

DIATOM BIOFUELS: OPTIMIZING NUTRIENT REQUIREMENTS FOR GROWTH
AND LIPID ACCUMULATION IN YNP ISOLATE RGd-1

by

Karen Margaret Moll

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Microbiology

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 2012

©COPYRIGHT

by

Karen Margaret Moll

2012

All Rights Reserved

APPROVAL

of a thesis submitted by

Karen Margaret Moll

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency and is ready for submission to The Graduate School.

Dr. Brent M. Peyton

Approved for the Department of Microbiology

Dr. Mark Jutila

Approved for The Graduate School

Dr. Ronald W. Larsen

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Karen Margaret Moll

November, 2012

DEDICATION

This thesis is dedicated to my father, Keith Moll.

TABLE OF CONTENTS

1. INTRODUCTION AND BACKGROUND.....	1
Introduction:	1
Background	3
The Diatoms	3
Silica Utilization.....	7
Carbon Utilization	11
Iron Utilization	12
Genetically Modified versus Natural Strains	13
Summary	15
2. OPTIMIZATION OF GROWTH AND LIPID ACCUMULATION.....	16
Introduction	16
Methods	20
Microorganism & Growth Systems.....	20
Growth Studies	21
Dry Cell Weight	22
Nitrate Utilization and Chlorophyll Concentration	22
Dissolved Inorganic Carbon (DIC)	23
Silicon and Iron Quantification	23
TAG/FAME Analyses	24
DNA Extraction, Amplification and Clone Libraries	25
Results & Discussion.....	27
Silica Optimization Experiments.....	27
Sodium Bicarbonate Addition and Nitrate Limitation	36
Lipid Accumulation.....	39
Conclusions:	42
Acknowledgements	44
3. CONCLUSIONS	46
Suggestions For Future Work.....	46
REFERENCES	48

TABLE OF CONTENTS-CONTINUED

APPENDICES	57
APPENDIX A: Experimental Data	58
APPENDIX B: Abiotic Controls.....	153
APPENDIX C: Growth Medium.....	163
APPENDIX D: Strain Identification Using Molecular Techniques.....	165

LIST OF TABLES

Table	Page
2.1 Final growth and lipid accumulation characteristics at the completion of each condition tested: cell yield (cells mL ⁻¹), Nile Red fluorescence, specific Nile Red fluorescence, Dry Cell Weight, % TAG and % biofuel potential (BP).....	30
A.1 Cell Concentration (cells mL ⁻¹) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).....	59
A.2 Cell Concentration (cells mL ⁻¹) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).....	60
A.3 Total Silicon Concentration (mM) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).....	61
A.4 Total Silicon Concentration (mM) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).....	62
A.5 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 0 Si.....	63
A.6 Raw Data (cps) measured using ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 0.5 mM Si.....	63
A.7 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 1.0 mM Si.....	64
A.8 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 1.5 mM Si.....	64
A.9 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 2.0 mM Si.....	65
A.10 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 2.5 mM Si.....	66
A.11 Measured pH for growth in each silica concentration (0, 0.5 and 1.0 mM Si).....	67

LIST OF TABLES-CONTINUED

Table	Page
A.12 Measured pH for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).	68
A.13 Total Nile Red Fluorescence for growth in each silica concentration (0, 0.5 and 1.0 mM Si).	69
A.14 Total Nile Red Fluorescence for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).	70
A.15 Specific Nile Red Fluorescence (Nile Red Fluorescence x 10000/cell count) for growth in each silica concentration (0, 0.5, 1.0 mM Si)	71
A.16 Specific Nile Red Fluorescence (Nile Red Fluorescence x 10000/cell count) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).	72
A.17 Measured Nitrate Concentration (mg/L) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).	75
A.18 Measured Nitrate Concentration (mg/L) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).	76
A.19 NO ₃ ⁻ Peak Areas run on Ion Chromatography for growth in each silica concentration (0, 0.5 and 1.0 mM Si).	77
A.20 NO ₃ ⁻ Peak Areas run on Ion Chromatography for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).	78
A.21 Chlorophyll Concentration (mg/L) in each silica concentration (0, 0.5 and 1.0 mM Si).	79
A.22 Chlorophyll Concentration (mg/L) in each silica concentration (1.5, 2.0 and 2.5 mM Si).	80
A.23 Iron concentration (uM) measured using ICP-MS for growth in each silica concentration (0, 0.5 and 1.0 mM Si).	81

LIST OF TABLES-CONTINUED

Table	Page
A.24 Iron concentration (μM) measured using ICP-MS for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).....	82
A.25 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 0 Si.	83
A.26 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 0.5 mM Si.....	83
A.27 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 1.0 mM Si.....	84
A.28 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 1.5 mM Si.....	84
A.29 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 2.0 mM Si.....	85
A.30 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 2.5 mM Si.....	86
A.31 Phosphate (mg/L) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).	87
A.32 Phosphate (mg/L) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).	88
A.33 Phosphate Peak Areas using Ion Chromatography for growth in each silica concentration (0, 0.5, 1.0 and 1.5 mM Si).....	90
A.34 Phosphate Peak Areas using Ion Chromatography for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).....	91
A.35 Cell concentration (cells mL^{-1}) when grown in 2 mM Si/2.94 mM NO_3^- with and without NaHCO_3 addition.....	92
A.36 Cell concentration (cells mL^{-1}) when grown in 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.....	93

LIST OF TABLES-CONTINUED

Table	Page
A.37 Measured pH for cells grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	94
A.38 Measured pH for cells grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	95
A.39 Si concentration (mM) when grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	96
A.40 Si concentration (mM) when grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	97
A.41 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 2.0 mM Si and 25 mM NaHCO ₃	98
A.42 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 2.0 mM Si and 1 mM NO ₃ ⁻	99
A.43 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si ₂₈ , Si ₂₉ and Si ₃₀) for growth in 2.0 mM Si, 1 mM NO ₃ ⁻ and 25 mM NaHCO ₃	100
A.44 Total Nile Red fluorescence intensity for cells grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	101
A.45 Total Nile Red fluorescence for cells grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	102
A.46 Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	103
A.47 Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	104
A.48 Measured nitrate concentration (mM) when grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	106

LIST OF TABLES-CONTINUED

Table	Page
A.49 Measured nitrate concentration (mM) when grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	107
A.50 Chlorophyll concentration (mg/L) when grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	108
A.51 Chlorophyll concentration (mg/L) when grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	109
A.52 Dissolved Inorganic Carbon (DIC) concentration (mg/L) when grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	110
A.53 Dissolved Inorganic Carbon (DIC) concentration (mg/L) when grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	111
A.54 Iron concentration (uM) for cells grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	113
A.55 Iron concentration (uM) for cells grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	114
A.56 Raw Data (cps) for Iron measured using ICP-MS for Isotopes (Fe ₅₆ and Fe ₅₇) for growth in 2.0 mM Si and 1 mM NO ₃ ⁻	116
A.57 Raw Data (cps) for Iron measured using ICP-MS for Isotopes (Fe ₅₆ and Fe ₅₇) for growth in 2.0 mM Si, 1 mM NO ₃ ⁻ and 25 mM NaHCO ₃	117
A.58 Raw Data (cps) for Iron measured using ICP-MS for Isotopes (Fe ₅₆ and Fe ₅₇) for growth in 2.0 mM Si.	118
A.59 Phosphate concentration (mM) when grown in 2 mM Si/2.94 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	119
A.60 Phosphate concentration (mM) for cells grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	120
A.61 Cell concentration (cells mL ⁻¹) when grown in 4 & 8 mM Si.	122

LIST OF TABLES-CONTINUED

Table	Page
A.62 Measured pH for cells grown in 4 and 8 mM Si.....	124
A.63 Total Nile Red Fluorescence for cells grown in 4 & 8 mM Si.	126
A.64 Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 4 & 8 mM Si.	128
A.65 Measured Nitrate concentration (mM) when grown 4 & 8 mM Si.....	130
A.66 Chlorophyll concentration (mg/L) when grown in 4 & 8 mM Si.	132
A.67 Dissolved Inorganic Carbon (DIC) (mg/L) for cells grown in 4 & 8 mM Si.	134
A. 68 Si concentration (mM) when grown in 4 & 8 mM Si.	136
A.69 Iron concentration (uM) for cells grown in 4 & 8 mM Si.	138
A.70 Phosphate concentration (mM) for cells grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	140
A.71 Cell concentrations (cells□mL ⁻¹) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	143
A.72 RGd-1 medium pH throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	145
A.73 RGd-1 Total Nile Red fluoresece throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	147
A.74 RGd-1 specific Nile Red fluorescence (Nile Red intensity/cell concentration * 10000) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	149

LIST OF TABLES-CONTINUED

Table	Page
A.75 RGd-1 growth in CHES buffered medium.	151
B.2 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 0 Si (filtered).	159
B.3 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 1 mM Si (unfiltered).	159
B.4 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 1 mM Si (filtered).	159
B.5 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 2.5 mM Si (unfiltered).	159
B.6 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 2.5 mM Si (filtered).	160
B.7 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 0 Si (unfiltered).	160
B.8 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 0 Si (filtered).	160
B.9 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 1.0 mM Si (unfiltered).	160
B.10 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 1.0 mM Si (filtered).	160
B.11 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 2.5 mM Si (unfiltered).	161
B.12 Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 2.5 mM Si (filtered).	161
C.1 Growth Medium: Bold's Basal Medium titrated to 8.7 (with added B_{12} and S3 medium).	164

LIST OF TABLES-CONTINUED

Table	Page
D.1 Thermocycle conditions for amplification of DNA.....	167
D.2 PCR Reaction Components.....	167

LIST OF FIGURES

Figure		Page
1.2	Silicic acid speciation across pH range 6-13 in Bold's Basal Medium with 2 mM Sodium metasilicate. Species distribution was determined using MINTEQ version 3.0.....	11
1.3	Iron speciation across pH range 6-13 in Bold's Basal Medium with 2 mM Sodium metasilicate, using 500 mV for redox potential. Species distribution was determined using MINTEQ version 3.0.....	13
2.1	Field Emission-Scanning Electron Microscopy (FE-SEM) image of RGd-1.....	20
2.2	(A) RGd-1 growth (cells mL ⁻¹) for 6 silica concentrations (B) silica concentrations in the growth medium over time (C) correlation of final dry cell weight produced with silica concentrations.	29
2.3	(A) Chlorophyll production for RGd-1 grown in each silica concentration (B) NO ₃ ⁻ removal from growth medium	31
2.4	(A) Medium pH for diatoms six silica concentrations (B) Nile Red intensity of RGd-1 grown in each of six silica concentrations.	34
2.5	Growth characteristics for RGd-1 grown in four conditions: standard BBM nitrate concentrations 2.94 mM, 2.94 mM NO ₃ ⁻ + 25 mM NaHCO ₃ , 1 mM NO ₃ ⁻ , and 1 mM NO ₃ ⁻ + 25 mM NaHCO ₃ . (A) Diatom cell growth (cells mL ⁻¹) (B) Silicon removal from the growth medium (C) NO ₃ ⁻ from the growth medium (mM) (D) Chlorophyll concentration (mg/L) (E) Medium pH (F) Nile Red fluorescence intensity.....	38
2.6	(A) Transmitted light images of RGd-1 (B) Epifluorescent microscopy of RGd-1 stained with Nile Red.....	39
2.7	Percent lipid (w/w) in biomass: % TAG and % Biofuel Potential (%BP).....	40
2.8	Correlation of C16, C18 and C20 to dry cell weight (DCW).	41

LIST OF FIGURES-CONTINUED

Figure	Page
2.9 Final lipid yields ($\text{g}\cdot\text{L}^{-1}$) of RGd-1 of grown in each condition tested. (A) MAG (blue) DAG (red) TAG (green) and total lipid (orange) of AFDW (B) FAME speciation as % total FAME in 2mM Si system.	41
2.10 RGd-1 cell aggregates following 15 days of growth in (A) 4 mM and (B) 8 mM Sodium metasilicate. Micrographs were obtained using transmitted light with a 40x water immersion objective.	42
A.1 Specific Nile Red Fluorescence (Nile Red Fluorescence x 10000/cell count) for growth in each silica concentration (0, 0.5, 1.0, 1.5, 1.0, 1.5, 2.0 and 2.5 mM Si).	74
A.2 Iron concentration (μM) measured using ICP-MS for growth in each silica concentration (0, 0.5 and 1.0 mM Si).	81
A.3 Phosphate (mg/L) for growth in each silica concentration (0, 0.5, 1.0, 1.5, 1.0, 1.5, 2.0 and 2.5 mM Si).	89
A.4 Specific Nile Red fluorescence (fluorescence per cell = Nile Red fluorescence x 10000/cell count) for cells grown in 2 mM Si/2.94 mM NO_3^- or 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.	105
A.5 Dissolved Inorganic Carbon (DIC) (mg/L) for cells grown in 2 mM Si/2.94 mM NO_3^- or 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.	113
A.6 Iron concentration (μM) for grown in 2 mM Si/2.94 mM NO_3^- or 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.	115
A.7 Phosphate concentration (mM) for cells grown in 2 mM Si/2.94 mM NO_3^- or 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.	121
A.8 Cell Concentration for cells grown in 4 & 8 mM Si.	123
A.9 Measured pH for cells grown in 4 and 8 mM Si.	125

LIST OF FIGURES-CONTINUED

Figure	Page
A.10 Total Nile Red fluorescence for cells grown in 4 & 8 mM Si.	127
A.11 Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 4 & 8 mM Si.	129
A.12 Measured Nitrate concentration (mM) when grown 4 & 8 mM Si.....	131
A.13 Chlorophyll concentration (mg/L) when grown in 4 & 8 mM Si.....	133
A.14 Dissolved Inorganic Carbon (DIC) (mg/L) for cells grown in 4 & 8 mM Si.	135
A.15 Si concentration (mM) when grown in 4 & 8 mM Si.	137
A.16 Iron concentration (uM) for cells grown in 4 & 8 mM Si.	139
A.17 Phosphate concentration (mM) for cells grown in 2 mM Si/1 mM NO ₃ ⁻ with and without NaHCO ₃ addition.	141
A.18 RGd-1 Cell concentrations (cells•mL ⁻¹) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	142
A.19 RGd-1 medium pH throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	144
A.20 RGd-1 Total Nile Red fluorescence throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	146
A.20 RGd-1 specific Nile Red fluorescence (Nile Red intensity/cell concentration * 10000) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.	148

LIST OF FIGURES-CONTINUED

Figure	Page
A.21 RGd-1 growth in B8.7SiS 25mM CHES buffered medium.	150
A.22 RGd-1 biomass yield per gram of Silica utilized.	152
B.1 Si concentration quantified over time in abiotic controls with 0 Si added. Samples were measured in triplicate.	156
B.2 Si concentration measured over time in abiotic controls with 1 mM Si. Samples were measured in triplicate.	156
B.3 Si concentration over time in abiotic controls with 2.5 mM Si. Samples were measured in triplicate.	157
B.4 Fe concentration measured over time in abiotic controls with 0 Si added. Samples were measured in triplicate.	157
B.5 Fe measured over time in abiotic controls with 1 mM Si. Samples were measured in triplicate.	158
B.6 Fe measured over time in abiotic controls with 2.5 mM Si. Samples were measured in triplicate.	158
B.7 Si measured over time in unfiltered abiotic controls with CHES, CAPS or Piperidine. Samples were measured in duplicate.	162
B.8 Si measured over time in filtered abiotic controls with CHES, CAPS or Piperidine. Samples were measured in duplicate.	162

ABSTRACT

The world's crude oil supply is decreasing at an alarming rate and no longer represents a long-term solution to meet energy needs. Development of renewable energy sources is required to meet transport fuel demands. Algal biofuels represent a potentially viable option. Diatom strain, RGd-1, isolated from Yellowstone National Park, produces high concentrations of lipids that can be used for biodiesel production. To increase cell numbers, RGd-1 was grown in six silica concentrations: without added silica, four silica concentrations within the soluble range (0.5-2mM), and one just above the soluble range (2.5 mM). Increasing the silica concentration resulted in an increase in total cell numbers and dry cell weight (DCW) with $R^2=0.965$. Silica depletion was verified by inductively coupled plasma mass spectrometry (ICP-MS). When grown in higher silica concentrations the medium reached a higher pH, which remained elevated. Nile Red fluorescence can be used as measurement of triacylglycerol (TAG). Once silica was depleted, Nile Red fluorescence increased. Unlike green algae and other diatoms, nitrate was never depleted when using the standard Bolds Basal Medium concentration (2.94 mM). RGd-1 never depleted nitrate from the growth medium and utilized only 1/3 of the original nitrate concentration (1 mM) by the time cells reached stationary phase. Therefore, the nitrate concentration was decreased to 1mM to induce a dual nitrate and silica stress. To increase the lipid content further, sodium bicarbonate was added to cells grown with each nitrate concentration (2.94 and 1 mM NO_3^-). Coupling nitrate limitation with sodium bicarbonate addition resulted in higher Nile Red fluorescence. RGd-1 fatty acids were primarily observed as C16:0, C16:1, C18:1-3 and C20:5, averaging at approximately 35, 30, 16 and 10%, respectively of the total lipid content. With exception of cells grown without added silica, the percent lipid content was approximately the same (30-40% (w/w) TAG (Triacylglycerol) and 70-80% (w/w) fatty acid methyl ester (FAME) grown under all conditions within the soluble range. However, when factoring in the dry cell weight from each system, it was observed that the TAG and FAME yields increased with silica concentration when normalized to DCW.

CHAPTER 1

INTRODUCTION AND BACKGROUND

Introduction

According to The World Research Institute (WRI), CO₂ emissions have increased 30,000 times since 1860. While atmospheric CO₂ remained stable during the Holocene (last 10,000 years) it has increased from 280 ppm to 390 ppm in last 150 years [1, 2]. Of additional concern is the predicted decline of crude oil between 2020 and 2040 [3]. According to the US Energy Information Administration (EIA) [4], the US annually consumes more than 18,000,000 barrels of crude oil per day, 8,736,000 of which are used to meet transportation fuel needs. Further, The International Energy Agency (IEA) claims global energy demand (including transport fuel, electric, etc.) will increase 55% between 2005-2030, with a 60% increase in oil demand alone by 2025. Algal biofuels are a potential alternative to petroleum-based fuels. Considered a “near carbon neutral” technology, any CO₂ emitted as a result of processing, transport or combustion is mitigated by CO₂ fixation, a requirement for algal growth. Increased demand coupled with decreasing availability of crude oil, it is imperative to explore alternative, renewable sources of fuel.

Development of first generation biofuels focused on converting cellulosic sources, such as corn into biofuel [5]. However, cellulosic biofuels require substantial land area for growth, have slow growth rates, and increase the prices of food-stocks [6]. According

to Chisti, 2007, ethanol production would require 846% of the current US crop area to meet 50% of all US transportation fuel needs, compared to 1-2.5% of the US crop area required to grow microalgae. Microalgae, therefore, offer a unique and promising biofuel alternative while circumventing problems incurred from cellulosic biofuels. The benefits of algal biofuels are numerous: algae fix CO_2 as a requirement for growth, have fast growth rates, require less land area than cellulosic biofuels, and do not require arable land for growth. Events such as drought, or other unforeseen catastrophic events, have the potential to destroy an entire season of corn growth, compared to 1-2 weeks of algal growth.

Additionally beneficial is the ability to couple algal growth for biofuel production with environmental remediation. Due to their ability to fix CO_2 , algae can utilize atmospheric CO_2 , thus mitigating levels that have increased significantly from anthropogenic activities. Algae do not require freshwater for growth, and are capable of growth on high concentrations CO_2 sources, such as flue gas. Additionally, algae have the potential to remediate wastewater through removal of NO_3^- and PO_4^{3-} [7]. Therefore, algal growth is duly advantageous through remediation activities, while producing biofuel that can be used to meet transport fuel needs.

To increase the viability of algal biofuels, potential strains must have fast growth rates and intrinsically high TAG content. Some strains of algae naturally accumulate high concentrations of lipids within intracellular lipid vacuoles, which can be converted to biofuel. From 1978-1996, substantial efforts were made by the Aquatic Species Program

(ASP) to screen more than 3,000 algal strains for biofuel production. Approximately 60% of these strains were diatoms [8, 9]. While the majority of these studies focused on nutrient stress to induce lipid accumulation, there was also a strong impetus to identify a “lipid trigger” to increase the rate of lipid accumulation.

The goals of this thesis are the following: (1) determine the optimal silica concentration for cell growth for the diatom, RGd-1 and (2) increase lipid content per cell through nitrate limitation and sodium bicarbonate addition for isolate RGd-1.

Background

The Diatoms

Currently, green algae receive most of the attention with regards to algal biofuel research and development. Notwithstanding, diatoms possess immense potential as contributors to biodiesel production. When faced with nutrient limitations such as silica, diatoms appear to redirect carbon storage as triacylglycerol (TAG), which can be used for biodiesel production [10]. The benefits of diatom biofuels are numerous: (1) Diatom cell walls are comprised of silica, as opposed to cellulose cell walls characteristic of green algae. Whereas cellulose cell walls are thick, rigid structures often requiring energy intensive procedures for lipid extraction, siliceous cell walls break easily [8]. In fact, diatom cells can become so swollen from lipid accumulation that some frustules break on their own [8]. (2) Diatoms produce comparatively less cellular starch. Consequently, fixed carbon has increased potential to be allocated to TAG accumulation rather than one of the two aforementioned carbon sinks (cell wall construction or starch accumulation)

[10, 11]. (3) Nitrate depletion is known to limit growth when depleted from experimental growth medium in green algae and some diatoms. However, nitrate depletion has the potential to limit protein synthesis required for essential or desired cell functions, such as lipid synthesis. Therefore, characterizing diatoms for lipid accumulation, whose limiting nutrient is silica, potentially circumvents inhibition of protein synthesis due to nitrate limitation. Notwithstanding, a combination of physiological stresses, (e.g. silica or nitrate limitation and sodium bicarbonate addition) have been individually shown to increase lipid accumulation, it is possible that a combined stress could increase lipid content further. The efforts of this work focus on the role of each physiological stress on lipid accumulation as well as determining whether combining these stresses can increase lipid accumulation further.

The evolutionary history of diatoms is complex. It is hypothesized that approximately 1.5 billion years ago, a photosynthetic cyanobacterium was engulfed by a heterotrophic eukaryote (Figure 1.1). This event led to the incorporation of approximately 10% cyanobacterial genes into the host's genome and creation of 3 lineages; the glaucophytes, red algae and green algae. Approximately 500 million years later, an additional endosymbiotic event occurred, whereby another heterotrophic eukaryote engulfed a red alga. Ultimately, the red algal nuclear and plastid genomes were transferred to the new host genome [12, 13]. It is hypothesized that this secondary endosymbiosis lead to diatom utilization of C_4 rather than C_3 metabolism for carbon fixation [14]. It has been suggested that some diatoms utilize C_4 metabolism due to the presence of phosphoenolpyruvate (PEPCK) in sequenced genomes of *Thalassiosira*

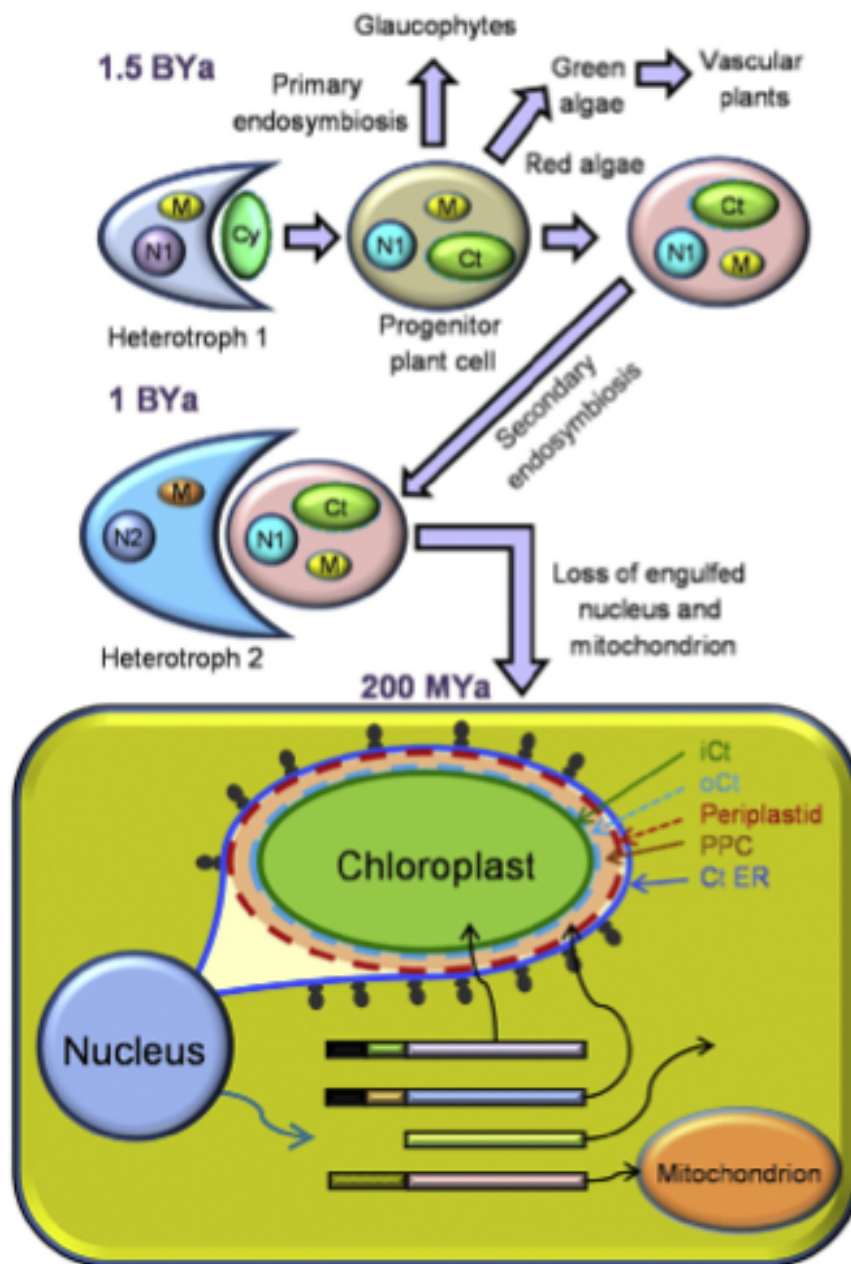


Figure 1.1: Endosymbiotic theory of diatom origins [11].¹

¹ Reprinted from [11] S.R. Smith, R.M. Abbriano, M. Hildebrand. Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways. *Algal Research* 1 (2012) 2-16.

pseudonana and *Phaeodactylum tricornutum* [12, 14]. Approximately 250 million years ago (Triassic Period) when diatoms arose, the oceanic concentration of silicic acid was approximately 700 times the current levels [15]. During the Cretaceous period (100 million years ago) diatoms split into two major lineages; the centrics (radial symmetry) and the pennates (bilateral symmetry) when atmospheric CO₂ was nearly five times higher than current levels [13]. Approximately 50 million years ago, the silicic acid concentration decreased to current levels (1-70 µM) [16] with a concomitant rise in atmospheric oxygen and fall of CO₂ and iron in marine systems, which was primarily due to diatom activity [15].

Diatoms play a pivotal role in the biogeochemical cycle with regards to silica and carbon recycling, specifically through biomineralization and dissolution of silica [17]. Existing in both soil and aqueous environments, silicates are the most abundant minerals on earth, comprising approximately 60% of the earth's crust. Silicate abundance and availability contributes to diatom success across diverse environments [18]. Oceanic silica is replenished due to coastal erosion, wind driven introduction and biogeochemical cycling. Diatoms thrive in the photic zone of freshwater and marine systems where they fix inorganic carbon as a part of photosynthesis. Once depleted of key nutrients, diatoms sink to benthic regions where they supply organic carbon to heterotrophic organisms. Specifically, iron depletion has been found to increase the rate of silica uptake making the cells more dense and settle to benthic regions [19].

Silica recycling by frustule dissolution is a slow process. Kamatani (1982) found that the rate of silica dissolution was a function of temperature, where increased

dissolution from frustules [20, 21]. At 27°C 75% silica frustules were dissolved by within 30 days [21]. Bidle and Azam (1999) investigated the role of bacterial assemblages disrupting the protective organic layer surrounding the diatom frustule expediting silica dissolution, which is ultimately returning the liberated silica to the pelagic zone, via upwelling [22].

Silica Utilization

Diatoms have siliceous cell walls known as frustules. Construction of diatom frustules is energetically less demanding than the cellulose cell walls of their green algal counterparts. Silicification requires 6.7% the energy required to manufacture a polysaccharide cell wall [8]. Frustules are shaped like a petri dish with one side overlapping the other like a box lid. Each half is referred to as a valve, with the larger and smaller valves known as the epivalve and hypovalve, respectively [13]. Diatoms store silica in the silica deposition vesicle (SDV) within which they can concentrate silica up to 1000 times compared to their environment (strain specific). During cellular replication, the SDV supplies the silica for new valve formation using the existing valve as a template to generate the new valve for completion of the two new daughter cells [13, 17, 23].

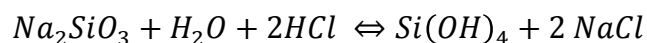
Silica uptake is closely tied to the cell cycle [23]. Frustule deposition begins just prior to cell division and is completed just prior to cytokinesis [24]. Hildebrand et al. (2007) synchronized *Thalassiosira pseudonana* cells by starving them of silica for 24 hours. Following inoculation into silica replete media, it was determined 80% of cells were in

G1 (during which cell expansion and growth take place) [25], and after 8 hours more than 95% were in G1. Two arrest points have been identified in the cell cycle, namely, the G₁/S and G₂/M boundaries. The first arrest point, G₁/S, has been argued to have an Si dependency on DNA synthesis [26], and arrest at G₂/M is due to Si limitation [24]. Other factors contributing to silicification include, temperature, salinity [24] and iron concentration [19].

Silica uptake is proposed to occur by three possible mechanisms: (1) surge uptake, which occurs when silica starved cells are exposed to silica replete conditions, (2) internally controlled uptake, whereby cellular uptake is regulated by the cell cycle with respect to silica required for cellular division and (3) externally controlled uptake, as occurs by the environmental silica concentration. Diatoms are known to increase frustule silicification when environmental silica concentrations are high [27]. Therefore, freshwater diatoms commonly have more heavily silicified frustules than marine diatoms [27].

Determination of K_s and V_{max} values for asynchronous cultures is problematic, since not all of the cells will be taking up silica at the same time. Brzezinski (1992) found that both values were underrepresented 8-fold when averaged across an asynchronous population of *Thalassiosira pseudonana*. The rate of uptake is very species dependent. Martin-Jézéquel (2000) lists saturation (V_{max} and μ_{max}) and half saturation constants (K_s and K_μ) for silicic acid uptake for a variety of diatoms in different growth conditions with values ranging from $K_s=0.2 \mu\text{M}$ (*Thalassiosira gravida*) to $97.4 \mu\text{M}$ (*Phaeodactylum tricornutum*).

In an aqueous solution silica is soluble up to 2 mM concentrations, above which polymers form. Crystalline silica (i.e., sand) has a relatively low solubility at 6 ppm Si(OH)_4 compared to 100-130 ppm of hydrated silica gels [28]. Diatoms utilize silica as silicic acid Si(OH)_4 . However, monomeric and dimeric silicic acids (biologically available silica) are in equilibrium with polymers eventually leading to dissipation of silica polymers [18]. The following represents silicic acid formation from sodium metasilicate where silicate ions are converted to silicic acid and sodium chloride in aqueous environments:



Medium pH plays a multifaceted role in silicate speciation. The solubility of silica increases significantly above pH 8 or 9. The silicomolybdate method detects the concentrations of monomeric and dimeric silicic acid, or biologically available silica in aqueous medium [29]. This method detects down to 0.4 mg SiO_2/L . through reaction of molybdate with silica and phosphate at pH 1.2 to produce heteropoly acids. Oxalic acid is added to disrupt the molybdophosphoric acid, so only the molybdosilicic acid contributes to the colorimetric reading [29]. Whereas monomeric silicic acid reacts rapidly, within 75 seconds, dimeric silicic acid reacts completely by ten minutes and silica polymers can take considerably longer depending on the extent of polymerization [18]. Using the molybdate method, it is possible to determine the measureable monomeric silicic acid in solution to determine solubility with varying pH. It was found silica solubility increased

rapidly above pH 10.26. Below pH 9, silica auto-polymerizes into polysilicic acids. Therefore, it is critical to understand the silica solubility for the pH of the system being measured.

At circum-neutral pH the solubility of silicic acid is largely unaffected. However, above pH 9, solubility increases and silicate ions and silicic acid are formed in solution [18]. Under low silica concentrations (10 μM Si) at pH 8, $\text{Si}(\text{OH})_4$ constitutes approximately 90% soluble silica. However, higher concentrations favor silica polymerization [30]. This species distribution can be verified using MINTEQA2 (figure 1.2), to determine the silicate speciation across pH range 6-13 in diatom growth medium containing 2 mM sodium metasilicate which is in agreement with the silicate speciation described by Wetherbee et. al, 2000 for unsaturated conditions [30]. Between pH 6-8, $\text{Si}(\text{OH})_4$ is the dominant silicic acid species. However, with increasing pH, silicic acid becomes deprotonated and exists primarily as $\text{Si}(\text{OH}_3)^-$ at pH 10.5 and above.

Carbon Utilization

Previous studies have shown that adding sodium bicarbonate to green algae and diatom cells during the late exponential growth phase can increase the extent of lipid accumulation for green algae (*Scenedesmus* sp. & *Chlamydomonas reinhardtii*) and the diatom (*Phaeodactylum tricornutum*) [31, 32]. An additional study by White, et al. (2012) observed increased total lipid per cell when two marine species; *Tetraselmis*

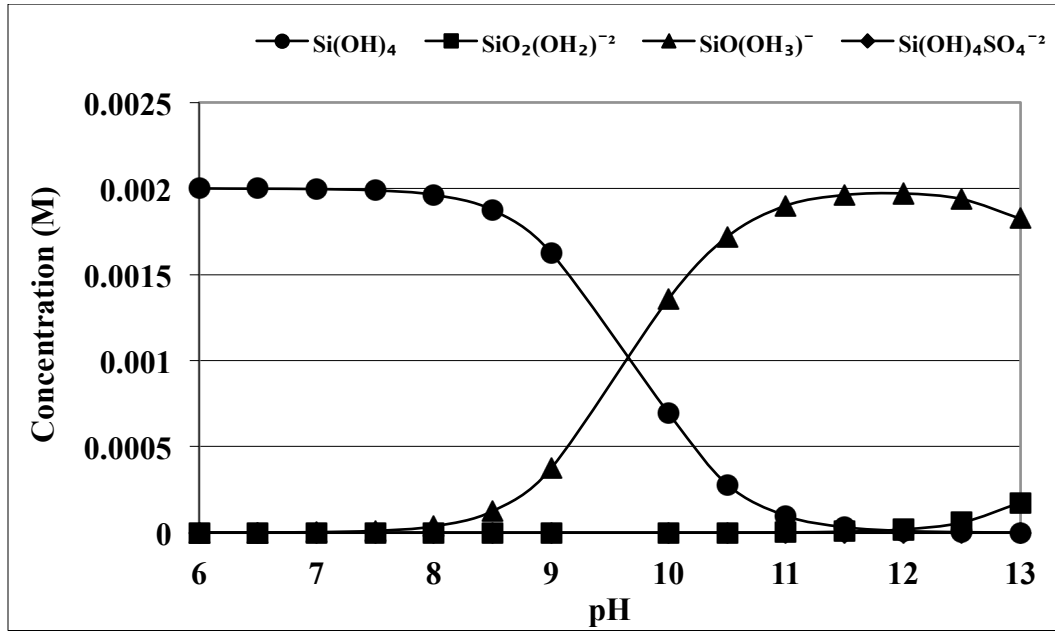


Figure 1.2: Silicic acid speciation across pH range 6-13 in Bold's Basal Medium with 2 mM Sodium metasilicate. Species distribution was determined using MINTEQA2 version 3.0.

suecica and *Nannochloropsis salina* were grown in two different concentrations of NaHCO_3 from the onset of each experiment [33]. While their data indicate increased lipid accumulation, it does not show the dramatic rate of increased as seen in Gardner et al. (2012) [31] when NaHCO_3 is added just prior to nutrient depletion. Additionally, Gardner et al, showed how the sodium bicarbonate lipid inducer has diverse application for freshwater and marine species of green algae and diatoms.

It is hypothesized that in diatoms, one mechanism making bicarbonate utilization may advantageous is their ability to utilize a C_4 pathway [31]. Pyruvate is formed via pyruvate kinase and converted to oxaloacetate by pyruvate carboxylase prior to conversion to malate, which is ultimately decarboxylated by malic enzyme supplying

CO₂ in proximity to ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) for fixation as part of the biochemical carbon concentrating mechanism (CCM) [11, 34-36]. An additional mechanism involves a biophysical mechanism for CCM, whereby inorganic carbon is brought into the plastid where it is in close proximity to Rubisco using membrane transporters and carbonic anhydrases to cross membranes [37].

Iron Utilization

Approximately 30-40% of the world's oceans are iron limited. Studies have investigated "iron fertilization" experiments whereby iron is added to High Nutrient Low Chlorophyll (HNLC) areas to induce phytoplankton growth, and consequent CO₂ fixation [38]. In fact, approximately 70% blooms stimulated by these events are comprised of diatoms. Iron limitation is known to induce chlorosis, as well as reduced carbon fixation rates, photosynthetic efficiency, and growth rates [39]. Iron limitation has also been linked to increased rates of silicification, thus increasing cell density and promoting cell sinking. Iron limitation is additionally known to decrease expression of β -carbonic anhydrase, and decreasing carbon fixation and nitrate uptake. According to Allen et al., 2008, cells grown under limited nitrate conditions fixed carbon at rates 14 times lower compared to cells grown in iron replete conditions [39]. Since iron limitation can result in detrimental physiological effects, it is pertinent to determine the potential for these phenomena to occur. Figure 1.3 illustrates the iron speciation in the experimental growth medium (B8.8 SiS). Within the medium pH range 8-13, iron is complexed to ETDA as

$\text{FeOH}^+\text{HEDTA}^-$, $\text{Fe}(\text{OH})_2^-\text{HEDTA}^{2-}$ and $\text{Fe}(\text{OH})_3^-\text{HEDTA}^{3-}$, indicating it is maintained in a soluble form and is biologically available within the pH range of RGd-1 experimentation (pH 8 - ~11).

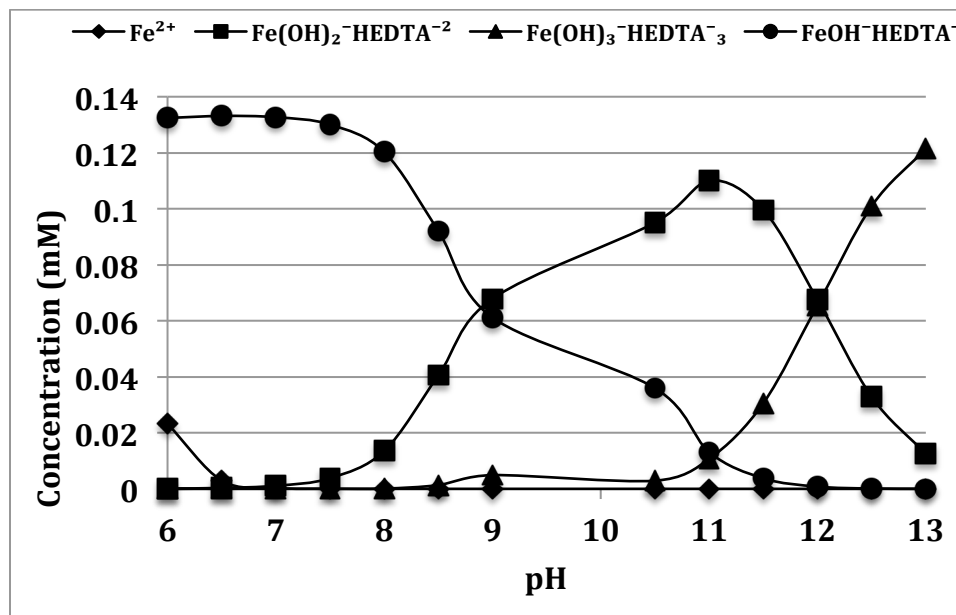


Figure 1.3: Iron speciation across pH range 6-13 in Bold's Basal Medium with 2 mM Sodium metasilicate, using 500 mV for redox potential. Species distribution was determined using MINTEQA2 version 3.0.

Genetically Modified versus Natural Strains

Many efforts focus on genetically modified algal strains to increase TAG content. *Chlamydomonas reinhardtii* is a model alga used in genetic modification by mutagenesis or transformation [40-42]. One method involves the *C. reinhardtii* starchless mutant, whereby a greater pool of fixed carbon can be redirected towards lipid rather than starch accumulation [43, 44]. In addition to physiological characterization, using growth studies, genetic engineering of key lipid accumulating control points have been examined. In a review by Dorval Courchesne et al. (2009) compared lipid

accumulation tendencies through biological, genetic and transcription factor engineering. TAG accumulation occurs by three major steps: (1) formation of malonyl-CoA by carboxylation of acetyl-CoA, (2) acyl chain elongation and (3) TAG formation. Overexpression of Acetyl-CoA Carboxylase (ACC) showed an increase in malonyl-CoA, but did not result in an increase in lipid accumulation, suggesting ACC alone may not result in increased lipid accumulation, but may work in concert with other enzyme(s). In filamentous fungi overexpression of malic enzyme was found to increase reduction of NADP⁺ to NADPH with a concomitant increase in lipid accumulation. While use of transcription factors to promote lipid accumulation is still in infancy, it has immense potential to increase lipid accumulating tendencies in green algae and diatoms [45].

Pursuit of natural algal strains is beneficial for biofuel production. Genetic modification requires prior knowledge of an organism's genome sequence information. While prices for genomic sequencing are declining rapidly, it is still an expensive endeavor. Extremophilic properties such as temperature, alkalinity or salinity can be pursued for algal biofuel production in raceway ponds. Organisms adapted to extreme environments are more likely to be successful in outdoor raceway ponds, by reducing the potential for competing (non-productive with respect to biofuel production) organisms to take over, resulting in a "pond crash." It is likely that utilizing both GMO and natural strains provide their own unique advantages. Discovery of novel strains with fast growth rates and high lipid contents will contribute immensely to improved viability of algal biofuels.

Summary

Diatoms have a complex evolutionary history resulting in unique growth requirements, specifically with regard to silica and carbon utilization. Diatoms require silica for frustule formation, without which growth is limited. RGd-1 is capable of growth at high pH, which is advantageous when HCO_3^- can be utilized as a carbon source, potentially through C_4 metabolism. Because RGd-1 stores high concentrations of lipids, it is a promising candidate for biofuel production. Characterization has the potential to improve diatom biofuel viability through identification of growth and nutrient conditions that will increase the growth rate and extent of lipid accumulation. Here, the alkaliphilic diatom strain, RGd-1 (isolated at pH 9.3) is optimized with regard to silica concentration, nitrate limitation, and sodium bicarbonate addition.

CHAPTER 2

OPTIMIZATION OF GROWTH AND LIPID ACCUMULATION

Introduction

Algal biofuels represent a promising alternative to petroleum-based transportation fuels. In addition to supplementing crude oil supplies, algal biofuels utilize atmospheric CO₂ as a carbon source for fuel production [5, 46] and, therefore, provide a unique and promising renewable energy source, as well as CO₂ sink [46], capturing 2g CO₂ per gram of biomass (approximately one-half of the biomass is comprised of carbon) [47]. Compared to first generation biofuels (eg. palm oil), algal biodiesel is among the most sustainable of biofuels with regards to food, energy and environmental impacts and has desirable fuel characteristics similar to petroleum based diesel [6]. Specifically, algal biofuels produce more oil per hectare, have a faster growth rates and do not require arable land, thus avoiding food-versus-fuel conflicts [5, 6, 46, 47]. The 2007 Energy Independence and Security Act (EISA) and Renewable Fuels Standard (RFS) sought to increase the renewable energy contribution to energy production and decrease greenhouse gas emissions. The RFS mandates “production of 36 billion gallons of renewable fuels by 2022, with at least 21 billion gallons of advanced biofuels, particularly those with higher-energy density than ethanol.” [47]

Some algal strains naturally contain high levels of lipids, in the form of triacylglycerol (TAG) [31, 48]. Promising strains for use in biofuel production must have fast growth rates and high TAG content [47, 49]. In most cases, substantial TAG

accumulation does not occur while cells are rapidly growing, rather, TAG tends to accumulate when cells are stressed by nutrient depletion, pH, temperature or light intensity/photoperiodicity [50-52]. Strain optimization has the added benefit identifying novel strains for biofuel production. The Aquatic Species Program (ASP) found through screening of approximately 3000 microalgae, no single strain succeeded in both maximal growth and lipid accumulation [53]. However, extremophilic organisms have the potential to grow successfully in outdoor raceway ponds by growing at conditions uninhabitable by other microorganisms [46, 54, 55]. Specifically, organisms with optimal growth conditions in extreme conditions such as salinity and pH may be more likely to be successful in raceway ponds. Alkaliphilic algae (organisms that thrive above pH 9) [56] have the added benefit of dissolved inorganic carbon (DIC) availability due to increased solubility at increased pH [57].

Paramount to algal biofuel feasibility is strain selection [6, 49, 58]. While many recent studies have focused on green algae, few have focused on diatoms for biofuel production. Compared to green algae, diatoms have fundamental differences that may be advantageous for biofuel production. As opposed to green algae with cell walls composed of cellulose, diatoms have silicious cell walls (frustules) which require comparatively less energy expenditure (6.7%) for cell wall formation [59, 60]. Once stressed, some diatom strains allocate a large portion of fixed carbon towards lipid accumulation [8]. Silicious cell walls provide an added advantage in lipid harvesting with their ability to break easily. There is evidence that diatoms utilize C_4 pathways, which have increased photosynthetic efficiency over organisms that utilize only C_3 pathways (e.g. most green

algae) [34, 35, 46, 61]. Pyruvate is formed via pyruvate kinase and converted to oxaloacetate by pyruvate carboxylase prior to conversion to malate, which is ultimately decarboxylated by malic enzyme supplying CO_2 in proximity to ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) for fixation as part of the biochemical carbon concentrating mechanism (CCM) [11, 34-36]. An additional mechanism involves a biophysical mechanism for CCM, whereby inorganic carbon is brought into the plastid where it is in close proximity to Rubisco using membrane transporters and carbonic anhydrases to cross membranes [37]. Therefore, select diatom strains are potentially more efficient at accumulating high concentrations of lipids as compared to green algae.

In nature, diatoms play a central role in the global carbon cycle where they fix dissolved CO_2 in the photic zone, ultimately sink once depleted of silica and supply benthic microorganisms with organic carbon and contribute ~40% of marine primary productivity [8]. Diatoms are responsible for approximately 20% of the global carbon fixation, which is estimated to be the photosynthetic equivalent of all rainforest carbon fixation combined [8, 25, 62].

Diatoms require silica for growth. Elemental silicon is one of the most abundant elements on earth, contributing approximately 28% of the earth's crust [63]. Diatoms incorporate monomeric or dimeric silicic acid $\text{Si}(\text{OH})_4$ or biogenic silica into frustules composed of amorphous silica [18]. The solubility of silica in water (pH 7) is 2mM [25, 28], beyond which, it auto-polymerizes into polymeric silicates [8]. At pH 8, silica exists as silicic acid (orthosilicate) $\text{Si}(\text{OH})_4$ [8] (97%) and $\text{SiO}(\text{OH})_3^-$ (3%) [16, 64], above which, silicic acid is deprotonated with $\text{pK}_{\text{a}1} = 9.8$ and $\text{pK}_{\text{a}2} = 12.3$.

Diatoms can concentrate silica intracellularly, up to 1000 times that of their environment. This concentration can sometimes sustain several cell divisions depending on the strain [16]. *Navicula pelliculosa* is known to pool 438-680 mM Si intracellularly [64]. Diatoms store silica in the silica deposition vesicle (SDV) until it is used for cell wall synthesis. Within the SDV, the pH is acidic and promotes silicic acid polymerization which eventually will be used for frustule formation [25].

When faced with nutrient limitation many algal strains increase the lipid content per cell. Specifically, silica, nitrate and phosphate are nutrients known to limit growth of diatoms [8, 50, 65]. Nitrate limitation is known to decrease chlorophyll concentration, which has been shown to decrease photosynthetic rates in *Chlamydomonas*, and can ultimately lead to chlorophyll degradation [8, 66]. While nitrate is a particularly important limiting nutrient in biofuel production for green algae [48, 67-72], nitrate limitation is known to cause stress in some diatoms as well. Additionally, nitrate depletion can limit protein synthesis required for desirable functions such as TAG accumulation [8]. Once depleted of silica, diatoms increase the rate and extent of lipid accumulation [8, 10, 47, 50, 73, 74]. Silica limitation has not been shown to directly contribute to the adverse affects associated with nitrate limitation (e.g. chlorosis or limitation of protein dependent lipid accumulation), and the combination of silica and nitrate limitation has the potential to increase TAG accumulation and could potentially be relevant on an industrial scale.

Here we focus on a locally isolated alkaliphilic, diatom strain, RGd-1, using nutrient and chemical stress (e.g. pH) to increase diatom cell yield and TAG

accumulation through optimization of silica and nitrate limitation and sodium bicarbonate addition.

Methods

Microorganism & Growth Systems

RGd-1 (Figure 2.1) was isolated from an alkaline stream in Yellowstone National Park (USA). Initial identity was determined by frustule morphology described by Round, et al. (1990) [13]. RGd-1 shared 94 % sequence similarity with *Sellophora pupila* with for both SSU rDNA and Internally Transcribed Spacer (ITS) Regions.

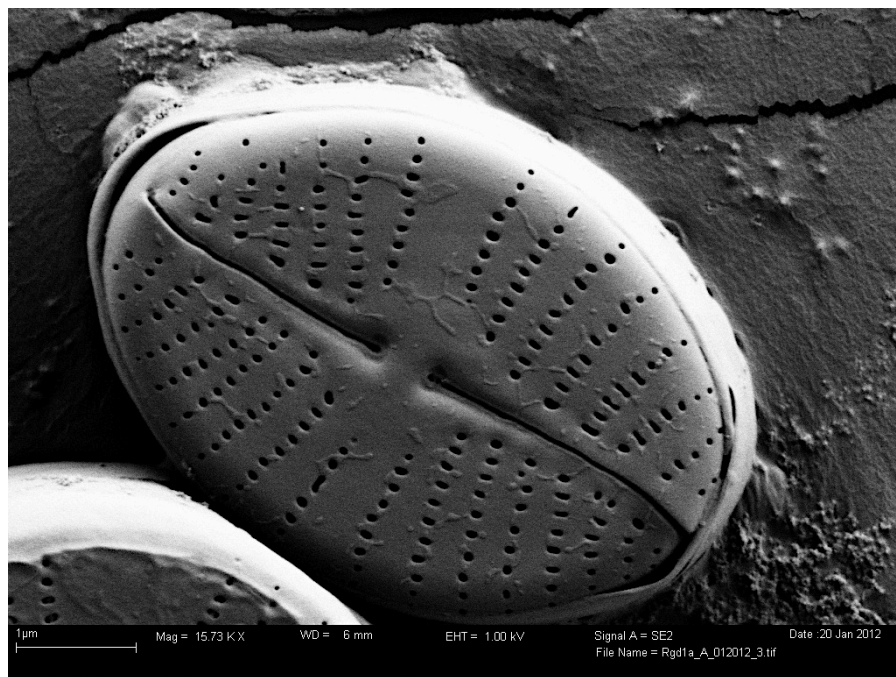


Figure 2.1: Field Emission-Scanning Electron Microscopy (FE-SEM) image of RGd-1.

Cultures were grown under 14:10 L/D cycle, aerated with ambient air (dissolved inorganic carbon (DIC) $\sim 8\text{-}9\text{ mg/L}$) at $27 \pm 1^\circ\text{C}$ in a temperature controlled water bath. RGd-1 was grown in modified Bolds Basal Medium titrated to pH 8.7 with added sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) (Sigma-Aldrich), S3 vitamin solution and Vitamin B₁₂, according to the ASP II medium recipe [75]. Diatom cultures were grown in triplicate 1.25 L photobioreactors illuminated with $400\text{ }\mu\text{moles m}^{-2}\text{s}^{-1}$ using 12 T5 4 ft fluorescent lights. Light intensity was measured using a photosynthetically active radiation (PAR) meter (LI-COR). Samples were collected daily just prior to the end of each light cycle. Growth measurements were quantified by direct cell counts using a Reichert hemacytometer. A minimum of 400 cells were enumerated from each sample for statistical significance [76]. Sample pH was measured using a standard benchtop pH meter (Accumet). Prior to analysis by inductively coupled plasma mass spectrometry (ICP-MS), ion chromatography (IC) or dissolved inorganic carbon (DIC) analysis samples were filtered using 0.22 μm filter (Whatman).

Growth Studies

To optimize diatom cell growth, the growth medium was modified with the following silica concentrations: 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mM Si; with the last being just above the soluble range. In addition, sodium bicarbonate (25mM) was added to adjacent 2.0 mM Si cultures to investigate if the addition of bicarbonate affected TAG accumulation based on Gardner's 2012 work [31].

To further increase the lipid content per cell, nitrate was limited. Approximately 1/3 of the Bold's Basal Medium nitrate concentration (1 mM) was utilized by the time cells reached stationary phase. Therefore, the nitrate concentration was decreased to 1 mM nitrate (compared to 2.94 mM nitrate in standard Bold's Basal Medium) to induce a dual silica and nitrate stress. Sodium bicarbonate was added to some cultures concentrations of 1 mM and 2.94 mM. Each with these experiments was grown using 2 mM Si. At the completion of each experiment, cultures were centrifuged at 5000 x g for 10 minutes. The pelleted biomass was lyophilized (Labconco Lyophilizer) and later used for TAG and FAME analyses.

Dry Cell Weight

Dry cell weight (DCW) was determined by filtering samples using GF/F Glass Microfiber Filters (Whatman). Samples were weighed after drying at 60°C for approximately 24 hours and re-weighed up to 48 hours to ensure all water had been evaporated.

Ash free dry weight (AFDW) was determined by heating DCW samples to 500°C for 4 hours [77, 78]. The difference between the DCW and ash remaining is the ash free dry weight. Samples were weighed immediately upon retrieval from the furnace.

Nitrate Utilization and Chlorophyll Concentration

Nitrate concentrations were measured by ion chromatography using an AS22 Anion-Exchange Column (Dionex) with 4.5 mM NaHCO₃ and 1.4 mM Na₂CO₃ buffer and 1.0 mL flow rate. Nitrate was detected using a CD20 conductivity detector, and

results were analyzed on PeakNet 5.2 Chromatography Workstation software. Chlorophyll was measured by centrifuging 1 mL of culture at 5000 x g for 10 minutes and re-suspending in methanol. Samples were intermittently vortexed, sonicated and incubated at 4°C for one hour. Following incubation, samples were centrifuged again at 5000 x g for 10 minutes and read at 632, 652 and 665 nm in a spectrophotometer (Genesis 10-S, Thermo Electron Corporation). Total chlorophyll concentrations were calculated by adding chlorophyll a and c according the methods outlined in Ritchie (2008) [79].

Dissolved Inorganic Carbon (DIC)

DIC was measured using ~7 mL of filtered sample supernatant and analyzed using a Skalar Formacs TOC/TN Analyzer with a LAS-160 autosampler (Skalar). DIC concentration was determined through injection of 100 µL of sample in 2% (v/v) Phosphoric acid and CO₂ peak area was measured with an IR detector, and peak area was calibrated with peak responses from sodium carbonate/bicarbonate standard solutions.

Silicon and Iron Quantification

Total elemental silicon and iron were quantified by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7500e,) in helium mode. Isotopes of Si₂₈, Si₂₉, Si₃₀ and Fe₅₆ and Fe₅₇ were measured simultaneously for each sample run on ICP-MS. The two most abundant isotopes, Si₂₈ and Fe₅₆ provided the most linear calibration curves, therefore, total Si and Fe were quantified using Si₂₈ and Fe₅₆, respectively.

TAG/FAME Analyses

TAG content was estimated daily by staining with Nile Red (9-diethylamino-5H-benzo(α) phenoxazine-5-one) (Kodak) (0.25 mg/mL suspended in acetone) by adding 4 μ L/mL sample [80] and quantified on a microplate reader (Bio-Tek) with 480/580 excitation/emission filters. Cultures were determined to have an optimal Nile Red exposure time of 4 minutes to achieve maximum fluorescence. If necessary, cell cultures were diluted at cell concentrations higher than 3.0×10^6 cells mL⁻¹ to maintain Nile Red fluorescence linearity. Nile Red has been shown to have a positive correlation with neutral lipids (e.g. TAG) [32, 80-82] and were quantified with gas chromatography - flame ionization detector (GC-FID; Agilent 6890N) [32]. Typically, biodiesel derived from algae is derived primarily from TAGs. However, in order to determine the fatty acid profile, the degree of saturation, and total biofuel potential obtained from direct transesterification, FAMES were quantified using gas chromatography mass spectrometry (GC-MS; Agilent 6890 and 5973 Network MS).

TAGs were extracted using 20 mg of dry diatom biomass in 5 mL 1:1:1 chloroform/hexane/tetrahydrofuran solution (triple solvent) [32]. Samples were sonicated for a total of 2 minutes, using four 30 second pulses in an ice bath. Extracts were centrifuged (1380 x g) for approximately 2 minutes. One mL was removed and placed in a GC vial with 10 μ L octacosane (10 mg•mL⁻¹ triple solvent) as an internal standard.

Samples were analyzed using GC-FID with 1 μ L injections for quantification of free fatty acids, monoacylglycerols, diacylglycerols and triacylglycerols on a 15 m RTX biodiesel column (Restek) according to Gardner et al. (2012) [32].

FAMES were extracted and quantified by direct in-situ transesterification [83] and GC-MS, respectively to determine total biofuel potential based on methods outlined by Bigelow, et al. (2011) [84]. Dry biomass (10 mg) was added to 1 mL toluene and 2 mL sodium methoxide (pure, titrated, (0.5M in Methanol) Acros Organics). Samples were placed in 5mL crimp cap serum vials and heated to 80 °C for 30 minutes with intermittent vortexing. After cooling, 2 mL 14% BF₃-methanol (Thermo-Scientific) was added and heated as described above. Once samples were cooled, 0.8 mL saturated NaCl and 1.0 mL hexane were added. Samples were centrifuged at 1380 x g for 2 minutes, after which, 1 mL was removed and placed in a GC vial with 10 μ L octacosane as an internal standard. Dilutions (1:10) were performed if peak areas were greater than the linear range of the calibration standards, namely for C-16 and C-18 FAMES. All other fatty acids were quantified by integrating peak areas of undiluted samples.

DNA Extraction, Amplification and Clone Libraries

Diatom identity was confirmed by small subunit (SSU) rDNA (18S), internally transcribed spacer (ITS) regions and rbcL (ribulose-bisphosphate carboxylase oxygenase (Rubisco)). RGd-1 was confirmed axenic by lack of growth following inoculation of Bold's Basal Medium supplemented with 0.05% glucose and 0.05% yeast extract and

incubated in the dark, as well as careful microscopic examination (Nikon eclipse 800) and confirmed unialgal by clone library analysis.

DNA was extracted from diatom samples by adding 200 μ L of extraction buffer (1M NaCl, 70 mM Tris, 30 mM NaEDTA, pH 8.6). Contents were centrifuged at 14,500 x g for one minute, and the supernatant discarded. Fresh extraction buffer (500 μ L), sterile glass beads, 125 μ L 2% (w/v) CTAB (Tris-Cl, pH 8.0, 1.4 M sodium chloride, Teknova) extraction solution and 200 μ L chloroform were added and cells disrupted by using a bead beater (Fastprep) at 6.5 for 45 seconds. Extracts were centrifuged at 12,000 x g for 15 minutes to separate the organic and aqueous phases. The aqueous phase was removed and dispensed into a sterile 1.5 mL centrifuge tube. To the extractant, 40 μ L of 3M sodium acetate. Five μ L RNase and 480 μ L isopropanol (Molecular Grade, Fisher) were added. The extract was incubated at 20°C overnight and centrifuged at 12,000 x g for 15 minutes at 4°C to pellet DNA. The pellet was washed with 200 μ L of 80% ethanol and centrifuged again. Once the pellet was air dried in a biosafety cabinet, the pellet was resuspended in 100 μ L of 10 mM Tris-HCl.

DNA was amplified using the following 18S primers UNI7F (5' ACCTGGTTGATCCTGCCAG 3') and 1534R (5' TGATCCTTCYGCAGGTTTCAC 3') [85, 86] as well ITS 1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4 (5' TCCTCCGCTTATTGATATGC 3') [86-90]. Additionally, *rbcL* primers were used to obtain greater specificity forward (5' GATGATGARAAAYATTAATC 3') and reverse (5' ATTTGDCCACAGTGDATACCA 3') [91]. Per 50 μ L PCR reaction, 1 μ M of each primer was added, 25 μ L of GoTAQ® Green Master Mix, 5 μ L sample and 15 μ L

DNase/RNase free water. Samples were amplified by PCR with the following parameters: initial denaturation of 2 min at 94°C, followed by 40 cycles of 30 sec at 94°C, 1 min at 52°C, 1.15 min at 72°C, and final extension of 7 min at 72°C.

Amplicons were run on a 0.7% agarose gel to verify size. The remaining amplification product was cleaned using a QIAquick PCR Purification Kit (Qiagen) and cloned using pGEM®-T Vector System II, grown in LB + ampicillin (100 µg/mL) and plasmids extracted using QIAprep Spin Miniprep Kit (Qiagen). Samples were submitted to Functional Biosciences (Madison, WI) for DNA sequencing, aligned using Jalview (version 2.8) and identity determined using BLAST queries.

Results & Discussion

Silica Optimization Experiments

To elucidate the effects of silica on growth and TAG accumulation for RGd-1, Bold's Basal Medium was modified with 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mM Si in addition to B₁₂ and S3 vitamin supplements and titrated to pH 8.7. Cell numbers increased with silica concentration and the doubling time for cells grown in each of the six silica concentrations remained consistent at 29.4 ± 1.4 hours. Figure 2.2 (A) and Table 2.1 show that with no added silica, RGd-1 grew to a final cell concentration of $1.1 \times 10^6 \pm 2.65 \times 10^5$ cells mL⁻¹ (average $\pm$ standard deviation) and $1.14 \times 10^7 \pm 1.28 \times 10^6$ cells/mL when grown in the presence of 2.5 mM Si. Therefore, increased silica concentration in the growth medium allowed for extended periods of growth until silica was depleted and

limited growth. As shown in Figure 2.2 (A) and 2.2 (B), this resulted in a net increase in the final cell concentration as the initial silica concentration increased. Final dry cell weight increased in proportion to silica concentration with a linear correlation coefficient $R^2=0.965$ (Figure 2.2 (C)).

Figure 2.2 (B) shows silica concentrations in the growth medium over time. Initially the silica concentration appeared to increase for the 2 and 2.5 mM Si through days 5 and 7, respectively (Figure 2.2 (A)). Two different abiotic controls were run to investigate (1) silica polymerization [18] and (2) whether borosilicate glass is a contributory silica source using three different synthetic buffers: CHES (pKa: 9.3), CAPS (pKa: 10.4) and Piperidine (pKa: 11.14). While the first round of abiotic controls did not indicate silica polymer retention on filters, it is possible silica polymers were non-homogeneously suspended within the media. Over time, the polymers were dissociated, potentially due to aeration, thus appearing as though the Si concentration increased between days 5-7. It is unlikely exogenous sources of Si were introduced into these abiotic controls since the pH did not exceed 9, the point at which Si can be leached from borosilicate glass [30]. However, when exposed to higher pH (> pH 9), in the second round of abiotic controls, there was up to 0.5 mM Si measured (Figure B.3), which is roughly equivalent to Day 11 in growth for 1.5-2.5 mM Si systems. ICP-MS results indicate silica depletion by Day 12. Therefore, the concentration of silica that may be leached from the borosilicate glass over a two-week period approximates to approximately one cell doubling. And while this concentration of silica may sustain cell growth, it is neither an unlimited nor strongly contributing silica source.

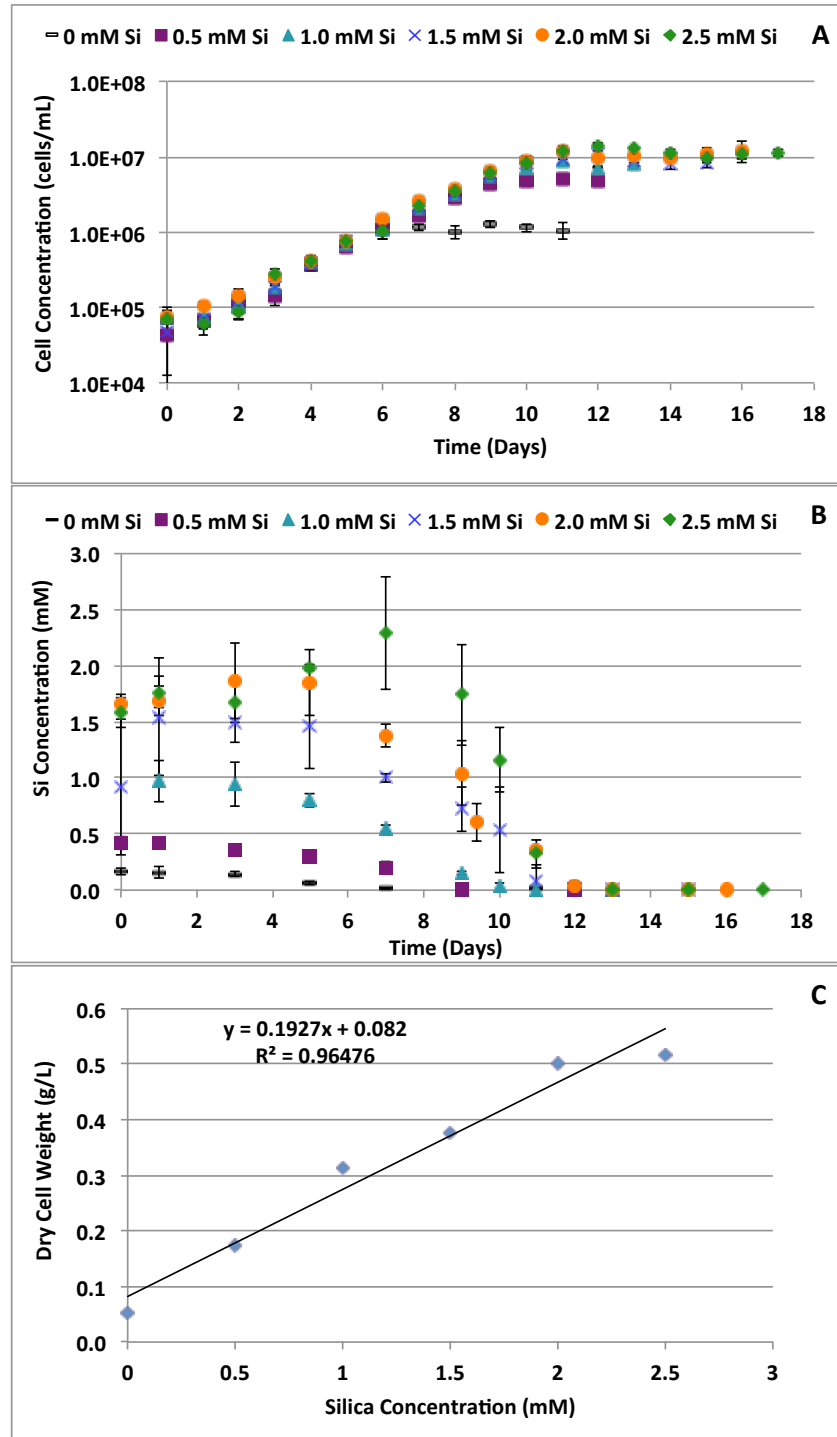


Figure 2.2 (A) RGd-1 growth (cells mL^{-1}) for 6 silica concentrations (B) silica concentrations in the growth medium over time (C) correlation of final dry cell weight produced with silica concentrations.

Table 2.1 Final growth and lipid accumulation characteristics at the completion of each condition tested: cell yield (cells•mL⁻¹), Nile Red fluorescence, specific Nile Red fluorescence, Dry Cell Weight, % TAG and % biofuel potential (BP).

Condition (mM)	Doubling Time (Hours)		Dry Cell Weight (g/L)		Final Cell Concentration (cells•mL ⁻¹)		Final Nile Red Fluorescence		Final Specific Fluorescence		% TAG (w/w)		% BP (w/w)		TAG Yield (g/L)	Biodiese l Yield (g/L)	
	Avg.	St. Dev.	Avg.	St. Dev.	Avg.	St. Dev.	Avg.	St. Dev.	Avg.	St. Dev.	Avg.	St Dev.	Avg.	St Dev.	Avg.	St Dev.	Avg.
0 Si	25.31	4.41	0.052	±0.011	1.07E+06	±2.60E+05	2522	±848	23	±3	7.17	±0.741	25.78	±2.90	0.004	0.000	0.014
0.5 mM Si	27.07	0.43	0.175	±0.006	4.87E+06	±4.23E+05	10273	±2495	21	±4	21.64	±1.386	52.58	±6.58	0.038	0.002	0.092
1.0 mM Si	27.30	0.15	0.315	±0.031	8.01E+06	±3.61E+05	15568	±1746	20	±3	21.71	±1.913	47.47	±4.47	0.068	0.006	0.151
1.5 mM Si	27.42	1.03	0.376	±0.039	8.23E+06	±1.12E+06	16940	±2909	21	±1	23.08	±7.141	49.54	±5.23	0.087	0.027	0.189
2.0 mM Si	28.28	0.70	0.503	±0.061	1.22E+07	±3.72E+06	29430	±2440	25	±6	24.68	±1.785	52.71	±2.42	0.124	0.009	0.266
2.5 mM Si	25.55	2.38	0.517	±0.065	1.14E+07	±1.28E+06	33030	±1235	29	±4	23.90	±53.03	50.63	±1.86	0.124	0.002	0.265
2.0 mM Si + HCO ₃ ⁻	35.87	5.1	0.62	±0.014	1.48E+07	±1.16E+06	50613	±3134	34	±2	25.83	±4.485	56.86	±2.33	0.160	0.008	0.353
2.0 mM Si 1 mM NO ₃ ⁻	29.89	2.9	0.46	±0.045	1.26E+07	±6.43E+05	36683	±9126	29	±6	25.37	±1.291	49.62	±6.39	0.117	0.021	0.230
2.0 mM 1 mM NO ₃ ⁻ +HCO ₃ ⁻	31.35	3.4	0.515	±0.008	1.36E+07	±2.10E+06	57122	±1870	43	±6	28.06	±1.479	52.72	±2.69	0.145	0.008	0.272
4.0 mM Si	33.45	0.63	0.757	±0.102	1.74E+07	±1.85E+06	62177	±7800	36	±1	32.81	±3.195	63.66	±11.70	0.181	0.018	0.486
8.0 mM Si	37.19	1.54	1.1	±0.035	2.14E+07	±2.21E+06	72533	±4543	34	±4	47.10	±5.513	53.45	±1.02	0.406	0.055	0.596

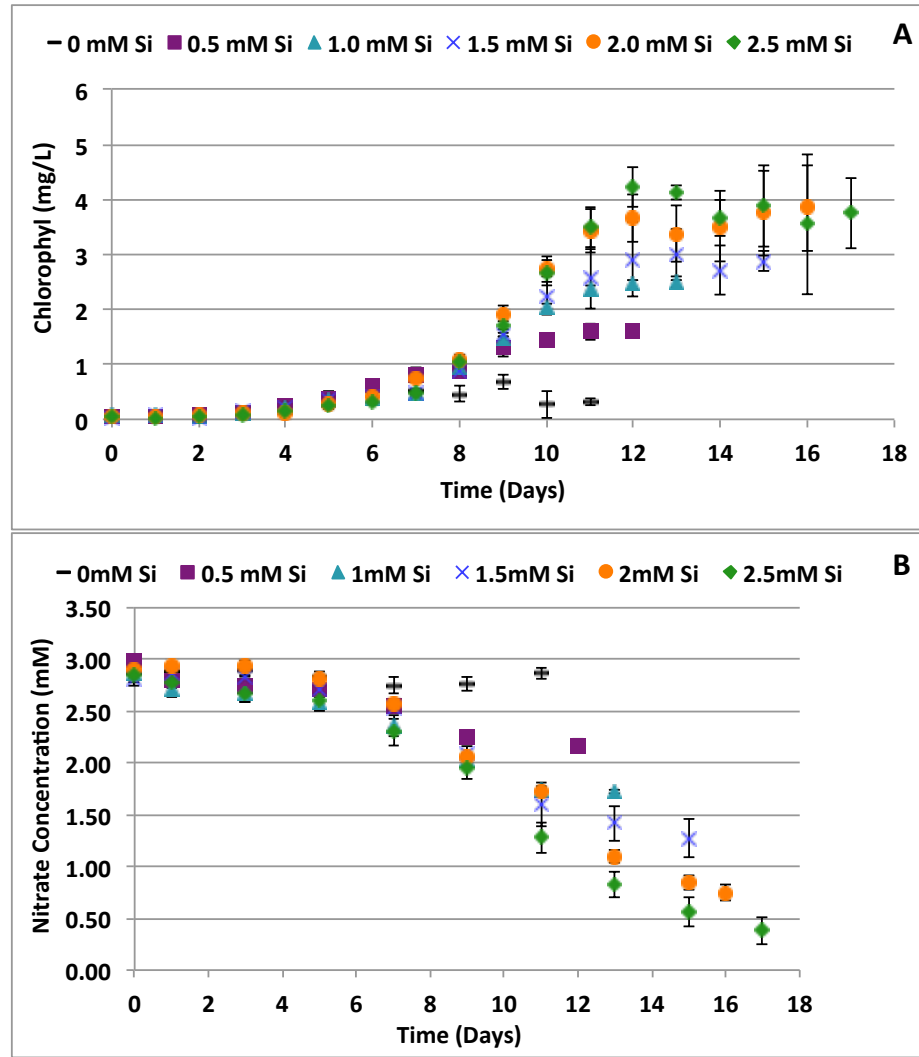


Figure 2.3 (A) Chlorophyll production for RGD-1 grown in each silica concentration (B) NO_3^- removal from growth medium.

As shown in Figure 2.3 (A), total chlorophyll (chlorophyll a + c) was monitored daily as a measure of diatom cell health (Figure 2.3 (A)). Increased silica concentrations resulted in an increased nitrate uptake, indicated by removal from the growth medium. Whereas cells grown without added silica utilized less than 1% nitrate, cells grown in 2.5 mM Silica, utilized approximately 87% nitrate (Figure 2.3 (B)). However, cultures grown in silica concentrations (0-2.5 mM) never depleted nitrate from the growth medium.

Cultures grown in higher silica concentrations reached higher maximum pH. When grown without added silica (0 Si), the medium pH remained constant throughout growth at approximately pH 7.8. Due to increased photosynthetic activity from higher cell numbers, cultures at higher silica concentrations reached higher pHs, which remained elevated for extended periods of time (Figure 2.3 (A)). This may be due to three independent factors; production of a hydroxyl ion through utilization of CO_2 , NO_3^- and silica. It is therefore likely that cultures grown in higher silica concentrations reached higher maximum pH due to silica utilization, as well as higher concentrations of cells utilizing more CO_2 , increasing the pH further. An increased maximum pH observed corresponded with increased silica concentrations such that cells grown with increased silica were potentially exposed to higher pH stress [31, 48, 52].

Gardner et al. (2011) observed the impact of two physiological stresses (pH and nitrate limitation) on lipid accumulation for two chlorophytes. Independently, each slightly increased lipid accumulation, but the combination of the two stresses together resulted in significantly increased total lipid accumulation. A similar phenomenon is evident with RGd-1 when comparing cell concentration, pH and Nile Red intensity (Figures 2.2 (A), 2.3 (A) and 2.3b (B)) for all but the silica free treatment. By day 7, cells were growing exponentially for all but the Si free treatment, the pH reached approximately 9, and as shown in Figure 2.3(B), the Nile Red fluorescence reached values between 2000-3000 for all silica concentrations tested. The pH for cells grown without additional silica remained consistent throughout the experiment at approximately

7.8. It is evident that when the $\text{pH} \geq 9$, Nile Red fluorescence increased, which occurred prior to Si depletion.

As shown in Figure 2.4 (B), Nile Red fluorescence increased with initial silica concentration. Cells grown without additional silica accumulated lipid with a final Nile Red fluorescence of 2522 ± 848 (average $\pm$ standard deviation), compared to cells grown in 2.5 mM Si which reached an average total fluorescence at the time of harvest of 33030 ± 2440 . There was a slight increase in specific Nile Red fluorescence per cell for 2 and 2.5 mM silica, at 25 and 29 (Table 2.1), respectively, compared to the lower silica concentrations (0-1.5 mM Si) with an average of 21.25. Increased lipid per cell is indicated by increased specific Nile Red fluorescence where:

$$\text{Specific Nile Red fluorescence} = \frac{\text{Total Nile Red fluorescence} \times 10,000}{\text{cell concentration}}$$

As shown in Figure 2.4 (B), Nile Red fluorescence for the six silica concentrations grouped into three clusters: (i) 0, 0.5 mM Si, (ii) 1, 1.5 mM Si and (iii) 2 and 2.5 mM Si. The two highest silica concentrations, 2 and 2.5 mM Si, reached the highest total Nile Red fluorescence at 29430 ± 1235 and 33030 ± 2440 and specific fluorescence at 25 ± 6 and 29 ± 4 , respectively (Table 2.1).

When grown with silica concentrations double or quadruple the aqueous solubility (4 and 8 mM Si) cultures reached higher cell densities and lipid content compared to those grown between 0-2.5 mM Si. RGd-1 utilized most of this silica and grew to higher cell numbers (Figure A.8), lipid accumulation (Figures 2.7, 2.6,

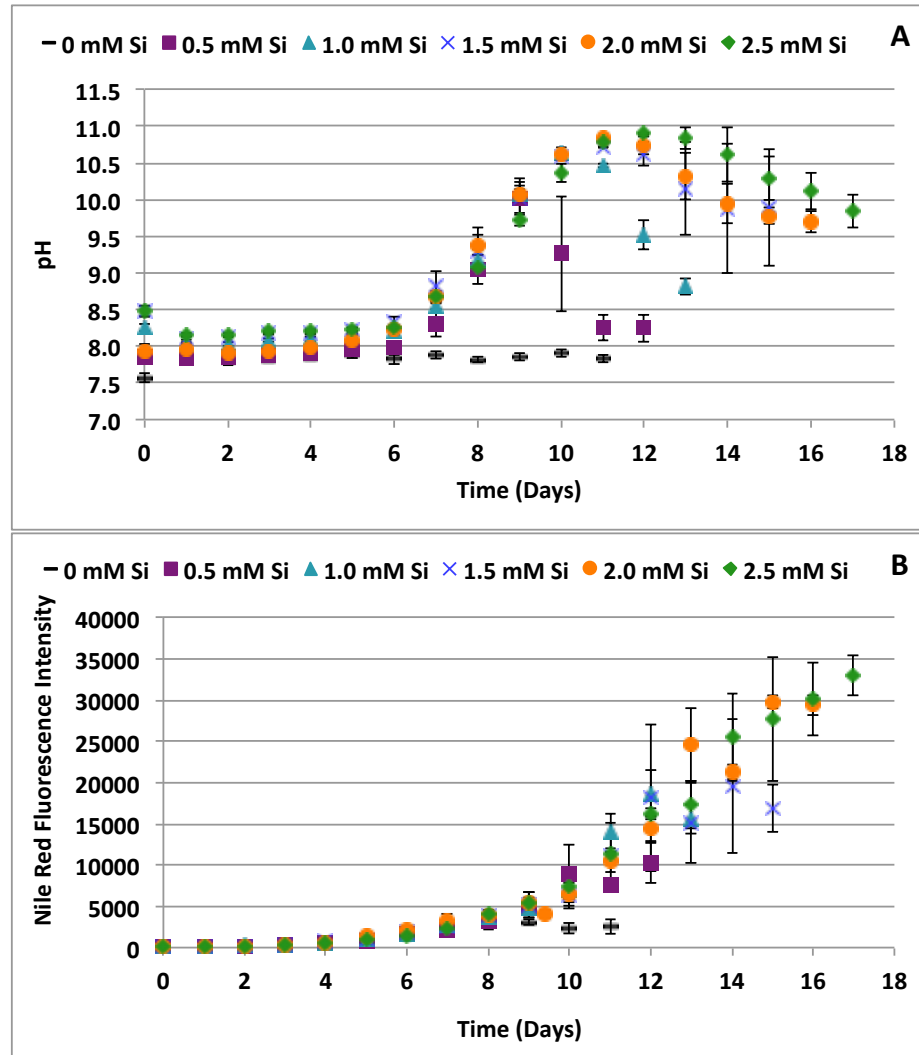


Figure 2.4 (A) Medium pH for diatoms six silica concentrations (B) Nile Red intensity of RGd-1 grown in each of six silica concentrations.

A.10 and A.11) and consequent dry cell weight (Table 2.1). However, cultures grown with 4 and 8 mM Si grew at a slower rate with 33.45 and 37.13 hour doubling times, respectively (Table 2.1). It is likely this decreased growth rate can be attributed to silica limitation imposed by silica polymerization [28]. This indicates that in spite of possible silica polymer formation at 4 and 8 mM Si, biologically available silica

(monomeric and dimeric silicic acid) was available from polymerized silica. However, there is an apparent tradeoff for growth rate and growth yield at these high silica concentrations. Results show that even though those cells were able to utilize the majority of the silica present at high concentrations (Figure A.15), faster growth rates were observed at silica concentrations below saturation.

When grown in 2mM Si, RGd-1 reached maximum cell growth rate without risk of silica polymerization associated with concentrations exceeding solubility (>2 mM) [28, 64]. Organisms with faster growth rates are much more likely to succeed in an outdoor raceway pond. Sub-optimal growth rates of desired organisms increases the potential for unwanted organisms with faster growth rates to take over the pond, resulting in poor TAG production or pond “crash” [46, 54]. Of the conditions tested here, diatoms grown in 2 mM silica yielded optimal cell concentrations, growth rates, dry cell weight and Nile Red fluorescence. Therefore, based on these results, 2 mM Si was chosen as the model silica concentration for further optimization of this strain. As verified by a student t-test ($\alpha = 0.05$ & $t_{\text{stat}} = 0.048$; $p = 0.481$ & $t_{\text{critical}} = 1.746$ and $p = 0.963$ & $t_{\text{critical}} = 2.120$ for one tailed and two tailed distributions about the mean, respectively), there was no significant difference between 2.0 and 2.5 mM Si. Therefore, 2 mM Si was chosen as the optimal silica concentration for RGd-1 growth and lipid accumulation to minimize cost associated with extra unnecessary silica.

Sodium Bicarbonate Addition and Nitrate Limitation

To increase the intracellular lipid content, cultures were grown under limited nitrate (1 mM) and with sodium bicarbonate (25 mM) added just prior to silica depletion to increase lipid per cell. Figure 2.5 (A) shows cell growth remained consistent across the four growth conditions tested: two nitrate concentrations (2.94 and 1 mM NO_3^-) each grown with and without sodium bicarbonate addition. Final cell concentrations were not statistically different, ranging from $1.22 \times 10^7 \pm 3.72 \times 10^6$ cells/mL (2.94 mM NO_3^- without NaHCO_3) to $1.48 \times 10^7 \pm 1.16 \times 10^6$ cells/mL (2.94 mM NO_3^- with NaHCO_3) (Table 2.1, Figure 2.5 (A)). Silica was depleted by approximately day 12 for each of the four growth systems (Figure 2.5 (B)). By day 12, cultures grown with 1.0 mM NO_3^- were found to contain less total chlorophyll than cultures grown with standard Bold's Basal Medium nitrate concentration (2.94 mM NO_3^-) (Figure 2.5 (D)). For the limited nitrate studies, once nitrate was depleted, chlorophyll began to degrade by day 11, but was stable when grown in 2.94 mM NO_3^- . DIC measured sodium bicarbonate addition was 210.80 ± 4.97 mg/L and 212.67 ± 2.94 mg/L for 2.94 mM and 1 mM NO_3^- , respectively compared to cultures that did not receive sodium bicarbonate addition; 0.99 ± 0.90 mg/L (2.94 mM NO_3^-) and 3.13 ± 3.10 mg/L (1 mM NO_3^-) (Figure A.5).

Cultures with added sodium bicarbonate maintained a slightly higher pH when compared to those that did not (Figure 2.5 (E)). This trend was consistent at both 1.0 mM NO_3^- and 2.94 mM NO_3^- with added sodium bicarbonate, indicating a possible pH stress, and DIC increase, with greater potential for lipid accumulation. There was an increase in Nile Red fluorescence observed with number of physiological stresses (1) silica

limitation alone, (2) silica and nitrate depletion or silica limitation with sodium bicarbonate addition or (3) silica and nitrate limitation with sodium bicarbonate addition. Cultures grown under silica limitation alone resulted in a total Nile Red fluorescence of 29430 ± 2440 (Figure 2.5 (F)). A combination of silica and nitrate limitation resulted in a slight increase in Nile Red fluorescence to 36683 ± 9126 . When combined with sodium bicarbonate addition, total Nile Red fluorescence exceeded the other two combinations. Silica depletion and sodium bicarbonate addition resulted in substantial Nile Red increase to 50613 ± 3134 . The most dramatic response was observed through the combination of three physiological stresses: silica with nitrate depletion and sodium bicarbonate addition with a total Nile Red fluorescence of 57122 ± 1870 . For cultures grown under one stress (silica depletion) the specific Nile Red fluorescence was 25 ± 6 . When grown under two stresses (e.g. silica and nitrate depletion or silica depletion with sodium bicarbonate addition), specific Nile Red fluorescence reached 29 ± 6 and 34 ± 2 , respectively. However, when the three stresses were combined (silica and nitrate depletion with added sodium bicarbonate) specific Nile Red fluorescence reached the highest values at 43 ± 6 and can be visualized microscopically by transmitted light microscopy and epifluorescent microscopy (Figures 2.6 (A) and 2.6 (B), respectively). These results indicate that additive stresses have significant potential to increase TAG production in these diatom cultures.

When grown under limited nitrate conditions, cultures run the risk of having insufficient amounts of nitrogen available to build essential enzymes and proteins

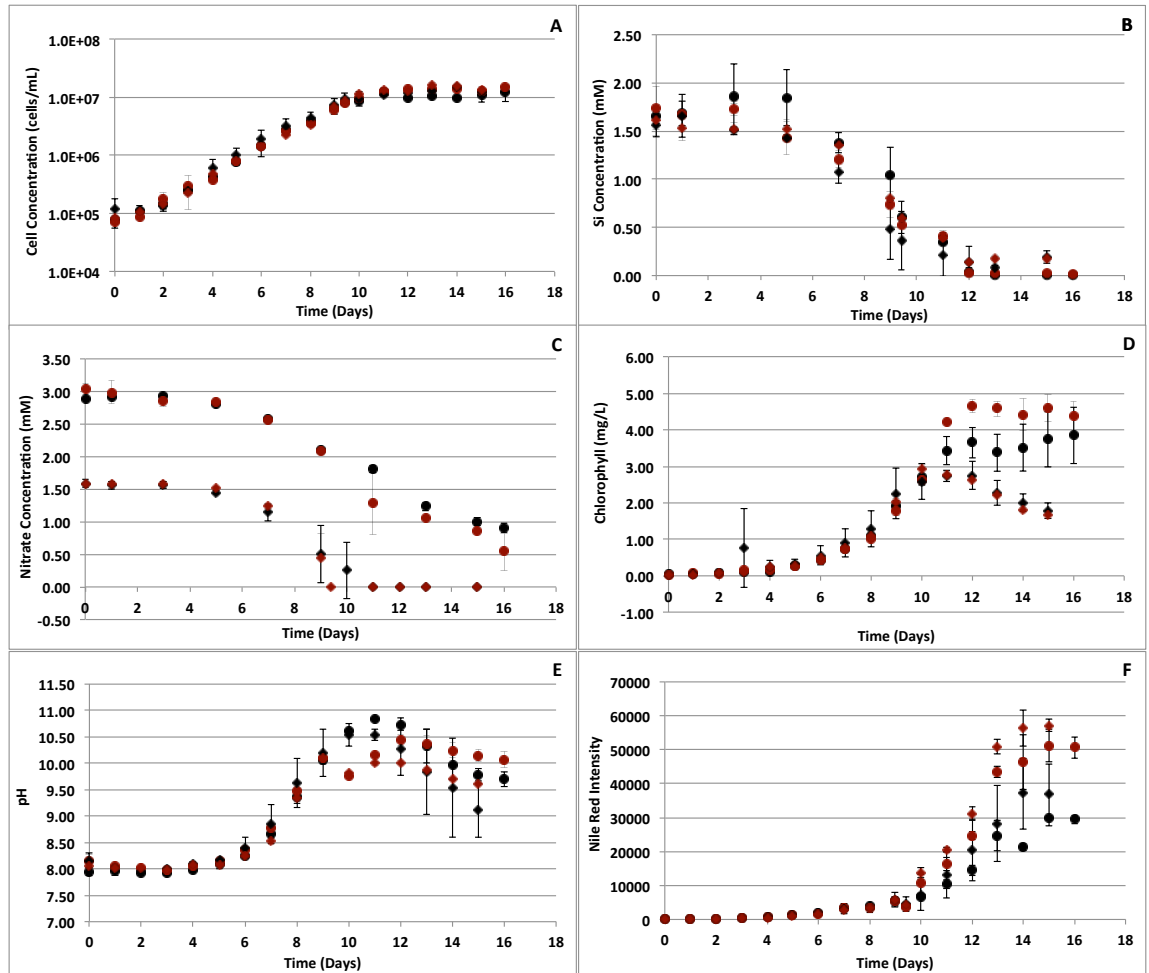


Figure 2.5 Growth characteristics for RGd-1 grown in four conditions: standard BBM nitrate concentrations (2.94 mM) ($\bullet$), 2.94 mM NO_3^- + 25 mM NaHCO_3 ($\bullet$), 1 mM NO_3^- ($\blacklozenge$), and 1 mM NO_3^- + 25 mM NaHCO_3 ($\blacklozenge$). (A) Diatom cell growth (cells mL^{-1}) (B) Silicon removal from the growth medium (C) NO_3^- from the growth medium (mM) (D) Chlorophyll concentration (mg/L) (E) Medium pH (F) Nile Red fluorescence intensity.

required for cellular activities such as lipid synthesis and storage [8, 49]. While chlorophyll degradation suggests diminished photosynthetic efficiency and consequent

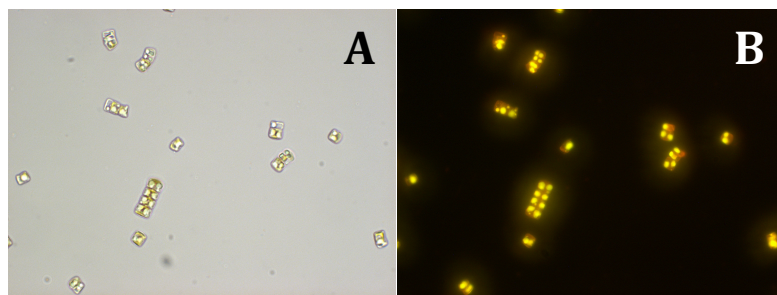


Figure 2.6 (A) Transmitted light images of RGd-1 (B) Epifluorescent microscopy of RGd-1 stained with Nile Red.

capacity to fix carbon, these results indicate total and specific lipid accumulation was not adversely affected. Therefore, this indicates a potential for decreased chlorophyll stability, however, it is still relevant to incorporate nitrate stress in large-scale systems, with careful monitoring of cell health. If cultures are harvested in a timely manner, this study indicates an increased capacity to obtain greater quantities of lipids with potentially lower costs.

Lipid Accumulation

When grown in the presence of silica, there was a similar lipid per weight of biomass for each condition tested. This result was not expected since the Nile Red fluorescence indicated increased total and specific TAG accumulation. With exception of 0 silica added, all conditions tested resulted in similar percent lipid per weight of biomass (30-40% (w/w) TAG and 70-80% (w/w) biofuel potential) for AFDW (Figure 2.7). However, it is possible to obtain the total TAG or biofuel yield in each system (Figure 2.9

(A, B) when the total lipid production in each system is examined, (i.e. % biofuel potential multiplied by the dry cell weight,). When viewed in this way, it is evident that although under most conditions there was approximately the same amount of lipid per weight of biomass (Figure 2.7, Table 2.1), there was a greater *total* lipid produced when grown with higher silica concentrations and when the cultures were amended with sodium bicarbonate. Figure 2.8 summarizes the composition of the fatty acids extracted from RGd-1 under the conditions tested. With exception of 0 silica added, approximately 94% of FAMES existed as C16:0, C16:1, C18:1-3 and C20:5, averaging at approximately 35, 30, 13 and 10%, respectively. Additionally, there is a direct correlation between dry cell weight and sum of C16, C18 and C20 (Figure 2.8) saturated and unsaturated fatty acids indicating the majority of the lipid content in the cells was comprised of the three fatty acids.

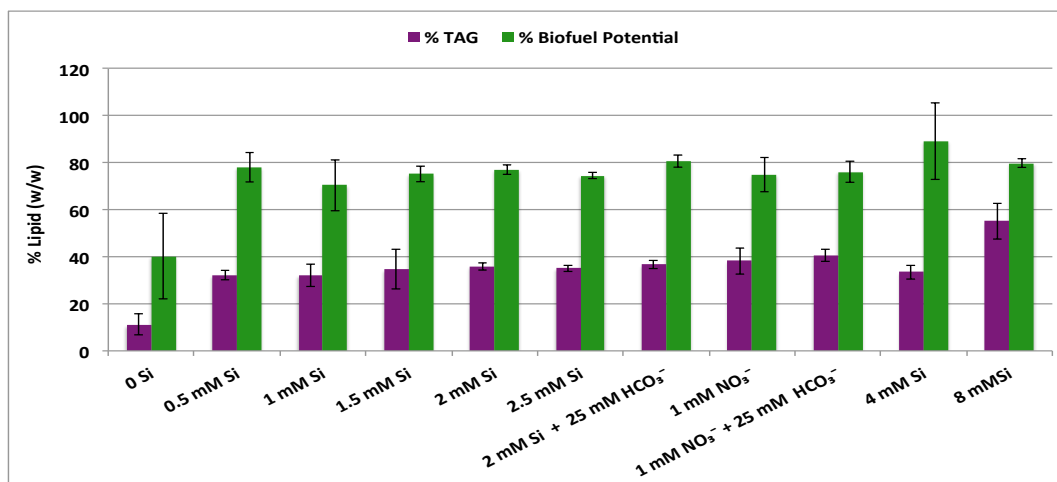


Figure 2.7 Percent lipid (w/w) in biomass: % TAG and % Biofuel Potential (%BP).

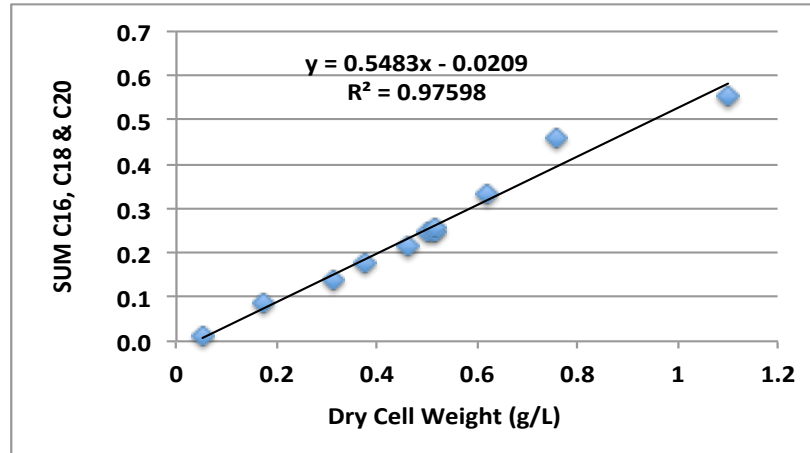


Figure 2.8 Correlation of C16, C18 and C20 to dry cell weight (DCW).

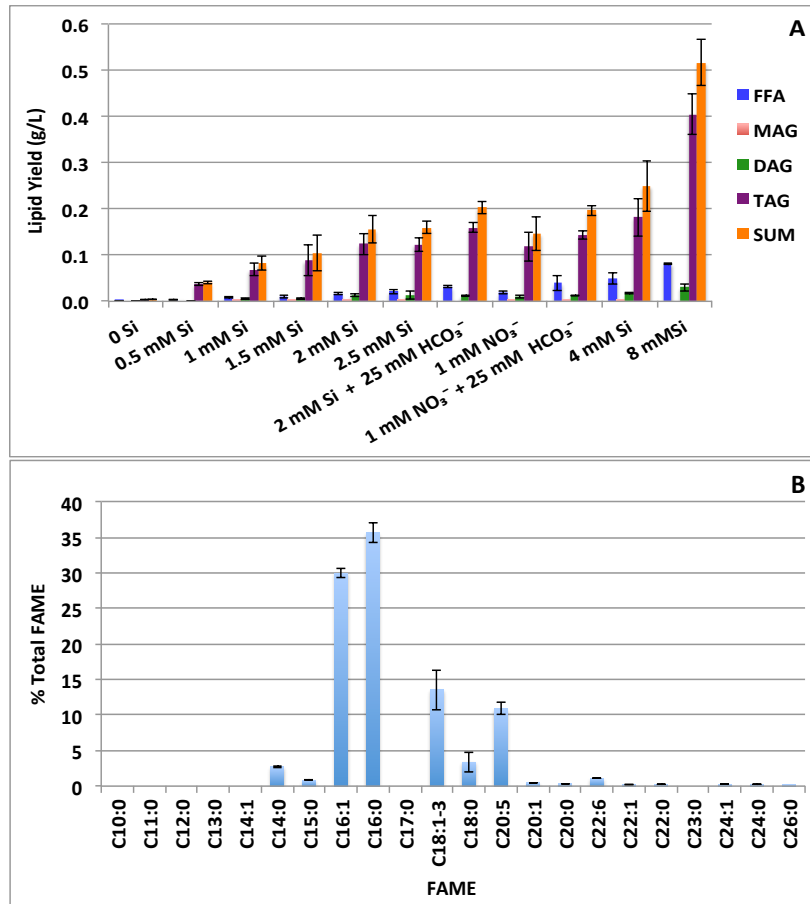


Figure 2.9 Final lipid yields ($\text{g}\cdot\text{L}^{-1}$) of RGd-1 of grown in each condition tested. (A) MAG (blue) DAG (red) TAG (green) and total lipid (orange) of AFDW (B) FAME speciation as % total FAME in 2mM Si system.

Conclusions

Based on doubling time, cell yield, dry cell weight and lipid content, RGd-1 growth on 2 mM Si was chosen as the optimum silica concentration for growth. It is evident that both nitrate limitation and sodium bicarbonate addition each resulted in an increase in Nile Red fluorescence. Cultures which received sodium bicarbonate remained at a higher pH than cultures that did not. Sodium bicarbonate addition, therefore, added a pH stress as well as an added source of inorganic carbon that can be used for lipid production [31]. Concentrations exceeding 2 mM run the risk of added complications resulting from polymerization of silicic acid, rendering it potentially less bioavailable. Decreased growth rates for 4 and 8 mM silica were likely due to polymerized silica. Silica depletion in over saturated systems was verified by ICP-MS. Transmitted light images indicate that polymerization may have occurred in 4 and 8 mM Silica systems.

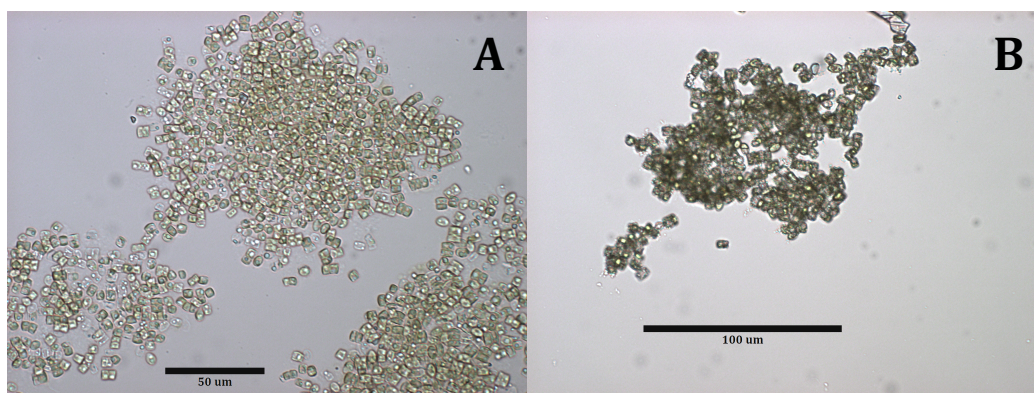


Figure 2.10 RGd-1 cell aggregates following 15 days of growth in (A) 4 mM and (B) 8 mM Sodium metasilicate. Micrographs were obtained using transmitted light with a 40x water immersion objective.

Increased initial silica concentrations were found to increase RGd-1 dry cell weight, cell concentration, pH, chlorophyll and nitrate utilization as well as total TAG and biodiesel potential. When taking into consideration these growth characteristics, 2 mM sodium metasilicate was found to optimize final cell concentration and lipid content. Increasing the silica concentration from 2 to 2.5 mM silica was marginal with no significant difference in cell concentration, dry cell weight, %TAG content or % biofuel potential. Silica concentrations exceeding solubility (4 and 8 mM Si) resulted in longer periods of growth and consequent elevated pH. However, once silica was depleted, total Nile Red fluorescence increased. Gardner, et al. (2011), found a similar effect with the Chlorophytes, *Scenedemus sp.* and *Coelastrrella sp.* under nitrate and pH stress. There was an initial increase in Nile Red fluorescence when under pH stress (>pH 10), but when cells faced nitrate depletion, Nile Red fluorescence increased further. A combined pH and nitrate stress resulted in the highest Nile Red fluorescence intensity, indicating that lipid accumulation was due to combined stresses. RGd-1 was exposed to the following stresses that have the potential to additively accumulate high quantities of lipids: pH stress, silica, nitrate and iron depletion as well as sodium bicarbonate addition. These results indicate a similar phenomenon, whereby, Nile Red fluorescence increases initially, with pH, but when cells face nutrient limitation such as silica, or nitrate depletion, Nile Red intensity exceeds those in nutrient replete conditions. Further, while cultures grown under nitrate replete conditions reached higher Nile Red fluorescence, cultures grown under limited nitrate conditions reached 1.5 times the maximal Nile Red fluorescence.

Lipid analysis by gas chromatography indicated that as long as silica is present, RGd-1 accumulated 30-40% TAG per weight and 70-80% biofuel potential. However, when lipid yield is evaluated for the entire system, it is clear more TAG and biofuel potential result. It is hypothesized that the discrepancy between % TAG and Nile Red fluorescence is due to frustule breakage during centrifugation as a part of the harvesting process, resulting in lipid loss and under-represented % neutral lipid and FAME. In addition, there is a notable increase in TAG yield when grown in 4 and 8 mM Si at ~ 1.5 and > 3 times the TAG yield compared to cultures grown in 2 mM Si. It is possible the RGd-1 grown at the highest silica concentrations had more silicified cell walls that were able to withstand cell breakage that is hypothesized to occur during centrifugation as a part of the harvesting process.

The results of this study indicate multiple stresses that can result in lipid accumulation: silica and nitrate depletion, sodium bicarbonate addition, and potential iron and pH stress. At this point it is unclear if each of these results in cumulative lipid production. Future work will attempt to determine the potential for additive metabolic pathways to induce lipid accumulation.

Acknowledgements

The authors acknowledge funding for the establishment and operation of the Environmental and Biofilm Mass Spectrometry Facility at Montana State University (MSU) through the Defense University Research Instrumentation Program (DURIP, Contract Number: W911NF0510255) and the MSU Thermal Biology Institute from the

NASA Exobiology Program (Project NAG5-8807). Funding Source: U.S. Department of Energy, Office of Biomass Programs grant DE-FG36-08G018161. This work was made possible by the microscopy facilities at the Center for Biofilm Engineering, which was supported by funding obtained from the NSF-MRI Program and the M.J. Murdock Charitable Trust.

CHAPTER 3

CONCLUSIONS

Suggestions For Future Work

Diatom strain, RGd-1 has strong potential as a contributor for biofuel production. Further optimization studies (growth and molecular) can elucidate key insight to RGd-1 growth and lipid accumulation.

1. To clarify the contribution of iron stress to lipid accumulation, additional growth studies are required. Specifically, varying Si:Fe ratios will help determine the contribution of each, as well as a potential to induce a dual stress resulting in lipid accumulation.
2. This organism does not have its genome sequenced. In fact, there are only two diatom genomes that are publically available, fully assembled and annotated. Having this information available will allow for transcriptomic, proteomic and metabolomic analyses. While growth studies are key to understanding an organism's physiology, in depth analyses are limited without detailed transcriptomic, proteomic and metabolomic work.
3. Lipid accumulation in this work involved five potential lipid-inducing stresses. It is important to determine the contribution of each physiological stress on lipid accumulation, which could be achieved using transcriptomic analyses. Specifically, determining how significant each physiological stress (e.g. silica or nitrate limitation as well as sodium bicarbonate addition is in their contribution

4. towards additive lipid accumulation and the extent to which genes are up or down expressed in response to physiological stress.
5. Diatoms are known to utilize bacterial siderophores [92]. Given the potential for iron to limit diatom growth, a mixed culture of diatom and siderophore producing bacteria could potentially result in increased diatom growth.
6. Preliminary work of growing RGd-1 in a raceway pond resulted in the cells becoming dense and sinking. Even though RGd-1 is well characterized to be a planktonic organism, it is possible that raceway ponds are not the most suitable method for growth for diatoms. Rather, it would be advantageous to develop an alternative growth system for diatom biofuel production. Potential systems could utilize sand as an inexpensive silica source or allow for diatom biofilm formation. Preliminary confocal microscopy imaging showed that using a combination of nucleic acid and lipophilic stains can show improved resolution to image diatom cells growing within a biofilm. Biofilms can be stained, cryosectioned and imaged for lipid accumulation with depth. This would provide an additional method to determine lipid content using an alternative growth system that may be more advantageous for improved diatom growth and biofuel viability.

REFERENCES

- [1] V. Smetacek. Diatoms and the Ocean Carbon Cycle. *Protist* 150 (1999) 25-32.
- [2] I.P.o.C.C. (IPCC). IPCC Fourth Assessment Report: Climate Change 2007. 2007.
- [3] J. MacKenzie. Oil as a finite resource. *Natural Resources Research* 7 (1998) 97-100.
- [4] U.S.E.I. Administration.
- [5] Y. Chisti. Biodiesel from microalgae. *Biotechnology Advances* 25 (2007) 294-306.
- [6] A.L. Ahmad, N.H.M. Yasin, C.J.C. Derek, J.K. Lim. Microalgae as a sustainable energy source for biodiesel production: A review. *Renewable and Sustainable Energy Reviews* 15 (2011) 584-93.
- [7] E. Eustance, Gardner, Robert D., Moll, Karen, Menicucci, Joseph, Gerlach, Robin & Peyton, Brent, M. . Growth, nitrogen utilization and biofuel potential for two Chlorophytes grown on ammonium or nitrate. *Journal of Applied Phycology* (2012).
- [8] M. Hildebrand, A.K. Davis, S.R. Smith, J.C. Traller, R. Abbriano. The place of diatoms in the biofuels industry. *Biofuels* 3 (2012) 221-40.
- [9] J. Sheehan, L. National Renewable Energy. A look back at the U.S. Department of Energy's Aquatic Species Program biodiesel from algae. National Renewable Energy Laboratory, Golden, Colo., 1998.
- [10] P.G. Roessler. Effects if Silicon Deficiency in Lipid Composition and Metabolism in the Diatom *Cyclotella Cryptica*. *Journal of Phycology* 24 (1988) 394-400.
- [11] S.R. Smith, R.M. Abbriano, M. Hildebrand. Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways. *Algal Research* 1 (2012) 2-16.
- [12] E.V. Armbrust, J.A. Berges, C. Bowler, B.R. Green, D. Martinez, N.H. Putnam, et al. The Genome of the Diatom *Thalassiosira Pseudonana*: Ecology, Evolution, and Metabolism. *Science* 306 (2004) 79-86.
- [13] F. Round, Mann, DG, Crawford, RM. *The Diatoms: Biology & Morphology of the Genera*. Cambridge, Cambridge University Press, 1990.
- [14] K. Roberts, E. Granum, R. Leegood, J. Raven. Carbon acquisition by diatoms. *Photosynthesis Research* 93 (2007) 79-88.

- [15] E.V. Armbrust. The life of diatoms in the world's oceans. *Nature* 459 (2009) 185-92.
- [16] V. Martin-Jézéquel, M. Hildebrand, M.A. Brzezinski. Silicon Metabolism in Diatoms: Implications for Growth. *Journal of Phycology* 36 (2000) 821-40.
- [17] N.S. Kroger, M. The Biochemistry of Silica Formation in Diatoms. In: E. Baeuerlein, (Ed.) *Biom mineralization: From Biology to Biotechnology and Medical Application*, Wiley-VCH, Weinheim, 2000. p. 151-70.
- [18] R.K. Iler. *The Colloid Chemistry of Silica and Silicates*. Ithaca, NY, Cornell University Press, 1955.
- [19] D.A. Hutchins, K.W. Bruland. Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. *Nature* 393 (1998) 561-4.
- [20] A. Kamatani, J.P. Riley. Rate of dissolution of diatom silica walls in seawater. *Marine Biology* 55 (1979) 29-35.
- [21] A. Kamatani. Dissolution rates of silica from diatoms decomposing at various temperatures. *Marine Biology* 68 (1982) 91-6.
- [22] K.D. Bidle, F. Azam. Accelerated dissolution of diatom silica by marine bacterial assemblages. *Nature* 397 (1999) 508-12.
- [23] M. Hildebrand, L.G. Frigeri, A.K. Davis. Synchronized Growth of *Thalassiosira pseudonana* (Bacillariophyceae) Provides Novel Insights Into Cell-Wall Synthesis Processes in Relation to the Cell Cycle. *Journal of Phycology* 43 (2007) 730-40.
- [24] M.A. Brzezinski, D.J. Conley. Silicon Deposition During The Cell Cycle Of *Thalassiosira weissflogii* (Bacillariophyceae) Determined Using Dual Rhodamine 123 And Propidium Iodide Staining. *Journal of Phycology* 30 (1994) 45-55.
- [25] M. Hildebrand. Diatoms, Biom mineralization Processes, and Genomics. *Chemical Reviews* 108 (2008) 4855-74.
- [26] W.M. Darley, B.E. Volcani. Role of silicon in diatom metabolism: A silicon requirement for deoxyribonucleic acid synthesis in the diatom *Cylindrotheca fusiformis* Reimann and Lewin. *Experimental Cell Research* 58 (1969) 334-42.
- [27] D.J. Conley, S.S. Kilham, E. Theriot. Differences in Silica Content Between Marine and Freshwater Diatoms. *Limnology and Oceanography* 34 (1989) 205-13.

- [28] R.K. Iler. The Chemistry of Silica: Solubility, polymerization, colloid and surface properties, and biochemistry, Wiley-Interscience, 1979.
- [29] 4500-SiO₂. Standard Methods for the Examination of Water and Wastewater, American Public Health Association 2005.
- [30] R. Wetherbee, Crawford, S. & Mulvaney, P. The Nanostructure and Development of Diatom Biosilica. In: E. Baeuerlein, (Ed.) Biomineralization: From Biology to Biotechnology and Medical Application, Wiley-VCH, Weinheim, 2000. p. 189-206.
- [31] R. Gardner, K. Cooksey, F. Mus, R. Macur, K. Moll, E. Eustance, et al. Use of sodium bicarbonate to stimulate triacylglycerol accumulation in the chlorophyte *Scenedesmus sp.* and the diatom & *Phaeodactylum tricornutum*. Journal of Applied Phycology (2012) 1-10.
- [32] R.D. Gardner, E. Lohman, R. Gerlach, K.E. Cooksey, B.M. Peyton. Comparison of CO₂ and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. Biotechnology and Bioengineering (2012).
- [33] D. White, A. Pagarette, P. Rooks, S. Ali. The effect of sodium bicarbonate supplementation on growth and biochemical composition of marine microalgae cultures. Journal of Applied Phycology 1-13.
- [34] J.R. Reinfelder, A.J. Milligan, F.o.M.M. Morel. The Role of the C₄ Pathway in Carbon Accumulation and Fixation in a Marine Diatom. Plant Physiology 135 (2004) 2106-11.
- [35] J.R. Reinfelder, A.M.L. Kraepiel, F.M.M. Morel. Unicellular C₄ photosynthesis in a marine diatom. Nature 407 (2000) 996-9.
- [36] J. Valenzuela, Mazurie, A., Carlson, R.P., Gerlach, R., Cooksey, K.E., Peyton, B.M & Fields, M.W. Potential role of multiple carbon fixation pathways during lipid accumulation in *Phaeodactylum tricornutum*. Biotechnology for biofuels 5 (2012) 10.1186/754-6834-5-40.
- [37] J.R. Reinfelder. Carbon Concentrating Mechanisms in Eukaryotic Marine Phytoplankton. Annual review of Marine Science 3 (2011) 291-315.
- [38] K.O. Buesseler, J.E. Andrews, S.M. Pike, M.A. Charette. The Effects of Iron Fertilization on Carbon Sequestration in the Southern Ocean. Science 304 (2004) 414-7.

- [39] A.E. Allen, J. LaRoche, U. Maheswari, M. Lommer, N. Schauer, P.J. Lopez, et al. Whole-cell response of the pennate diatom *Phaeodactylum tricornutum* to iron starvation. *Proceedings of the National Academy of Sciences* 105 (2008) 10438-43.
- [40] P. Kroth. Molecular Biology and the Biotechnological Potential of Diatoms. In: R. León, A. Galván, E. Fernández, (Eds.), *Transgenic Microalgae as Green Cell Factories*. Vol. 616, Springer New York 2007. p. 23-33.
- [41] R.M. Dent, C.M. Haglund, B.L. Chin, M.C. Kobayashi, K.K. Niyogi. Functional Genomics of Eukaryotic Photosynthesis Using Insertional Mutagenesis of *Chlamydomonas reinhardtii*. *Plant Physiology* 137 (2005) 545-56.
- [42] K.L. Kindle. High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. In: M. Lee, (Ed.) *Methods in Enzymology*. Vol. Volume 297, Academic Press 1998. p. 27-38.
- [43] A. Villarejo, F. Martinez, M. del Pino Plumed, Z. Ramazanov. The induction of the CO₂ concentrating mechanism in a starch-less mutant of *Chlamydomonas reinhardtii*. *Physiologia Plantarum* 98 (1996) 798-802.
- [44] C. Zabawinski, N. Van Den Koornhuyse, C. D'Hulst, R. Schlichting, C. Giersch, B. Delrue, et al. Starchless Mutants of *Chlamydomonas reinhardtii* Lack the Small Subunit of a Heterotetrameric ADP-Glucose Pyrophosphorylase. *J Bacteriol* 183 (2001) 1069-77.
- [45] N.m.M. Dorval Courchesne, A. Parisien, B. Wang, C.Q. Lan. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. *Journal of Biotechnology* 141 (2009) 31-41.
- [46] P. Schenk, S. Thomas-Hall, E. Stephens, U. Marx, J. Mussgnug, C. Posten, et al. Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *BioEnergy Research* 1 (2008) 20-43.
- [47] P.T. Pienkos, A. Darzins. The promise and challenges of microalgal-derived biofuels. *Biofuels, Bioproducts and Biorefining* 3 (2009) 431-40.
- [48] R. Gardner, P. Peters, B. Peyton, K. Cooksey. Medium pH and nitrate concentration effects on accumulation of triacylglycerol in two members of the chlorophyta. *Journal of Applied Phycology* 23 (2011) 1005-16.
- [49] M. Griffiths, S. Harrison. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology* 21 (2009) 493-507.

- [50] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal* 54 (2008) 621-39.
- [51] Q. Hu. Environmental Effects on Cell Composition. In: A. Richmond, (Ed.) *Handbook of Microalgal Culture*, Blackwell, Oxford, 2004. p. 83-93.
- [52] J.B. Guckert, K.E. Cooksey. Triglyceride Accumulation and Fatty Acid Profile Changes in *Chlorella* (Chlorophyta) During High pH-Induced Cell Cycle Inhibition. *Journal of Phycology* 26 (1990) 72-9.
- [53] J. Sheehan, Dunahay, T. Benemann, J. & Roessler, P. A look back at the U.S. Department of Energy's Aquatic Species Program- biodiesel from algae. National Renewable Energy Laboratory. (1998).
- [54] O. Pulz, W. Gross. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology* 65 (2004) 635-48.
- [55] L. Rodolfi, G. Chini Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, et al. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering* 102 (2009) 100-12.
- [56] M.T. Madigan, Martinko, John M., Stahl, David, A. & Clark, David P. Brock *Biology of Microorganisms*. 13 ed. Boston, Benjamin Cummings, 2012.
- [57] W.M. Strumm, James J. *Aquatic Chemistry*, Wiley, 1996.
- [58] T.M. Mata, A.n.A. Martins, N.S. Caetano. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews* 14 (2010) 217-32.
- [59] J.A. Raven. The Transport and Function of Silicon in Plants. *Biological Reviews* 58 (1983) 179-207.
- [60] N. Kroger, N. Poulsen. Diatoms, From Cell Wall Biogenesis to Nanotechnology. *Annual Review of Genetics* 42 (2008) 83-107.
- [61] R. Radakovits, R.E. Jinkerson, S.I. Fuerstenberg, H. Tae, R.E. Settlege, J.L. Boore, et al. Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. *Nat Commun* 3 (2012) 686.
- [62] C.B. Field, M.J. Behrenfeld, J.T. Randerson, P. Falkowski. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science* 281 (1998) 237-40.

- [63] F.I. Inagaki, Y.M. Motomura, S.O. Ogata. Microbial silica deposition in geothermal hot waters. *Applied Microbiology and Biotechnology* 60 (2003) 605-11.
- [64] M. Hildebrand. Silicic Acid Transport and Its Control During Cell Wall Silicification in Diatoms. In: E. Baeuerlein, (Ed.) *Biomineralization: From Biology to Biotechnology and Medical Application*, Wiley-VCH, Weinheim, 2000. p. 171-88.
- [65] M.T. Croft, A.D. Lawrence, E. Raux-Deery, M.J. Warren, A.G. Smith. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. *Nature* 438 (2005) 90-3.
- [66] J.A. Berges, D.O. Charlebois, D.C. Mauzerall, P.G. Falkowski. Differential Effects of Nitrogen Limitation on Photosynthetic Efficiency of Photosystems I and II in Microalgae. *Plant Physiology* 110 (1996) 689-96.
- [67] G.E. FOGG. Photosynthesis and Formation of Fats in a Diatom. *Annals of Botany* 20 (1956) 265-85.
- [68] A. Ben-Amotz, T.G. Tornabene, W.H. Thomas. Chemical Profile of Selected Species of Microalgae with Emphasis on Lipids. *Journal of Phycology* 21 (1985) 72-81.
- [69] M. Piorreck, K.-H. Baasch, P. Pohl. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry* 23 (1984) 207-16.
- [70] N.S. Shifrin, S.W. Chisholm. Phytoplankton Lipids: Interspecific Differences and Effects of Nitrate, Silicate and Light-Dark Cycles. *Journal of Phycology* 17 (1981) 374-84.
- [71] H.A. Spoehr, H.W. Milner. The Chemical Composition of *Chlorella*; Effect of Environmental Conditions. *Plant Physiology* 24 (1949) 120-49.
- [72] Y. Suen, J.S. Hubbard, G. Holzer, T.G. Tornabene. Total Lipid Production of the Green Alga *Nannochloropsis* sp. Under Different Nitrogen Regimes. *Journal of Phycology* 23 (1987) 289-96.
- [73] S. Taguchi, J.A. Hirata, E.A. Laws. Silicate Deficiency and Lipid Synthesis of Marine Diatoms. *Journal of Phycology* 23 (1987) 260-7.

- [74] J. Coombs, P.J. Halicki, O. Holm-Hansen, B.E. Volcani. Studies on the biochemistry and fine structure of silica shell formation in diatoms : Changes in concentration of nucleoside triphosphates during synchronized division of *Cylindrotheca fusiformis* Reimann and Lewin. *Experimental Cell Research* 47 (1967) 302-14.
- [75] L. Provasoli, J.J.A. McLaughlin, M.R. Droop. The development of artificial media for marine algae. *Archives of Microbiology* 25 (1957) 392-428.
- [76] R.e. Andersen. *Algal Culturing Techniques*. San Francisco, Academic Press, 2005.
- [77] C. Zhu, Y. Lee. Determination of biomass dry weight of marine microalgae. *Journal of Applied Phycology* 9 (1997) 189-94.
- [78] P. Chelf. Environmental control of lipid and biomass production in two diatom species. *Journal of Applied Phycology* 2 (1990) 121-9.
- [79] R. Ritchie. Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. *Photosynthetica* 46 (2008) 115-26.
- [80] K.E. Cooksey, J.B. Guckert, S.A. Williams, P.R. Callis. Fluorometric determination of the neutral lipid content of microalgal cells using Nile Red. *Journal of Microbiological Methods* 6 (1987) 333-45.
- [81] D. Elsey, D. Jameson, B. Raleigh, M.J. Cooney. Fluorescent measurement of microalgal neutral lipids. *Journal of Microbiological Methods* 68 (2007) 639-42.
- [82] W. Chen, C. Zhang, L. Song, M. Sommerfeld, Q. Hu. A high throughput Nile red method for quantitative measurement of neutral lipids in microalgae. *Journal of Microbiological Methods* 77 (2009) 41-7.
- [83] M. Griffiths, R. van Hille, S. Harrison. Selection of Direct Transesterification as the Preferred Method for Assay of Fatty Acid Content of Microalgae. *Lipids* 45 (2010) 1053-60.
- [84] N. Bigelow, W. Hardin, J. Barker, S. Ryken, A. MacRae, R. Cattolico. A Comprehensive GC-MS Sub-Microscale Assay for Fatty Acids and its Applications. *Journal of the American Oil Chemists' Society* 88 (2011) 1329-38.
- [85] M. Majaneva, J.-M. Rintala, M. Piisilä, D. Fewer, J. Blomster. Comparison of wintertime eukaryotic community from sea ice and open water in the Baltic Sea, based on sequencing of the 18S rRNA gene. *Polar Biology* 35 (2012) 875-89.

- [86] S.Y. Moon-van der Staay, R. De Wachter, D. Vaultot. Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature* 409 (2001) 607-10.
- [87] T.J. White, Bruns, T., Lee, S., & Taylor, J. . Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: M.A. Innis, Gelfand, D.H., Sninsky, J.J. & White, T.J., (Ed.) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, Inc., San Diego, 1990. p. 315-22.
- [88] M. Gardes, T.J. White, J.A. Fortin, T.D. Bruns, J.W. Taylor. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Canadian Journal of Botany* 69 (1991) 180-90.
- [89] A.W. Coleman, A. Suarez, L.J. Goff. Molecular Delineation of Species and Syngens in Volvocacean Green Algae (Chlorophyta). *Journal of Phycology* 30 (1994) 80-90.
- [90] T.G. Mitchell, E.Z. Freedman, T.J. White, J.W. Taylor. Unique oligonucleotide primers in PCR for identification of *Cryptococcus neoformans*. *Journal of Clinical Microbiology* 32 (1994) 253-5.
- [91] B. Wawrik, J.H. Paul, F.R. Tabita. Real-Time PCR Quantification of *rbcL* (Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase) mRNA in Diatoms and Pelagophytes. *Applied and Environmental Microbiology* 68 (2002) 3771-9.
- [92] S. Soria-Dengg, U. Horstmann. Ferrioxamines B and E as iron sources for the marine diatom *Phaeodactylum tricornutum*. *Marine Ecology Progress Series* 127 (1995) 269-77.
- [93] N.R. Pace. A Molecular View of Microbial Diversity and the Biosphere. *Science* 276 (1997) 734-40.
- [94] C.R. Woese. Bacterial Evolution. *Microbiological Reviews* 51 (1987) 221-71.
- [95] M. Gardes, T.D. Bruns. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2 (1993) 113-8.

APPENDICES

APPENDIX A

EXPERIMENTAL DATA

Table A.1 Cell Concentration (cells mL⁻¹) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	9.60E+04	4.60E+04	5.00E+04	6.40E+04	2.78E+04	1.00E+04	3.60E+04	7.90E+04	4.17E+04	3.48E+04	9.75E+04	4.60E+04	7.50E+04	7.28E+04	2.58E+04
1	6.50E+04	5.90E+04	5.25E+04	5.88E+04	6.25E+03	7.30E+04	6.10E+04	6.90E+04	6.77E+04	6.11E+03	8.50E+04	4.90E+04	9.50E+04	7.63E+04	2.42E+04
2	7.90E+04	1.40E+05	1.20E+05	1.13E+05	3.11E+04	8.40E+04	9.50E+04	1.70E+05	1.16E+05	4.68E+04	8.50E+04	1.10E+05	1.43E+05	1.13E+05	2.91E+04
3	1.03E+05	1.50E+05	1.40E+05	1.31E+05	2.48E+04	1.60E+05	1.30E+05	1.36E+05	1.42E+05	1.59E+04	1.63E+05	1.90E+05	1.98E+05	1.84E+05	1.83E+04
4	3.10E+05	3.20E+05	3.50E+05	3.27E+05	2.08E+04	3.80E+05	3.60E+05	4.00E+05	3.80E+05	2.00E+04	3.20E+05	4.30E+05	4.60E+05	4.03E+05	7.37E+04
5	6.30E+05	5.70E+05	5.60E+05	5.87E+05	3.79E+04	7.20E+05	6.10E+05	5.70E+05	6.33E+05	7.77E+04	7.10E+05	6.80E+05	6.90E+05	6.93E+05	1.53E+04
6	1.11E+06	8.00E+05	1.02E+06	9.77E+05	1.59E+05	1.12E+06	1.16E+06	9.70E+05	1.08E+06	1.00E+05	1.08E+06	1.14E+06	1.12E+06	1.11E+06	3.06E+04
7	1.18E+06	1.05E+06	1.26E+06	1.16E+06	1.06E+05	1.80E+06	1.58E+06	1.59E+06	1.66E+06	1.24E+05	1.85E+06	2.21E+06	2.25E+06	2.10E+06	2.20E+05
8	1.24E+06	8.50E+05	9.70E+05	1.02E+06	2.00E+05	3.33E+06	2.91E+06	2.55E+06	2.93E+06	3.90E+05	2.84E+06	3.20E+06	3.64E+06	3.23E+06	4.01E+05
9	1.21E+06	1.44E+06	1.18E+06	1.28E+06	1.42E+05	4.47E+06	4.45E+06	4.38E+06	4.43E+06	4.73E+04	5.27E+06	5.36E+06	6.17E+06	5.60E+06	4.96E+05
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1.23E+06	1.00E+06	1.19E+06	1.14E+06	1.23E+05	5.68E+06	4.23E+06	4.65E+06	4.85E+06	7.46E+05	7.43E+06	6.45E+06	7.48E+06	7.12E+06	5.81E+05
11	1.32E+06	8.00E+05	1.08E+06	1.07E+06	2.60E+05	4.67E+06	4.90E+06	5.83E+06	5.13E+06	6.14E+05	8.33E+06	8.55E+06	9.23E+06	8.70E+06	4.69E+05
12	-	-	-	-	-	5.15E+06	4.38E+06	5.07E+06	4.87E+06	4.23E+05	7.45E+06	7.13E+06	7.43E+06	7.34E+06	1.79E+05
13	-	-	-	-	-	-	-	-	-	-	8.15E+06	7.60E+06	8.28E+06	8.01E+06	3.61E+05

Table A.2 Cell Concentration (cells mL⁻¹) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	2.40E+04	8.90E+04	3.10E+04	4.80E+04	3.57E+04	8.25E+04	7.25E+04	7.00E+04	7.50E+04	6.61E+03	6.50E+04	9.50E+04	5.10E+04	7.03E+04	2.25E+04
1	7.90E+04	6.80E+04	6.10E+04	6.93E+04	9.07E+03	1.10E+05	1.13E+05	9.75E+04	1.07E+05	8.22E+03	7.80E+04	4.90E+04	5.10E+04	5.93E+04	1.62E+04
2	1.40E+05	9.40E+04	7.60E+04	1.03E+05	3.30E+04	1.59E+05	1.66E+05	1.03E+05	1.43E+05	3.45E+04	9.60E+04	6.60E+04	9.80E+04	8.67E+04	1.79E+04
3	1.93E+05	1.73E+05	1.84E+05	1.83E+05	1.00E+04	2.40E+05	2.40E+05	2.80E+05	2.53E+05	2.31E+04	3.40E+05	2.65E+05	2.48E+05	2.84E+05	4.90E+04
4	3.90E+05	3.70E+05	3.90E+05	3.83E+05	1.15E+04	4.37E+05	3.95E+05	4.20E+05	4.17E+05	2.11E+04	3.50E+05	4.20E+05	4.60E+05	4.10E+05	5.57E+04
5	8.10E+05	7.30E+05	7.30E+05	7.57E+05	4.62E+04	8.60E+05	6.90E+05	7.90E+05	7.80E+05	8.54E+04	6.40E+05	7.40E+05	8.80E+05	7.53E+05	1.21E+05
6	1.20E+06	1.20E+06	1.30E+06	1.23E+06	5.77E+04	1.33E+06	1.44E+06	1.64E+06	1.47E+06	1.57E+05	7.90E+05	1.10E+06	1.23E+06	1.04E+06	2.26E+05
7	1.99E+06	1.99E+06	2.27E+06	2.08E+06	1.62E+05	2.58E+06	2.64E+06	2.76E+06	2.66E+06	9.17E+04	2.12E+06	2.53E+06	2.14E+06	2.26E+06	2.31E+05
8	3.16E+06	3.48E+06	3.25E+06	3.30E+06	1.65E+05	3.66E+06	3.69E+06	4.09E+06	3.81E+06	2.40E+05	3.54E+06	3.68E+06	3.49E+06	3.57E+06	9.85E+04
9	5.36E+06	5.54E+06	6.01E+06	5.64E+06	3.36E+05	6.95E+06	5.73E+06	7.05E+06	6.58E+06	7.35E+05	6.37E+06	6.42E+06	6.22E+06	6.34E+06	1.04E+05
9.4	-	-	-	-	-	8.25E+06	8.50E+06	8.50E+06	8.42E+06	1.44E+05	-	-	-	-	-
10	6.48E+06	8.65E+06	9.98E+06	8.37E+06	1.77E+06	8.70E+06	8.03E+06	9.23E+06	8.65E+06	6.01E+05	7.83E+06	8.40E+06	9.18E+06	8.47E+06	6.78E+05
11	7.88E+06	1.04E+07	1.01E+07	9.46E+06	1.38E+06	1.41E+07	1.09E+07	1.19E+07	1.23E+07	1.64E+06	1.22E+07	1.10E+07	1.22E+07	1.18E+07	6.93E+05
12	7.58E+06	9.98E+06	1.49E+07	1.08E+07	3.73E+06	9.48E+06	9.70E+06	1.00E+07	9.73E+06	2.61E+05	1.53E+07	1.23E+07	1.34E+07	1.37E+07	1.52E+06
13	7.65E+06	8.88E+06	9.60E+06	8.71E+06	9.86E+05	1.07E+07	1.00E+07	1.08E+07	1.05E+07	4.36E+05	1.34E+07	1.24E+07	1.33E+07	1.30E+07	5.51E+05
14	6.73E+06	8.33E+06	8.63E+06	7.90E+06	1.02E+06	1.04E+07	9.00E+06	1.01E+07	9.83E+06	7.37E+05	1.22E+07	1.06E+07	1.23E+07	1.17E+07	9.54E+05
15	6.98E+06	9.12E+06	8.60E+06	8.23E+06	1.12E+06	1.13E+07	8.05E+06	1.28E+07	1.07E+07	2.43E+06	1.12E+07	9.13E+06	9.75E+06	1.00E+07	1.06E+06
16	-	-	-	-	-	1.62E+07	8.85E+06	1.15E+07	1.22E+07	3.72E+06	1.17E+07	9.30E+06	1.11E+07	1.07E+07	1.25E+06
17	-	-	-	-	-	-	-	-	-	-	1.25E+07	1.00E+07	1.17E+07	1.14E+07	1.28E+06

Table A.3 Total Silicon Concentration (mM) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	0.201	0.146	0.149	0.165	0.031	0.355	0.456	0.456	0.423	0.058	0.823	0.886	1.746	1.151	0.516
1	0.213	0.116	0.143	0.157	0.050	0.406	0.405	0.420	0.410	0.008	0.834	0.900	1.181	0.972	0.185
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	0.152	0.121	0.162	0.145	0.021	0.344	0.359	0.383	0.362	0.019	0.811	1.168	0.852	0.944	0.195
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	0.078	0.043	0.071	0.064	0.018	0.290	0.272	0.328	0.297	0.029	0.796	0.742	0.866	0.802	0.062
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	0.012	0.017	0.011	0.013	0.004	0.142	0.255	0.200	0.199	0.057	0.570	0.555	0.518	0.547	0.027
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	0.012	0.015	0.011	0.013	0.002	0.000	0.000	0.000	0.000	0.000	0.167	0.162	0.135	0.155	0.017
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	0.013	0.014	0.011	0.013	0.001						0.000	0.000	0.000	0.000	0.000
12	-	-	-	-	-	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	0.000	0.000	0.000	0.000	0.000

Table A.4 Total Silicon Concentration (mM) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	0.220	1.277	1.248	0.915	0.602	1.704	1.708	1.568	1.660	0.079	1.482	1.524	1.729	1.578	0.133
1	1.289	1.190	2.142	1.540	0.523	1.589	1.634	1.834	1.686	0.131	1.847	1.833	1.601	1.760	0.138
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1.581	1.632	1.287	1.500	0.186	1.554	2.218	1.817	1.863	0.334	1.814	1.475	1.710	1.666	0.173
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	1.901	1.354	1.154	1.470	0.386	1.654	2.178	1.704	1.845	0.289	1.988	1.954	2.010	1.984	0.028
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1.029	0.952	1.020	1.000	0.042	1.464	1.264	1.406	1.378	0.103	1.875	2.138	2.843	2.285	0.500
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	0.739	0.517	0.914	0.723	0.199	0.733	1.079	1.305	1.039	0.288	1.762	2.177	1.280	1.740	0.449
9.4	-	-	-	-	-	0.514	0.794	0.501	0.603	0.166	-	-	-	-	-
10	-	-	-	-	-	0.323	0.359	0.380	0.354	0.029	-	-	-	-	-
11	0.242	0.000	0.000	0.081	0.140	0.000	0.094	0.000	0.031	0.054	0.383	0.408	0.179	0.323	0.126
12	-	-	-	-	-	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-
13	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-	0.000	0.000	0.000	0.000	0.000
14	-	-	-	-	-	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-
15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
16	0.220	1.277	1.248	0.915	0.602	1.704	1.708	1.568	1.660	0.079					
17	-	-	-	-	-	-	-	-	-	-	0.000	0.000	0.000	0.000	0.000

Table A.5 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si₂₈, Si₂₉ and Si₃₀) for growth in 0 Si.

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	669508.81	486052.74	497911.89	40820.92	30310.56	30792.15	353414.02	315125.97	286976.04
1	709454.94	386302.11	477550.38	43437.92	24156.38	29727.49	374364.45	269128.29	283759.98
2	-	-	-	-	-	-	-	-	-
3	507544.50	403414.11	539242.62	31591.70	25469.92	33648.87	299272.68	334871.55	363411.07
4	-	-	-	-	-	-	-	-	-
5	259261.00	145438.66	237636.83	17343.94	10608.37	16016.96	274419.13	287127.30	298529.34
6	-	-	-	-	-	-	-	-	-
7	38827.52	58113.16	37026.92	4631.95	6682.61	4501.39	260408.66	276439.26	266635.36
8	-	-	-	-	-	-	-	-	-
9	39616.68	50859.20	38701.21	4705.47	6325.78	4503.26	272627.58	359788.84	253507.46
10	-	-	-	-	-	-	-	-	-
11	44365.42	45737.35	38033.48	5159.58	5338.60	4627.58	286565.88	296190.41	262969.30

Table A.6 Raw Data (cps) measured using ICP-MS for each of the three Si isotopes (Si₂₈, Si₂₉ and Si₃₀) for growth in 0.5 mM Si.

2

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	4295.75	1946.82	1946.88	28.02	127.56	127.64	1043.26	369.64	355.52
1	4691.35	4684.35	4797.41	30.66	30.46	31.30	1062.97	1122.94	1167.27
2	-	-	-	-	-	-	-	-	-
3	4210.46	4324.97	4509.17	27.60	28.10	29.43	1035.79	1030.08	1068.34
4	-	-	-	-	-	-	-	-	-
5	3788.40	3650.19	4087.37	24.96	23.96	26.79	1012.50	985.55	1060.15
6	-	-	-	-	-	-	-	-	-
7	2639.11	3519.94	3092.56	17.77	23.64	20.59	995.15	1303.86	1082.30
8	-	-	-	-	-	-	-	-	-
9	94.74	91.67	181.37	2.48	2.33	2.83	996.64	941.96	962.43
10	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-
12	108.69	91.38	113.76	2.26	2.24	2.94	881.91	875.27	1201.20

Table A.7: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for growth in 1.0 mM Si.

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	7920.48	8408.90	15076.99	50.37	53.50	95.52	1227.79	1267.33	2189.53
1	8006.91	8522.65	10702.80	50.98	54.14	68.13	1282.16	1289.15	1600.69
2	-	-	-	-	-	-	-	-	-
3	7831.81	10596.01	8151.58	49.87	67.73	51.77	1220.17	1619.64	1253.20
4	-	-	-	-	-	-	-	-	-
5	7717.21	7295.77	8258.18	49.23	46.72	52.75	1281.98	1200.57	1371.04
6	-	-	-	-	-	-	-	-	-
7	5961.57	5842.59	5557.24	38.60	37.79	36.10	1182.02	1145.63	1164.82
8	-	-	-	-	-	-	-	-	-
9	2836.98	2797.69	2591.89	18.97	18.88	17.58	1021.41	1095.49	1085.92
10	-	-	-	-	-	-	-	-	-
11	116.91	96.67	111.33	2.76	2.47	2.61	1032.64	935.07	993.60
12	-	-	-	-	-	-	-	-	-
13	109.03	106.82	96.76	2.58	2.87	2.52	965.52	1160.41	980.21

Table A.8: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for growth in 1.5 mM Si.

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	3244.71	11441.69	11217.21	21.75	72.53	70.87	1198.02	1483.27	1467.31
1	11537.72	10770.90	18152.54	72.94	68.13	114.38	1453.26	1459.40	2352.46
2	-	-	-	-	-	-	-	-	-
3	13803.12	14196.87	11521.64	87.17	89.91	72.69	1823.61	1954.47	1559.80
4	-	-	-	-	-	-	-	-	-
5	16280.58	12040.76	10493.77	102.99	76.59	66.12	2234.65	1671.58	1404.26
6	-	-	-	-	-	-	-	-	-
7	9518.75	8925.54	9448.73	60.71	56.95	60.43	1465.97	1397.80	1422.45
8	-	-	-	-	-	-	-	-	-
9	7272.31	5547.83	8631.71	47.53	36.48	56.58	1660.13	1373.30	2045.81
10	-	-	-	-	-	-	-	-	-
11	3417.49	163.30	128.63	23.48	3.16	3.18	1517.33	1075.81	1133.97
12	-	-	-	-	-	-	-	-	-
13	649.42	133.78	249.07	5.38	3.06	6.23	950.80	1070.93	2176.47
14	-	-	-	-	-	-	-	-	-
15	245.24	169.78	144.42	4.18	3.59	2.90	1339.89	1279.69	985.44

Table A.9: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for growth in 2.0 mM Si.

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	6107.36	6120.76	5656.93	412.48	410.87	381.74	591.44	591.98	545.21
1	5725.80	5874.85	6543.34	383.77	396.01	442.95	550.20	569.46	627.71
2	-	-	-	-	-	-	-	-	-
3	5607.86	7823.42	6485.57	377.43	523.63	435.71	552.07	768.16	639.64
4	-	-	-	-	-	-	-	-	-
5	5941.52	7689.30	6107.62	399.84	517.23	412.66	575.89	740.64	590.61
6	-	-	-	-	-	-	-	-	-
7	5309.95	4642.61	5114.03	357.19	313.15	345.27	571.99	491.87	540.54
8	-	-	-	-	-	-	-	-	-
9	2870.53	4025.33	4776.38	192.53	270.05	321.09	407.57	553.94	691.65
9.4	2139.88	3074.14	2095.07	142.83	204.21	138.69	371.99	505.18	374.25
11	1503.04	1622.11	1692.75	100.65	107.67	113.53	365.02	354.41	434.40
12	174.98	736.75	95.51	12.25	44.32	7.84	275.61	340.50	279.28
13	76.74	68.81	84.80	7.10	6.41	8.27	280.12	270.58	393.83
14	-	-	-	-	-	-	-	-	-
15	67.85	106.98	86.84	6.59	9.18	7.88	291.24	347.86	311.12
16	99.22	77.42	78.13	8.76	6.88	7.01	339.44	293.37	290.92

Table A.10: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for growth in 2.5 mM Si.

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	13029.77	13359.13	14951.32	78.10	76.34	81.61	1603.05	1548.41	1662.79
1	15864.89	15753.94	13958.14	85.61	84.73	80.15	1809.57	1855.11	1592.96
2	-	-	-	-	-	-	-	-	-
3	15606.34	12982.89	14799.71	83.98	73.84	84.44	1784.96	1479.07	1693.76
4	-	-	-	-	-	-	-	-	-
5	16954.67	16693.98	17131.25	92.93	90.50	92.64	1930.16	1822.70	1970.73
6	-	-	-	-	-	-	-	-	-
7	16082.98	18118.91	23583.14	87.24	98.75	128.18	1817.06	2027.15	2660.02
8	-	-	-	-	-	-	-	-	-
9	15201.33	18425.95	11465.68	96.04	116.83	72.54	2038.19	2508.16	1625.19
10	-	-	-	-	-	-	-	-	-
11	4513.96	4704.18	2927.12	30.12	31.51	20.29	1321.53	1360.48	1302.82
12	-	-	-	-	-	-	-	-	-
13	389.09	443.92	116.46	5.21	5.09	3.15	1343.25	1212.69	1101.40
14	-	-	-	-	-	-	-	-	-
15	149.07	132.99	161.31	3.62	3.40	4.38	1194.29	1152.04	1513.65
16	-	-	-	-	-	-	-	-	-
17	194.15	129.54	138.52	4.62	3.29	3.59	1541.00	1099.99	1204.85

Table A.11: Measured pH for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	7.51	7.54	7.63	7.56	0.06	7.77	7.89	7.92	7.86	0.08	8.20	8.28	8.30	8.26	0.05
1	7.77	7.79	7.79	7.78	0.01	7.84	7.84	7.83	7.84	0.01	7.98	8.03	8.05	8.02	0.04
2	7.74	7.78	7.80	7.77	0.03	7.85	7.85	7.85	7.85	0.00	7.99	8.05	8.05	8.03	0.03
3	7.79	7.82	7.84	7.82	0.03	7.88	7.89	7.89	7.89	0.01	8.06	8.09	8.10	8.08	0.02
4	7.82	7.85	7.85	7.84	0.02	7.91	7.90	7.91	7.91	0.01	8.06	8.11	8.12	8.10	0.03
5	7.84	7.87	7.90	7.87	0.03	7.93	7.96	7.95	7.95	0.02	8.10	8.17	8.17	8.15	0.04
6	7.75	7.83	7.89	7.82	0.07	7.92	8.00	7.99	7.97	0.04	8.15	8.23	8.22	8.20	0.04
7	7.83	7.87	7.94	7.88	0.06	8.14	8.28	8.46	8.29	0.16	8.49	8.58	8.62	8.56	0.07
8	7.80	7.79	7.85	7.81	0.03	8.96	8.90	9.28	9.05	0.20	9.04	9.21	9.30	9.18	0.13
9	7.80	7.88	7.90	7.86	0.05	9.67	10.13	10.22	10.01	0.30	9.94	10.12	10.17	10.08	0.12
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	7.85	7.90	7.95	7.90	0.05	8.48	9.25	10.06	9.26	0.79	10.52	10.70	10.66	10.63	0.09
11	7.78	7.83	7.88	7.83	0.05	8.10	8.22	8.44	8.25	0.17	10.43	10.48	10.45	10.45	0.03
12	-	-	-	-	-	8.05	8.27	8.41	8.24	0.18	9.75	9.43	9.38	9.52	0.20
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table A.12: Measured pH for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	8.54	8.52	8.40	8.49	0.08	7.86	7.96	8.01	7.94	0.08	8.39	8.47	8.54	8.47	0.08
1	8.09	8.11	8.13	8.11	0.02	7.87	7.98	8.03	7.96	0.08	8.14	8.15	8.17	8.15	0.02
2	8.12	8.13	8.13	8.13	0.01	7.89	7.89	7.97	7.92	0.05	8.14	8.16	8.17	8.16	0.02
3	8.16	8.18	8.18	8.17	0.01	7.87	7.94	7.97	7.93	0.05	8.19	8.21	8.21	8.20	0.01
4	8.18	8.16	8.18	8.17	0.01	7.93	7.97	8.03	7.98	0.05	8.21	8.21	8.23	8.22	0.01
5	8.26	8.24	8.21	8.24	0.03	7.98	8.12	8.14	8.08	0.09	8.21	8.24	8.24	8.23	0.02
6	8.42	8.29	8.26	8.32	0.09	8.20	8.23	8.27	8.23	0.04	8.23	8.27	8.27	8.26	0.02
7	9.04	8.75	8.65	8.81	0.20	8.61	8.76	8.63	8.67	0.08	8.60	8.72	8.68	8.67	0.06
8	9.67	9.12	9.08	9.29	0.33	9.23	9.50	9.40	9.38	0.14	9.03	9.14	9.07	9.08	0.06
9	10.28	9.95	9.86	10.03	0.22	9.98	10.13	10.08	10.06	0.08	9.63	9.73	9.78	9.71	0.08
9	-	-	-	-	-	8.15	8.33	8.20	8.23	0.09	-	-	-	-	-
10	10.58	10.58	10.57	10.58	0.01	10.57	10.62	10.62	10.60	0.03	10.25	10.35	10.51	10.37	0.13
11	10.70	10.72	10.72	10.71	0.01	10.83	10.83	10.82	10.83	0.01	10.74	10.77	10.87	10.79	0.07
12	10.82	10.52	10.53	10.62	0.17	10.72	10.87	10.63	10.74	0.12	10.92	10.91	10.88	10.90	0.02
13	10.88	9.85	9.72	10.15	0.64	10.33	10.64	10.00	10.32	0.32	10.91	10.93	10.67	10.84	0.14
14	10.88	9.55	9.21	9.88	0.88	9.81	10.27	9.78	9.95	0.27	10.83	10.81	10.19	10.61	0.36
15	10.79	9.54	9.33	9.89	0.79	9.72	9.91	9.70	9.78	0.12	10.46	10.47	9.95	10.29	0.30
16	-	-	-	-	-	9.71	9.84	9.55	9.70	0.15	10.27	10.24	9.83	10.11	0.25
17	-	-	-	-	-	-	-	-	-	-	9.97	9.98	9.58	9.84	0.23

Table A.13: Total Nile Red Fluorescence for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	88	0	64	51	45	123	119	46	96	123	0	15	31	15	16
1	55	24	104	61	40	98	92	107	99	98	186	98	128	137	45
2	211	152	67	143	72	134	253	214	200	134	305	254	128	229	91
3	416	357	241	338	89	415	445	330	397	415	296	311	504	370	116
4	577	617	604	599	20	592	610	470	557	592	564	574	683	607	66
5	971	690	845	835	141	727	937	672	779	727	1065	818	1197	1027	192
6	1813	1587	1810	1737	130	1489	1687	1548	1575	1489	1962	1486	1645	1698	242
7	2692	1948	1889	2176	448	2335	1983	1999	2106	2335	2484	2524	3022	2677	300
8	3192	2158	2823	2724	524	3662	3256	2774	3231	3662	3269	3857	3934	3687	364
9	3411	2741	3021	3058	337	6134	4685	3610	4810	6134	4373	4740	4932	4682	284
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	2270	1764	3039	2358	642	12970	7220	6730	8973	12970	4975	7220	8790	6995	1917
11	3479	1865	2222	2522	848	7725	7050	7885	7553	7725	11715	13615	16465	13932	2391
12	-	-	-	-	-	11215	7445	12160	10273	11215	15155	19730	20830	18572	3010
13	-	-	-	-	-	-	-	-	-	-	15625	17285	13795	15568	1746

Table A.14: Total Nile Red Fluorescence for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	31	77	28	45	27	123	79	64	89	31	21	107	55	61	43
1	101	174	155	143	38	153	39	64	85	60	122	177	76	125	51
2	43	204	131	126	81	204	134	156	165	36	122	104	128	118	12
3	369	372	488	410	68	244	305	229	259	40	275	244	223	247	26
4	650	693	675	673	22	668	543	733	648	97	461	702	470	544	137
5	866	1047	882	932	100	1334	1432	1389	1385	49	839	968	909	905	65
6	1819	2143	1904	1955	168	2273	1984	1764	2007	255	1196	1612	1459	1422	210
7	2335	2820	2396	2517	264	3632	2148	3798	3193	909	2273	2588	2280	2380	180
8	3360	4105	4425	3963	546	3950	3260	4450	3887	598	3970	4550	3955	4158	339
9	3480	5540	6395	5138	1498	4580	4530	6950	5353	1383	6740	5230	4320	5430	1222
9.4	-	-	-	-	-	3995	3585	4515	4032	466	-	-	-	-	-
10	4505	7385	6885	6258	1539	7035	6390	6090	6505	483	8625	5690	8105	7473	1566
11	6410	13230	13705	11115	4082	10480	9065	11595	10380	1268	11905	10650	11415	11323	633
12	7965	22250	24185	18133	8859	13855	13290	16295	14480	1597	16815	15880	16295	16330	468
13	10180	19850	15185	15072	4836	25515	19745	28535	24598	4466	16350	15010	20660	17340	2952
14	11305	19930	27515	19583	8111	20965	20525	22370	21287	964	25850	19990	30490	25443	5262
15	14345	20085	16390	16940	2909	30595	29760	29040	29798	778	24480	22310	36260	27683	7506
16	-	-	-	-	-	30550	28105	29635	29430	1235	31400	25240	33790	30143	4411
17	-	-	-	-	-	-	-	-	-	-	30580	33050	35460	33030	2440

Table A.15: Specific Nile Red Fluorescence (Nile Red Fluorescence x 10000/cell count) for growth in each silica concentration (0, 0.5, 1.0 mM Si)

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	9	0	13	7	7	123	33	6	54	61	0	3	4	2	2
1	8	4	20	11	8	13	15	16	15	1	22	20	13	18	4
2	27	11	6	14	11	16	27	13	18	7	36	23	9	23	13
3	40	24	17	27	12	26	34	24	28	5	18	16	25	20	5
4	19	19	17	18	1	16	17	12	15	3	18	13	15	15	2
5	15	12	15	14	2	10	15	12	12	3	15	12	17	15	3
6	16	20	18	18	2	13	15	16	15	1	18	13	15	15	3
7	23	19	15	19	4	13	13	13	13	0	13	11	13	13	1
8	26	25	29	27	2	11	11	11	11	0	12	12	11	11	1
9	28	19	26	24	5	14	11	8	11	3	8	9	8	8	0
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	18	18	26	21	4	23	17	14	18	4	7	11	12	10	3
11	26	23	21	23	3	17	14	14	15	2	14	16	18	16	2
12	-	-	-	-	-	22	17	24	21	4	20	28	28	25	4
13	-	-	-	-	-	-	-	-	-	-	19	23	17	20	3

Table A.16: Specific Nile Red Fluorescence (Nile Red Fluorescence x 10000/cell count) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	13	9	9	10	2	15	11	9	12	3	3	11	11	8	5
1	13	26	25	21	7	14	3	7	8	5	16	36	15	22	12
2	3	22	17	14	10	13	8	15	12	4	13	16	13	14	2
3	19	22	27	22	4	10	13	8	10	2	8	9	9	9	1
4	17	19	17	18	1	15	14	17	15	2	13	17	10	13	3
5	11	14	12	12	2	16	21	18	18	3	13	13	10	12	2
6	15	18	15	16	2	17	14	11	14	3	15	15	12	14	2
7	12	14	11	12	2	14	8	14	12	3	11	10	11	11	0
8	11	12	14	12	2	11	9	11	10	1	11	12	11	12	1
9	6	10	11	9	2	7	8	10	8	2	11	8	7	9	2
9.4	-	-	-	-	-	5	4	5	5	1	-	-	-	-	-
10	7	9	7	7	1	8	8	7	8	1	11	7	9	9	2
11	8	13	14	11	3	7	8	10	8	1	10	10	9	10	0
12	11	22	16	16	6	15	14	16	15	1	11	13	12	12	1
13	13	22	16	17	5	24	20	26	23	3	12	12	16	13	2
14	17	24	32	24	8	20	23	22	22	1	21	19	25	22	3
15	21	22	19	21	1	27	37	23	29	7	22	24	37	28	8
16	-	-	-	-	-	19	32	26	25	6	27	27	30	28	2
17	-	-	-	-	-	-	-	-	-	-	24	33	30	29	4

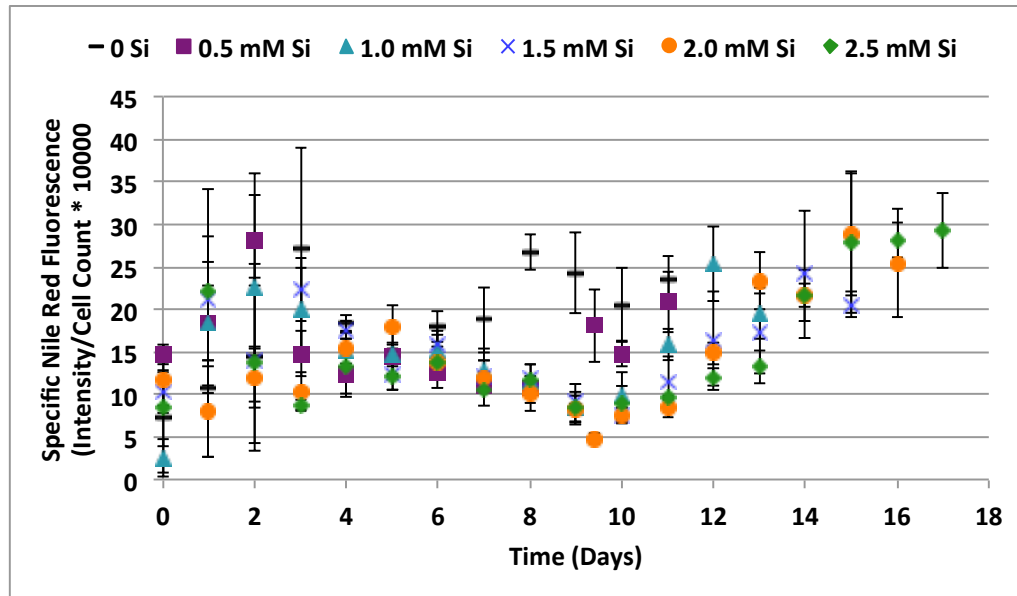


Figure A.1: Specific Nile Red Fluorescence (Nile Red Fluorescence x 10000/cell count) for growth in each silica concentration (0, 0.5, 1.0, 1.5, 1.0, 1.5, 2.0 and 2.5 mM Si).

Table A.17: Measured Nitrate Concentration (mg/L) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	2.80	2.94	2.87	2.87	0.07	2.91	2.98	3.03	2.98	0.06	2.88	2.84	2.86	2.86	0.02
1	2.91	2.89	2.86	2.88	0.03	2.79	2.77	2.80	2.79	0.02	2.63	2.77	2.69	2.70	0.07
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2.97	2.87	2.85	2.89	0.06	2.77	2.73	2.72	2.74	0.03	2.75	2.59	2.66	2.67	0.08
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	2.86	2.86	2.77	2.83	0.05	2.73	2.67	2.71	2.70	0.04	2.63	2.51	2.59	2.58	0.06
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2.72	2.84	2.68	2.74	0.08	2.57	2.53	2.56	2.55	0.02	2.46	2.26	2.34	2.35	0.10
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	2.75	2.82	2.70	2.76	0.06	2.27	2.22	2.24	2.24	0.02	2.08	1.95	2.04	2.02	0.06
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	2.83	2.92	2.82	2.86	0.06	-	-	-	-	-	1.75	1.72	1.74	1.74	0.01
12	-	-	-	-	-	2.24	2.11	2.14	2.16	0.07	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	1.70	1.72	1.74	1.72	0.02

Table A.18: Measured Nitrate Concentration (mg/L) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	2.87	2.80	2.74	2.80	2.90	2.88	2.91	2.89	0.01	2.81	2.93	2.80	2.85	0.07
1	2.85	2.84	2.87	2.85	2.94	2.91	2.94	2.93	0.02	2.82	2.76	2.72	2.76	0.05
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2.85	2.81	2.79	2.82	2.89	2.98	2.93	2.94	0.04	2.75	2.65	2.60	2.67	0.08
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	2.76	2.69	2.66	2.70	2.82	2.81	2.82	2.81	0.01	2.68	2.49	2.63	2.60	0.10
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2.55	2.55	2.50	2.53	2.55	2.59	2.55	2.56	0.02	2.43	2.26	2.18	2.29	0.13
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	2.16	2.06	2.07	2.10	2.06	2.06	2.03	2.05	0.02	2.07	1.91	1.86	1.95	0.11
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1.84	1.49	1.46	1.60	1.71	1.78	1.67	1.72	0.06	1.42	1.27	1.13	1.28	0.15
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	1.61	1.32	1.31	1.41	1.10	1.16	1.03	1.10	0.07	0.92	0.85	0.68	0.82	0.12
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	1.48	1.13	1.19	1.27	0.84	0.91	0.78	0.84	0.07	0.67	0.62	0.40	0.56	0.14
16	-	-	-	-	0.69	0.83	0.72	0.75	0.07	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	0.50	0.42	0.24	0.38	0.13

Table A.19: NO₃⁻ Peak Areas run on Ion Chromatography for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	9520814	9978268	9753064	9750715	228736	9900248	10121838	10308670	10110252	204457	9792682	9666894	9724338	9727971	62973
1	9882526	9805892	9710226	9799548	86325	9483572	9408498	9517450	9469840	55759	8940772	9427340	9124636	9164249	245691
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	10075304	9745806	9667320	9829477	216479	9427112	9267986	9248556	9314551	97963	9357546	8795756	9054660	9069321	281182
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9715152	9714652	9406356	9612053	178139	9293030	9055778	9202004	9183604	119691	8936770	8540570	8802030	8759790	201449
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9237518	9634546	9098336	9323467	278246	8716836	8608462	8685000	8670099	55702	8344952	7680912	7964826	7996897	333180
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9334926	9591030	9167946	9364634	213101	7696984	7528572	7608148	7611235	84248	7055550	6630400	6930278	6872076	218469
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9619944	9919608	9575930	9705161	187016	7624900	7159682	7281472	7355351	241248	5946516	5859910	5908714	5905047	43419
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	5763860	5836530	5896858	5832416	66594

Table A.20: NO₃⁻ Peak Areas run on Ion Chromatography for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	9744198	9504646	9326996	9525280	209365	4562535	4537475	4578819	4559610	20827	9553758	9945408	9507722	9668963	240513
1	9693530	9640188	9751454	9695057	55649	4634677	4587526	4630206	4617470	26028	9571048	9368942	9245610	9395200	164300
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9692418	9560612	9475622	9576217	109237	4558230	4699593	4622772	4626865	70770	9358916	8990846	8819554	9056439	275599
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9387088	9140290	9037210	9188196	179791	4441597	4422578	4438690	4434288	10245	9107632	8450020	8936176	8831276	341125
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	8652114	8659038	8487350	8599501	97187	4016532	4072608	4022421	4037187	30816	8260660	7674482	7423304	7786149	429701
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	7353328	6989030	7032808	7125055	198898	3244106	3249838	3195642	3229862	29774	7041562	6502250	6327946	6623919	372041
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	6255106	5057000	4968370	5426825	718680	2701087	2809032	2634101	2714740	88261	4838338	4323322	3846214	4335958	496183
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	5462504	4490490	4458516	4803837	570647	1739610	1826250	1618936	1728265	104122	3133310	2898814	2325808	2785977	415408
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	5026604	3850102	4031960	4302889	633317	1322538	1438989	1224574	1328700	107340	2283888	2096598	1365972	1915486	485019
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	1690498	1410216	803282	1301332	453519

Table A.21: Chlorophyll Concentration (mg/L) in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	0.040	0.055	0.070	0.055	0.015	0.070	0.070	0.042	0.060	0.016	0.074	0.152	0.042	0.089	0.056
1	0.042	0.070	0.070	0.060	0.016	0.082	0.042	0.042	0.056	0.023	0.129	0.055	0.074	0.086	0.038
2	0.084	0.070	0.092	0.082	0.012	0.102	0.089	0.097	0.096	0.006	0.057	0.124	0.015	0.065	0.055
3	0.080	0.092	0.065	0.079	0.014	0.162	0.115	0.107	0.128	0.030	0.126	0.139	0.120	0.128	0.010
4	0.177	0.236	0.194	0.202	0.031	0.318	0.259	0.217	0.265	0.051	0.192	0.264	0.227	0.227	0.036
5	0.286	0.232	0.343	0.287	0.056	0.527	0.286	0.316	0.376	0.131	0.343	0.311	0.358	0.338	0.024
6	0.664	0.473	0.387	0.508	0.142	0.697	0.562	0.575	0.611	0.074	0.385	0.413	0.366	0.388	0.023
7	0.463	0.516	0.528	0.502	0.035	0.741	0.953	0.703	0.799	0.135	0.443	0.490	0.550	0.494	0.054
8	0.525	0.304	0.558	0.463	0.138	1.007	0.881	0.777	0.888	0.115	0.969	0.819	1.071	0.953	0.127
9	0.753	0.535	0.739	0.676	0.122	1.517	1.175	1.250	1.314	0.180	1.414	1.399	1.607	1.473	0.116
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	0.338	0.000	0.482	0.273	0.248	1.468	1.456	1.408	1.444	0.032	1.998	1.973	2.116	2.029	0.076
11	0.344	0.250	0.357	0.317	0.059	1.526	1.494	1.764	1.595	0.148	2.265	2.345	2.446	2.352	0.091
12	-	-	-	-	-	1.631	1.562	1.587	1.593	0.035	2.470	2.363	2.525	2.453	0.082
13	-	-	-	-	-	0.070	0.070	0.042	0.060	0.016	2.538	2.552	2.372	2.487	0.100

Table A.22: Chlorophyll Concentration (mg/L) in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	0.015	0.070	0.010	0.032	0.033	0.057	0.042	0.042	0.047	0.009	0.010	0.042	0.056	0.036	0.024
1	0.070	0.070	0.070	0.070	0.000	0.055	0.038	0.063	0.052	0.013	0.015	0.042	0.042	0.033	0.016
2	0.030	0.010	0.015	0.018	0.010	0.080	0.065	0.084	0.076	0.010	0.025	0.015	0.103	0.048	0.049
3	0.194	0.135	0.122	0.150	0.038	0.154	0.107	0.103	0.121	0.028	0.112	0.052	0.083	0.083	0.030
4	0.266	0.227	0.192	0.228	0.037	0.132	0.105	0.132	0.123	0.016	0.164	0.122	0.136	0.141	0.021
5	0.316	0.400	0.303	0.340	0.053	0.284	0.246	0.320	0.283	0.037	0.271	0.185	0.246	0.234	0.045
6	0.463	0.438	0.351	0.417	0.059	0.428	0.423	0.435	0.429	0.006	0.269	0.323	0.331	0.308	0.034
7	0.458	0.490	0.517	0.488	0.030	0.782	0.710	0.739	0.744	0.036	0.430	0.468	0.567	0.488	0.071
8	0.829	0.846	0.990	0.888	0.088	1.105	1.015	1.157	1.092	0.072	1.013	0.985	1.147	1.048	0.087
9	1.408	1.578	1.627	1.537	0.115	2.023	1.769	1.968	1.920	0.134	1.584	1.582	1.951	1.706	0.212
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	1.836	2.503	2.406	2.249	0.360	2.858	2.453	2.846	2.719	0.231	2.650	2.448	2.915	2.671	0.235
11	1.934	2.910	2.815	2.553	0.538	3.784	3.008	3.488	3.427	0.392	3.543	3.113	3.839	3.498	0.365
12	2.125	3.289	3.322	2.912	0.681	3.951	3.160	3.834	3.648	0.427	4.224	3.865	4.580	4.223	0.358
13	2.451	3.327	3.190	2.989	0.471	3.764	2.799	3.568	3.377	0.510	4.183	3.981	4.237	4.134	0.135
14	2.776	2.225	3.125	2.709	0.454	4.006	2.792	3.732	3.510	0.637	3.866	3.286	3.833	3.662	0.326
15	3.096	2.781	2.761	2.880	0.188	4.289	2.869	4.071	3.743	0.765	3.980	3.097	4.564	3.881	0.739
16	-	-	-	-	-	4.371	2.947	4.209	3.842	0.779	3.653	2.229	4.7815	3.555	1.279
17	-	-	-	-	-	0.057	0.042	0.042	0.047	0.009	3.903	3.049	4.294	3.749	0.637

Table A.23: Iron concentration (uM) measured using ICP-MS for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	8.103	8.167	6.630	7.634	0.869	10.532	5.829	5.433	7.265	2.837	6.624	6.753	13.480	8.953	3.921
1	8.143	5.764	6.136	6.681	1.280	9.865	10.165	9.922	9.984	0.159	5.547	5.574	7.107	6.076	0.893
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	5.666	6.676	7.250	6.530	0.802	6.582	7.041	7.013	6.878	0.257	4.686	6.666	4.246	5.200	1.289
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	4.375	4.927	4.934	4.745	0.320	5.490	5.125	6.060	5.558	0.471	4.028	3.534	3.723	3.762	0.249
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	3.707	14.046	3.887	7.213	5.918	4.428	6.139	4.673	5.080	0.925	3.004	2.616	2.293	2.638	0.356
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	4.419	5.649	3.464	4.511	1.095	1.219	0.022	-0.120	0.374	0.736	0.311	0.000	0.000	0.104	0.180
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	3.718	3.843	3.114	3.558	0.390						0.617	0.000	0.000	0.206	0.356
12	-	-	-	-	-	2.644	2.211	3.287	2.714	0.541	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	0.421	0.851	0.621	0.631	0.215

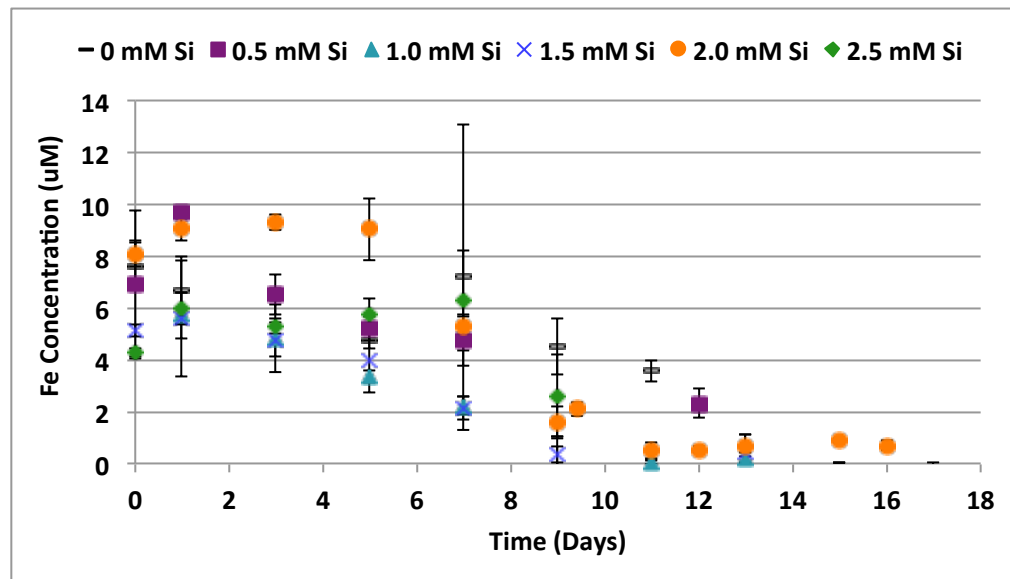


Figure A.2: Iron concentration (uM) measured using ICP-MS for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Table A.24: Iron concentration (uM) measured using ICP-MS for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	5.207	5.607	5.640	5.485	0.241	8.402	8.402	7.523	8.109	0.507	4.548	4.573	4.851	4.657	0.168
1	4.530	4.813	8.506	5.950	2.219	8.801	8.801	9.567	9.057	0.442	6.797	6.610	5.602	6.336	0.643
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	5.414	5.618	4.406	5.146	0.649	9.482	9.482	8.982	9.315	0.289	6.002	5.602	5.421	5.675	0.297
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	5.852	3.812	3.513	4.392	1.273	9.733	9.733	7.670	9.045	1.191	5.950	5.605	6.756	6.104	0.591
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2.217	2.358	3.035	2.537	0.437	5.073	5.073	5.830	5.325	0.437	4.995	6.144	8.712	6.617	1.903
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	0.067	0.784	1.416	0.755	0.675	1.225	1.225	2.272	1.574	0.605	3.716	4.093	1.213	3.007	1.565
9.4	-	-	-	-	-	2.245	2.245	1.798	2.096	0.258	-	-	-	-	-
10	-	-	-	-	-	0.295	0.295	0.871	0.487	0.332	-	-	-	-	-
11	0.018	0.000	0.000	0.006	0.010	0.583	0.583	0.406	0.524	0.102	0.000	0.005	0.097	0.034	0.054
12	-	-	-	-	-	0.441	0.441	1.171	0.685	0.422	-	-	-	-	-
13	0.000	0.000	1.669	0.556	0.964	-	-	-	-	-	0.237	0.000	0.000	0.079	0.137
14	-	-	-	-	-	0.944	0.944	0.796	0.895	0.085	-	-	-	-	-
-	0.000	0.361	0.255	0.205	0.186	0.589	0.589	0.899	0.692	0.179	0.000	0.000	0.371	0.124	0.214
16	-	-	-	-	-	8.402	8.402	7.523	8.109	0.507	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	0.404	0.000	0.220	0.208	0.202

Table A.25: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 0 Si.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	704.30	697.09	596.73	17.15	17.27	14.32
1	715.89	530.80	555.76	17.23	12.65	13.37
2	-	-	-	-	-	-
3	510.65	602.85	638.69	12.46	14.40	15.51
4	-	-	-	-	-	-
5	419.21	458.71	466.83	9.98	11.04	11.05
6	-	-	-	-	-	-
7	377.44	1115.49	374.92	8.69	28.59	9.04
8	-	-	-	-	-	-
9	420.58	519.19	344.08	10.06	12.43	8.23
10	-	-	-	-	-	-
11	366.71	381.59	313.96	8.71	8.95	7.55

Table A.26: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 0.5 mM Si.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	1711.17	1070.24	1016.38	48.83	16.15	14.82
1	1620.33	1661.14	1628.00	46.33	47.88	45.96
2	-	-	-	-	-	-
3	1172.92	1235.40	1231.58	33.28	34.84	35.05
4	-	-	-	-	-	-
5	1024.16	974.37	1101.75	28.76	27.36	31.51
6	-	-	-	-	-	-
7	879.37	1112.55	912.71	24.86	31.72	24.58
8	-	-	-	-	-	-
9	442.13	278.95	259.64	10.56	6.57	5.85
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	636.29	577.31	723.85	15.57	13.88	17.65

Table A.27: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 1.0 mM Si.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	1178.69	1196.27	2112.81	33.91	34.32	59.81
1	1031.93	1035.51	1244.38	29.40	29.64	35.50
2	-	-	-	-	-	-
3	914.57	1184.40	854.64	22.38	28.86	21.04
4	-	-	-	-	-	-
5	824.88	757.61	783.36	19.93	18.34	19.16
6	-	-	-	-	-	-
7	685.40	632.49	588.44	16.89	15.39	14.29
8	-	-	-	-	-	-
9	318.44	270.08	251.79	7.63	6.31	5.63
10	-	-	-	-	-	-
11	360.13	231.02	238.84	8.93	5.29	5.42
12	-	-	-	-	-	-
13	333.34	391.91	360.60	8.14	9.37	9.10

Table A.28: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 1.5 mM Si.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	985.56	1040.07	1044.56	23.69	30.06	29.79
1	893.28	931.84	1435.14	26.02	23.98	41.15
2	-	-	-	-	-	-
3	1013.83	1041.60	876.35	24.81	25.78	21.86
4	-	-	-	-	-	-
5	1073.55	795.46	754.70	26.74	19.69	18.85
6	-	-	-	-	-	-
7	578.10	597.34	689.56	14.45	14.82	16.97
8	-	-	-	-	-	-
9	285.08	382.77	469.01	6.59	9.19	11.40
10	-	-	-	-	-	-
11	278.41	234.68	251.87	6.40	5.41	5.78
12	-	-	-	-	-	-
13	201.72	234.66	503.43	4.53	5.89	12.26
14	-	-	-	-	-	-
15	275.03	325.18	310.77	6.38	7.87	7.63

Table A.29: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 2.0 mM Si.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	676.78	671.72	613.14	16.37	16.15	14.82
1	667.42	698.34	749.37	16.04	17.26	18.09
2	-	-	-	-	-	-
3	557.72	743.72	710.37	13.56	17.76	17.05
4	-	-	-	-	-	-
5	624.68	760.42	622.89	14.76	17.98	15.45
6	-	-	-	-	-	-
7	562.39	449.79	500.29	13.40	10.65	12.10
8	-	-	-	-	-	-
9	189.76	193.27	263.07	4.76	4.57	6.01
9.4	227.11	261.26	231.49	5.58	6.43	5.67
11	171.79	131.28	169.66	4.00	2.87	3.94
12	143.37	150.50	138.67	3.22	3.36	3.13
13	143.18	141.02	189.70	3.28	3.14	4.26
14	-	-	-	-	-	-
15	262.23	174.55	164.69	6.10	3.84	3.81
16	191.99	150.85	171.55	4.61	3.43	4.10

Table A.30: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for growth in 2.5 mM Si.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	895.71	899.12	937.07	23.08	25.60	26.69
1	1202.16	1176.75	1039.37	34.44	33.43	29.74
2	-	-	-	-	-	-
3	1093.93	1039.37	1014.76	31.33	26.93	28.90
4	-	-	-	-	-	-
5	1086.73	1039.80	1196.60	29.62	28.48	30.97
6	-	-	-	-	-	-
7	956.66	1113.19	1463.08	26.11	28.93	38.22
8	-	-	-	-	-	-
9	782.43	833.68	441.35	19.54	20.44	10.81
10	-	-	-	-	-	-
11	238.75	276.74	289.20	5.71	6.56	7.14
12	-	-	-	-	-	-
13	308.31	270.00	217.41	7.40	6.19	5.11
14	-	-	-	-	-	-
15	260.75	246.09	326.60	6.32	5.92	8.01
16	-	-	-	-	-	-
17	331.02	238.01	305.92	8.03	5.67	7.77

Table A.31: Phosphate (mg/L) for growth in each silica concentration (0, 0.5 and 1.0 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	1.606	1.600	1.579	1.595	0.014	1.558	1.589	1.619	1.589	0.031	1.594	1.562	1.575	1.577	0.016
1	1.653	1.594	1.578	1.608	0.040	1.511	1.520	1.553	1.528	0.022	1.515	1.603	1.564	1.561	0.044
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1.760	1.588	1.579	1.642	0.102	1.562	1.539	1.533	1.544	0.015	1.621	1.514	1.560	1.565	0.054
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	1.683	1.616	1.549	1.616	0.067	1.581	1.531	1.559	1.557	0.025	1.568	1.502	1.544	1.538	0.033
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1.724	1.622	1.524	1.623	0.100	1.558	1.527	1.566	1.550	0.021	1.552	1.440	1.491	1.494	0.056
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1.717	1.595	1.534	1.615	0.093	1.531	1.477	1.462	1.490	0.036	1.484	1.401	1.473	1.452	0.045
9.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1.770	1.651	1.594	1.671	0.090	-	-	-	-	-	1.397	1.375	1.472	1.415	0.051
12	-	-	-	-	-	1.796	1.500	1.513	1.603	0.167	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	1.772	1.555	1.562	1.629	0.123

Table A.32: Phosphate (mg/L) for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	1.555	1.525	1.511	1.530	0.022	1.636	1.472	1.483	1.530	0.091	1.563	1.631	1.549	1.581	0.044
1	1.609	1.623	1.650	1.627	0.021	1.519	1.497	1.480	1.499	0.019	1.625	1.580	1.568	1.591	0.030
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	1.641	1.623	1.617	1.627	0.012	1.716	1.497	1.465	1.559	0.137	1.604	1.542	1.520	1.555	0.044
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	1.626	1.586	1.568	1.593	0.030	1.552	1.447	1.445	1.481	0.061	1.525	1.485	1.566	1.525	0.040
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1.588	1.593	1.560	1.581	0.018	1.471	1.415	1.412	1.433	0.033	1.529	1.436	1.395	1.453	0.069
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1.480	1.502	1.511	1.498	0.016	1.427	1.302	1.315	1.348	0.069	1.513	1.424	1.448	1.462	0.046
9.4	-	-	-		-	1.500	1.348	1.337	1.395	0.091	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1.410	1.388	1.394	1.397	0.011	1.433	1.226	1.234	1.298	0.117	1.389	1.298	1.347	1.344	0.046
12	-	-	-	-	-	1.364	1.207	1.192	1.254	0.095	-	-	-	-	-
13	1.384	1.459	1.463	1.435	0.045	1.327	1.212	1.247	1.262	0.059	1.313	1.335	1.334	1.328	0.012
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	1.619	1.459	1.466	1.514	0.090	1.346	1.183	1.218	1.249	0.086	1.343	1.360	1.308	1.337	0.026
16	-		-	-	-	1.231	1.229	1.264	1.241	0.020	-		-	-	-
17	-	-	-	-	-	-	-	-	-	-	1.565	1.427	1.444	1.479	0.075

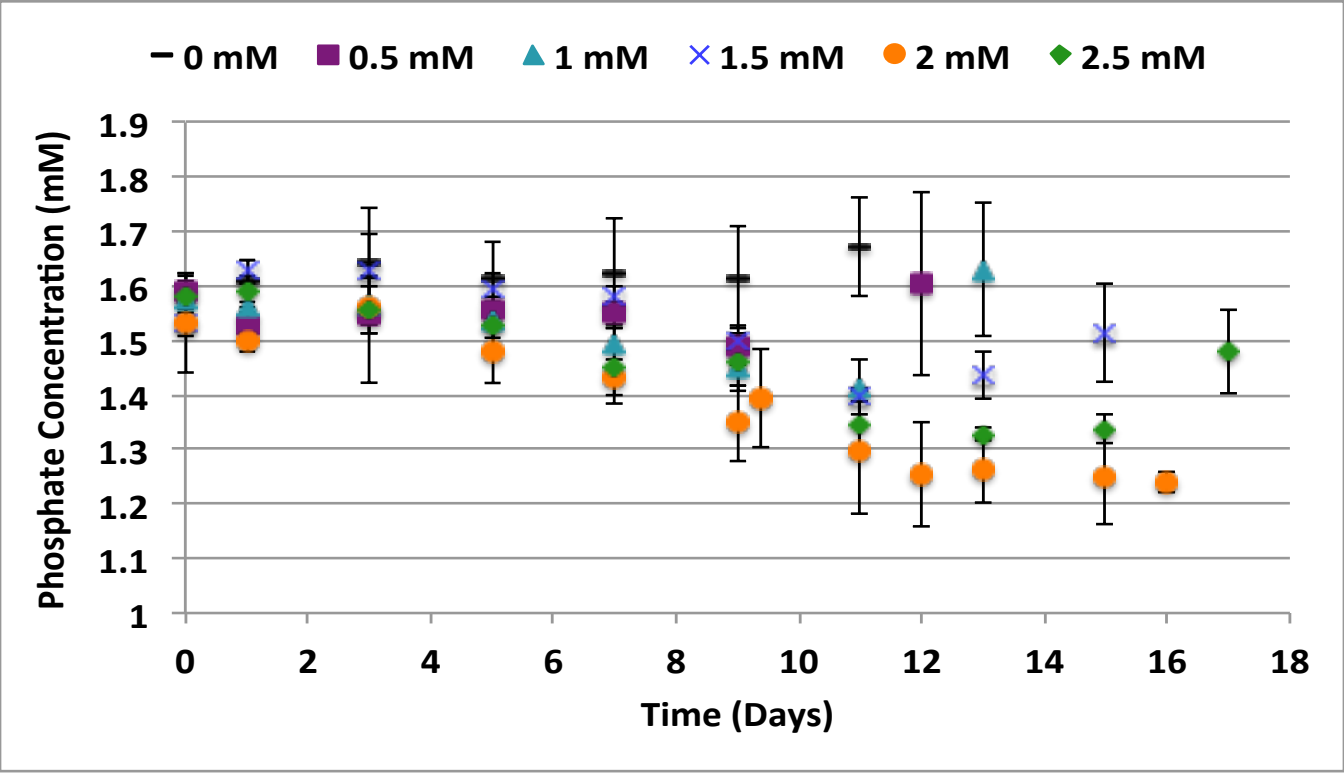


Figure A.3: Phosphate (mg/L) for growth in each silica concentration (0, 0.5, 1.0, 1.5, 1.0, 1.5, 2.0 and 2.5 mM Si).

Table A.33: Phosphate Peak Areas using Ion Chromatography for growth in each silica concentration (0, 0.5, 1.0 and 1.5 mM Si).

Time (Days)	0-1	0-2	0-3	Average	Standard Deviation	0.5-1	0.5-2	0.5-3	Average	Standard Deviation	1-1	1-2	1-3	Average	Standard Deviation
0	1975985	1967639	1942473	1962032	17445	1916127	1954413	1991980	1954173	37927	1961009	1921409	1936830	1939749	19961
1	2033504	1960615	1941261	1978460	48642	1858721	1869631	1910615	1879656	27361	1863859	1971563	1924183	1919868	53981
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2165215	1953877	1942061	2020384	125566	1920912	1893096	1885405	1899804	18680	1993924	1861959	1918512	1924798	66207
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	2070053	1988417	1905039	1987836	82509	1944790	1883531	1917760	1915360	30700	1928726	1848186	1898814	1891909	40712
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2120736	1995143	1874451	1996777	123151	1916218	1877839	1925784	1906614	25374	1908722	1771788	1833497	1838002	68578
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	2111484	1961532	1886701	1986572	114464	1883436	1817344	1798294	1833025	44684	1825739	1722797	1811458	1786665	55770
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	2176853	2030442	1960542	2055946	110388	-	-	-	-	-	1718783	1691320	1810585	1740229	62458
12	-	-	-	-	-	2209031	1844537	1861239	1971602	205789	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	2179249	1912401	1921339	2004330	151550

Table A.34: Phosphate Peak Areas using Ion Chromatography for growth in each silica concentration (1.5, 2.0 and 2.5 mM Si).

Time (Days)	1.5-1	1.5-2	1.5-3	Average	Standard Deviation	2-1	2-2	2-3	Average	Standard Deviation	2.5-1	2.5-2	2.5-3	Average	Standard Deviation
0	1912386	1875458	1859108	1882317	27293	2109999	1911509	1924551	1982020	111025	1923007	2005916	1905556	1944826	53620
1	1979035	1996579	2029295	2001636	25509	1968344	1941640	1921192	1943725	23645	1998595	1944139	1928307	1957014	36870
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2018358	1995885	1989452	2001232	15177	2207157	1941179	1902324	2016887	165920	1973517	1896418	1870255	1913397	53684
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	2000204	1950706	1928912	1959941	36532	2008646	1880988	1877834	1922489	74631	1875342	1826828	1926220	1876130	49701
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1953503	1959859	1919439	1944267	21735	1909291	1842282	1838302	1863292	39886	1880679	1766081	1715517	1787426	84625
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1820489	1847629	1859275	1842464	19902	1856441	1704238	1720141	1760273	83662	1860887	1752249	1781466	1798201	56219
10	-	-	-	-	-	1944504	1761004	1747275	-	-	-	-	-	-	-
11	1733869	1707958	1714357	1718728	13497	1863219	1612771	1622582	1699524	141849	1708434	1596233	1656933	1653867	56163
12	-	-	-	-	-	1780009	1589615	1571043	-	-	-	-	-	-	-
13	1702590	1794708	1799922	1765740	54752	1734686	1595093	1637477	1655752	71568	1615511	1642352	1641353	1633072	15216
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	1990957	1795051	1802793	1862934	110939	1757753	1559882	1602632	1640089	104118	1652377	1672471	1609194	1644681	32333
16	-	-	-	-	-	1618929	1616001	1659033	1631321	24044	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	1925327	1755209	1775745	1818760	92859

Table A.35: Cell concentration (cells mL⁻¹) when grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	8.25E+04	7.25E+04	7.00E+04	7.50E+04	6.6E+03	6.40E+04	6.63E+04	7.50E+04	6.84E+04	5.8E+03
1	1.10E+05	1.13E+05	9.75E+04	1.07E+05	8.2E+03	9.25E+04	8.25E+04	8.40E+04	8.63E+04	5.4E+03
2	1.59E+05	1.66E+05	1.03E+05	1.43E+05	3.5E+04	2.30E+05	1.24E+05	1.74E+05	1.76E+05	5.3E+04
3	2.40E+05	2.40E+05	2.80E+05	2.53E+05	2.3E+04	5.90E+05	2.80E+05	3.00E+05	2.90E+05	1.7E+05
4	4.37E+05	3.95E+05	4.20E+05	4.17E+05	2.1E+04	4.00E+05	3.53E+05	4.00E+05	3.84E+05	2.7E+04
5	8.60E+05	6.90E+05	7.90E+05	7.80E+05	8.5E+04	7.50E+05	8.60E+05	8.20E+05	8.10E+05	5.6E+04
6	1.33E+06	1.44E+06	1.64E+06	1.47E+06	1.6E+05	1.45E+06	1.56E+06	1.40E+06	1.47E+06	8.2E+04
7	2.58E+06	2.64E+06	2.76E+06	2.66E+06	9.2E+04	2.53E+06	2.72E+06	2.37E+06	2.54E+06	1.8E+05
8	3.66E+06	3.69E+06	4.09E+06	3.81E+06	2.4E+05	3.34E+06	3.64E+06	3.52E+06	3.50E+06	1.5E+05
9	6.95E+06	5.73E+06	7.05E+06	6.58E+06	7.3E+05	6.45E+06	5.85E+06	6.15E+06	6.15E+06	3.0E+05
9.4	8.25E+06	8.50E+06	8.50E+06	8.42E+06	1.4E+05	7.75E+06	7.83E+06	7.83E+06	7.80E+06	4.6E+04
10	8.70E+06	8.03E+06	9.23E+06	8.65E+06	6.0E+05	9.98E+06	9.95E+06	9.83E+06	9.92E+06	7.9E+04
11	1.41E+07	1.09E+07	1.19E+07	1.23E+07	1.6E+06	1.13E+07	1.21E+07	1.38E+07	1.24E+07	1.3E+06
12	9.48E+06	9.70E+06	1.00E+07	9.73E+06	2.6E+05	1.28E+07	1.53E+07	1.41E+07	1.41E+07	1.3E+06
13	1.07E+07	1.00E+07	1.08E+07	1.05E+07	4.4E+05	1.46E+07	1.32E+07	1.43E+07	1.40E+07	7.4E+05
14	1.04E+07	9.00E+06	1.01E+07	9.83E+06	7.4E+05	1.39E+07	1.49E+07	1.34E+07	1.41E+07	7.6E+05
15	1.13E+07	8.05E+06	1.28E+07	1.07E+07	2.4E+06	1.23E+07	1.26E+07	1.34E+07	1.28E+07	5.7E+05
16	1.62E+07	8.85E+06	1.15E+07	1.22E+07	3.7E+06	1.54E+07	1.56E+07	1.35E+07	1.48E+07	1.2E+06

Table A.36: Cell concentration (cells mL⁻¹) when grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	8.88E+04	7.60E+04	1.90E+05	1.18E+05	6.25E+04	8.50E+04	7.90E+04	8.90E+04	8.43E+04	5.03E+03
1	8.40E+04	1.01E+05	1.36E+05	1.07E+05	2.65E+04	9.00E+04	1.09E+05	1.18E+05	1.06E+05	1.43E+04
2	1.38E+05	1.50E+05	1.18E+05	1.35E+05	1.62E+04	1.36E+05	1.60E+05	1.23E+05	1.40E+05	1.88E+04
3	2.20E+05	2.70E+05	2.50E+05	2.47E+05	2.52E+04	2.20E+05	2.40E+05	2.00E+05	2.20E+05	2.00E+04
4	4.43E+05	4.52E+05	8.80E+05	5.92E+05	2.50E+05	4.57E+05	5.07E+05	4.88E+05	4.84E+05	2.52E+04
5	8.80E+05	7.60E+05	1.40E+06	1.01E+06	3.40E+05	8.30E+05	8.00E+05	8.40E+05	8.23E+05	2.08E+04
6	1.25E+06	1.42E+06	2.91E+06	1.86E+06	9.13E+05	1.36E+06	1.48E+06	1.41E+06	1.42E+06	6.03E+04
7	3.08E+06	2.44E+06	4.24E+06	3.25E+06	9.12E+05	2.29E+06	2.21E+06	2.23E+06	2.24E+06	4.16E+04
8	3.67E+06	3.65E+06	5.84E+06	4.39E+06	1.26E+06	3.88E+06	3.32E+06	3.20E+06	3.47E+06	3.63E+05
9	6.60E+06	5.55E+06	9.65E+06	7.27E+06	2.13E+06	6.58E+06	7.13E+06	7.20E+06	6.97E+06	3.40E+05
9.4	8.38E+06	7.83E+06	1.20E+07	9.40E+06	2.27E+06	9.48E+06	8.45E+06	8.38E+06	8.77E+06	6.16E+05
10	8.05E+06	8.28E+06	1.26E+07	9.64E+06	2.56E+06	1.23E+07	9.55E+06	1.17E+07	1.12E+07	1.45E+06
11	1.17E+07	1.10E+07	1.04E+07	1.10E+07	6.51E+05	1.25E+07	1.39E+07	1.43E+07	1.36E+07	9.45E+05
12	1.10E+07	1.11E+07	1.34E+07	1.18E+07	1.36E+06	1.16E+07	1.29E+07	1.38E+07	1.28E+07	1.11E+06
13	1.35E+07	1.21E+07	1.32E+07	1.29E+07	7.37E+05	1.65E+07	1.77E+07	1.53E+07	1.65E+07	1.20E+06
14	1.49E+07	1.45E+07	1.55E+07	1.50E+07	5.03E+05	1.49E+07	1.64E+07	1.57E+07	1.57E+07	7.51E+05
15	1.23E+07	1.21E+07	1.33E+07	1.26E+07	6.43E+05	1.14E+07	1.56E+07	1.37E+07	1.36E+07	2.10E+06

Table A.37: Measured pH for cells grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	7.86	7.96	8.01	7.94	0.08	8.04	8.30	8.09	8.14	0.14
1	7.87	7.98	8.03	7.96	0.08	8.04	8.01	8.07	8.04	0.03
2	7.89	7.89	7.97	7.92	0.05	8.01	7.99	8.06	8.02	0.04
3	7.87	7.94	7.97	7.93	0.05	7.99	7.96	8.02	7.99	0.03
4	7.93	7.97	8.03	7.98	0.05	8.05	8.02	8.09	8.05	0.04
5	7.98	8.12	8.14	8.08	0.09	8.14	8.13	8.16	8.14	0.02
6	8.20	8.23	8.27	8.23	0.04	8.32	8.35	8.41	8.36	0.05
7	8.61	8.76	8.63	8.67	0.08	8.71	8.78	8.84	8.78	0.07
8	9.23	9.50	9.40	9.38	0.14	9.41	9.50	9.52	9.48	0.06
9	9.98	10.13	10.08	10.06	0.08	10.06	10.07	10.11	10.08	0.03
9.4	8.15	8.33	8.20	8.23	0.09	8.47	8.47	8.50	8.48	0.02
10	10.57	10.62	10.62	10.60	0.03	9.75	9.75	9.74	9.75	0.01
11	10.83	10.83	10.82	10.83	0.01	10.16	10.14	10.18	10.16	0.02
12	10.72	10.87	10.63	10.74	0.12	10.33	10.43	10.53	10.43	0.10
13	10.33	10.64	10.00	10.32	0.32	10.21	10.43	10.47	10.37	0.14
14	9.81	10.27	9.78	9.95	0.27	10.08	10.31	10.33	10.24	0.14
15	9.72	9.91	9.70	9.78	0.12	10.00	10.20	10.23	10.14	0.13
16	9.71	9.84	9.55	9.70	0.15	9.90	10.19	10.10	10.06	0.15

Table A.38: Measured pH for cells grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	8.03	8.30	8.14	8.16	0.14	8.05	8.03	8.03	8.04	0.01
1	8.03	8.03	8.04	8.03	0.01	8.03	8.03	8.03	8.03	0.00
2	7.94	7.95	8.00	7.96	0.03	8.01	8.02	8.03	8.02	0.01
3	8.00	8.00	8.01	8.00	0.01	8.00	7.98	7.97	7.98	0.02
4	8.04	8.08	8.12	8.08	0.04	8.05	8.09	8.05	8.06	0.02
5	8.12	8.17	8.20	8.16	0.04	8.07	8.08	8.08	8.08	0.01
6	8.20	8.37	8.61	8.39	0.21	8.25	8.23	8.23	8.24	0.01
7	8.49	8.82	9.23	8.85	0.37	8.54	8.57	8.52	8.54	0.03
8	9.24	9.50	10.15	9.63	0.47	9.29	9.40	9.32	9.34	0.06
9	9.97	9.90	10.72	10.20	0.45	10.07	10.09	10.08	10.08	0.01
9.4	8.24	8.57	8.42	8.41	0.17	8.48	8.51	8.53	8.51	0.03
10	10.48	10.35	10.78	10.54	0.22	9.83	9.82	9.80	9.82	0.02
11	10.57	10.63	10.42	10.54	0.11	10.00	10.03	9.96	10.00	0.04
12	10.19	10.79	9.80	10.26	0.50	9.99	10.05	9.98	10.01	0.04
13	9.45	10.77	9.30	9.84	0.81	9.85	9.93	9.85	9.88	0.05
14	9.00	10.61	8.99	9.53	0.93	9.66	9.74	9.69	9.70	0.04
15	8.93	9.69	8.71	9.11	0.51	9.58	9.63	9.62	9.61	0.03

Table A.39: Si concentration (mM) when grown in 2 mM Si/2.94 mM NO3- with and without NaHCO3 addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	1.70	1.71	1.57	1.66	0.08	1.49	1.81	1.92	1.74	0.23
1	1.59	1.63	1.83	1.69	0.13	1.70	1.65	1.66	1.67	0.03
2	-	-	-	-	-	-	-	-	-	-
3	1.55	2.22	1.82	1.86	0.33	1.80	1.68	1.70	1.73	0.07
4	-	-	-	-	-	-	-	-	-	-
5	1.65	2.18	1.70	1.85	0.29	1.61	1.26	1.41	1.43	0.17
6	-	-	-	-	-	-	-	-	-	-
7	1.46	1.26	1.41	1.38	0.10	1.27	1.25	1.11	1.21	0.08
8	-	-	-	-	-	-	-	-	-	-
9	0.73	1.08	1.30	1.04	0.29	0.89	0.62	0.71	0.74	0.14
9.4	0.51	0.79	0.50	0.60	0.17	0.51	0.57	0.51	0.53	0.03
10	0.32	0.36	0.38	0.35	0.03	0.36	0.38	0.48	0.41	0.06
11	0.00	0.09	0.00	0.03	0.05	0.02	0.03	0.02	0.02	0.01
12	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.00
13	-	-	-	-	-	-	-	-	-	-
14	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.00
15	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.01	0.02	0.00

Table A.40: Si concentration (mM) when grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	1.72	1.51	1.57	0.13	1.72	1.79	1.58	1.62	0.16	1.79
1	1.91	1.50	1.66	0.22	1.91	1.38	1.60	1.53	0.13	1.38
2	-	-	-	-	-	-	-	-	-	-
3	1.52	1.52	1.50	0.04	1.52	1.45	1.58	1.52	0.07	1.45
4	-	-	-	-	-	-	-	-	-	-
5	1.39	1.43	1.43	0.03	1.39	1.48	1.45	1.52	0.10	1.48
6	-	-	-	-	-	-	-	-	-	-
7	1.14	0.94	1.07	0.11	1.14	1.22	1.44	1.36	0.12	1.22
8	-	-	-	-	-	-	-	-	-	-
9	0.66	0.12	0.47	0.31	0.66	0.81	0.80	0.80	0.01	0.81
9.4	0.62	0.03	0.36	0.30	0.62	0.63	0.55	0.60	0.04	0.63
10	-	-	-	-	-	-	-	-	-	-
11	0.44	0.00	0.22	0.22	0.44	0.38	0.37	0.39	0.03	0.38
12	0.33	0.02	0.14	0.17	0.33	0.14	0.14	0.14	0.00	0.14
13	0.06	0.07	0.08	0.02	0.06	0.15	0.20	0.18	0.02	0.15
14	-	-	-	-	-	-	-	-	-	-
15	0.12	0.23	0.19	0.06	0.12	0.14	0.19	0.17	0.02	0.14

Table A.41: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si₂₈, Si₂₉ and Si₃₀) for growth in 2.0 mM Si and 25 mM NaHCO₃.

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	6120.44	7469.06	7901.53	413.20	499.72	531.58	560.89	693.03	735.81
1	7015.11	6811.91	6837.28	471.26	457.22	463.43	651.50	644.18	643.47
2	-	-	-	-	-	-	-	-	-
3	7417.92	6925.24	6987.92	500.07	467.22	468.98	688.07	636.38	645.20
4	-	-	-	-	-	-	-	-	-
5	6627.78	5209.76	5816.99	445.80	350.52	391.77	630.40	497.14	575.48
6	-	-	-	-	-	-	-	-	-
7	5211.62	5144.82	4588.43	351.34	346.06	307.59	548.47	547.80	479.25
8	-	-	-	-	-	-	-	-	-
9	3651.02	2540.95	2933.49	244.17	170.65	196.27	511.61	372.36	402.06
9.4	2122.83	2355.40	2097.96	142.48	156.71	140.25	364.92	416.26	348.77
11	1499.27	1580.82	1976.88	99.30	105.26	131.73	351.98	389.60	438.89
12	89.52	149.81	73.25	7.79	11.94	6.52	268.78	340.70	237.21
13	89.80	96.82	77.83	8.10	8.13	6.97	267.80	286.35	260.53
14	-	-	-	-	-	-	-	-	-
15	108.78	80.32	87.61	8.93	6.97	7.59	292.01	251.38	283.11
16	90.26	69.94	72.14	8.52	6.13	6.57	325.70	219.03	246.68

Table A.42: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si₂₈, Si₂₉ and Si₃₀) for growth in 2.0 mM Si and 1 mM NO₃⁻.

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	5851.83	6134.73	5346.61	399.01	414.25	362.86	600.95	625.90	541.56
1	6092.73	7336.64	5938.82	416.29	501.75	406.99	627.07	762.20	617.89
2	-	-	-	-	-	-	-	-	-
3	5788.36	6009.25	6000.48	393.11	406.80	407.67	602.99	617.45	622.18
4	-	-	-	-	-	-	-	-	-
5	5760.46	5562.84	5687.34	392.73	379.96	386.92	612.48	609.66	626.75
6	-	-	-	-	-	-	-	-	-
7	4690.64	4683.26	4013.91	319.73	318.16	273.24	545.23	548.18	533.52
8	-	-	-	-	-	-	-	-	-
9	2981.48	3059.80	1206.66	202.52	207.34	82.76	470.03	465.30	394.68
9.4	2261.43	2926.93	895.94	152.66	199.91	59.93	446.53	501.52	380.73
11	1489.34	2311.00	328.08	101.66	157.06	22.37	397.71	473.43	334.02
12	244.83	1374.16	100.65	16.98	91.30	7.90	321.33	370.51	219.24
13	434.71	276.05	308.47	28.10	18.43	20.31	375.25	258.79	287.00
15	910.31	496.35	972.18	56.47	30.63	58.74	325.71	301.92	351.58

Table A.43: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for growth in 2.0 mM Si, 1 mM NO_3^- and 25 mM NaHCO_3 .

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	5199.78	6472.06	5768.20	352.64	437.77	388.37	519.19	645.95	579.62
1	5743.70	4830.19	5692.19	389.12	328.17	385.67	571.53	484.54	568.96
2	-	-	-	-	-	-	-	-	-
3	5411.97	5097.98	5591.43	366.32	344.34	378.07	546.55	517.74	559.22
4	-	-	-	-	-	-	-	-	-
5	5816.66	5220.54	5093.55	394.56	351.32	343.98	593.75	519.09	523.17
6	-	-	-	-	-	-	-	-	-
7	4992.49	4228.59	5045.84	337.24	284.43	339.55	550.84	473.01	560.12
8	-	-	-	-	-	-	-	-	-
9	2573.35	2665.52	2611.98	173.77	178.68	175.44	406.11	416.49	407.35
9.4	1906.35	1969.03	1664.62	128.30	132.24	110.93	396.25	420.81	371.57
11	1146.03	1010.29	956.31	77.70	69.25	64.25	353.49	340.16	316.51
12	100.60	88.52	104.45	8.73	7.91	9.02	302.85	294.56	329.86
13	254.62	141.09	310.64	17.76	11.18	21.37	340.49	299.97	417.37
14	-	-	-	-	-	-	-	-	-
15	205.14	105.14	293.30	14.00	8.80	19.49	230.27	305.58	287.72

Table A.44: Total Nile Red fluorescence intensity for cells grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	123	79	64	89	31	31	43	40	38	6
1	153	39	64	85	60	76	73	113	87	22
2	204	134	156	165	36	290	49	116	152	124
3	244	305	229	259	40	647	284	226	386	228
4	668	543	733	648	97	531	671	479	560	99
5	1334	1432	1389	1385	49	1490	1443	1410	1448	40
6	2273	1984	1764	2007	255	1706	1780	1602	1696	89
7	3632	2148	3798	3193	909	3430	3572	3112	3371	236
8	3950	3260	4450	3887	598	3320	3742	2796	3286	474
9	4580	4530	6950	5353	1383	4915	5525	5660	5367	397
9.4	3995	3585	4515	4032	466	3540	3235	3830	3535	298
10	7035	6390	6090	6505	483	11990	12360	7250	10533	2849
11	10480	9065	11595	10380	1268	16570	14255	17945	16257	1865
12	13855	13290	16295	14480	1597	23315	25680	24815	24603	1197
13	25515	19745	28535	24598	4466	41715	44865	43595	43392	1585
14	20965	20525	22370	21287	964	46895	53990	37995	46293	8014
15	30595	29760	29040	29798	778	53165	54900	45580	51215	4957
16	30550	28105	29635	29430	1235	49330	54185	48325	50613	3134

Table A.45: Total Nile Red fluorescence for cells grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	52	34	37	41	10	24	27	18	23	5
1	193	67	97	119	66	65	67	82	71	9
2	223	192	58	158	88	211	183	76	157	71
3	262	223	403	296	95	339	379	342	353	22
4	559	360	1383	767	542	272	439	396	369	87
5	955	867	2151	1324	717	1029	1243	1148	1140	107
6	1718	1383	2890	1997	791	1694	2002	1371	1689	316
7	2876	1850	4750	3159	1471	2276	3204	2770	2750	464
8	3022	2222	4718	3321	1275	3204	3082	3638	3308	292
9	6225	3385	7690	5767	2189	4825	6165	5710	5567	681
9.4	4455	2425	6650	4510	2113	4350	4595	4255	4400	175
10	5985	3480	12850	7438	4851	12420	15610	12405	13478	1846
11	17395	5170	16660	13075	6856	20570	19855	21090	20505	620
12	25435	9980	25940	20452	9072	28930	31540	33020	31163	2071
13	32075	15655	36865	28198	11124	49075	53150	50205	50810	2104
14	40620	25300	45290	37070	10457	60960	50465	57770	56398	5380
15	39075	26600	44375	36683	9126	55195	57240	58930	57122	1870

Table A.46: Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	15	11	9	12	3	5	6	5	6	1
1	14	3	7	8	5	8	9	13	10	3
2	13	8	15	12	4	13	4	7	8	4
3	10	13	8	10	2	11	10	8	10	2
4	15	14	17	15	2	13	19	12	15	4
5	16	21	18	18	3	20	17	17	18	2
6	17	14	11	14	3	12	11	11	12	0
7	14	8	14	12	3	14	13	13	13	0
8	11	9	11	10	1	10	10	8	9	1
9	7	8	10	8	2	8	9	9	9	1
9.4	5	4	5	5	1	5	4	5	5	0
10	8	8	7	8	1	12	12	7	11	3
11	7	8	10	8	1	15	12	13	13	1
12	15	14	16	15	1	18	17	18	18	1
13	24	20	26	23	3	29	34	30	31	3
14	20	23	22	22	1	34	36	28	33	4
15	27	37	23	29	7	43	44	34	40	5
16	19	32	26	25	6	32	35	36	34	2

Table A.47: Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	6	4	2	4	2	3	3	2	3	1
1	23	7	7	12	9	7	6	7	7	1
2	16	13	5	11	6	16	11	6	11	5
3	12	8	16	12	4	15	16	17	16	1
4	13	8	16	12	4	6	9	8	8	1
5	11	11	15	13	2	12	16	14	14	2
6	14	10	10	11	2	12	14	10	12	2
7	9	8	11	9	2	10	14	12	12	2
8	8	6	8	7	1	8	9	11	10	2
9	9	6	8	8	2	7	9	8	8	1
9.4	5	3	6	5	1	5	5	5	5	0
10	7	4	10	7	3	10	16	11	12	3
11	15	5	16	12	6	16	14	15	15	1
12	23	9	19	17	7	25	24	24	24	1
13	24	13	28	22	8	30	30	33	31	2
14	27	17	29	25	6	41	31	37	36	5
15	32	22	33	29	6	48	37	43	43	6

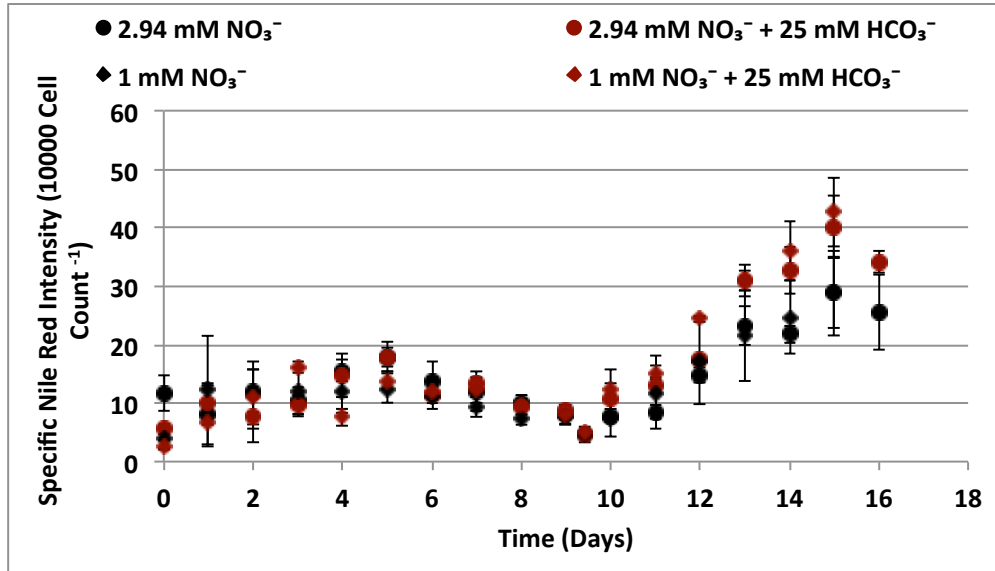


Figure A.4: Specific Nile Red fluorescence (fluorescence per cell = Nile Red fluorescence x 10000/cell count) for cells grown in 2 mM Si/2.94 mM NO₃⁻ or 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Table A.48: Measured nitrate concentration (mM) when grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	2.88	2.87	2.89	2.88	0.01	3.03	3.12	2.94	3.03	0.09
1	2.93	2.90	2.92	2.92	0.02	3.19	2.89	2.87	2.98	0.18
2	-	-	-	-	-	-	-	-	-	-
3	2.88	2.96	2.92	2.92	0.04	2.97	2.82	2.80	2.86	0.09
4	-	-	-	-	-	-	-	-	-	-
5	2.81	2.80	2.81	2.81	0.01	2.89	2.85	2.77	2.84	0.06
6	-	-	-	-	-	-	-	-	-	-
7	2.57	2.60	2.57	2.58	0.02	2.56	2.56	2.56	2.56	0.00
8	-	-	-	-	-	-	-	-	-	-
9	2.12	2.12	2.09	2.11	0.02	2.10	2.09	2.04	2.08	0.03
9.4	-	-	-	-	-	-	-	-	-	-
10	1.80	1.86	1.76	1.81	0.05	0.74	1.60	1.50	1.28	0.47
11	-	-	-	-	-	-	-	-	-	-
12	1.24	1.29	1.17	1.23	0.06	1.07	1.06	1.07	1.07	0.01
13	-	-	-	-	-	-	-	-	-	-
14	0.99	1.06	0.94	1.00	0.06	0.85	0.81	0.92	0.86	0.05
15	0.85	0.98	0.89	0.91	0.07	0.73	0.68	0.20	0.54	0.29

Table A.49: Measured nitrate concentration (mM) when grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	1.64	1.53	1.59	1.59	0.06	1.57	1.60	1.57	1.58	0.02
1	1.62	1.51	1.55	1.56	0.06	1.58	1.55	1.61	1.58	0.03
2	-	-	-	-	-	-	-	-	-	-
3	1.61	1.50	1.58	1.56	0.05	1.59	1.56	1.57	1.57	0.01
4	-	-	-	-	-	-	-	-	-	-
5	1.42	1.44	1.48	1.45	0.03	1.50	1.56	1.47	1.51	0.04
6	-	-	-	-	-	-	-	-	-	-
7	1.25	1.18	0.99	1.14	0.14	1.26	1.22	1.21	1.23	0.02
8	-	-	-	-	-	-	-	-	-	-
9	0.71	0.80	0.00	0.50	0.44	0.66	0.66	0.00	0.44	0.38
9.4	0.00	0.76	0.00	0.25	0.44	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	-	-	-	-	-	-	-	-	-	-
14	0.00	0.00	0.00	0.00	0.00	1.57	1.60	1.57	1.58	0.02
15	1.64	1.53	1.59	1.59	0.06	1.58	1.55	1.61	1.58	0.03

Table A.50: Chlorophyll concentration (mg/L) when grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	0.057	0.042	0.042	0.047	0.009	0.030	0.010	0.010	0.017	0.011
1	0.055	0.038	0.063	0.052	0.013	0.065	0.078	0.082	0.075	0.009
2	0.080	0.065	0.084	0.076	0.010	0.097	0.038	0.033	0.056	0.036
3	0.154	0.107	0.103	0.121	0.028	0.171	0.114	0.144	0.143	0.029
4	0.132	0.105	0.132	0.123	0.016	0.151	0.132	0.132	0.139	0.011
5	0.284	0.246	0.320	0.283	0.037	0.328	0.256	0.219	0.268	0.056
6	0.428	0.423	0.435	0.429	0.006	0.450	0.426	0.371	0.416	0.040
7	0.782	0.710	0.739	0.744	0.036	0.769	0.767	0.706	0.747	0.036
8	1.105	1.015	1.157	1.092	0.072	1.013	1.023	0.958	0.998	0.035
9	2.023	1.769	1.968	1.920	0.134	1.911	1.712	1.706	1.776	0.117
9.4	2.858	2.453	2.846	2.719	0.231	2.535	2.766	2.518	2.606	0.139
10	3.784	3.008	3.488	3.427	0.392	4.257	4.291	4.113	4.220	0.095
11	3.951	3.160	3.834	3.648	0.427	4.811	4.677	4.456	4.648	0.179
12	3.764	2.799	3.568	3.377	0.510	4.591	4.774	4.371	4.579	0.202
13	4.006	2.792	3.732	3.510	0.637	4.605	4.727	3.897	4.410	0.448
14	4.289	2.869	4.071	3.743	0.765	4.715	4.864	4.156	4.578	0.373
15	4.371	2.947	4.209	3.842	0.779	4.418	4.743	3.929	4.363	0.410
16	0.057	0.042	0.042	0.047	0.009	0.030	0.010	0.010	0.017	0.011

Table A.51: Chlorophyll concentration (mg/L) when grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	0.010	0.010	0.067	0.029	0.033	0.010	0.038	0.065	0.038	0.027
1	0.070	0.070	0.070	0.070	0.000	0.027	0.038	0.038	0.034	0.006
2	0.048	0.038	0.188	0.091	0.084	0.084	0.052	0.084	0.074	0.018
3	0.141	0.135	2.002	0.759	1.076	0.129	0.122	0.122	0.124	0.004
4	0.174	0.128	0.433	0.245	0.165	0.202	0.208	0.263	0.224	0.034
5	0.286	0.288	0.465	0.346	0.103	0.244	0.307	0.271	0.274	0.032
6	0.445	0.366	0.855	0.555	0.263	0.475	0.483	0.479	0.479	0.004
7	0.754	0.599	1.353	0.902	0.398	0.736	0.773	0.777	0.762	0.023
8	1.090	0.905	1.844	1.280	0.497	1.035	1.117	1.162	1.105	0.064
9	2.212	1.575	2.982	2.257	0.705	1.978	1.995	2.150	2.041	0.095
9.4	2.978	2.037	2.743	2.586	0.490	2.993	2.790	3.021	2.935	0.126
10	2.814	2.839	2.563	2.739	0.153	2.869	2.695	2.760	2.775	0.088
11	2.645	3.167	2.433	2.748	0.378	2.582	2.657	2.605	2.615	0.039
12	2.220	2.642	1.958	2.273	0.345	2.204	2.192	2.198	2.198	0.006
13	2.067	2.192	1.736	1.998	0.236	1.838	1.849	1.729	1.805	0.066
14	1.737	2.003	1.597	1.779	0.207	1.609	1.781	1.620	1.670	0.096
15	0.010	0.010	0.067	0.029	0.033	0.010	0.038	0.065	0.038	0.027

Table A.52: Dissolved Inorganic Carbon (DIC) concentration (mg/L) when grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	8.83	8.3	8.64	8.59	0.27	8.66	3.97	8.75	7.13	2.73
1	6.5	6.32	6.42	6.41	0.09	6.32	5	6.33	5.88	0.77
2	6.59	6.58	6.55	6.57	0.02	6.38	5.19	6.49	6.02	0.72
3	7.4	7.68	7.63	7.57	0.15	7.64	6.04	7.54	7.07	0.90
4	7.44	7.52	7.8	7.59	0.19	7.51	6.06	7.34	6.97	0.79
5	8.72	8.21	8.54	8.49	0.26	8.2	6.43	8.01	7.55	0.97
6	8.13	8.01	8.4	8.18	0.20	7.61	6.21	7.71	7.18	0.84
7	8.38	6.72	8.59	7.90	1.02	7.55	5.43	6.72	6.57	1.07
8	5.09	3.67	5.3	4.69	0.89	0.036	-0.14	5.36	1.75	3.13
9	2.08	-0.18	1.72	1.21	1.21	0.73	-0.34	-0.01	0.13	0.55
9.4	21.31	19.2	21.97	20.83	1.45	316	313.2	298.4	309.20	9.46
10	1.21	-0.027	1.77	0.98	0.92	210.56	215.88	205.95	210.80	4.97
11	4.8	2.38	6.13	4.44	1.90	163.47	169.05	164.31	165.61	3.01
12	6.18	3.62	8.2	6.00	2.30	105	107.29	100.01	104.10	3.72
13	12.68	7.27	16.66	12.20	4.71	116.19	110.39	103.33	109.97	6.44
14	16.94	11.4	18.64	15.66	3.79	113.38	109.05	105.03	109.15	4.18
15	20.06	15.32	21.14	18.84	3.10	148.3	140.19	133.72	140.74	7.31
16	20.66	16.71	22.21	19.86	2.84	121.88	112.2	114.89	116.32	5.00

Table A.53: Dissolved Inorganic Carbon (DIC) concentration (mg/L) when grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	8.93	4.66	6.8	6.80	2.14	6.88	7.28	6.77	6.98	0.27
1	4.77	4.68	4.84	4.76	0.08	4.89	4.75	4.96	4.87	0.11
2	5.3	5.06	5.3	5.22	0.14	5.1	5.08	5.03	5.07	0.04
3	6.28	5.87	6.41	6.19	0.28	6.2	6.19	6.17	6.19	0.02
4	6.21	5.58	6.42	6.07	0.44	6.22	5.99	6.36	6.19	0.19
5	7.24	6.65	7.87	7.25	0.61	7.35	7.5	7.76	7.54	0.21
6	6.91	5.51	6.79	6.40	0.78	7.08	6.96	7	7.01	0.06
7	7.54	4.52	4.65	5.57	1.71	7.33	7.29	8.12	7.58	0.47
8	3.02	1.53	4.54	3.03	1.51	5.04	2.96	6.7	4.90	1.87
9	0.93	-0.64	0.4	0.23	0.80	0.89	0.18	0.62	0.56	0.36
9.4	25.88	13.78	22.98	20.88	6.32	299.3	219.4	217.8	245.50	46.60
10	6.17	-0.22	3.13	3.03	3.20	209.34	213.76	214.92	212.67	2.94
11	7.33	1.52	8.22	5.69	3.64	176.53	177.03	182.46	178.67	3.29
12	10.23	1.44	11.23	7.63	5.39	124.07	122.61	130.09	125.59	3.96
13	17.19	2	13.92	11.04	7.99	130.53	126.59	135.59	130.90	4.51
14	17.74	3.88	14.84	12.15	7.31	134.5	129.41	137.81	133.91	4.23
15	20.34	9.45	17.08	15.62	5.59	166.15	163.46	172.84	167.48	4.83

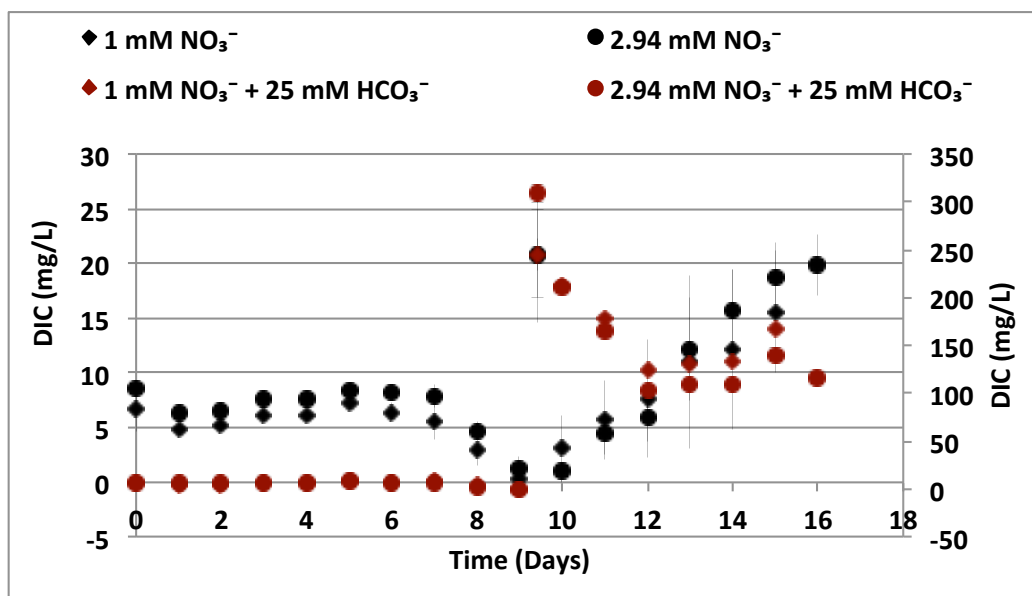


Figure A.5: Dissolved Inorganic Carbon (DIC) (mg/L) for cells grown in 2 mM Si/2.94 mM NO₃⁻ or 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Table A.54: Iron concentration (uM) for cells grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	8.48	8.40	7.52	8.13	0.53	8.71	15.54	12.42	12.22	3.42
1	8.34	8.80	9.57	8.90	0.62	10.08	15.38	9.87	11.78	3.12
2	-	-	-	-	-	-	-	-	-	-
3	6.69	9.48	8.98	8.39	1.49	8.80	14.22	8.38	10.47	3.26
4	-	-	-	-	-	-	-	-	-	-
5	7.70	9.73	7.67	8.37	1.18	7.34	5.71	11.74	8.26	3.12
6	-	-	-	-	-	-	-	-	-	-
7	6.76	5.07	5.83	5.89	0.85	5.48	10.18	4.89	6.85	2.90
8	-	-	-	-	-	-	-	-	-	-
9	1.17	1.22	2.27	1.56	0.62	1.89	3.40	1.01	2.10	1.21
9.4	1.73	2.24	1.80	1.93	0.28	1.95	5.94	1.53	3.14	2.43
10	0.90	0.30	0.87	0.69	0.34	1.68	6.21	2.55	3.48	2.40
11	0.48	0.58	0.41	0.49	0.09	0.89	5.13	0.70	2.24	2.50
12	0.47	0.44	1.17	0.70	0.41	0.66	1.24	0.73	0.88	0.31
13	-	-	-	-	-	-	-	-	-	-
14	2.26	0.94	0.80	1.33	0.81	1.05	0.73	1.32	1.03	0.29
15	1.21	0.59	0.90	0.90	0.31	1.54	0.48	0.69	0.90	0.56
16	8.48	8.40	7.52	8.13	0.53	8.71	15.54	12.42	12.22	3.42

Table A.55: Iron concentration (uM) for cells grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	11.09	10.37	10.27	10.58	0.45	10.08	12.39	11.14	1.16	10.08
1	13.18	15.56	12.46	13.73	1.62	10.08	12.39	11.57	1.29	10.08
2	-	-	-	-	-	-	-	-	-	-
3	11.28	10.97	12.00	11.42	0.53	10.11	11.27	10.68	0.58	10.11
4	-	-	-	-	-	-	-	-	-	-
5	10.57	10.32	11.41	10.76	0.57	10.18	10.08	10.57	0.77	10.18
6	-	-	-	-	-	-	-	-	-	-
7	9.01	8.74	9.00	8.92	0.15	8.22	10.02	9.38	1.00	8.22
8	-	-	-	-	-	-	-	-	-	-
9	4.92	4.61	4.69	4.74	0.16	3.63	4.45	3.84	0.53	3.63
9.4	6.50	6.09	6.41	6.33	0.21	5.44	4.59	5.03	0.43	5.44
10	4.00	4.46	4.14	4.20	0.24	4.12	3.86	4.13	0.29	4.12
11	4.19	4.32	3.97	4.16	0.18	2.99	3.65	3.30	0.33	2.99
12	3.04	4.34	3.79	3.72	0.65	2.13	3.36	2.69	0.62	2.13
13	-	-	-	-	-	-	-	-	-	-
14	2.83	1.78	3.88	2.83	1.05	2.26	1.98	1.87	0.45	2.26
15	11.09	10.37	10.27	10.58	0.45	10.08	12.39	11.14	1.16	10.08

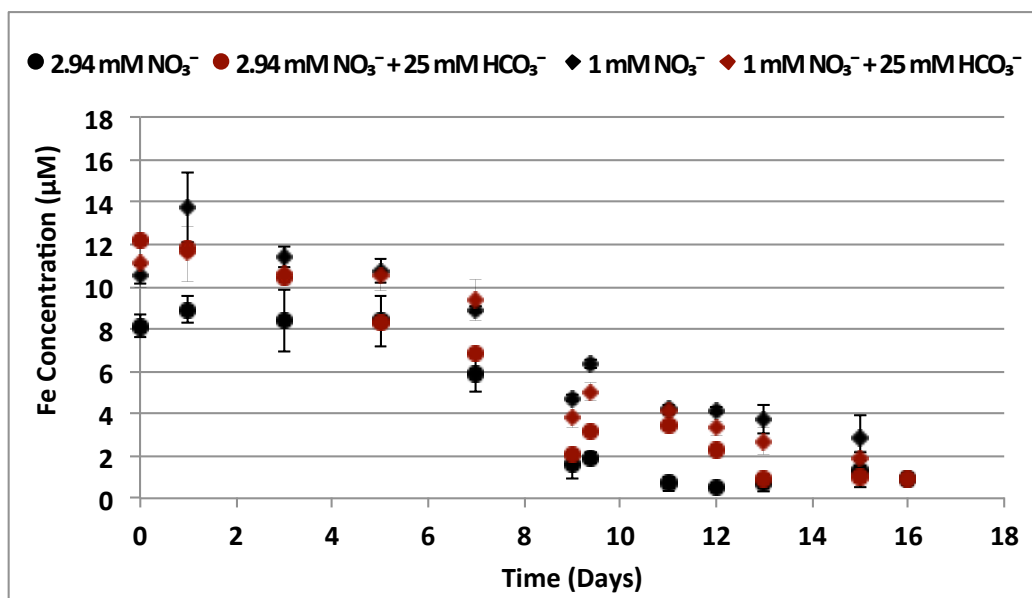


Figure A.6: Iron concentration (uM) for grown in 2 mM Si/2.94 mM NO_3^- or 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.

Table A.56: Raw Data (cps) for Iron measured using ICP-MS for Isotopes (Fe₅₆ and Fe₅₇) for growth in 2.0 mM Si and 1 mM NO₃⁻.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	697.04	1142.71	939.49	17.176	27.70	22.88
1	786.59	1132.32	772.86	19.38	27.61	18.71
2	-	-	-	-	-	-
3	703.06	1057.04	675.96	17.20	26.08	16.26
4	-	-	-	-	-	-
5	607.60	501.33	894.67	15.09	12.33	21.72
6	-	-	-	-	-	-
7	486.37	793.22	447.82	11.95	19.43	10.66
8	-	-	-	-	-	-
9	252.54	350.97	194.64	5.85	8.49	4.63
9.4	255.81	516.56	229.04	6.19	12.46	5.45
11	238.72	533.86	295.55	5.75	12.99	7.06
12	186.77	463.37	174.78	4.29	11.21	4.13
13	172.26	209.91	176.84	4.02	5.163	4.22
14	-	-	-	-	-	-
15	197.32	176.59	214.88	4.69	4.26	5.12
16	229.46	159.99	174.11	5.63	3.87	4.14302911

Table A.57: Raw Data (cps) for Iron measured using ICP-MS for Isotopes (Fe₅₆ and Fe₅₇) for growth in 2.0 mM Si, 1 mM NO₃⁻ and 25 mM NaHCO₃.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	827.47	778.68	772.30	20.22	18.54	18.62
1	882.04	1043.61	833.23	21.31	25.44	20.41
2	-	-	-	-	-	-
3	753.13	732.39	802.35	18.30	18.23	19.63
4	-	-	-	-	-	-
5	705.38	688.25	761.97	17.26	17.18	18.36
6	-	-	-	-	-	-
7	599.80	581.51	598.65	14.76	14.05	14.78
8	-	-	-	-	-	-
9	322.39	300.91	306.36	7.614	7.31	7.34
9.4	429.22	401.58	422.94	10.45	9.49	10.31
11	259.62	290.96	269.34	6.13	6.97	6.39
12	272.45	281.81	257.54	6.69	6.48	6.07
13	326.92	282.66	245.89	7.89	4.92	6.26
14	-	-	-	-	-	-
15	313.54	244.86	382.13	7.55	5.81	9.04

Table A.58: Raw Data (cps) for Iron measured using ICP-MS for Isotopes (Fe₅₆ and Fe₅₇) for growth in 2.0 mM Si.

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	732.35	869.35	782.35	17.63	21.38	19.27
1	807.94	678.96	816.68	19.77	16.55	19.87
2	-	-	-	-	-	-
3	714.53	680.76	750.26	17.53	16.57	18.37
4	-	-	-	-	-	-
5	761.19	685.06	679.30	18.91	17.02	16.45
6	-	-	-	-	-	-
7	667.81	568.42	675.40	16.24	13.74	16.52
8	-	-	-	-	-	-
9	283.61	294.58	343.23	6.83	6.92	8.16
9.4	379.55	402.66	351.97	9.04	9.87	8.50
11	342.14	323.56	308.07	8.44	7.99	7.47
12	273.39	256.27	295.39	6.58	6.22	7.21
13	230.78	205.27	278.50	5.49	4.97	6.53
14	-	-	-	-	-	-
15	160.42	212.66	196.07	3.77	5.16	4.64

Table A.59: Phosphate concentration (mM) when grown in 2 mM Si/2.94 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2-1	2-2	2-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	1.64	1.47	1.48	1.53	0.09	1.51	1.53	1.46	1.50	0.04
1	1.52	1.50	1.48	1.50	0.02	1.59	1.37	1.33	1.43	0.14
2	-	-	-	-	-	-	-	-	-	-
3	1.72	1.50	1.46	1.56	0.14	1.38	1.34	1.30	1.34	0.04
4	-	-	-	-	-	-	-	-	-	-
5	1.55	1.45	1.44	1.48	0.06	1.38	1.37	1.34	1.36	0.02
6	-	-	-	-	-	-	-	-	-	-
7	1.47	1.42	1.41	1.43	0.03	1.28	1.30	1.28	1.29	0.01
8	-	-	-	-	-	-	-	-	-	-
9	1.43	1.30	1.31	1.35	0.07	1.17	1.16	1.09	1.14	0.04
9.4	1.50	1.35	1.34	1.39	0.09	-	-	-	-	-
10	1.43	1.23	1.23	1.30	0.12	1.26	1.09	1.00	1.12	0.13
11	1.36	1.21	1.19	1.25	0.10	-	-	-	-	-
12	1.33	1.21	1.25	1.26	0.06	1.17	1.21	1.17	1.18	0.02
13	-	-	-	-	-	-	-	-	-	-
14	1.35	1.18	1.22	1.25	0.09	1.17	1.19	1.17	1.18	0.01
15	1.23	1.23	1.26	1.24	0.02	1.11	1.08	1.16	1.12	0.04
16	1.64	1.47	1.48	1.53	0.09	1.51	1.53	1.46	1.50	0.04

Table A.60: Phosphate concentration (mM) for cells grown in 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Time (Days)	2Si:N-1	2Si:N-2	2Si:N-3	Average	Standard Deviation	2+HCO3-1	2+HCO3-2	2+HCO3-3	Average	Standard Deviation
0	1.52	1.46	1.49	1.49	0.03	1.40	1.44	1.42	1.42	0.02
1	1.53	1.48	1.50	1.50	0.03	1.44	1.41	1.44	1.43	0.02
2	-	-	-	-	-	-	-	-	-	-
3	1.56	1.49	1.53	1.53	0.04	1.48	1.45	1.46	1.46	0.01
4	-	-	-	-	-	-	-	-	-	-
5	1.57	1.52	1.54	1.55	0.02	1.46	1.57	1.48	1.50	0.06
6	-	-	-	-	-	-	-	-	-	-
7	1.52	1.41	1.45	1.46	0.06	1.48	1.43	1.44	1.45	0.03
8	-	-	-	-	-	-	-	-	-	-
9	1.43	1.30	1.33	1.35	0.07	1.35	1.32	1.34	1.34	0.01
9.4	1.46	1.34	1.31	1.37	0.08	1.33	1.32	1.35	1.33	0.02
10	1.27	1.40	1.41	1.36	0.08	1.34	1.32	1.36	1.34	0.02
11	1.39	1.36	1.40	1.38	0.02	1.34	1.29	1.30	1.31	0.03
12	1.48	1.34	1.46	1.43	0.08	1.32	1.29	1.33	1.31	0.02
13	-	-	-	-	-	1.35	1.30	1.31	1.32	0.03
14	2.33	2.11	2.36	2.27	0.13	1.40	1.44	1.42	1.42	0.02
15	1.52	1.46	1.49	1.49	0.03	1.44	1.41	1.44	1.43	0.02

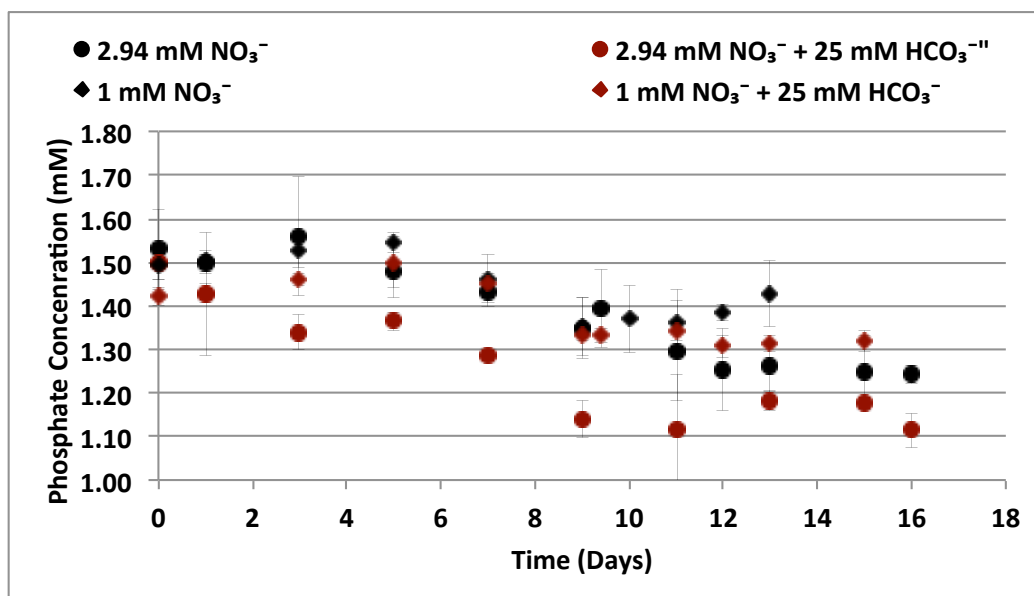


Figure A.7: Phosphate concentration (mM) for cells grown in 2 mM Si/2.94 mM NO₃⁻ or 2 mM Si/1 mM NO₃⁻ with and without NaHCO₃ addition.

Table A.61: Cell concentration (cells mL⁻¹) when grown in 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	1.66E05	1.35E05	1.21E05	1.41E05	2.30E04	1.04E05	8.00E04	7.25E04	8.55E04	1.65E04
2	2.70E05	2.46E05	2.41E05	2.52E05	1.55E04	2.40E05	2.26E05	2.41E05	2.36E05	8.39E03
4	8.60E05	6.50E05	7.30E05	7.47E05	1.06E05	5.58E05	6.24E05	5.93E05	5.92E05	3.30E04
6	2.10E06	2.24E06	1.70E06	2.01E06	2.80E05	1.24E06	1.10E06	1.13E06	1.16E06	7.37E04
8	5.03E06	5.12E06	4.81E06	4.99E06	1.59E05	3.39E06	3.77E06	3.24E06	3.47E06	2.73E05
10	1.05E07	9.93E06	8.25E06	9.56E06	1.17E06	8.65E06	8.15E06	6.78E06	7.86E06	9.68E05
12	1.22E07	1.26E07	1.03E07	1.17E07	1.23E06	1.03E07	1.26E07	1.23E07	1.17E07	1.25E06
14	1.27E07	1.13E07	1.47E07	1.29E07	1.71E06	1.03E07	1.25E07	1.21E07	1.16E07	1.17E06
16	1.69E07	1.36E07	1.87E07	1.64E07	2.59E06	1.42E07	1.42E07	1.70E07	1.51E07	1.62E06
18	1.69E07	1.88E07	1.76E07	1.78E07	9.61E05	1.80E07	1.99E07	2.10E07	1.96E07	1.52E06
20	1.60E07	2.14E07	2.13E07	1.96E07	3.09E06	2.07E07	2.05E07	1.92E07	2.01E07	8.14E05
22	1.77E07	2.22E07	2.02E07	2.00E07	2.25E06	2.60E07	2.63E07	2.46E07	2.56E07	9.07E05
24	1.56E07	1.73E07	1.93E07	1.74E07	1.85E06	1.94E07	2.24E07	1.73E07	1.97E07	2.56E06
26	-	-	-	-	-	2.66E07	3.01E07	2.29E07	2.65E07	3.60E06
27	-	-	-	-	-	1.97E07	2.06E07	2.39E07	2.14E07	2.21E06

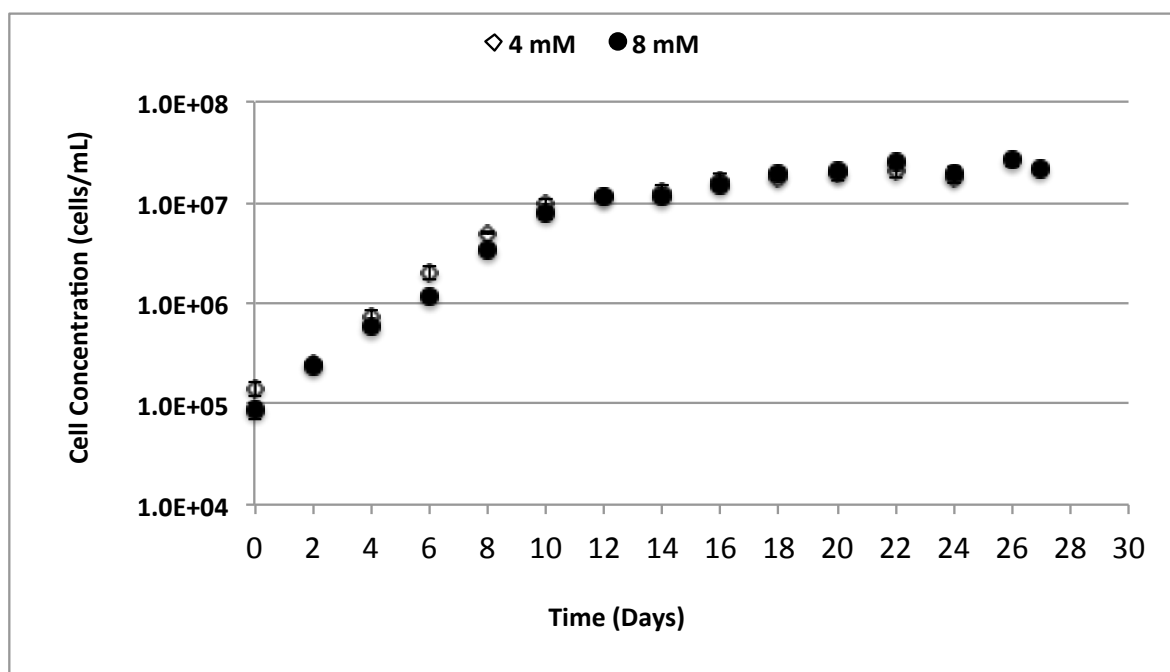


Figure A.8: Cell Concentration for cells grown in 4 & 8 mM Si.

Table A.62: Measured pH for cells grown in 4 and 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	8.52	8.54	8.89	8.65	0.21	9.07	9.11	9.05	9.08	0.03
2	8.01	8.07	8.11	8.06	0.05	8.42	8.47	8.48	8.46	0.03
4	8.28	8.35	8.37	8.33	0.05	8.58	8.61	8.62	8.60	0.02
6	8.76	8.75	8.82	8.78	0.04	8.74	8.74	8.72	8.73	0.01
8	9.60	9.54	9.60	9.58	0.03	9.20	9.22	9.14	9.19	0.04
10	10.27	10.18	9.96	10.14	0.16	9.85	9.87	9.80	9.84	0.04
12	10.56	10.51	10.48	10.52	0.04	10.03	10.05	10.04	10.04	0.01
14	10.71	10.69	10.87	10.76	0.10	10.12	10.18	10.16	10.15	0.03
16	10.85	10.84	11.05	10.91	0.12	10.28	10.34	10.32	10.31	0.03
18	10.89	10.92	10.93	10.91	0.02	10.43	10.50	10.46	10.46	0.04
20	10.91	10.94	10.33	10.73	0.34	10.54	10.56	10.59	10.56	0.03
22	10.48	10.54	9.73	10.25	0.45	10.41	10.34	10.39	10.38	0.04
24	9.56	9.73	9.12	9.47	0.31	10.02	9.91	10.01	9.98	0.06
26	-	-	-	-	-	9.74	9.71	9.73	9.73	0.02
27	-	-	-	-	-	9.53	9.52	9.48	9.51	0.03

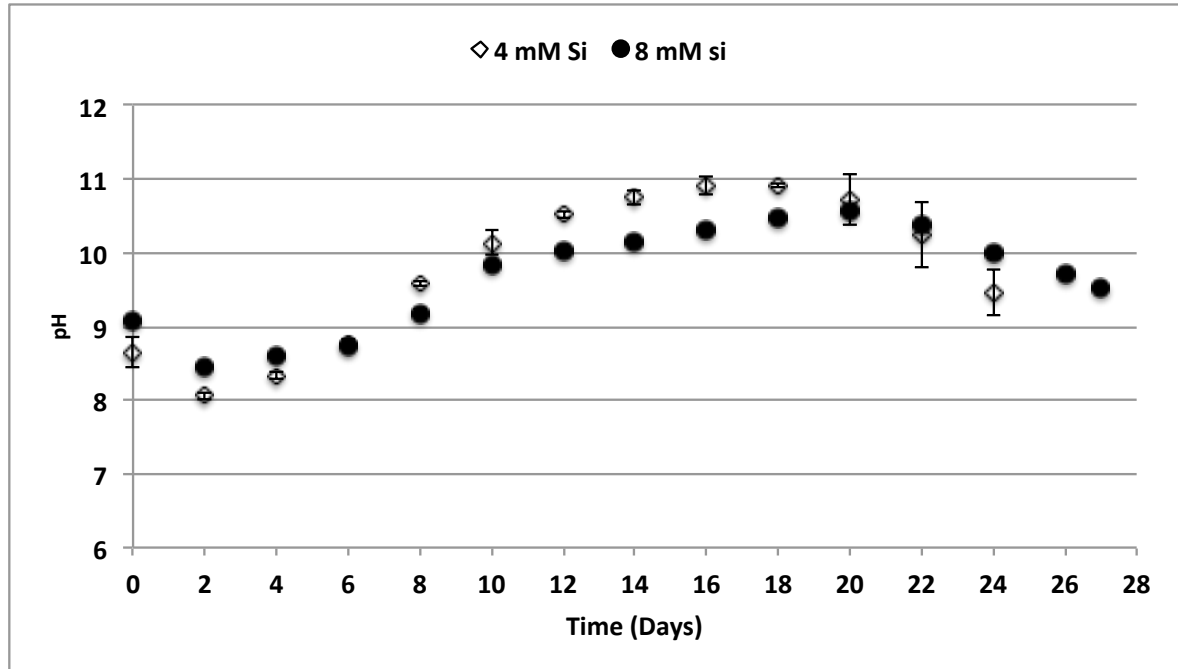


Figure A.9: Measured pH for cells grown in 4 and 8 mM Si.

Table A.63: Total Nile Red Fluorescence for cells grown in 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	83	67	12	54	37	0	43	104	49	52
2	9	27	9	9	18	33	37	28	33	5
4	31	43	34	36	6	18	36	34	17	31
6	168	110	174	151	35	110	150	104	121	25
8	865	1420	1070	1118	281	2425	2125	1895	2148	266
10	4640	4275	3875	4263	383	6805	6015	4820	5880	999
12	10740	9100	11910	10583	1412	12180	13610	12090	12627	853
14	13950	17120	20540	17203	3296	19140	15900	14070	16370	2567
16	23620	26090	32470	27393	4567	32040	27580	28960	29527	2283
18	29790	32200	44430	35473	7850	41600	43430	39300	41443	2069
20	44950	49410	68660	54340	12600	53290	57290	54540	55040	2046
22	53620	61920	73580	63040	10027	68640	75140	68240	70673	3873
24	54380	62170	69980	62177	7800	70460	73550	62750	68920	5562
26	-	-	-	-	-	84810	83100	84290	84067	877
27	-	-	-	-	-	75870	67360	74370	72533	4543

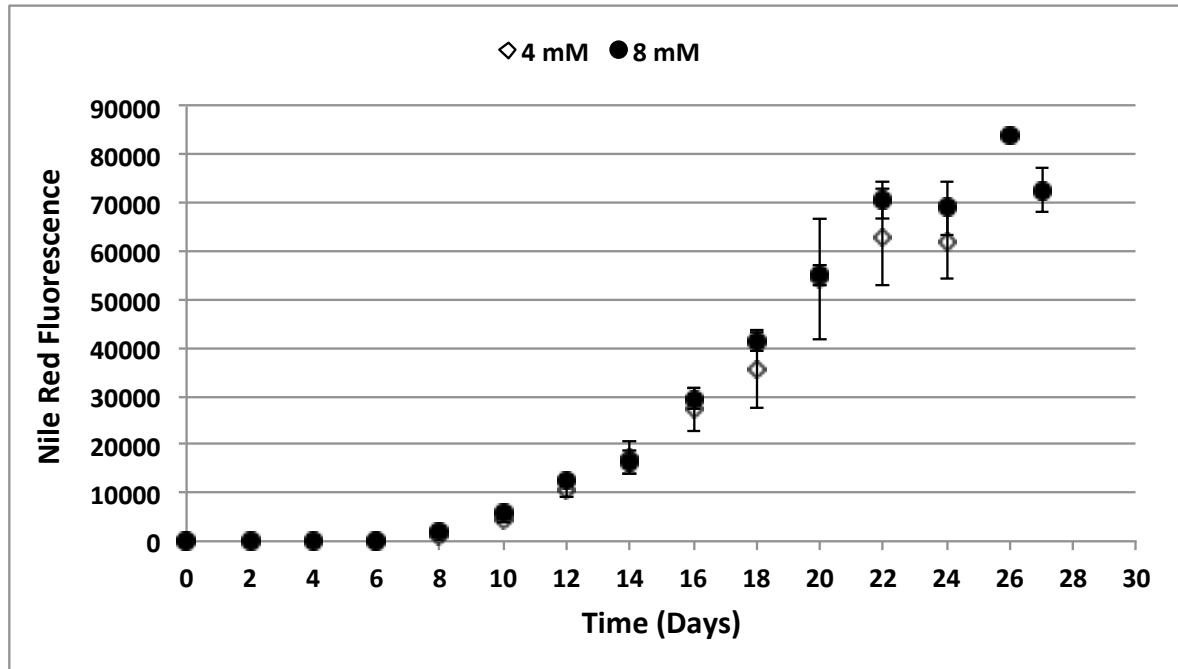


Figure A.10: Total Nile Red fluorescence for cells grown in 4 & 8 mM Si.

Table A.64: Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	5	5	1	4	2	0	5	14	7	7
2	0	1	0	0	1	1	2	1	1	0
4	0	1	0	0	0	0	1	1	0	1
6	1	0	1	1	0	1	1	1	1	0
8	2	3	2	2	1	7	6	6	6	1
10	4	4	5	4	0	8	7	7	7	0
12	9	7	12	9	2	12	11	10	11	1
14	11	15	14	13	2	19	13	12	14	4
16	14	19	17	17	3	23	19	17	20	3
18	18	17	25	20	5	23	22	19	21	2
20	28	23	32	28	5	26	28	28	27	1
22	30	28	36	32	4	26	29	28	28	1
24	35	36	36	36	1	36	33	36	35	2
26	-	-	-	-	-	32	28	37	32	5
27	-	-	-	-	-	39	33	31	34	4

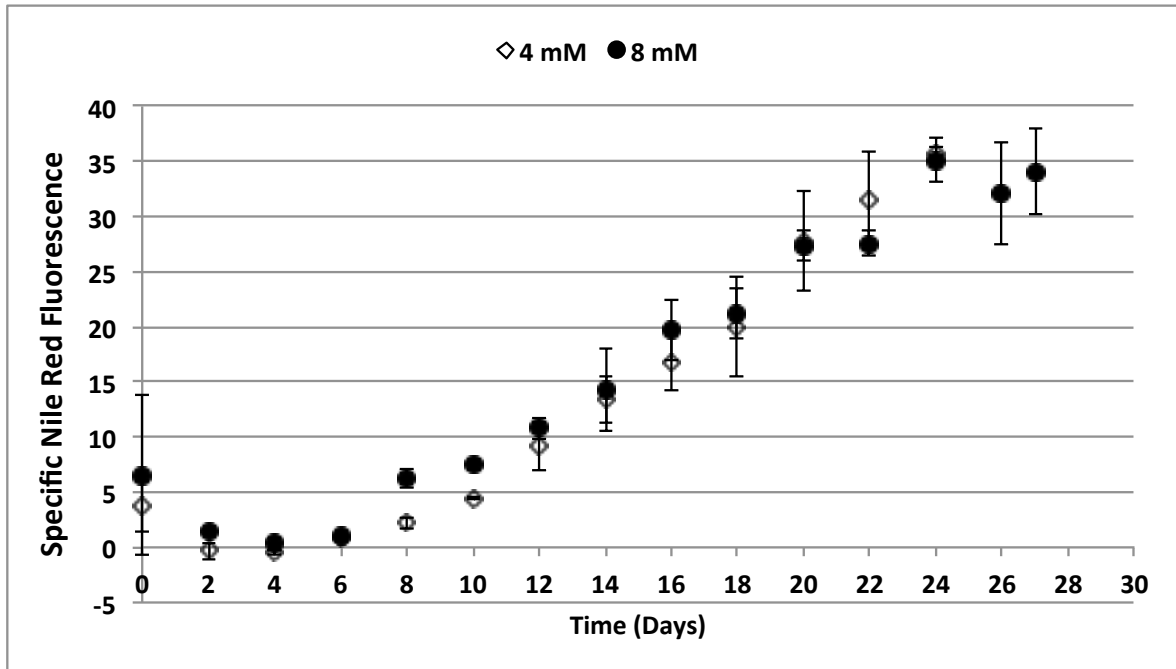


Figure A.11: Specific Nile Red Fluorescence (fluorescence per cells = Nile Red Intensity x 10000/cell count) for cells grown in 4 & 8 mM Si.

Table A.65: Measured Nitrate concentration (mM) when grown 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	2.502	2.436	2.385	2.441	0.059	2.487	2.550	2.551	2.529	0.036
2	2.380	2.353	2.373	2.368	0.014	2.384	2.505	2.477	2.455	0.063
4	1.835	1.853	1.747	1.811	0.057	2.145	2.203	2.212	2.187	0.036
6	0.985	0.950	0.836	0.924	0.078	1.278	1.145	1.255	1.226	0.071
8	0.270	0.316	0.000	0.195	0.171	0.548	0.390	0.456	0.465	0.079
10	0.000	0.000	0.000	0.000	0.000	0.185	0.000	0.000	0.062	0.107
12	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
14	2.502	2.436	2.385	2.441	0.059	0.000	0.000	0.000	0.000	0.000
16	2.380	2.353	2.373	2.368	0.014	0.000	0.000	0.000	0.000	0.000
18	1.835	1.853	1.747	1.811	0.057	2.487	2.550	2.551	2.529	0.036
20	0.985	0.950	0.836	0.924	0.078	2.384	2.505	2.477	2.455	0.063
22	0.270	0.316	0.000	0.195	0.171	2.145	2.203	2.212	2.187	0.036
24	0.000	0.000	0.000	0.000	0.000	1.278	1.145	1.255	1.226	0.071
26	-	-	-	-	-	0.548	0.390	0.456	0.465	0.079
27	-	-	-	-	-	0.185	0.000	0.000	0.062	0.107

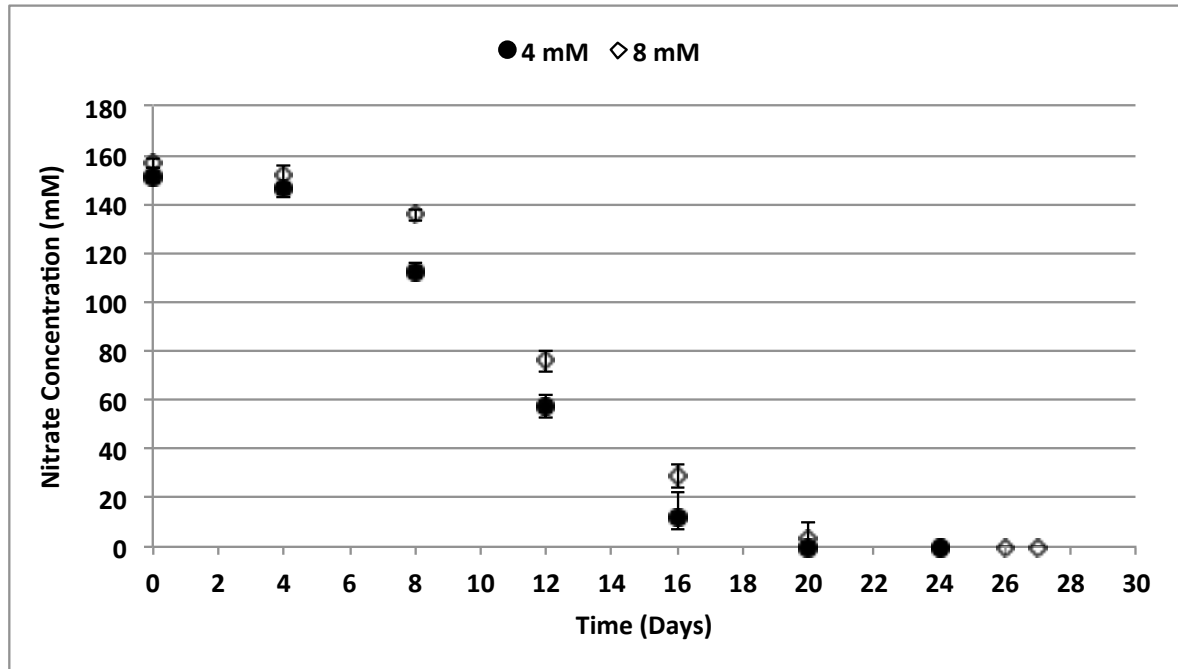


Figure A.12: Measured Nitrate concentration (mM) when grown 4 & 8 mM Si.

Table A.66: Chlorophyll concentration (mg/L) when grown in 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	0.027	-0.005	0.027	0.017	0.018	0.084	0.099	0.084	0.089	0.009
2	0.095	0.109	0.126	0.110	0.016	0.244	0.441	0.189	0.291	0.132
4	0.343	0.360	0.237	0.313	0.067	0.147	0.174	0.179	0.167	0.017
6	0.727	0.714	0.801	0.747	0.047	1.421	0.645	0.735	0.934	0.425
8	1.224	1.895	1.592	1.571	0.336	0.870	0.928	0.960	0.919	0.046
10	3.522	3.119	2.505	3.049	0.513	2.010	2.227	2.008	2.081	0.126
12	4.152	3.637	3.668	3.819	0.289	3.276	3.808	3.604	3.563	0.268
14	5.007	4.751	5.636	5.131	0.455	3.982	4.468	4.562	4.337	0.311
16	5.980	6.060	7.744	6.594	0.996	4.983	5.771	6.847	5.867	0.935
18	6.152	6.443	6.548	6.381	0.205	6.178	6.851	7.151	6.727	0.498
20	5.000	5.846	4.861	5.235	0.533	7.241	7.385	8.092	7.572	0.455
22	3.617	4.540	3.470	3.876	0.580	6.594	6.174	7.280	6.683	0.559
24	2.711	3.157	2.626	2.831	0.285	5.357	4.666	5.191	5.072	0.361
26	-	-	-	-	-	4.343	3.696	4.306	4.115	0.363
27	-	-	-	-	-	3.353	3.223	3.532	3.369	0.155

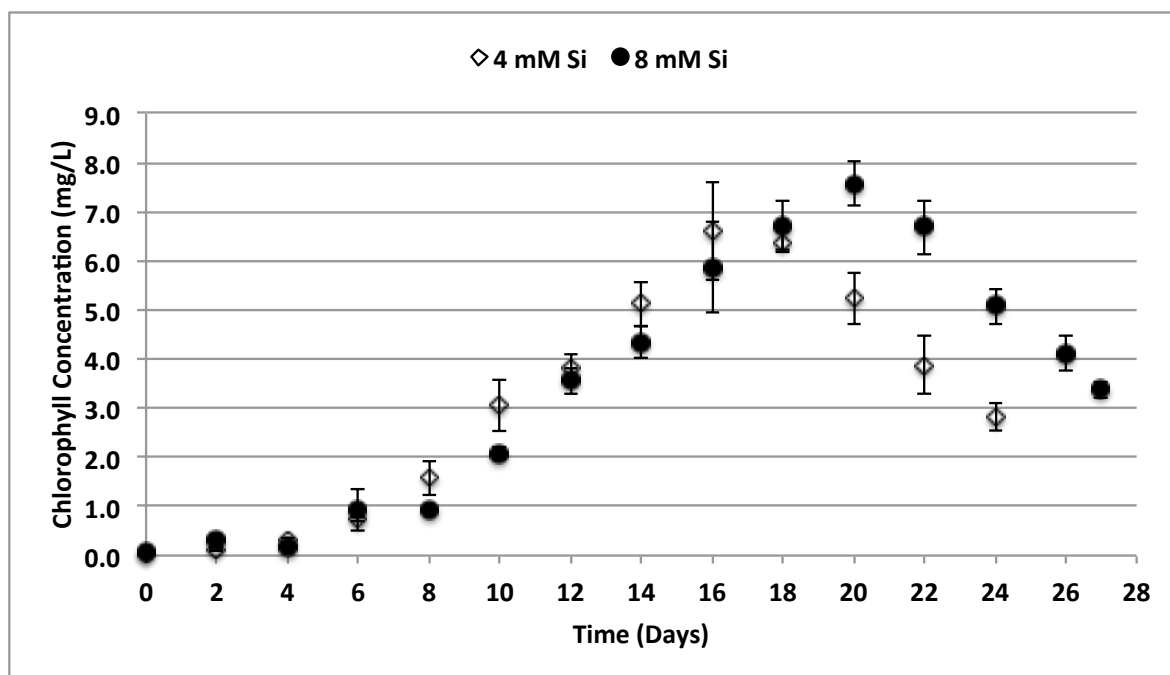


Figure A.13: Chlorophyll concentration (mg/L) when grown in 4 & 8 mM Si.

Table A.67 Dissolved Inorganic Carbon (DIC) (mg/L) for cells grown in 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
4	8.49	8.13	7.99	8.20	0.26	17.75	18.24	18.21	18.07	0.27
6	8.21	7.82	7.38	7.80	0.42	19.29	19.89	19.79	19.66	0.32
8	1.23	2.16	0.64	1.34	0.77	17.66	17.51	18.02	17.73	0.26
10	0.07	0.06	0.05	0.06	0.01	7.80	7.84	11.19	8.94	1.95
12	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0
14	0.73	0.66	2.50	1.30	1.04	0.47	0.38	0.41	0.42	0.05
16	1.17	1.49	2.59	1.75	0.74	0.91	0.77	0.86	0.85	0.07
18	2.11	1.79	4.38	2.76	1.41	0.81	1.09	1.16	1.02	0.19
20	3.69	3.91	13.89	7.16	5.83	2.02	2.47	1.92	2.14	0.29
22	9.24	9.13	23.32	13.90	8.16	3.87	5.77	4.34	4.66	0.99
24	23.98	21.44	33.08	26.17	6.12	13.16	16.48	12.91	14.18	1.99
26	-	-	-	-	-	21.00	22.12	21.62	21.58	0.56
27	-	-	-	-	-	28.38	27.84	28.64	28.29	0.41

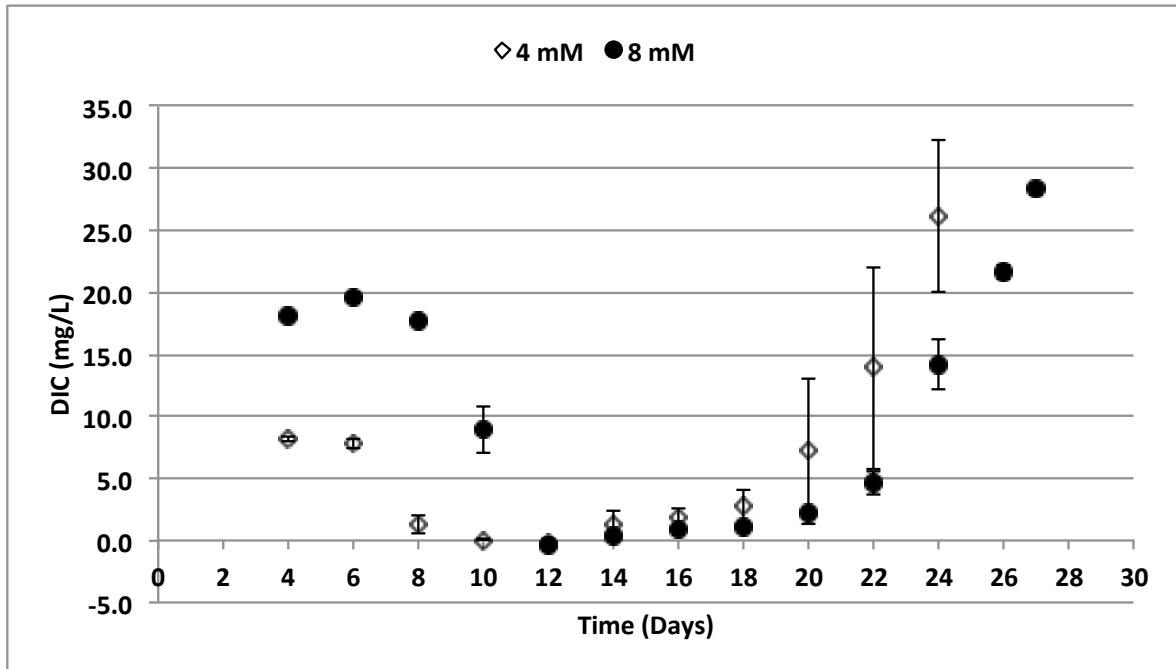


Figure A.14: Dissolved Inorganic Carbon (DIC) (mg/L) for cells grown in 4 & 8 mM Si.

Table A. ⁶⁸Si concentration (mM) when grown in 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	3.64	3.73	4.00	3.79	0.19	6.92	5.92	6.06	6.30	0.54
4	2.18	2.18	1.81	2.06	0.21	4.03	4.58	4.00	4.20	0.32
8	1.61	1.62	1.55	1.59	0.04	3.54	4.01	4.11	3.89	0.30
12	1.19	1.20	1.32	1.24	0.07	3.06	2.80	4.14	3.33	0.71
16	1.17	1.20	1.08	1.15	0.06	3.07	3.30	2.39	2.92	0.47
20	1.04	1.30	1.27	1.21	0.14	3.25	3.38	2.67	3.10	0.38
24	1.03	1.08	1.06	1.05	0.02	3.15	3.14	2.49	2.93	0.38
26	-	-	-	-	-	2.79	3.10	2.89	2.93	0.16
27	-	-	-	-	-	3.24	3.17	4.05	3.49	0.49

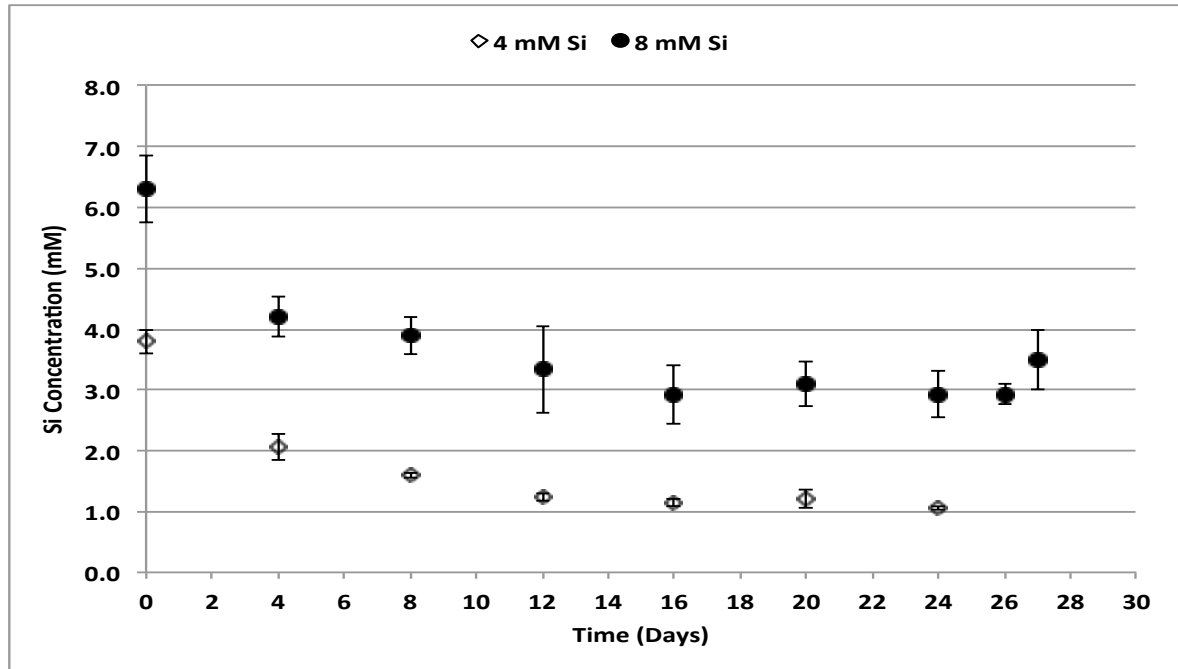


Figure A.15: Si concentration (mM) when grown in 4 & 8 mM Si.

Table A.69: Iron concentration (uM) for cells grown in 4 & 8 mM Si.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	3.20	3.34	2.79	3.11	0.29	8.43	7.24	7.24	7.64	0.69
4	3.63	3.94	2.96	3.51	0.50	8.26	9.08	7.75	8.37	0.67
8	2.71	2.99	3.05	2.92	0.18	7.93	8.41	8.52	8.28	0.31
12	2.21	2.15	2.41	2.26	0.14	5.45	5.54	7.52	6.17	1.17
16	1.66	1.84	1.93	1.81	0.14	4.11	3.62	3.62	3.78	0.29
20	2.04	2.52	2.03	2.20	0.28	3.68	3.43	3.28	3.46	0.20
24	1.97	1.85	1.52	1.78	0.23	3.45	2.98	2.71	3.05	0.38
26	-	-	-	-	-	2.77	2.83	2.65	2.75	0.09
27	-	-	-	-	-	3.28	2.96	3.87	3.37	0.46

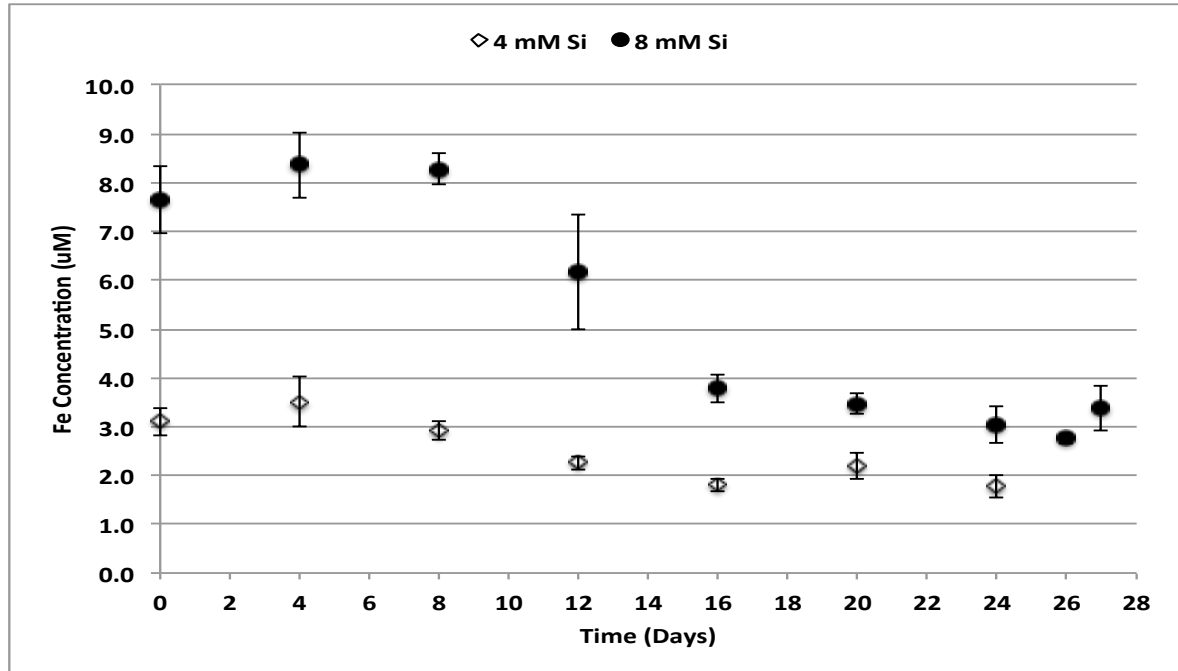


Figure A.16: Iron concentration (uM) for cells grown in 4 & 8 mM Si.

Table A.70: Phosphate concentration (mM) for cells grown in 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.

Time (Days)	4-1	4-2	4-3	Average	Standard Deviation	8-1	8-2	8-3	Average	Standard Deviation
0	1.45	1.42	1.37	1.41	0.04	1.38	1.48	1.41	1.43	0.05
4	1.45	1.43	1.54	1.48	0.06	1.35	1.43	1.40	1.39	0.04
8	1.43	1.46	1.58	1.49	0.08	1.38	1.45	1.42	1.42	0.04
12	1.52	1.34	1.36	1.41	0.10	1.33	1.32	1.31	1.32	0.01
16	1.34	1.24	1.23	1.27	0.06	1.26	1.27	1.25	1.26	0.01
20	1.19	1.15	1.19	1.18	0.02	0.12	1.20	0.07	0.46	0.64
24	1.24	1.14	1.21	1.20	0.05	1.21	1.19	1.17	1.19	0.02
26						1.42	1.16	1.13	1.24	0.16
27						1.23	1.14	1.12	1.16	0.06

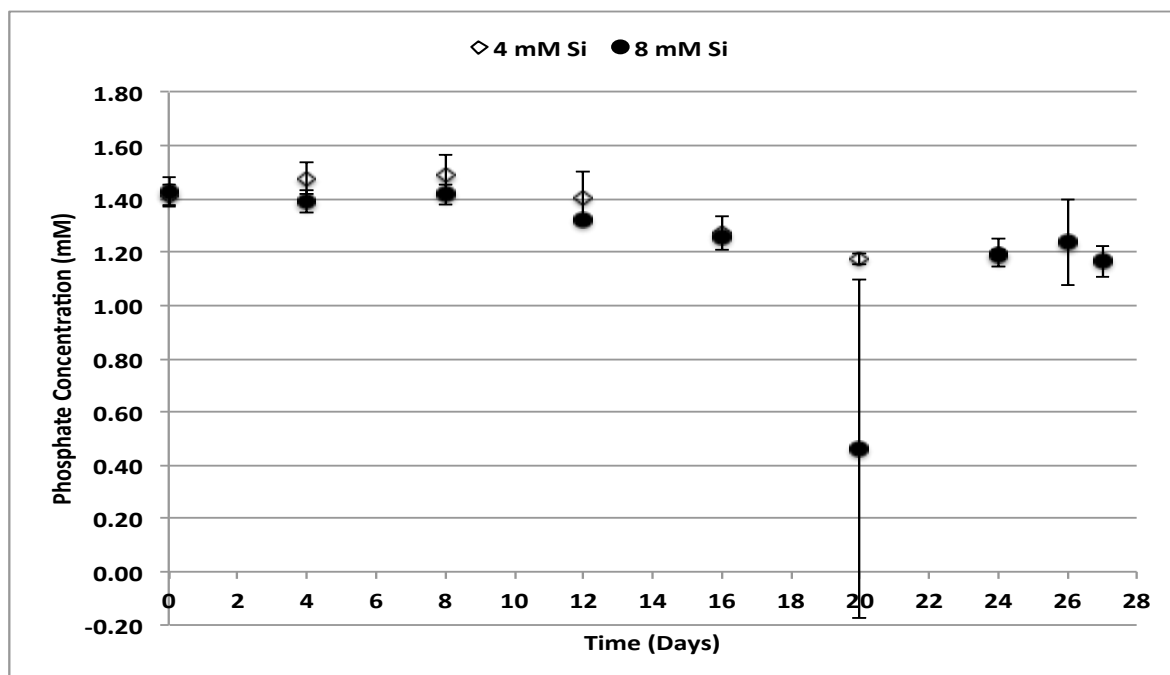


Figure A.17: Phosphate concentration (mM) for cells grown in 2 mM Si/1 mM NO_3^- with and without NaHCO_3 addition.

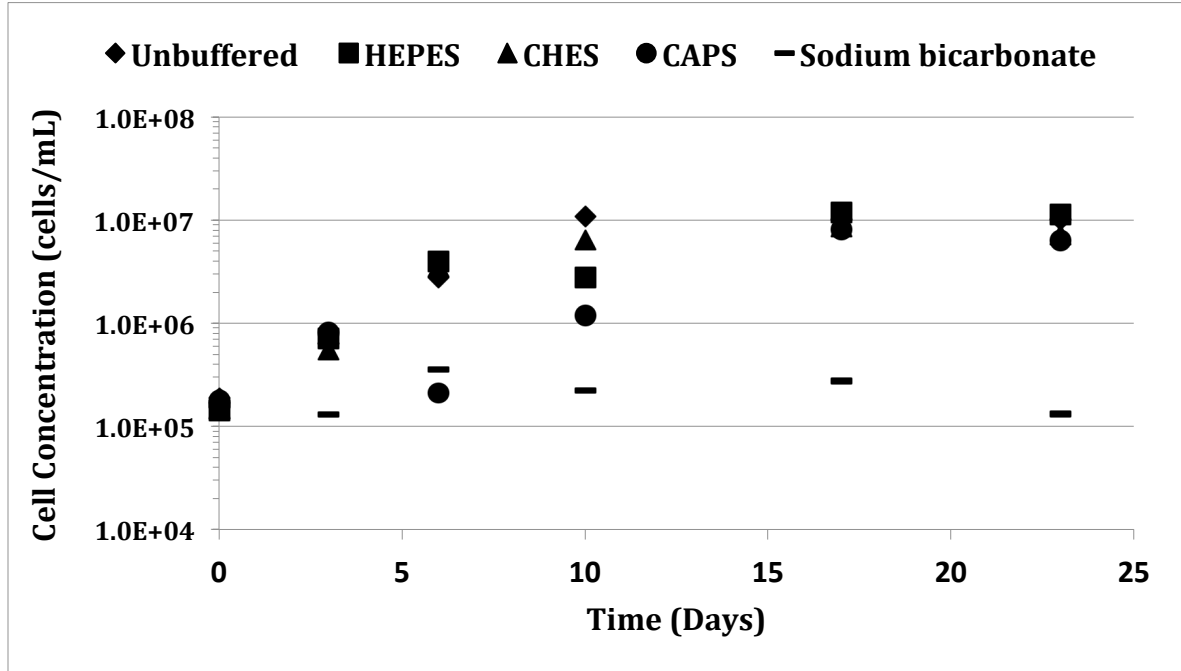


Figure A.18 RGd-1 Cell concentrations ($\text{cells} \cdot \text{mL}^{-1}$) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Table A.71 Cell concentrations (cells•mL⁻¹) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Time (Days)	Unbuffered	HEPES pKa: 7.4	CHES pKa: 9.3	CAPS pKa: 10.4	Sodium bicarbonate pKa: 10.3
0	1.87E+05	1.43E+05	1.49E+05	1.78E+05	1.61E+05
3	6.33E+05	7.08E+05	5.50E+05	8.18E+05	1.29E+05
6	2.75E+06	3.92E+06	3.52E+06	2.10E+05	3.58E+05
10	1.09E+07	2.80E+06	6.40E+06	1.18E+06	2.23E+05
17	1.02E+07	1.19E+07	8.63E+06	8.08E+06	2.75E+05
23	1.02E+07	1.13E+07	7.18E+06	6.37E+06	1.31E+05

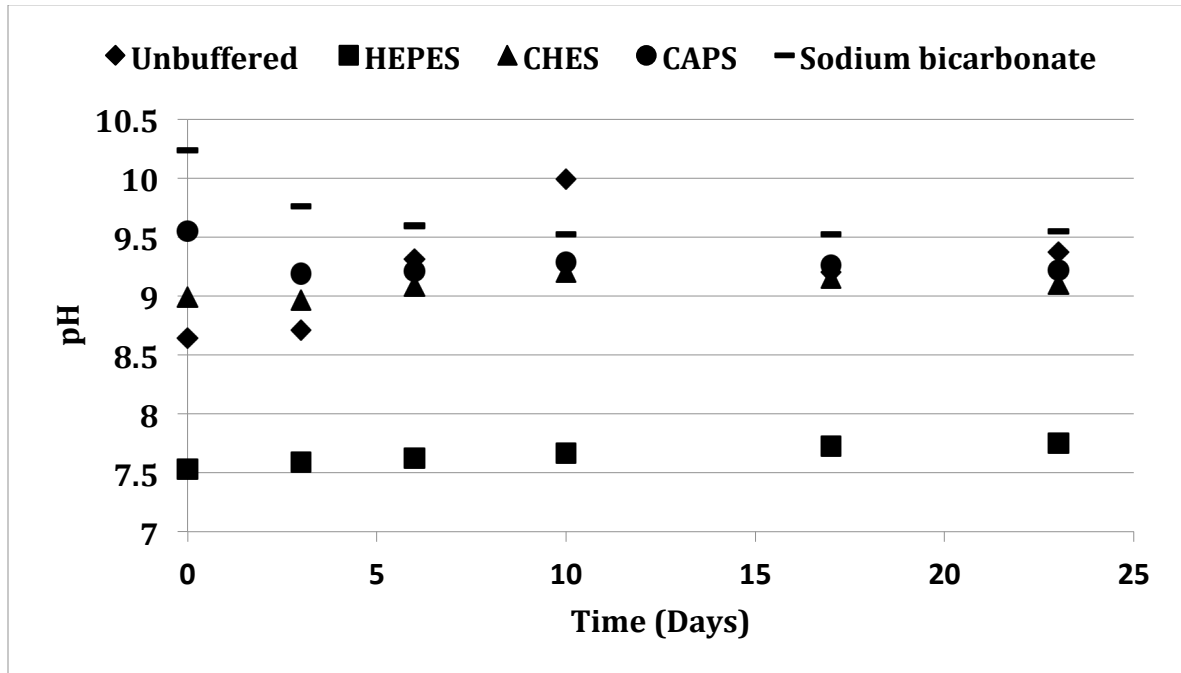


Figure A.19 RGd-1 medium pH throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Table A.72 RGd-1 medium pH throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Time (Days)	Unbuffered	HEPES pKa: 7.4	CHES pKa: 9.3	CAPS pKa: 10.4	Sodium bicarbonate pKa: 10.3
0	8.64	7.53	8.99	9.55	10.24
3	8.71	7.59	8.96	9.19	9.76
6	9.31	7.62	9.08	9.21	9.6
10	9.99	7.67	9.2	9.29	9.52
17	9.2	7.73	9.15	9.26	9.52
23	9.37	7.75	9.1	9.22	9.55

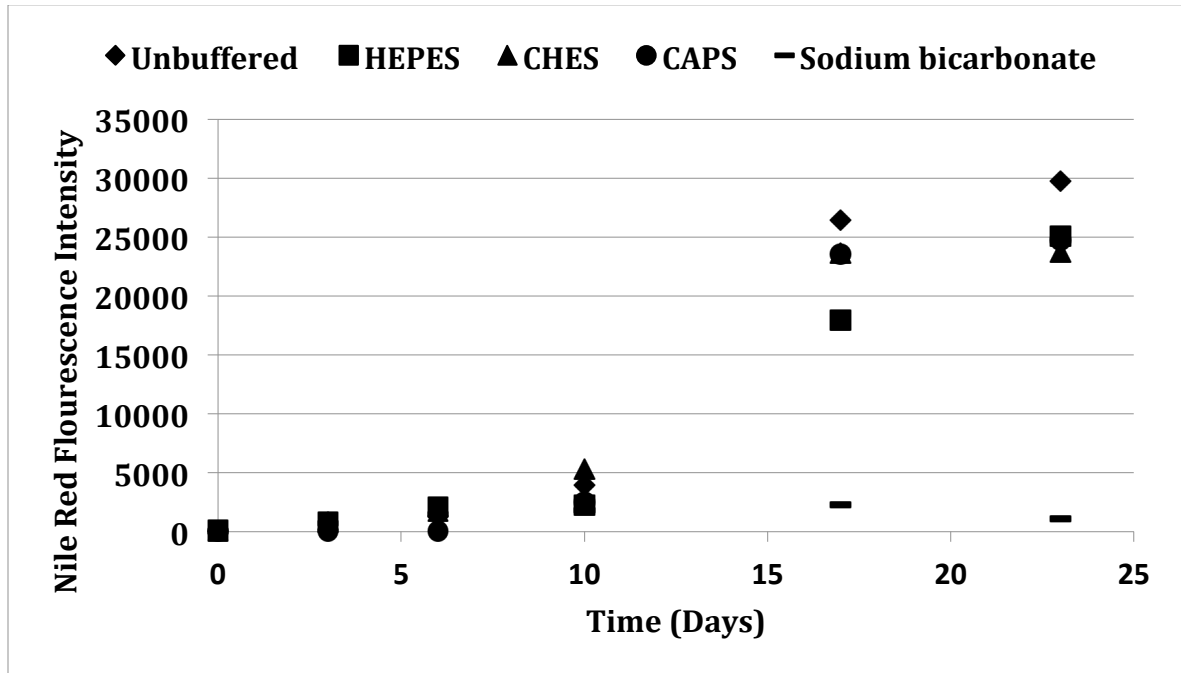


Figure A.20 RGd-1 Total Nile Red fluorescence throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Table A.73 RGd-1 Total Nile Red fluorescence throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Time (Days)	Unbuffered	HEPES pKa: 7.4	CHES pKa: 9.3	CAPS pKa: 10.4	Sodium bicarbonate pKa: 10.3
0	85	147	0	73	0
3	161	775	793	0	711
6	1301	2063	1776	31	1477
10	3925	2240	5344	2506	1782
17	26393	17917	23646	23572	2274
23	29756	25031	23725	24744	1111

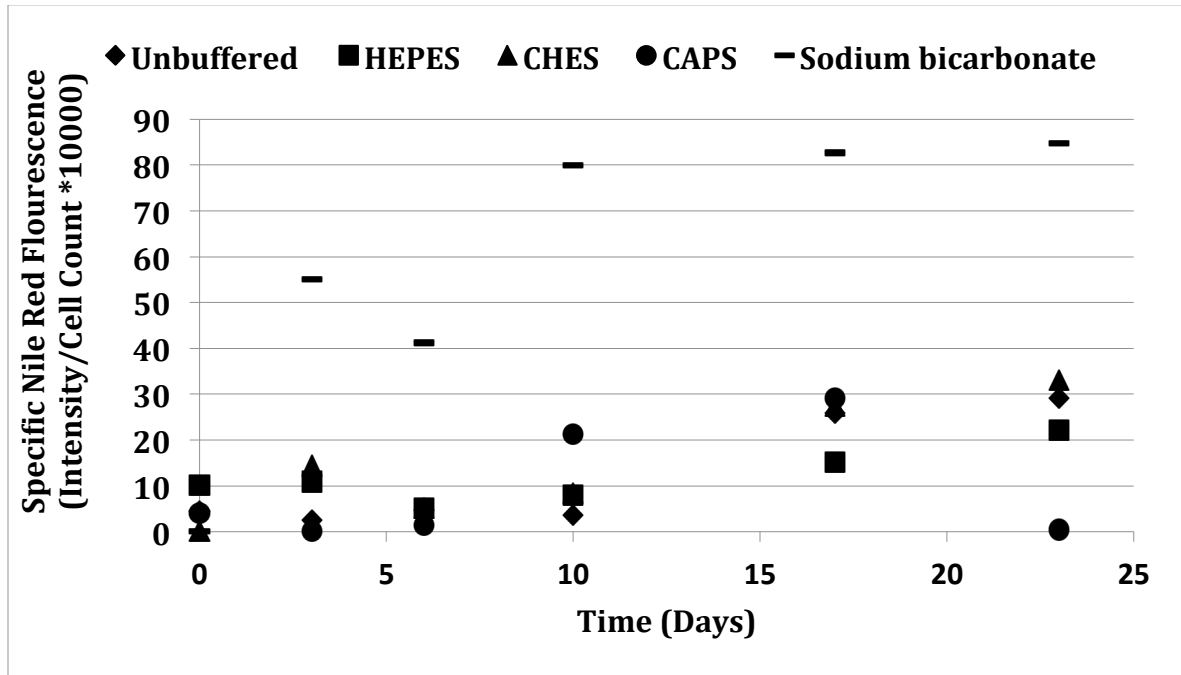


Figure A.20 RGd-1 specific Nile Red fluorescence (Nile Red intensity/cell concentration * 10000) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Table A.74 RGd-1 specific Nile Red fluorescence (Nile Red intensity/cell concentration * 10000) throughout screening experiments to determine pH for optimal cells growth using 3 biological buffers (HEPES, CHES and CAPS) and Sodium bicarbonate.

Time (Days)	Unbuffered	HEPES pKa: 7.4	CHES pKa: 9.3	CAPS pKa: 10.4	Sodium bicarbonate pKa: 10.3
0	5	10	0	4	0
3	3	11	14	0	55
6	5	5	5	1	41
10	4	8	8	21	80
17	26	15	27	29	83
23	29	22	33	0	85

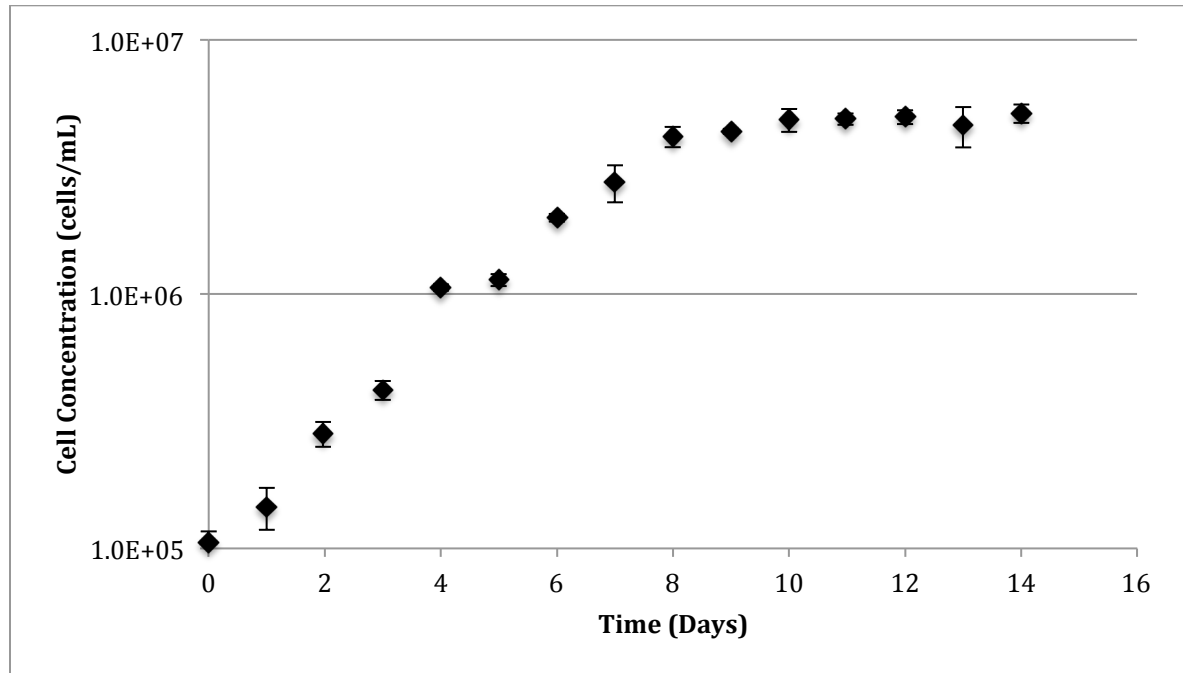


Figure A.21 RGd-1 growth in B8.7SiS 25mM CHES buffered medium.

Table A.75 RGd-1 growth in CHES buffered medium.

Time (Days)	CHES-1	CHES-2	CHES-3	Average	Standard Deviation
0	1.13E+05	9.30E+04	1.11E+05	1.06E+05	1.10E+04
1	1.75E+05	1.21E+05	1.41E+05	1.46E+05	2.73E+04
2	3.10E+05	2.48E+05	2.90E+05	2.83E+05	3.16E+04
3	4.30E+05	4.50E+05	3.80E+05	4.20E+05	3.61E+04
4	1.05E+06	1.10E+06	1.04E+06	1.06E+06	3.21E+04
5	1.13E+06	1.20E+06	1.08E+06	1.14E+06	6.03E+04
6	1.94E+06	2.07E+06	1.98E+06	2.00E+06	6.66E+04
7	2.85E+06	2.26E+06	3.17E+06	2.76E+06	4.62E+05
8	4.35E+06	4.41E+06	3.73E+06	4.16E+06	3.76E+05
9	4.43E+06	4.43E+06	4.26E+06	4.37E+06	9.81E+04
10	5.44E+06	4.53E+06	4.60E+06	4.86E+06	5.06E+05
11	4.64E+06	4.89E+06	5.15E+06	4.89E+06	2.55E+05
12	4.64E+06	5.05E+06	5.25E+06	4.98E+06	3.11E+05
13	5.56E+06	4.36E+06	3.94E+06	4.62E+06	8.41E+05
14	5.61E+06	4.80E+06	5.01E+06	5.14E+06	4.20E+05

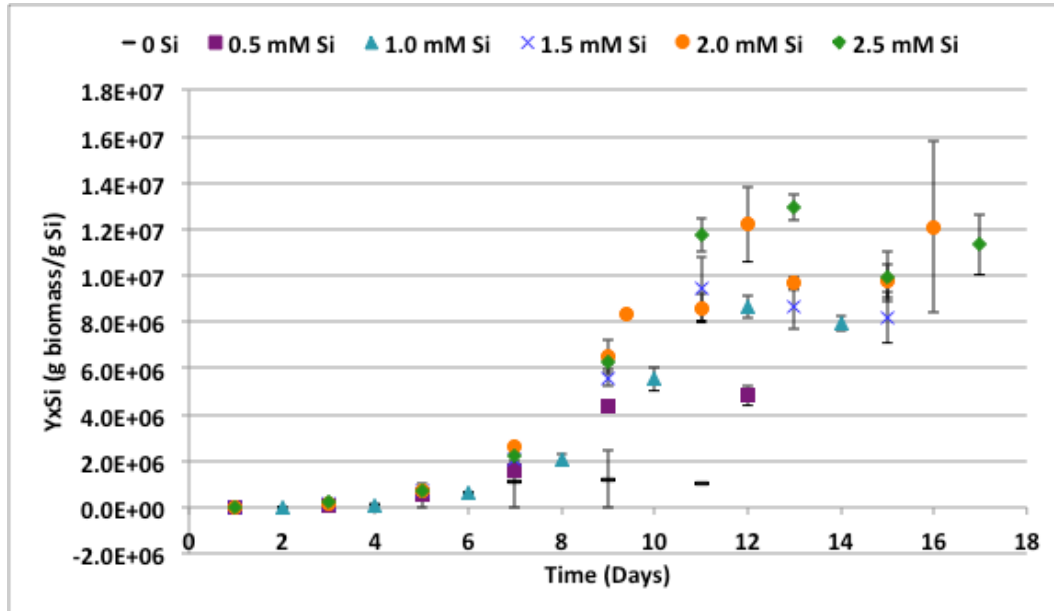


Figure A.22 RGd-1 biomass yield per gram of Silica utilized.

APPENDIX B

ABIOTIC CONTROLS

To discern whether silica polymers were formed when exposed to high temperatures as seen during autoclaving, filtered and unfiltered 0.5, 1.0 and 2.5 mM (B.1, B.2 & B.3, respectively) Si abiotic samples were analyzed on ICP-MS. Investigation of these abiotic controls allowed for the determination of whether autoclaving induced silica polymers were being retained on the filter when preparing samples for further analyses, thus appearing as an underestimated silica concentration. Whereas the concentrations for abiotic controls consisting of 0.5 and 1.0 mM Si remained consistent over time, the 2.5 mM Si abiotic control increased approximately 20 mg/L from day 0 to 7. Over time, with aerated mixing, the polymers were likely dissociated and re-suspended homogeneously within media, thus increasing the concentration throughout the bulk media. However, the increase in silica concentration for 2 and 2.5 mM Si were not observed until days 5 and 7 when the pH was 8.23 and 8.67, respectively. Therefore, if silica was scavenged from the bioreactor tubes, it would not have occurred prior to late exponential phase. Another potential silica source is from scavenged diatom frustules. Kamatani (1982) found that at 27°C (pH 8), 75% diatom frustules were consumed by 30 days [21]. While our studies are not as long, it is still possible diatoms could utilize some silica recycled from dead diatoms.

It is additionally possible that silica may have been scavenged from the borosilicate glass bioreactor tubes when the medium pH exceeded 9 [30]. To investigate the extent to which silica was leached from the borosilicate glass of the tube reactors used in growth studies, abiotic controls were run in duplicate for 15 days with synthetic

buffers CHES, CAPS and piperidine with pKa's 9.3, 10.4 and 11.15, respectively. It was found that approximately 0.15 g/L by day 15. CHES and CAPS buffered media were autoclaved to ensure sterility. However, Piperidine is known to produce flammable volatiles. To avoid this potential problem, Piperidine was filter sterilized and added post-autoclaving. Whereas there was no silica present on day 0 for the Piperidine samples, there was ≥ 0.1 g silica present for CHES and CAPS buffered media that had been autoclaved. Over the course of the 15 day experiment, Piperidine revealed a very clear increase in silica concentration over time from 0 Si to approximately 0.100 g/L (0.35 mM), values typically seen at day 11 for silica concentrations 1.5 mM and higher. This indicates that when cultures are growing at high pH, there is a potential for some silica leaching from the borosilicate glass over time, and may provide enough silica from approximately one cell doubling.

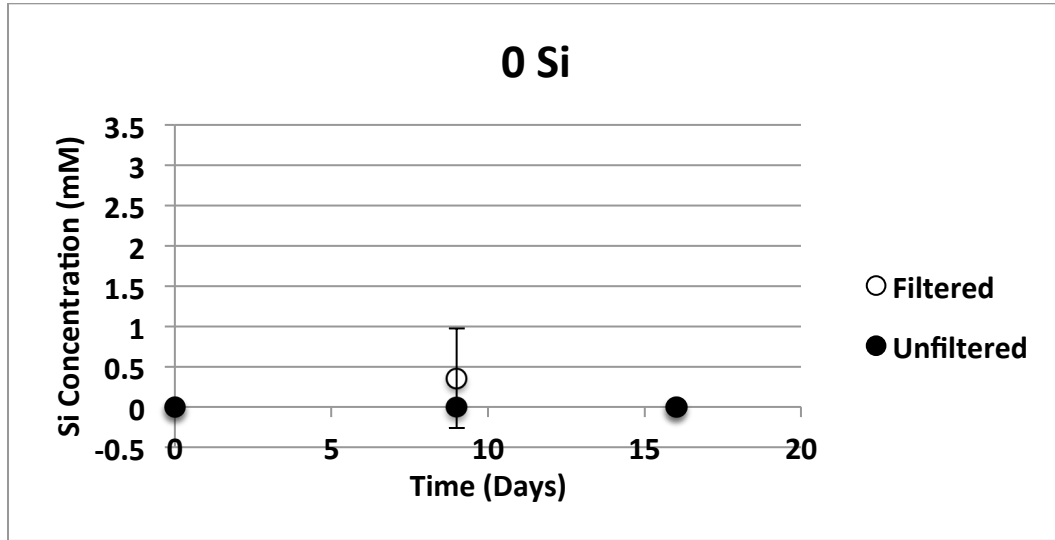


Figure B.1: Si concentration quantified over time in abiotic controls with 0 Si added. Samples were measured in triplicate.

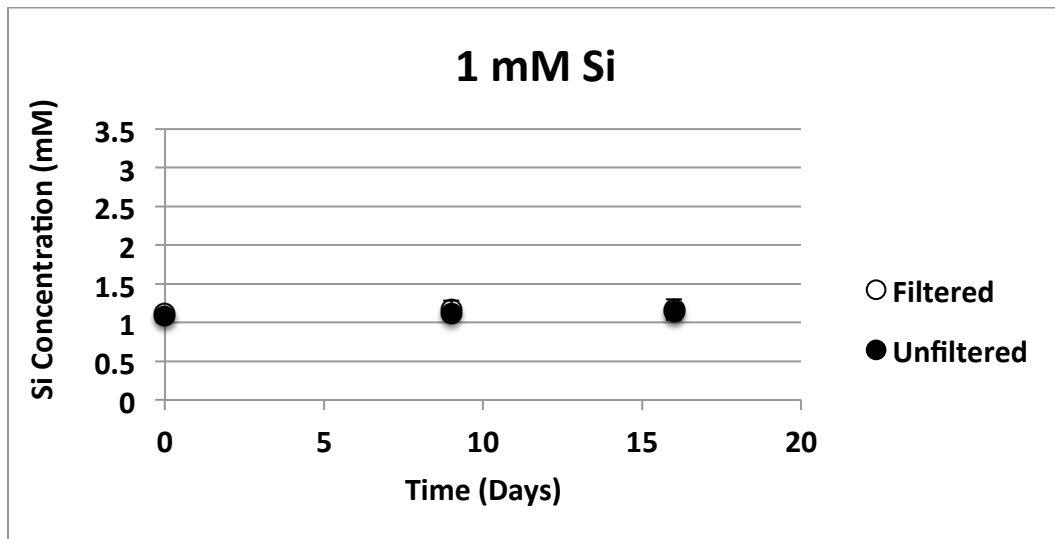


Figure B.2: Si concentration measured over time in abiotic controls with 1 mM Si. Samples were measured in triplicate.

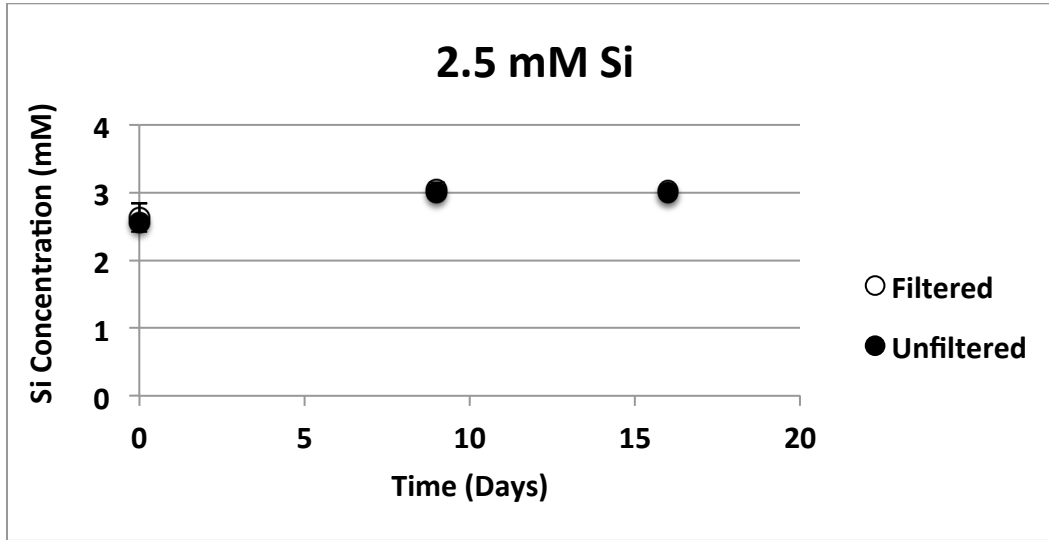


Figure B.3: Si concentration over time in abiotic controls with 2.5 mM Si. Samples were measured in triplicate.

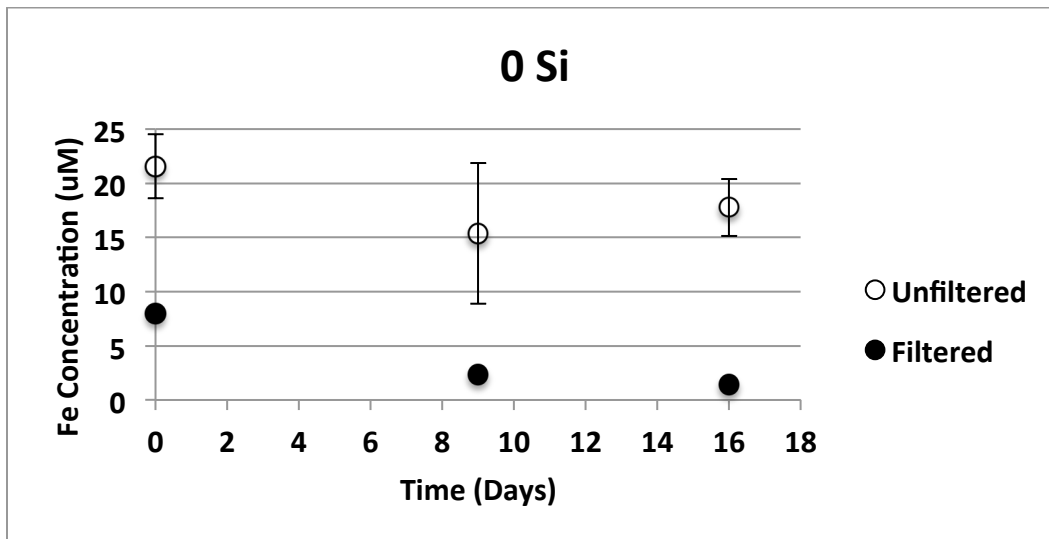


Figure B.4: Fe concentration measured over time in abiotic controls with 0 Si added. Samples were measured in triplicate.

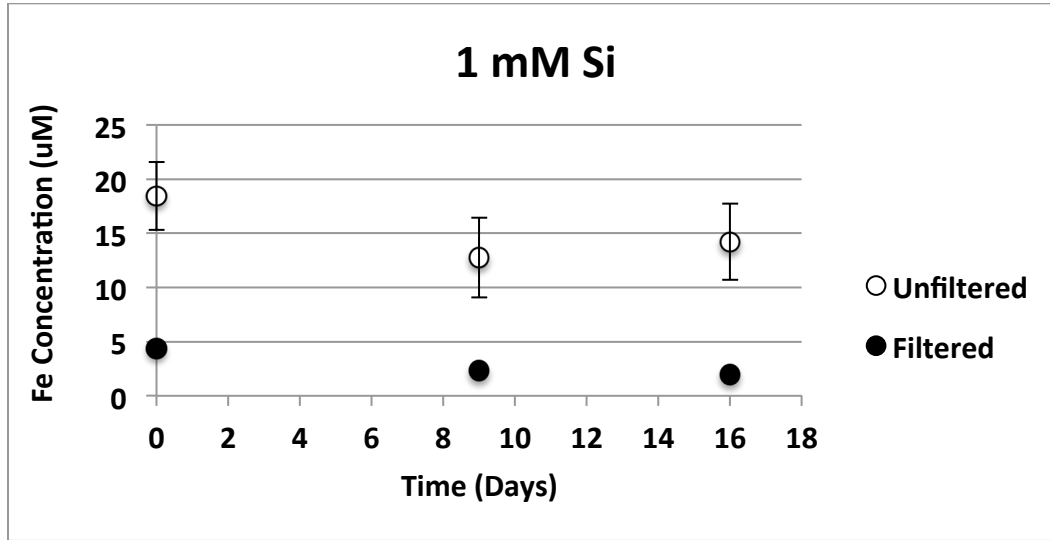


Figure B.5: Fe measured over time in abiotic controls with 1 mM Si. Samples were measured in triplicate.

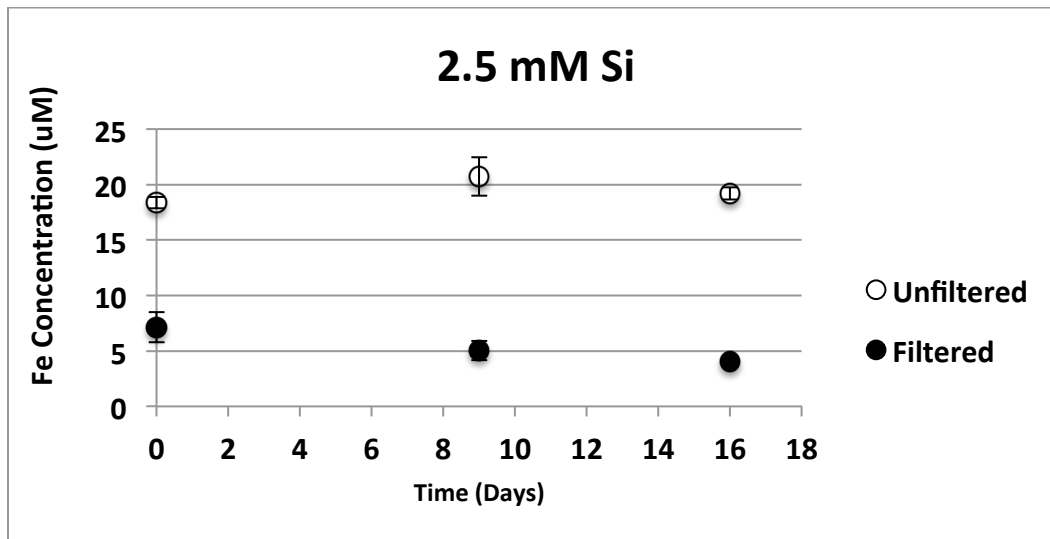


Figure B.6: Fe measured over time in abiotic controls with 2.5 mM Si. Samples were measured in triplicate.

Table B.1: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 0 Si (unfiltered).

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	370.02	439.54	356.74	43.50	48.00	42.56	518.15	543.35	523.66
9	363.12	420.27	-	40.06	43.32	172.31	488.62	478.76	575.16
16	379.51	413.02	350.67	39.53	41.97	38.78	462.60	480.91	483.97

Table B.2: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 0 Si (filtered).

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	336.67	381.80	328.23	36.54	38.79	36.79	455.60	453.59	472.68
9	322.32	405.98	314.47	33.25	39.23	32.81	418.25	457.25	428.70
16	314.36	381.55	315.35	31.21	34.61	31.48	398.80	410.88	414.67

Table B.3: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 1 mM Si (unfiltered).

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	2509.67	2441.83	2416.13	183.13	178.25	175.45	607.75	586.50	589.07
9	2673.51	2343.23	2586.08	190.83	168.57	184.29	568.01	561.34	565.72
16	2717.19	2558.49	2322.93	194.86	182.73	165.89	593.02	546.67	510.58

Table B.4: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 1 mM Si (filtered).

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	2425.48	2408.03	2388.50	172.28	169.14	168.06	530.80	512.33	512.14
9	2349.28	2539.27	2483.96	164.41	177.19	172.40	480.73	521.23	533.11
16	2625.95	2454.64	2404.22	181.76	170.19	165.76	521.96	494.92	494.61

Table B.5: Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 2.5 mM Si (unfiltered).

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	4759.60	4925.08	4332.00	335.07	344.34	301.89	684.32	673.83	646.21
9	5174.22	5453.24	5225.02	357.82	373.81	362.18	665.16	702.02	675.43
16	5253.31	5274.65	5246.15	362.00	363.22	361.94	656.05	662.68	660.45

Table B.6 Raw Data (cps) ICP-MS for each of the three Si isotopes (Si_{28} , Si_{29} and Si_{30}) for abiotic control, 2.5 mM Si (filtered).

Time (Days)	28-1	28-2	28-3	29-1	29-2	29-3	30-1	30-2	30-3
0	4636.25	4956.03	4115.43	319.09	338.38	285.81	590.11	627.74	597.44
9	5320.20	5009.99	5299.93	363.18	340.98	359.14	662.14	618.81	632.28
16	5150.22	5381.01	5117.10	350.57	365.81	347.90	652.14	639.17	606.43

Table B.7: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 0 Si (unfiltered).

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	1242.56	1354.24	1586.65	35.23	37.44	44.44
9	1058.00	1397.06	631.38	26.22	39.37	15.63
16	990.33	1259.37	1259.69	24.21	35.12	35.10

Table B.8: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 0 Si (filtered).

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	601.49	580.90	591.68	14.68	14.17	14.40
9	252.14	276.84	247.15	6.36	6.81	5.79
16	202.50	209.93	204.00	4.94	5.24	4.86

Table B.9: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 1.0 mM Si (unfiltered).

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	1358.01	1002.63	1265.97	38.56	28.55	35.92
9	1006.43	623.32	990.83	24.92	15.47	27.51
16	1099.34	721.17	1056.68	31.00	17.62	30.01

Table B.10: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 1.0 mM Si (filtered).

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	377.58	380.08	378.79	9.20	9.22	9.16
9	240.74	261.73	264.68	5.99	6.34	6.41
16	244.41	232.19	234.41	6.10	5.69	5.75

Table B.11: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 2.5 mM Si (unfiltered).

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	1172.61	1233.26	1211.43	33.12	34.80	33.54
9	1257.98	1318.89	1458.84	35.64	36.64	40.91
16	1223.98	1288.40	1246.78	34.09	36.10	34.80

Table B.12: Raw Data (cps) for Iron measured using ICP-MS for isotopes (Fe_{56} and Fe_{57}) for Abiotic Controls with 2.5 mM Si (filtered).

Time (Days)	56-1	56-2	56-3	57-1	57-2	57-3
0	482.00	510.51	633.02	11.70	12.61	15.54
9	385.82	389.42	476.38	9.49	9.81	11.65
16	347.48	371.31	365.55	8.57	9.07	9.00

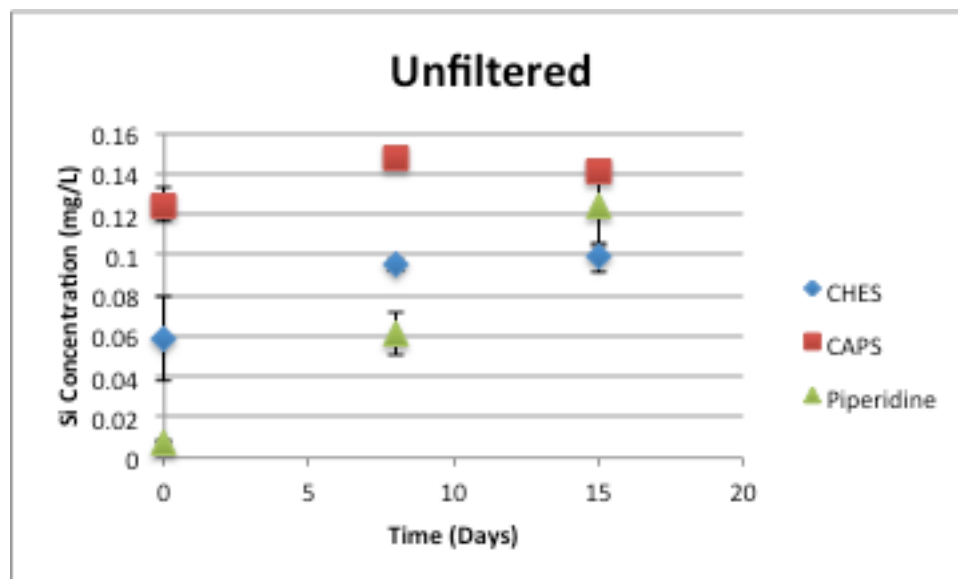


Figure B.7 Si measured over time in unfiltered abiotic controls with CHES, CAPS or Piperidine. Samples were measured in duplicate.

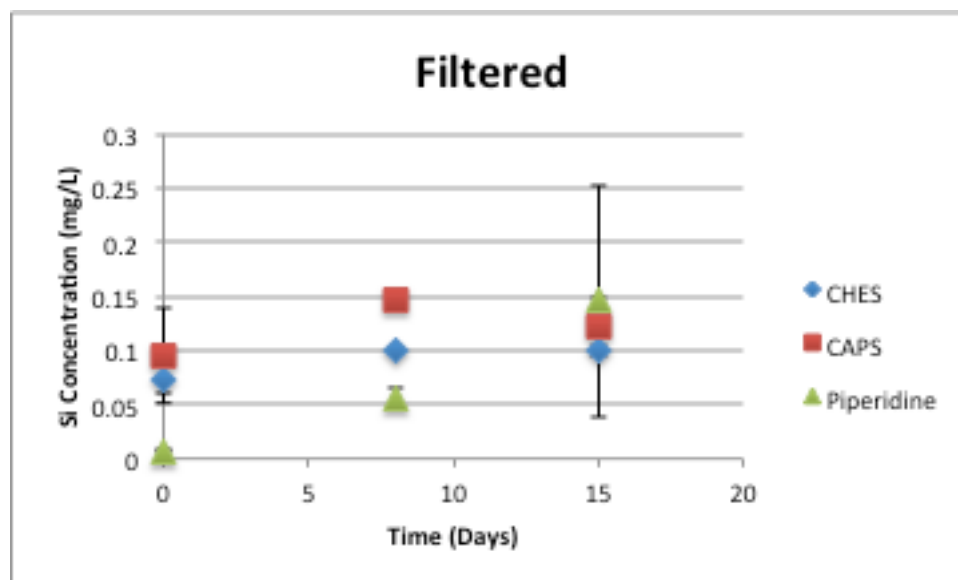


Figure B.8 Si measured over time in filtered abiotic controls with CHES, CAPS or Piperidine. Samples were measured in duplicate.

APPENDIX C

GROWTH MEDIUM

Table C.1 Growth Medium: Bold's Basal Medium titrated to 8.7 (with added B₁₂ and S3 medium).

Media Component	Per Liter	Concentration (M)
KH ₂ PO ₄	175 mg	1.27×10^{-3}
CaCl ₂ *2H ₂ O	25 mg	1.70×10^{-4}
MgSO ₄ *7H ₂ O	75 mg	3.04×10^{-4}
NaNO ₃	250 mg	2.94×10^{-4}
K ₂ HPO ₄	75 mg	4.31×10^{-4}
NaCl	25 mg	4.28×10^{-4}
H ₃ BO ₃	11.42 mg	1.85×10^{-4}
Microelement stock solution	1 mL	
Solution 1	1 mL	
Solution 2	1 mL	
Microelement Stock		
ZnSO ₄ *7H ₂ O	8.82 g	3.06×10^{-5}
MnCl ₂ *4H ₂ O	1.44 g	7.27×10^{-6}
MoO ₃	0.71 g	4.93×10^{-6}
CuSO ₄ *5H ₂ O	1.57 g	6.28×10^{-6}
Co(NO ₃) ₂ *6H ₂ O	0.49 g	1.68×10^{-6}
Solution 1		
Na ₂ EDTA	50 g	1.34×10^{-4}
KOH	3.1 g	5.52×10^{-5}
Solution 2		
FeSO ₄ *7H ₂ O	4.98 g	1.79×10^{-5}
Cyanocobalamin (B ₁₂)	0.002	1.48×10^{-9}
S3 Vitamin Solution		
Inositol	5 g	
Thymine	3 g	
Thymine HCl (B ₁)	0.5 g	
Nicotinic acid (niacin)	0.1 g	
Ca pantothenate	0.01 g	
p-Aminobenzoic acid	0.001 g	
Biotin (vitamin H)	0.002 g	
Folic acid		

APPENDIX D

STRAIN IDENTIFICATION USING MOLECULAR TECHNIQUES

Previously, strain identification was determined by careful examination of valve morphology [13]. However, polymorphic strains run the risk of being misidentified or there are multiple strains when there should be one. Here, identification of strain RGd-1 was determined by confirming morphological characteristics with molecular techniques. Genus and species level identification was determined using 18S rDNA [85, 86] and Internally Transcribed Spacer region (ITS), respectively. While 18S rDNA sequence is known to be well conserved, and can serve to identify organisms to the genus level [93, 94]. However, 18S rDNA sequences are often so conserved, that there is insufficient resolution to classify eukaryotes on a species level. Internally transcribed spacer regions (ITS) are hyper-variable regions between the small and large ribosomal subunits, incorporating the 5.8S region, and are known to have enough variation to identify strains to the species level [87, 89, 90, 95]. Of additional interest is the use of functional genes to gain sufficient resolution, to obtain species or subspecies identification. As such, *rbcL* primers were used to obtain further classification criteria using by targeting ribulose-bisphosphate carboxylase oxygenase (Rubisco) [91]. See the methods section in Chapter 2 for detailed methods of molecular techniques.

Table D.1: Thermocycle conditions for amplification of DNA.

Condition	Temperature	Time
1	94 °C	2 min
2	94 °C	1 min
3	52 °C	1 min
4	72 °C	1.15 min
5	72 °C	7 min
6	4 °C	Hold
Repeat steps 2-4 x 40 cycles		

Table D.2: PCR Reaction Components.

Component	(-) Control	Sample
DNase/RNase free H ₂ O	15 µL	10 µL
Mastermix	25 µL	25 µL
1xBSA	5 µL	5 µL
Forward Primer (1 µM)	2.5 µL	25 µL
Reverse Primer (1 µM)	2.5 µL	2.5 µL
Sample	5 µL	5 µL
Total	50 µL	50 µL

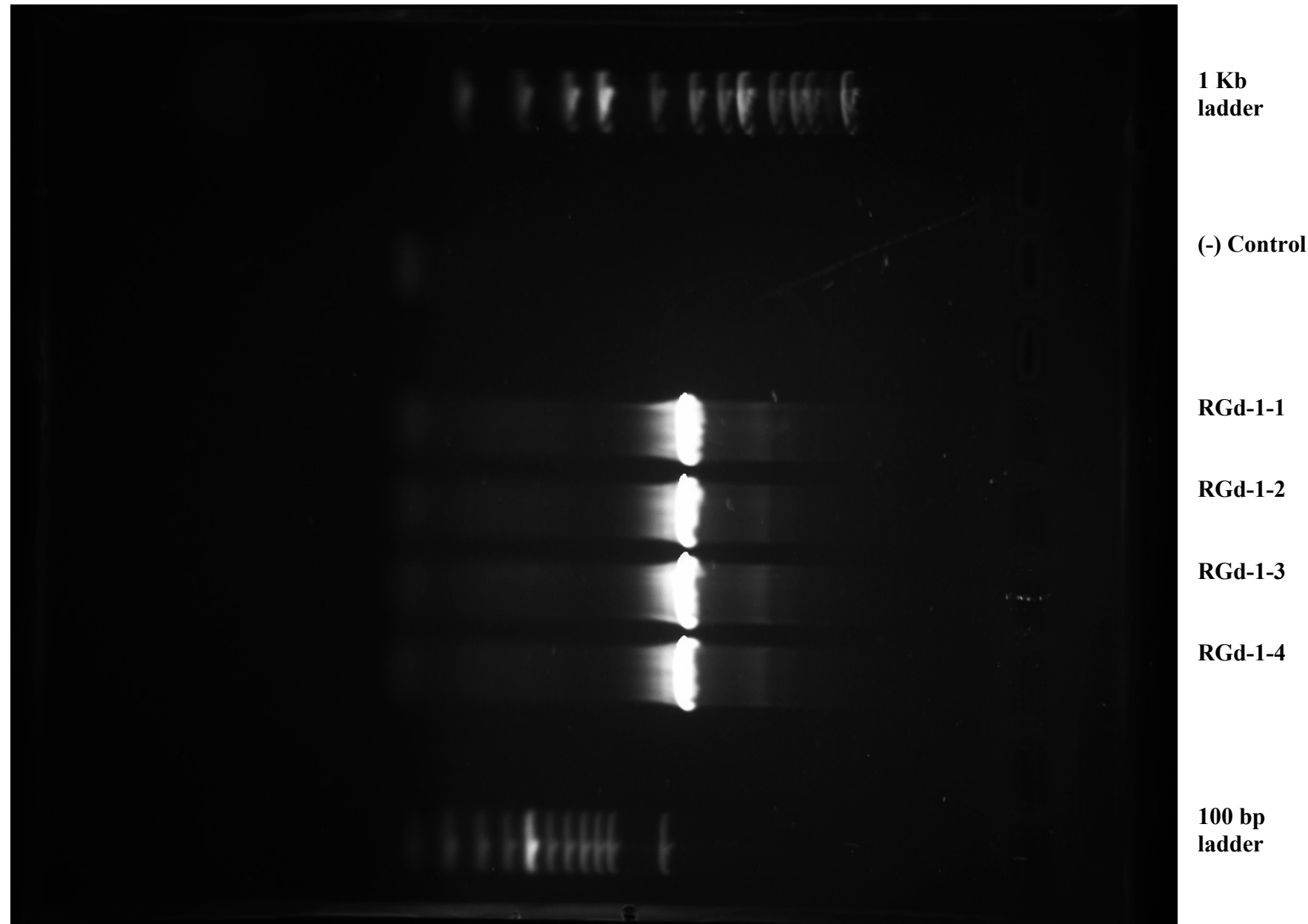


Figure D.1: Amplification of partial SSU rDNA (18S) using primers UNI 7F and 1534R. DNA was run on 0.7% Agarose gel.

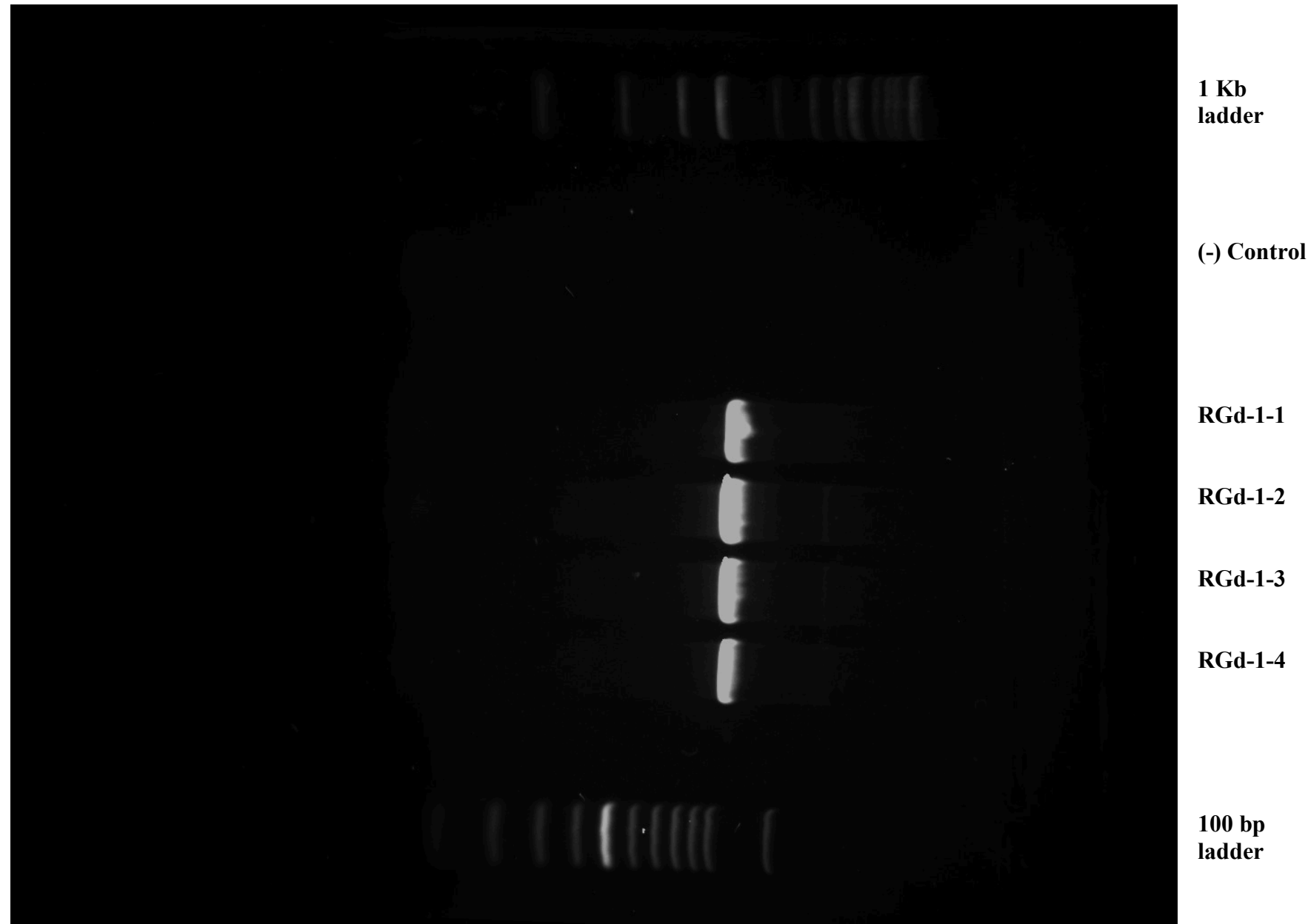


Figure D.2: Amplification of ITS regions using primers ITS1 and ITS4. DNA was run on 1.0% Agarose gel.

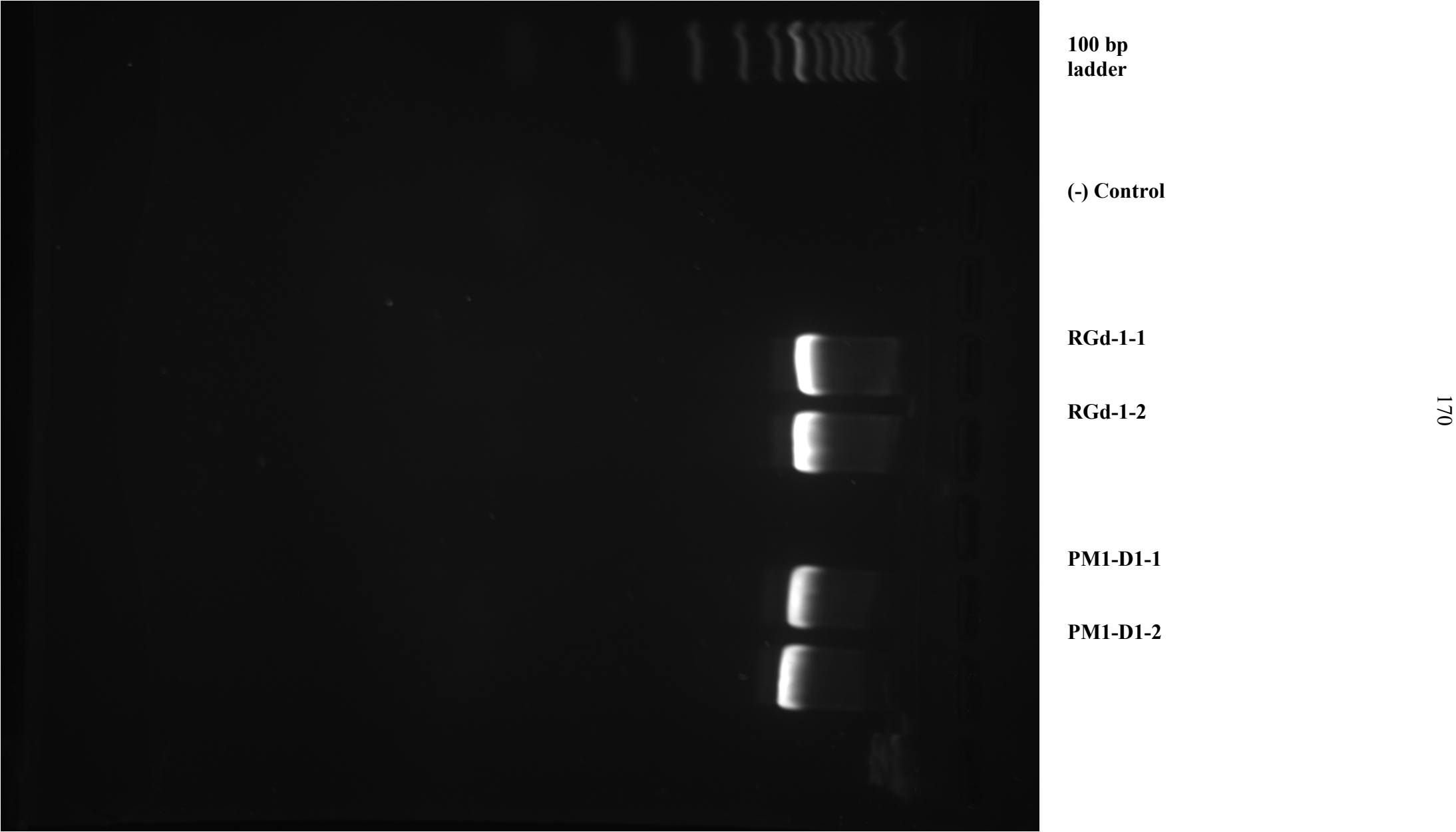


Figure D.3: Amplification of rbcL diatom specific primers for Rubisco. DNA was run on 2.0% Agarose gel.