tr>
</tbody>
</table>

\(^1\)Final pH required from calculation of 25, 50, 75, 100, 12.5, 37.5, 62.5, and 87.5% C reactors were 10.90, 11.04, 11.16, 11.23, 10.74, 10.93, 11.16, and 11.36, respectively.

\(^2\)\Delta(TIC)_{\text{pH}} = 0; \text{ therefore, } (TIC)u = TIC_{t1} - TIC_{t2}. 

APPENDIX D

BATCH GROWTH CURVES

CLOSED REACTORS

Sample batch growth curves displaying the relationship between biomass growth and inorganic carbon utilization are presented in the body of this paper; however, Appendix C presents these charts for each reactor in closed batch reactor experiments (Figures C1 through C12).

Figure D.1: Relationship between biomass, TIC, and carbonate species concentrations in 25% C reactor (Run 1C: initial pH not adjusted).
Figure D.2: Relationship between biomass, TIC, and carbonate species concentrations in 50% C reactor (Run 1C: initial pH not adjusted).

Figure C.3: Relationship between biomass, TIC, and carbonate species concentrations in 75% C reactor (Run 1C: initial pH not adjusted).
Figure D.4: Relationship between biomass, TIC, and carbonate species concentrations in 100% C reactor (Run 1C: initial pH not adjusted).

Figure D.5: Relationship between biomass, TIC, and carbonate species concentrations in 25% C reactor (Run 2C: initial pH adjusted to 10.3).
Figure D.6: Relationship between biomass, TIC, and carbonate species concentrations in 50% C reactor (Run 2C: initial pH adjusted to 10.3).

Figure D.7: Relationship between biomass, TIC, and carbonate species concentrations in 75% C reactor (Run 2C: initial pH adjusted to 10.3).
Figure D.8: Relationship between biomass, TIC, and carbonate species concentrations in 100% C reactor (Run 2C: initial pH adjusted to 10.3).

Figure D.9: Relationship between biomass, TIC, and carbonate species concentrations in 12.5% C reactor (Run 3C: initial pH adjusted to 10.3).
Figure D.10: Relationship between biomass, TIC, and carbonate species concentrations in 37.5% C reactor (Run 3C: initial pH adjusted to 10.3).

Figure D.11: Relationship between biomass, TIC, and carbonate species concentrations in 62.5% C reactor (Run 3C: initial pH adjusted to 10.3).
Figure D.12: Relationship between biomass, TIC, and carbonate species concentrations in 87.5% C reactor (Run 3C: initial pH adjusted to 10.3).

OPEN REACTORS

Fluctuations in pH within open batch algal reactors were observed to generally correspond with changes in biomass concentration (Figures D.13 through D.16).
Figure D.13: Relationship between biomass and pH in 25% C reactor (Run 10).

Figure D.14: Relationship between biomass and pH in 50% C reactor (Run 10).
Figure D.15: Relationship between biomass and pH in 75% C reactor (Run 1O).

Figure D.16: Relationship between biomass and pH in 100% C reactor (Run 1O).
APPENDIX E

PRELIMINARY DATA FOR OPEN BATCH REACTORS

A preliminary experiment was conducted to characterize algal growth in open batch reactors containing 25, 50, 75, or 100% C. Similar trends were observed for data from Prelim. 1O and Run 1O. Biomass dry weights increased over time (Figure E.1), while specific growth rates increased with increasing initial TIC concentration (Figure E.2). In addition, alkalinity (Figure E.3) and TIC (Figure E.5) increased over time due to diffusion of CO\(_2\) into reactors. Due to algal growth, pH in reactors increased over time (Figure E.4).

![Figure E.1: Dry weights of open batch algal reactors supplied with varying initial amounts of inorganic carbon (Prelim. 1O).](image-url)
Figure E.2: Natural log of biomass concentration versus time used to determine specific growth rates of open algal cultures supplied with various amounts of TIC (Prelim. 1O).

Figure E.3: Alkalinity of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon (Prelim. 1O).
Figure E.4: pH of algal cultures in open batch reactors supplied with varying initial amounts of inorganic carbon (Prelim. 1O).

Figure E.5: Total inorganic carbon concentrations within open batch algal cultures supplied with varying initial amounts of inorganic carbon (Prelim. 1O).
HETEROTROPHIC PLATE COUNTS

METHODS

Heterotrophic bacterial cultures in open reactors were quantified using the Pour Plate Method, as per Method 9215 B [1]. Distilled water samples were plated onto R2A agar to serve as experimental blanks. Petri dishes were incubated at 35°C in a Precision Gravity Convection Incubator (Model 6).

Colonies were counted according to Method 9215 A after 48 hr [1], as summarized in Table F.1.

Table F.1: Summary of method for reporting CFU from heterotrophic plate counts.

<table>
<thead>
<tr>
<th>Number of Colonies per Plate</th>
<th>Method for Reporting CFU and/or Concentration of Heterotrophic Bacteria.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• No colonies</td>
<td>• Number of colonies reported as &lt; 1 CFU.</td>
</tr>
<tr>
<td>• Less than or equal to 300 CFU/plate</td>
<td>• All colonies on plate counted, with concentration calculated according to equation F.1.</td>
</tr>
<tr>
<td>• Greater than 10 CFU/cm² (&gt; 300 CFU/plate)</td>
<td>• Colonies counted in four representative 1 cm² sections, with concentration calculated according to equation F.2.</td>
</tr>
<tr>
<td>• Greater than 100 CFU/cm² (&gt; 300 CFU/plate)</td>
<td>• Number of colonies reported as &gt; 6500 CFU for glass plates and &gt; 5700 CFU for plastic plates.</td>
</tr>
</tbody>
</table>

\[
C_HB = \frac{CFU}{V_s}. \tag{F.1}
\]
\[ C_{HB} = \frac{\text{CFU} \cdot \text{PF}}{V_S}. \]  

(F.2)

Where, \( C_{HB} \) = Concentration of heterotrophic bacteria (CFU/mL), \( \text{CFU} \) = Number of CFU counted (--), \( V_S \) = Volume of sample plate plated (mL), and \( \text{PF} \) = plate factor (57 for disposable plates; 65 for glass plates).

RESULTS

At 734 hr, white colonies were seen on plates from all reactors, while orange colonies also appeared on plates from the 100% C reactor (Figures F.1 through F.4).

Figure F.1: Concentration of heterotrophic bacteria in open algal batch reactor initially supplied with 25% C at 734 hr.
Figure F.2: Concentration of heterotrophic bacteria in open algal batch reactor initially supplied with 50% C at 734 hr.

Figure F.3: Concentration of heterotrophic bacteria in open algal batch reactor initially supplied with 75% C at 734 hr.
Comparison of results from all reactors showed heterotrophic bacteria concentration to be highest in the 75% C reactor at 734 hr (Figure F.5). Average concentrations were calculated using only data from plates containing \( \leq 300 \) CFU; however, since no plates from 75% C reactor met this criterion, data from 0.001 mL plates were used. This may explain why concentrations appear to be considerably higher in this reactor.
Figure F.5: Average concentration of heterotrophic bacteria in open algal batch reactors initially supplied with various amounts of inorganic carbon at 734 hr.

At 836 hr, white and orange colonies were seen on nearly all plates (Figure F.6) from all reactors (Figures F.7 to F.10).
Figures F.6: Heterotrophic plates from 25 (top left), 50 (top right), 75 (bottom left) and 100 (bottom right) %C reactors at 836 hr (Row 1: 0.01 mL; Row 2: 0.001 mL; Row 3: 0.0001 mL; Row 4: 0.00001 mL).
Figure F.7: Concentration of heterotrophic bacteria in open algal batch reactor initially supplied with 25% C at 836 hr.

Figure F.8: Concentration of heterotrophic bacteria in open algal batch reactor initially supplied with 50% C at 836 hr.
Figure F.9: Concentration of heterotrophic bacteria in open algal batch reactor initially supplied with 75% C at 836 hr.

Figure F.10: Concentration of heterotrophic bacteria in open algal batch reactor initially supplied with 100% C at 836 hr.
Concentrations of heterotrophic bacteria were compared for four open reactors at 836 hr. (Figure F.11). Average concentration for each reactor was calculated using concentrations from plates containing less than or equal to 300 colonies. It was observed that the 100% C reactor contained that highest concentration of heterotrophic bacteria.

![Figure F.11: Average concentration of heterotrophic bacteria in open algal batch reactors initially supplied with various amounts of inorganic carbon at 836 hr. Averages were calculated using results from plates containing ≤ 300 CFU.](image)

**REFERENCE**

APPENDIX G

IMPACT OF CARBON MITIGATION BY FRESHWATER ALGAE

Appendix G displays calculations used to estimate required pond dimensions to abate Clemson University CO₂ emissions. In 2008, Clemson University (CU) used 200,000 MMBTU derived from coal and 400,000 MMBTU originating from natural gas [1]. The carbon content coefficients for coal and natural gas are 25.49 and 14.47, respectively [2]. Based on the results of Chapter Three, approximately 400 mg/L C can be sequestered by the studied freshwater algae in 1100 hr of light. A pond volume of $8.14 \times 10^6 \text{ m}^3$ (6600 acre-ft) would be required to offset CU emissions.

Annual CO₂ emissions from coal usage:

$$200,000 \times 10^6 \, \frac{\text{BTU}}{\text{yr}} \left( \frac{25.49 \, \text{g C}}{1000 \, \text{BTU}} \right) \left( \frac{99}{100} \right) \left( \frac{44 \, \text{g CO}_2}{12 \, \text{g C}} \right) \left( \frac{\text{kg}}{1000 \, \text{g}} \right) = 1.85 \times 10^7 \, \frac{\text{kg CO}_2}{\text{yr}}$$

Annual CO₂ emissions from natural gas usage:

$$400,000 \times 10^6 \, \frac{\text{BTU}}{\text{yr}} \left( \frac{14.47 \, \text{g C}}{1000 \, \text{BTU}} \right) \left( \frac{99.5}{100} \right) \left( \frac{44 \, \text{g CO}_2}{12 \, \text{g C}} \right) \left( \frac{\text{kg}}{1000 \, \text{g}} \right) = 2.11 \times 10^7 \, \frac{\text{kg CO}_2}{\text{yr}}$$

Total CO₂ emissions from fossil fuel usage by Clemson University:

$$\left( 1.85 \times 10^7 + 2.11 \times 10^7 \, \frac{\text{kg CO}_2}{\text{yr}} \right) = 3.96 \times 10^7 \, \frac{\text{kg CO}_2}{\text{yr}}$$

Potential CO₂ mitigation by studied freshwater algal culture (assuming 10 hr light):

$$\frac{400 \, \text{mg C}}{\text{L} - 1100 \, \text{hr}} \left( \frac{10 \, \text{hr}}{\text{day}} \right) \left( \frac{365 \, \text{day}}{\text{yr}} \right) \left( \frac{g}{1000 \, \text{mg}} \right) \left( \frac{1000 \, \text{L}}{\text{m}^3} \right) \left( \frac{44 \, \text{g CO}_2}{12 \, \text{g C}} \right) = 4.87 \times 10^3 \, \frac{\text{g CO}_2}{\text{m}^3 - \text{yr}}$$
Required pond volume to abate CO$_2$ emissions from coal:

$$\left( \frac{4.87 \times 10^3 \text{ g CO}_2}{\text{m}^3 \text{ - yr}} \right) \cdot \text{Volume}_{\text{coal}} = \frac{1.85 \times 10^7 \text{ kg CO}_2}{\text{yr}} \left( \frac{1000 \text{ g}}{\text{kg}} \right)$$

$$\Rightarrow \text{Volume}_{\text{coal}} = 3.80 \times 10^6 \text{ m}^3$$

Required pond volume to abate CO$_2$ emissions from natural gas:

$$\left( \frac{4.87 \times 10^3 \text{ g CO}_2}{\text{m}^3 \text{ - yr}} \right) \cdot \text{Volume}_{\text{NG}} = \frac{2.11 \times 10^7 \text{ kg CO}_2}{\text{yr}} \left( \frac{1000 \text{ g}}{\text{kg}} \right)$$

$$\Rightarrow \text{Volume}_{\text{NG}} = 4.34 \times 10^6 \text{ m}^3$$

Total pond volume required to abate Clemson University emissions:

$$\text{Volume}_{\text{total}} = \left( 3.80 \times 10^6 + 4.34 \times 10^6 \right) \text{ m}^3 = 8.14 \times 10^6 \text{ m}^3$$

$$\text{Volume}_{\text{total}} = 8.14 \times 10^6 \text{ m}^3 \left( \frac{\text{acre}}{4046.9 \text{ m}^2} \right) \left( \frac{\text{ft}}{0.3048 \text{ m}} \right) = 6600 \text{ acre-ft}$$

Required pond area for depth of 0.9144 m (3 feet):

$$8.14 \times 10^6 \text{ m}^3 = \text{Area}_{\text{total}} (0.9144 \text{ m})$$

$$\Rightarrow \text{Area}_{\text{total}} = 8.90 \times 10^6 \text{ m}^2$$

$$6,600 \text{ acre-ft} = \text{Area}_{\text{total}} (3 \text{ ft})$$

$$\Rightarrow \text{Area}_{\text{total}} = 2200 \text{ acre}$$

REFERENCES


APPENDIX H

MATLAB® CODE FOR ALGAL GROWTH MODELS

Dynamic algal growth models intended to predict algal biomass and carbonate species concentrations in closed and open batch reactors were developed using Matlab® software. For each model, two files were created. Differential equations governing the system were described in a “model file.” Second, an “executable file” was used to solve the system of MBEs for user-defined initial conditions and either ODE15s or ODE23tb solvers provided by Matlab®. Only the models employing the CO$_2$/HCO$_3$/CO$_3^{2-}$ substitutable substrates model to estimate TIC-limited algal specific growth rates are included in Appendix H.

CLOSED ALGAL GROWTH MODEL

Model File

The following code was used to describe the system of differential equations describing a closed batch algal reactor. Data for the 50% C reactor from Run 2C is shown.
function yp = algalModelCarbonateClosed(t,y);

%*****************************************************************************Equilibrium Constants*****************************************************************************%

%%Note: Temperatures in Kelvin. K1, K2, K3, and KW are specified for 25C, but temperature-dependent relationships may also be used.

T = 25+273.15;
KH2CO3 = 1.72e-4;
KW = 1E-14;
K1 = 4.73151e-7;
K2 = 4.68813e-11;
K3 = 4645.15;

%K1 = exp(290.9097-(14554.21/T)-(45.0575*log(T)));
%K2 = exp(207.6548-(11843.79/T)-(33.6485*log(T)));
%K3 = 10^((1568.94/T)+0.4134-(0.006737*T));

%*****************************************************************************Carbonate Kinetic Constants*****************************************************************************%

%%Note: Temperatures in Kelvin.
%%Note: kplus6 and kminus6 not used in model simulations.
%%Note: kminus may be calculated using an experimentally-determined temperature-dependent relationship OR using its relationship to K1

kplus = 10^(10.685-(3618/T))*3600; % (1/hr)
kH2CO3 = 10^(13.770-(3699/T))*3600; % (1/hr)
kminus = kplus/K1; % (1/M-hr)
%kminus = %kh2CO3/KH2CO3; % (1/hr)
kplus4 = 10^(13.589-(2887/T))*3600; % (1/M-hr)
kminus4 = 10^(14.88-(5524/T))*3600; % (1/hr)
kplus7 = 1.411e-3 *3600; % (M/hr)
kminus7 = kplus7/KW; % (1/M-hr)
kminus5 = 5e10 * 3600; % (1/M-hr)
kplus5 = kminus5 *K2; % (1/M-hr)
%kplus6 = 3e6 *3600 % (1/M-sec), Eigen, 1964 (I = 1)
%kminus6 = kplus6/K3; % (1/hr)

%*****************************************************************************Carbonate Rate Definitions*****************************************************************************%

%%Note: rf5 and rr5 not used in model simulations.

rf1 = kplus*y(1);
rr1 = kminus*y(3)*y(4);
rf2 = kplus4*y(1)*y(5);
rr2 = kminus4*y(4);
rf3 = kplus7;
rr3 = kminus7*y(3)*y(5);
rf4 = kminus5*y(2)*y(3);
rr4 = kplus5*y(4);
%rf5 = kplus6*y(4)*y(5); %rr5 = kminus6*y(2);
%*************TIC-limited Algal Growth Kinetic Constants*************%

\[ b = 0.00285; \quad \text{(1/hr)} \]
\[ K_{\text{CO}_2} = 4.47 \times 10^{-8}; \quad \text{(mol C/L)} \]
\[ K_{\text{HCO}_3} = 3.88 \times 10^{-4}; \quad \text{(mol/L C)} \]
\[ K_{\text{CO}_3} = 8.7 \times 10^{-4}; \quad \text{(mol/L C)} \]
\[ \mu_{\text{Max}} = 0.0726; \quad \text{(hr}^{-1}) \]

%**********TIC-Limited Algal Growth Stoichiometric Constants**********%

%%Note: Choose Nfactor, Pfactor, Cfactor based on TIC treatment. Be sure C:N:P ratios are also specified correctly in the demo file.

\[ N_{\text{factor}} = 0.947; \quad \text{(mol N/mol X)} \]
\[ P_{\text{factor}} = 1; \quad \text{(mol P/mol X)} \]
\[ C_{\text{factor}} = 6.18; \quad \text{(mol C/mol X)} \]

%%Note: Molecular weight of algae calculated based on C:N:P ratios and general stoichiometric equation for algal growth

\[ \text{CH}_2\text{O} = C_{\text{factor}} \times (12.0107 + (2 \times 1.00794) + 15.9994); \]
\[ \text{NH}_3 = N_{\text{factor}} \times (14.0067 + (3 \times 1.00794)); \]
\[ \text{H}_3\text{PO}_4 = P_{\text{factor}} \times (3 \times 1.00794) + 30.9738 + 15.9994; \]
\[ \text{MW}_{\text{algae}} = \text{CH}_2\text{O} + \text{NH}_3 + \text{H}_3\text{PO}_4; \quad \text{(g/mol X)} \]

%%Note: Photosynthetic oxygen production (p) can be calculated based on Redfield equation, or specified.

\[ p = 0.5 \times \left( \frac{212}{106} \times C_{\text{factor}} \right) + (4 \times N_{\text{factor}}); \]

%%Note: H2Ofactors (mol H2O/mol X) and Hfactors (mol H/mol X) are calculated based on C:N:P ratios and general stoichiometric equation for algal growth.

\[ H_{2\text{O}_{\text{factor}}} \text{CO}_2 = -C_{\text{factor}} - (3 \times N_{\text{factor}}) + (2 \times p); \]
\[ H_{\text{factor}} \text{CO}_2 = (2 \times C_{\text{factor}}) + (3 \times N_{\text{factor}}) + 2 - (2 \times H_{2\text{O}_{\text{factor}}} \text{CO}_2); \]

\[ H_{2\text{O}_{\text{factor}}} \text{HCO}_3 = -(C_{\text{factor}} \times 2) - (3 \times N_{\text{factor}}) + (2 \times p); \]
\[ H_{\text{factor}} \text{HCO}_3 = C_{\text{factor}} + (3 \times N_{\text{factor}}) + 3 - 1 - (2 \times H_{2\text{O}_{\text{factor}}} \text{HCO}_3); \]

\[ H_{2\text{O}_{\text{factor}}} \text{CO}_3 = -(2 \times C_{\text{factor}}) - (3 \times N_{\text{factor}}) + (2 \times p); \]
\[ H_{\text{factor}} \text{CO}_3 = (2 \times C_{\text{factor}}) + (3 \times N_{\text{factor}}) + 2 - (2 \times H_{2\text{O}_{\text{factor}}} \text{CO}_3); \]

%*************Light-Limited Algal Growth Kinetic Constants*************%

\[ k_1 = 45.9 \times 3600; \quad \text{(micro-E/m}^2\text{hr)} \]
\[ I_0 = 121 \times 3600; \quad \text{(micro-E/m}^2\text{hr)} \]
\[ TSS = y(7) \times \text{MW}_{\text{algae}} \times 1000; \quad \text{(g/m}^3\text{)} \text{ or (mg/L)} \]
\[ K = 1.97 \times 0.0575 \times TSS; \quad \text{(1/m)} \]
\[ h = 0.2032; \quad \text{(m)} \text{-- 8 in.} \]
\[ I = \left( I_0 \times (1 - \exp(-K \times (h))) \right) / (K \times (h)); \quad \text{(micro-E/m}^2\text{hr)} \]
%**********TIC and Light-Limited Algal Specific Growth Rates***********%

\[
\begin{align*}
\mu_{\text{CO}_2} &= \mu_{\text{Max}} \times \left( \frac{y(1)}{(K_{s\text{CO}_2} + y(1))} \right) \times \left( \frac{I}{K_{sl} + I} \right), \\
\mu_{\text{HCO}_3} &= \mu_{\text{Max}} \times \left( \frac{y(4)}{(K_{s\text{HCO}_3} + y(4))} \right) \times \left( \frac{K_{s\text{CO}_2}}{(K_{s\text{CO}_2} + y(1))} \right) \times \left( \frac{I}{K_{sl} + I} \right), \\
\mu_{\text{CO}_3} &= \mu_{\text{Max}} \times \left( \frac{y(2)}{(K_{s\text{CO}_3} + y(2))} \right) \times \left( \frac{K_{s\text{CO}_2}}{(K_{s\text{CO}_2} + y(1))} \right) \times \left( \frac{I}{K_{sl} + I} \right),
\end{align*}
\]

%********************Nutrient Utilization Rates**************************%

%%Note: Nutrient utilization rates calculated based on specific growth rates and stoichiometric relationships.

\[
\begin{align*}
C_{\text{utilization CO}_2} &= C_{\text{factor}} \times \mu_{\text{CO}_2} \times y(7), \\
C_{\text{utilization HCO}_3} &= C_{\text{factor}} \times \mu_{\text{HCO}_3} \times y(7), \\
C_{\text{utilization CO}_3} &= C_{\text{factor}} \times \mu_{\text{CO}_3} \times y(7), \\
H_{\text{utilization CO}_2} &= H_{\text{factor CO}_2} \times \mu_{\text{CO}_2} \times y(7), \\
H_{\text{utilization HCO}_3} &= H_{\text{factor HCO}_3} \times \mu_{\text{HCO}_3} \times y(7), \\
H_{\text{utilization CO}_3} &= H_{\text{factor CO}_3} \times \mu_{\text{CO}_3} \times y(7), \\
H_{\text{2Outilization CO}_2} &= H_{\text{2Ofactor CO}_2} \times \mu_{\text{CO}_2} \times y(7), \\
H_{\text{2Outilization HCO}_3} &= H_{\text{2Ofactor HCO}_3} \times \mu_{\text{HCO}_3} \times y(7),
\end{align*}
\]

%**********************Differential Equations***************************%

% CO2 -- y(1)
\[
\text{CO2\_balance} = -r_{f1} + r_{r1} - r_{f2} + r_{r2} - C_{\text{utilization CO}_2};
\]

% CO3 -- y(2)
\[
\text{CO3\_balance} = -r_{f4} + r_{r4} - C_{\text{utilization CO}_3}; \% + r_{f5} - r_{r5};
\]

% H -- y(3)
\[
\text{H\_balance} = r_{f1} - r_{r1} + r_{f3} - r_{r3} - r_{f4} + r_{r4} - H_{\text{utilization CO}_2} - H_{\text{utilization HCO}_3} - H_{\text{utilization CO}_3};
\]

% HCO3 -- y(4)
\[
\text{HCO3\_balance} = r_{f1} - r_{r1} + r_{f2} - r_{r2} + r_{f4} - r_{r4} - C_{\text{utilization HCO}_3}; \% r_{f5} + r_{r5};
\]

% OH -- y(5)
\[
\text{OH\_balance} = -r_{f2} + r_{r2} + r_{f3} - r_{r3}; \% - r_{f5} + r_{r5};
\]

% H2O -- y(6)
\[
\text{H2O\_balance} = -r_{f1} + r_{r1} - r_{f3} + r_{r3} - H_{\text{2Outilization CO}_2} - H_{\text{2Outilization HCO}_3}; \% + r_{f5} - r_{r5};
\]

% Biomass -- y(7)
\[
\text{XformCO}_2 = \mu_{\text{CO}_2} \times y(7); \\
\text{XformHCO}_3 = \mu_{\text{HCO}_3} \times y(7); \\
\text{XformCO}_3 = \mu_{\text{CO}_3} \times y(7); \\
\text{Xdecay} = b \times y(7); \\
\text{Xbalance} = \text{XformCO}_2 + \text{XformHCO}_3 + \text{XformCO}_3 - \text{Xdecay};
\]

% TIC -- y(8)
\[
\text{CarbonBalance} = (\text{CO2\_balance} + \text{CO3\_balance} + \text{HCO3\_balance});
\]
**System of Differential Equations**

yp = [CO2_balance
      CO3_balance
      H_balance
      HCO3_balance
      OH_balance
      H2O_balance
      Xbalance
      CarbonBalance];

**Executable File**

The following code was used to solve the system of differential equations describing a closed batch algal reactor. Only the initial value vector for the 50% C reactor from Run 2C is shown.

type algalModelCarbonateClosed;

%NOTE: THIS FILE SOLVES THE SET OF DIFFERENTIAL EQUATIONS USING %USER-DEFINED INITIAL CONDITIONS. OUTPUT PLOTS INCLUDE CARBONATE SPECIES, BIOMASS CONCENTRATION (mg/L), pH, and ALKALINITY.

%**********Simulate the System of Differential Equations**********
[t,y] = ode23tb(@algalModelCarbonateClosed,[t0 tfinal],y0);

%**********Other Values**********
alk = (y(:,4))+(2*y(:,2)) + y(:,5) - y(:,3);
pH = -log10(y(:,3));
totalcarbon = (y(:,1)) + (y(:,2)) + (y(:,4));
Nfactor = 0.947; \quad \% \text{ (mol N/mol X)}
Pfactor = 1; \quad \% \text{ (mol P/mol X)}
Cfactor = 6.18; \quad \% \text{ (mol C/mol X)}

CH\textsubscript{2}O = Cfactor*(12.0107+(2*1.00794)+15.9994);
NH\textsubscript{3} = Nfactor*(14.0067+(3*1.00794));
H\textsubscript{3}PO\textsubscript{4} = Pfactor*(3*1.00794)+30.9738+15.9994;
MW\textsubscript{algae} = CH\textsubscript{2}O + NH\textsubscript{3} + H\textsubscript{3}PO\textsubscript{4}; \quad \% \text{ (g/mol X)}

figure(1);
plot(t, y(:,7)*MWalgae*1000, '-.
', 'Color', [0.48,0.06,0.89], 'LineWidth',3)
hold on
plot(Time, Biomass\_mgperL, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0.0,100]);
xlim ([0,300]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Biomass (mol/L)', 'FontSize', 25, 'FontName', 'Times');
h\_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h\_legend, 'FontName', 'Times', 'FontSize', 25);

figure(2);
plot(t, y(:,8), '-.', 'Color', [0.48,0.06,0.89], 'LineWidth',3)
hold on
plot(Time, TIC\_molperL, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0.0,0.003]);
xlim ([0,300]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Total Inorganic Carbon (mol/L C)', 'FontSize', 25, 'FontName', 'Times');
h\_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h\_legend, 'FontName', 'Times', 'FontSize', 20);

figure(3);
plot(t, y(:,1), '-.', 'Color', [0.48,0.06,0.89], 'LineWidth',3)
hold on
plot(Time, Carbon\_Dioxide, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0.0,4e-7]);
xlim ([0,300]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Carbon Dioxide (mol/L C)', 'FontSize', 25, 'FontName', 'Times');
h\_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h\_legend, 'FontName', 'Times', 'FontSize', 20);
figure(4);
plot(t, y(:,4), '-.', 'Color', [0.48, 0.06, 0.89], 'LineWidth', 3)
hold on
plot(Time, Bicarbonate, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0.0, 0.0014]);
xlim ([0, 300]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Bicarbonate (mol/L C)', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize', 20);

figure(5);
plot(t, y(:,2), '-.', 'Color', [0.48, 0.06, 0.89], 'LineWidth', 3)
hold on
plot(Time, Carbonate, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0.0, 0.0018]);
xlim ([0, 300]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Carbonate (mol/L C)', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize', 20);

figure(6);
plot(t, alk, '-.', 'Color', [0.48, 0.06, 0.89], 'LineWidth', 3)
hold on
plot(Time, Alk_molperL, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0, 0.005]);
xlim ([0, 300]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Alkalinity (mol/L)', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize', 20);

figure(7);
plot(t, pH, '-.', 'Color', [0.48, 0.06, 0.89], 'LineWidth', 3)
ylim ([9.5, 12]);
xlim ([0, 300]);
hold on
plot(Time, pHexperimental, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('pH', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize', 20);
OPEN ALGAL GROWTH MODEL

Model File

The following code was used to describe the system of differential equations describing an open batch algal reactor. Data for the 50% C reactor of Run 10 is included.

```matlab
function yp = algalModelCarbonateOpen(t,y);

%******************************************************************************%
%Equilibrium Constants******************************************************************************%

%Note: Temperatures in Kelvin. K1, K2, K3, and KW are specified for 25C, but temperature-dependent relationships may alternatively be used.

T = 25+273.15;
KH2CO3 = 1.72e-4;
KW = 1E-14;
K1 = 4.73151e-7;
K2 = 4.68813e-11;
K3 = 4645.15;

%K1 = exp(290.9097-(14554.21/T)-(45.0575*log(T)));
%K2 = exp(207.6548-(11843.79/T)-(33.6485*log(T)));
%K3 = 10^((1568.94/T)+0.4134-(0.006737*T));

%******************************************************************************Carbonate Kinetic Constants******************************************************************************%

%Note: Temperatures in Kelvin.
%Note: kplus6 and kminus6 not used in model simulations.
%Note: kminus may be calculated using an experimentally-determined temperature-dependent relationship OR using its relationship to K1

kplus = 10^(10.685-(3618/T))*3600;                % (1/hr)
kh2CO3 = 10^(13.770-(3699/T))*3600;               % (1/hr)
kminus = kplus/K1;                                % (1/M-hr)
%kminus = %kh2CO3/KH2CO3;                         % (1/hr)
kplus4 = 10^(13.589-(2887/T))*3600;              % (1/hr)
kminus4 = 10^(14.88-(5524/T))*3600;              % (1/hr)
kplus7 = 1.411e-3 *3600;                         % (M/hr)
kminus7 = kplus7/KW;                              % (1/M-hr)
kminus5 = 5e10 * 3600;                            % (1/M-hr)
kplus5 = kminus5 *K2;                            % (1/M-hr)
%kplus6 = 3e6 *3600                                % (1/M-sec), Eigen, 1964 (I = 1)
%kminus6 = kplus6/K3;                             % (1/hr)
```
%**************************Carbonate Rate Definitions**************************%

%Note: rf5 and rr5 not used in model simulations.

rf1 = kplus*y(1);
rr1 = kminus*y(3)*y(4);
rf2 = kplus4*y(1)*y(5);
rr2 = kminus4*y(4);
rf3 = kplus7;
rr3 = kminus7*y(3)*y(5);
rr4 = kminus5*y(2)*y(3);
rf4 = kplus5*y(4);
%rf5 = kplus6*y(4)*y(5);
%rr5 = kminus6*y(2);

%*************TIC-limited Algal Growth Kinetic Constants*************%

b = 0.00285; % (1/hr)
KsCO2 = 4.47e-8; % (mol C/L)
KsHCO3 = 3.88e-4; % (mol/L C)
KsCO3 = 8.70e-4; % (mol/L C)
MuMax = 0.0726; % (hr^-1)

%**********TIC-Limited Algal Growth Stoichiometric Constants**********%

%Note: Choose Nfactor, Pfactor, Cfactor based on TIC treatment. Be sure C:N:P ratios are also specified correctly in the demo file.

Nfactor = 5.26; % (mol N/mol X)
Pfactor = 1; % (mol P/mol X)
Cfactor = 45.09; % (mol C/mol X)

%Note: Molecular weight of algae calculated based on C:N:P ratios and general stoichiometric equation for algal growth

CH2O = Cfactor*(12.0107+(2*1.00794)+15.9994);
NH3 = Nfactor*(14.0067+(3*1.00794));
H3PO4 = Pfactor*(3*1.00794)+30.9738+15.9994;
MWalgae = CH2O + NH3 + H3PO4; % (g/mol X)

%Note: Photosynthetic oxygen production (p) can be calculated based on Redfield equation, or specified.

p = 0.5* ((212/106*Cfactor)+(4*Nfactor));

%Note: H2Ofactors (mol H2O/mol X) and Hfactors (mol H/mol X) are calculated based on C:N:P ratios and general stoichiometric equation for algal growth.

H2OfactorCO2 = -Cfactor-(3*Nfactor)+(2*p);
HfactorCO2 = (2*Cfactor)+(3*Nfactor)+2-(2*H2OfactorCO2);
\[ \text{H}_2\text{OfactorHCO}_3 = (-C\text{factor} \times 2) - (3 \times N\text{factor}) + (2 \times p) ; \]
\[ \text{HfactorHCO}_3 = C\text{factor} + (3 \times N\text{factor}) + 3 - 1 - (2 \times \text{H}_2\text{OfactorHCO}_3) ; \]

\[ \text{H}_2\text{OfactorCO}_3 = (-2 \times C\text{factor}) - (3 \times N\text{factor}) + (2 \times p) ; \]
\[ \text{HfactorCO}_3 = (2 \times C\text{factor}) + (3 \times N\text{factor}) + 2 - (2 \times \text{H}_2\text{OfactorCO}_3) ; \]

%************Light-Limited Algal Growth Kinetic Constants*************%

\[ K_{sl} = 45.9 \times 3600 ; \quad \% \text{(micro-E/m}^2\text{hr)} \]
\[ I_o = 121 \times 3600 ; \quad \% \text{(micro-E/m}^2\text{hr)} \]
\[ \text{TSS} = y(7) \times \text{MWalgae} \times 1000 ; \quad \% \text{(g/m}^3\text{)} \text{ or (mg/L)} \]
\[ K = 1.97 + (0.0575 \times \text{TSS}) ; \quad \% \text{(1/m)} \]
\[ h = 0.2032 ; \quad \% \text{(m)--8 in.} \]
\[ I = \frac{I_o \times (1 - \exp(-K \times (h)))}{K \times (h)} ; \quad \% \text{(micro-E/m}^2\text{hr)} \]

%**********TIC and Light-Limited Algal Specific Growth Rates***********%

%Note: Specific growth rates (1/hr) calculated based on Monod models.

\[ \text{MuCO}_2 = \text{MuMax} \times \left( \frac{(y(1)/(KsCO_2+y(1))) \times (I/(Ksl+I)))}{(I/(Ksl+I)))} \right) ; \]
\[ \text{MuHCO}_3 = \text{MuMax} \times \left( \frac{(y(4)/(KsHCO_3+y(4))) \times (KsCO_2/(KsCO_2+y(1))) \times (I/(Ksl+I)))}{(I/(Ksl+I)))} \right) ; \]
\[ \text{MuCO}_3 = \text{MuMax} \times \left( \frac{(y(2)/(KsCO_3+y(2))) \times (KsCO_2/(KsCO_2+y(1))) \times (I/(Ksl+I)))}{(I/(Ksl+I)))} \right) ; \]

%*******************Nutrient Utilization Rates**************************%

%Note: Nutrient utilization rates calculated based on specific growth rates and stoichiometric relationships.

\[ \text{CutilizationCO}_2 = C\text{factor} \times \text{MuCO}_2 \times y(7) ; \]
\[ \text{CutilizationHCO}_3 = C\text{factor} \times \text{MuHCO}_3 \times y(7) ; \]
\[ \text{CutilizationCO}_3 = C\text{factor} \times \text{MuCO}_3 \times y(7) ; \]
\[ \text{HutilizationCO}_2 = \text{HfactorCO}_2 \times \text{MuCO}_2 \times y(7) ; \]
\[ \text{HutilizationHCO}_3 = \text{HfactorHCO}_3 \times \text{MuHCO}_3 \times y(7) ; \]
\[ \text{HutilizationCO}_3 = \text{HfactorCO}_3 \times \text{MuCO}_3 \times y(7) ; \]
\[ \text{H2OutilizationCO}_2 = \text{H2OfactorCO}_2 \times \text{MuCO}_2 \times y(7) ; \]
\[ \text{H2OutilizationHCO}_3 = \text{H2OfactorHCO}_3 \times \text{MuHCO}_3 \times y(7) ; \]

%******************Diffusion Constants/Equations**************************%

%FICK'S LAW
\[ A = 5019e-9 \times 3600 ; \quad \% m^2/hr \]
\[ E_a = 19.51 ; \quad \% kJ/mol \]
\[ R = 8.3142/1000 ; \quad \% kJ/K-mol \]
\[ D\text{CO}_2 = A \times \exp(-E_a/(R \times T)) ; \quad \% (m^2/hr) \]
\[ L = 117e-6 ; \quad \% (m) \]
\[ keff = kplus+(kplus4 \times y(5)) ; \quad \% (1/sec) \]
\[ ak = (D\text{CO}_2/keff)^0.5 ; \quad \% (m) \]
%Henry's Law
pCO2 = 3.16e-4 % (atm)
KhCO2 = 3.3884e-2 % (M/atm), Stumm pg. 204
CO2eq = KhCO2 * pCO2;
SAV = 4.92; % (1/m)

%DIFFUSION EQUATION
rdiff = (DCO2/ak)*(CO2eq - y(1))*coth(L/ak)*SAV; % (M/s)

%********************Differential Equations**************************%

%CO2 -- y(1)
CO2_balance = -rf1 +rr1 -rf2 +rr2 -CutilizationCO2+rdiff;

%CO3 -- y(2)
CO3_balance = -rr4 +rf4 -CutilizationCO3;% +rf5 -rr5;

%H -- y(3)
H_balance = rf1 -rr1 +rf3 -rr3 -rr4 +rf4 -HutilizationCO2 -
HutilizationHCO3 -HutilizationCO3;

%HCO3 -- y(4)
HCO3_balance = rf1 -rr1 +rf2 -rr2 +rr4 -rf4 -CutilizationHCO3;

%OH -- y(5)
OH_balance = -rf2 +rr2 +rf3 -rr3; % -rf5 +rr5;

%H2O -- y(6)
H2O_balance = -rf1 +rr1 -rf3 +rr3 -H2OutilizationCO2 -
H2OutilizationHCO3;% +rf5-rr5;

%Biomass -- y(7)
XformCO2 = MuCO2*y(7);
XformHCO3 = MuHCO3*y(7);
XformCO3 = MuCO3*y(7);
Xdecay = b*y(7);
Xbalance = XformCO2 + XformHCO3+XformCO3 - Xdecay;

% TIC -- y(8)
CarbonBalance = (CO2_balance + CO3_balance + HCO3_balance);
%System of Differential Equations

yp = [CO2_balance
     CO3_balance
     H_balance
     HCO3_balance
     OH_balance
     H2O_balance
     Xbalance
     CarbonBalance];

Executable File

The following code was used to solve the system of differential equations describing an open batch algal reactor. Only the initial value vector for the 50% C reactor from Run 10 is shown.

type algalModelCarbonateOpen;

%NOTE: THIS FILE SOLVES THE SET OF DIFFERENTIAL EQUATIONS USING USER-DEFINED INITIAL CONDITIONS. OUTPUT PLOTS INCLUDE CARBONATE SPECIES, BIOMASS CONCENTRATION (mg/L), pH, and ALKALINITY.

%Format Chart Axes

set (0, 'defaultaxesfontsize',25);
set (0, 'defaultaxesfontname','Times');

%Define Initial Conditions

%%Note: Choose initial value vector or input a new one.
t0 = 0;
tfinal = 1500;
y0 = [1.54922e-7 0.000660866 8.12831e-11 0.00095 5242 0.000123027 55.5 3.707E-6 0.001616262]; %50C

%Simulate the System of Differential Equations

[t,y] = ode23tb(@algalModelCarbonateOpen,[t0 tfinal],y0);

%Other Values

alk = (y(:,4))+(2*y(:,2)) + y(:,5) - y(:,3);
pH = -log10(y(:,3));
totalcarbon = (y(:,1)) + (y(:,2)) + (y(:,4));
Nfactor = 5.26; % (mol N/mol X)
Pfactor = 1; % (mol P/mol X)
Cfactor = 45.09; % (mol C/mol X)

CH2O = Cfactor*(12.0107+(2*1.00794)+15.9994);
NH3 = Nfactor*(14.0067+(3*1.00794));
H3PO4 = Pfactor*(3*1.00794)+30.9738+15.9994;

MWalgae = CH2O + NH3 + H3PO4; % (g/mol X)

%********************Create Formatted Output Plots*******************%
```matlab
figure(4);
plot(t, y(:,4), '-.', 'Color', [0.48,0.06,0.89], 'LineWidth',3)
hold on
plot(Time, Bicarbonate, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0.0,2e-3]);
xlim ([0,1500]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Bicarbonate (mol/L C)', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize',20);

figure(5);
plot(t, y(:,2), '-.', 'Color', [0.48,0.06,0.89], 'LineWidth',3)
hold on
plot(Time, Carbonate, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0.0,5e-3]);
xlim ([0,1500]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Carbonate (mol/L C)', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize', 20);

figure(6);
plot(t, alk, '-.', 'Color', [0.48,0.06,0.89], 'LineWidth',3)
hold on
plot(Time, Alk_molperL, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
ylim ([0,10e-3]);
xlim ([0,1500]);
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('Alkalinity (mol/L)', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize', 20);

figure(7);
plot(t, pH, '-.', 'Color', [0.48,0.06,0.89], 'LineWidth',3)
ylim ([7,14]);
xlim ([0,1500]);
hold on
plot(Time, pHexperimental, 'ko', 'MarkerSize', 10, 'MarkerFaceColor', 'k')
xlabel('Time (hr)', 'FontSize', 25, 'FontName', 'Times');
ylabel('pH', 'FontSize', 25, 'FontName', 'Times');
h_legend=legend('Model Prediction', 'Experimental Results', 1);
set(h_legend, 'FontName', 'Times', 'FontSize', 20);
```
The closed algal growth model was calibrated and verified using data from unadjusted initial pH reactors (Run 1C) and adjusted initial pH reactors (Run 2C), with initial values shown in Table I.1. Results using data from Run 2C are included in Chapter 4, while results using data from Run 1C are presented in Appendix I. After calibration for the stoichiometric coefficient for oxygen production (p) (Figures I.1 through I.8), it was found that $1.25*p_r$ best represented data from 25 and 75% C reactors of Run 1C. Verification of the model was completed using data from the 50% C reactor of Run 1C (Figures I.9 through I.22). As with data from Run 2C, the model reasonably predicts biomass, CO$_2$, and HCO$_3^-$ concentrations, while it under-predicts CO$_3^{2-}$ concentrations near the end of exponential growth.

Table I.1. Initial values used for comparison, calibration, and verification of closed and open algal growth models.

<table>
<thead>
<tr>
<th>Species</th>
<th>Units</th>
<th>Reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25% C</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>(mol/L C)</td>
<td>3.870E-07</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>(mol/L C)</td>
<td>8.865E-04</td>
</tr>
<tr>
<td>CO$_3^{2-}$</td>
<td>(mol/L C)</td>
<td>2.279E-04</td>
</tr>
<tr>
<td>H$^+$</td>
<td>(mol/L C)</td>
<td>2.188E-10</td>
</tr>
<tr>
<td>OH$^-$</td>
<td>(mol/L C)</td>
<td>4.571E-05</td>
</tr>
<tr>
<td>TIC</td>
<td>(mol/L C)</td>
<td>1.115E-03</td>
</tr>
<tr>
<td>X$^1$</td>
<td>(mol/L X)</td>
<td>2.571E-05</td>
</tr>
</tbody>
</table>

$^1$Initial mass-based biomass concentrations were converted to a molar basis using experimentally determined MW values.
Figures I.1 - I.2: Effect of $p$ on pH (top left) and residual plots (bottom left) (25% reactor; Run 1C).
Figures I.3 - I.4: Effect of $p$ on alkalinity (top right) and residual plots (top left) (25% reactor; Run 1C).
Figures I.5 - I.6: Effect of p on pH (top left) and residual plots (bottom left) (75% reactor; Run 1C).
Figures I.7 - I.8: Effect of p on alkalinity (top right) and residual plots (top left) (75% reactor; Run 1C).
Figures I.9 - I.10: Biomass (top left) and residual plots (bottom left) for calibrated closed model (50% reactor; Run 1C).

Figures I.11 - I.12: TIC (top left) and residual plots (bottom left) for calibrated closed model (50% reactor; Run 1C).
Figures I.13 - I.14: pH (top left) and residual plots (bottom left) for calibrated closed model (50% reactor; Run 1C).
Figures I.15 - I.16: Alk (top left) and residual plots (bottom left) for calibrated closed model (50% reactor; Run 1C).
Figures I.17 - I.18: \( \text{CO}_2 \) (top left) and residual plots (bottom left) for calibrated closed model (50% reactor; Run 1C). Figures I.19 - I.20: \( \text{CO}_3^{2-} \) (top left) and residual plots (bottom left) for calibrated closed model (50% reactor; Run 1C).
Figures I.21 - I.22: CO$_3^{2-}$ (top left) and residual plots (bottom left) for calibrated closed model (50% reactor; Run 1C).