

INVESTIGATION OF AMMONIA AND NITRATE REMOVAL FROM MUNICIPAL
WASTEWATER USING BIOWISH™

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Civil and Environmental Engineering

by

Emily Holland

August 2017

© 2017

Emily Holland

ALL RIGHTS RESERVED

COMMITTEE MEMBERSHIP

TITLE: Investigation of Ammonia and Nitrate
Removal from Municipal Wastewater Using
BiOWiSH™

AUTHOR: Emily Holland

DATE SUBMITTED: August 2017

COMMITTEE CHAIR: Nirupam Pal, Ph.D.
Professor of Civil and Environmental
Engineering

COMMITTEE MEMBER: Rebekah Oulton, Ph.D.
Assistant Professor of Civil and
Environmental Engineering

COMMITTEE MEMBER: Amro El Badawy, Ph.D.
Research Scholar and Lecturer of Civil and
Environmental Engineering

ABSTRACT

Investigation of Ammonia and Nitrate Removal from Municipal Wastewater Using

BiOWiSH™

Emily Holland

This research entails investigation of ammonia, nitrate, and nitrite removal from wastewater using a proprietary blend of bacteria known as BiOWiSH™. The degradation rates of ammonia, nitrate, and nitrite for Aqua were determined using wastewater at the San Luis Obispo Water Resource Recovery Facility (SLO WRRF). Laboratory and field experiments were conducted to test how Aqua compared with natural bacteria for removal of nitrogen compounds. Preliminary data suggested that Aqua performed nitrate removal best in SLO WRRF wastewater at the secondary clarifier. Aqua could perform anoxic and aerobic denitrification in secondary clarifier wastewater. In mineral media, Aqua removed 6.6 mg NO₃-N/L/hr. In partially sterilized wastewater, Aqua removed 2.67 mg NO₃-N/L/hr. Field experiments using a batch reactor suggested that Aqua aided in nitrate removal when dosed above 25 ppm in secondary clarifier wastewater. A dose of 25 ppm Aqua resulted in a 0.1 mg NO₃-N/L/hr removal rate. A dose of 50 ppm Aqua resulted in a 0.15 mg NO₃-N/L/hr. Aqua did not aid in ammonia or nitrate removal in sludgewash at the SLO WRRF likely due to high concentrations of nitrate and ammonia existing in the wastewater were toxic to Aqua. Aqua removed about 5 ppm more nitrate than a competitor bacteria blend in a laboratory setting. Activating Aqua to increase initial cell count before inoculation did not have any effect on removal. Providing partial aeration did not help nitrification rates and inhibited nitrate removal for Aqua. Laboratory experiments showed

that Aqua did not remove nitrate in final clarifier wastewater most likely due to a limited carbon source. Aqua can perform nitrification in mineral media. Aerobic activation of Aqua inhibited denitrification. Aqua activated anoxically can perform denitrification. Using a powder with 70% microbial cultures, instead of the 1% found in Aqua, resulted in quicker nitrate removal. Inoculating as a concentrated liquid versus a dry powder did not affect nitrate removal rates. Use of trace mineral media did not affect nitrate removal rates.

ACKNOWLEDGMENTS

I would like to thank BiOWiSH™ Technologies for funding this research project and providing the necessary bacterial mixes needed for experimentation. I would also like to thank them for providing this amazing opportunity and for their guidance throughout this project.

I would also like to thank the San Luis Obispo Water Resource Recovery Facility for providing wastewater samples and allowing me to set my batch reactors up at the plant. They have been extremely nice and understanding throughout this project.

This thesis research would not have been possible without the help of Dr. Nirupam Pal. Thank you for allowing me to participate in this amazing research opportunity and thank you for all of your guidance throughout my research.

I would like to thank Dr. Rebekah Oulton and Dr. Amro El Badawy for being in my thesis committee and answering any questions I had about my thesis. I appreciate all the guidance you both have given me.

I would also like to thank all the professors and students that allowed me to use their laboratories and laboratory equipment. I really appreciate you taking the time to train and work with me on using the equipment.

This research would not have been possible without the help of Jennifer Manning, Joelle Arakaki, Kimberly Lamar, TJ Tamura, and Patrick Kalvass. I am truly grateful that they were there to help me with my continuous long hours of sampling.

TABLE OF CONTENTS

	Page
LIST OF TABLES	xiii
LIST OF FIGURES	xxi
LIST OF ABBREVIATIONS.....	xxvii
 CHAPTER	
1 INTRODUCTION	1
1.1 Sources and Transport of Nitrogen	1
1.2 Impacts on Aquatic Life.....	2
1.3 Impacts on Human Health	3
1.4 Objective	4
2 LITERATURE REVIEW	5
2.1 Mechanisms of Nitrogen Removal	5
2.2 Typical Industrial Nitrogen Removal Processes.....	12
2.2.1 Biological Suspended Growth Processes	12
2.2.2 Biological Attached Growth Processes	14
2.2.3 Physio-Chemical Treatment Processes	16
2.3 Bioaugmentation	17
2.4 BiOWiSH™ Bioaugmentation	19
3 METHODS AND MATERIALS.....	22
3.1 Recipes	22
3.2 Growth Media and Control Solution Preparation	22
3.3 Aqua Activation Method.....	23

3.4	Inoculation Methods	23
3.5	Incubation Method	24
3.6	Sample Set-Up	24
3.7	Sample Analysis.....	25
3.8	Laboratory Method	26
3.8.1	Experiment 1 – Effect of <i>Bacillus</i> Strains and Aqua on Denitrification	29
3.8.2	Experiment 2a & 2b – Effect of 50 ppm Aqua on Wastewater Collected from DAFT and Primary, Secondary, and Final Clarifiers	30
3.8.3	Experiment 3 –Effect of 50 ppm Aqua on Wastewater Collected from DAFT, Sludgewash, Primary Clarifier, and Secondary Clarifier	34
3.8.4	Experiment 4 – Effect of 500 ppm Aqua with New Inoculation Method on Secondary Clarifier Wastewater	35
3.9	San Luis Obispo Water Reclamation Facility Description	37
3.10	Bioreactor Set-Up	40
3.11	Field Method.....	43
3.11.1	Experiment 5 – Effect of 5 ppm Aqua on Secondary Clarifier Wastewater	45
3.11.2	Experiment 6 – Effect of 2.5 ppm Aqua on Secondary Clarifier Wastewater	46

3.11.3	Experiment 7 – Effect of 50 ppm Aqua on Secondary Clarifier	
	Wastewater	47
3.11.4	Experiment 8 – Effect of 25 ppm Aqua on Sludgewash	
	Wastewater	49
3.11.5	Experiment 9 – Effect of 10 ppm Aqua on Sludgewash	
	Wastewater	50
3.11.6	Experiment 10 – Effect of 5 ppm Aqua on Sludgewash	
	Wastewater	52
3.11.7	Experiment 11 – Effect of 2.5 ppm Aqua on Sludgewash	
	Wastewater	54
3.11.8	Experiment 12 – Effect of 25 ppm Activated Aqua on Secondary	
	Clarifier Wastewater.....	55
3.11.9	Experiment 13 – Effect of 25 ppm Activated Aqua under Partial	
	Aeration on Secondary Clarifier Wastewater.....	57
3.11.10	Experiment 14 – Effect of 25 ppm Biogenesis on Secondary	
	Clarifier Wastewater.....	59
3.12	Laboratory Method on Field Wastewater During Cold Weather.....	61
3.12.1	Experiment 15 – Effect of 25 ppm Biogenesis on Secondary	
	Clarifier Wastewater Conducted in Lab	61
3.12.2	Experiment 16 – Effect of 25 ppm Activated Aqua on Final	
	Clarifier Wastewater.....	63
3.12.3	Experiment 17 – Effect of 25 ppm Aqua on Final Clarifier Plus	
	5% Primary Clarifier Wastewater	65

3.12.4	Experiment 18 – Effect of 500 ppm Activated Aqua under High Aeration on Growth Media.....	67
4	RESULTS AND DISCUSSION.....	69
4.1	Laboratory Results.....	70
4.1.1	Experiment 1 – Effect of <i>Bacillus</i> Strains and Aqua on Denitrification	70
4.1.2	Experiment 2a & 2b – Effect of 50 ppm Aqua on Wastewater Collected from DAFT and Primary, Secondary, and Final Clarifiers.....	77
4.1.3	Experiment 3 – Effect of 50 ppm Aqua on Wastewater Collected from DAFT, Sludgewash, Primary Clarifier, and Secondary Clarifier	91
4.1.4	Experiment 4 - Effect of 500 ppm Aqua with New Inoculation Method on Secondary Clarifier Wastewater	108
4.2	Field Results.....	119
4.2.1	Experiment 5 – Effect of 5 ppm Aqua on Secondary Clarifier Wastewater	119
4.2.2	Experiment 6 – Effect of 2.5 ppm Aqua on Secondary Clarifier Wastewater	129
4.2.3	Experiment 7 – Effect of 50 ppm Aqua on Secondary Clarifier Wastewater	139
4.2.4	Experiment 8 – Effect of 25 ppm Aqua on Sludgewash Wastewater	148

4.2.5	Experiment 9 – Effect of 10 ppm Aqua on Sludgewash Wastewater	157
4.2.6	Experiment 10 – Effect of 5 ppm Aqua on Sludgewash Wastewater	165
4.2.7	Experiment 11 – Effect of 2.5 ppm Aqua on Sludgewash Wastewater	173
4.2.8	Experiment 12 – Effect of 25 ppm Activated Aqua on Secondary Clarifier Wastewater	181
4.2.9	Experiment 13 – Effect of 25 ppm Activated Aqua under Partial Aeration on Secondary Clarifier Wastewater	193
4.2.10	Experiment 14 – Effect of 25 ppm Biogenesis on Secondary Clarifier Wastewater	202
4.3	Laboratory Results for Field Wastewater During Cold Weather	211
4.3.1	Experiment 15 – Effect of 25 ppm Biogenesis on Secondary Clarifier Wastewater Conducted in Lab	211
4.3.2	Experiment 16 – Effect of 25 ppm Activated Aqua on Final Clarifier Wastewater	220
4.3.3	Experiment 17 – Effect of 25 ppm Aqua on Final Clarifier Plus 5% Primary Clarifier Wastewater	229
4.3.4	Experiment 18 – Effect of 500 ppm Activated Aqua under High Aeration on Growth Media	236
5	CONCLUSIONS	245
6	RECOMMENDATIONS	250

REFERENCES	253
APPENDICIES	
Appendix A: Other Experiments	260
A.1 Experiment A.1 – Secondary Wastewater with 25 ppm Aqua in Field	260
A.1.1 Methods and Materials	260
A.1.2 Results	261
A.2 Experiment A.2 – Secondary Wastewater with 10 ppm Aqua in Field	269
A.2.1 Methods and Materials	269
A.2.2 Results	271
Appendix B: Rate Constant Calculations	279
Appendix C: Samples Calculations for Oxygen and Alkalinity Required for Nitrogen Removal	282
Appendix D: Sample Calculations for Nitrogen Content and Eluent	284

LIST OF TABLES

Table	Page
3.1 Characteristics of SLO WRRF wastewater	27
3.2 Weights and concentrations for experiment 4	37
3.3 Weights and concentrations for experiment 5	46
3.4 Weights and concentrations for experiment 6	47
3.5 Weights and concentrations for experiment 7	48
3.6 Weights and concentrations for experiment 8	50
3.7 Weights and concentrations for experiment 9	52
3.8 Weights and concentrations for experiment 10	53
3.9 Weights and concentrations for experiment 11	55
3.10 Weights and concentrations for experiment 12	57
3.11 Weights and concentrations for experiment 13	59
3.12 Weights and concentrations for experiment 14	61
3.13 Weights and concentrations for experiment 15	63
3.14 Weights and concentrations for experiment 16	65
3.15 Weights and concentrations for experiment 17	66
3.16 Weights and concentrations for experiment 18	68
4.1 Nitrate concentrations in mg/L NO ₃ -N for experiment 1	71
4.2 Nitrite concentrations in mg/L NO ₂ -N for experiment 1	72
4.3 Denitrification rates for experiment 1	74
4.4 Total nitrate removed and removal rates for experiment 1	75
4.5 Labeling for experiment 2	77

4.6	Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 2	78
4.7	Nitrification rates for experiment 2	81
4.8	Total ammonia removed and removal rates for experiment 2	82
4.9	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 2	82
4.10	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 2	83
4.11	Denitrification rates for experiment 2	86
4.12	Total nitrate removed and removal rates for experiment 2	87
4.13	Carbon to nitrogen ratios for experiment 2	89
4.14	Labeling for experiment 3	92
4.15	pH measurements for experiment 3	93
4.16	Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 3	94
4.17	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 3	96
4.18	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 3	98
4.19	Nitrification rates for experiment 3	102
4.20	Total ammonia removed and removal rates for experiment 3	102
4.21	Carbon to nitrogen ratios for experiment 3	105
4.22	Labeling for experiment 4	109
4.23	pH measurements for experiment 4	110
4.24	Temperature measurements for experiment 4	110
4.25	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 4	111
4.26	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 4	113
4.27	Denitrification rates for experiment 4	116
4.28	Total nitrate removed and removal rates for experiment 4	117

4.29	Carbon to nitrogen ratios for experiment 4	118
4.30	Labeling for experiment 5	120
4.31	pH measurements for experiment 5.....	121
4.32	Temperature measurements for experiment 5	122
4.33	Ammonia concentrations in mg/L NH ₄ -N for experiment 5	122
4.34	Nitrate concentrations in mg/L NO ₃ -N for experiment 5	123
4.35	Nitrite concentrations in mg/L NO ₂ -N for experiment 5	124
4.36	Denitrification rates for experiment 5	127
4.37	Total nitrate removed and removal rates for experiment 5	127
4.38	Carbon to nitrogen ratios for experiment 5	128
4.39	Labeling for experiment 6	130
4.40	pH measurements for experiment 6.....	130
4.41	Temperature measurements for experiment 6	131
4.42	Ammonia concentrations in mg/L NH ₄ -N for experiment 6.....	132
4.43	Nitrate concentrations in mg/L NO ₃ -N for experiment 6.....	133
4.44	Nitrite concentrations in mg/L NO ₂ -N for experiment 6	134
4.45	Denitrification rates for experiment 6	136
4.46	Total nitrate removed and removal rates for experiment 6	137
4.47	Carbon to nitrogen ratios for experiment 6	138
4.48	Labeling for experiment 7	139
4.49	pH measurements for experiment 7.....	140
4.50	Temperature measurements for experiment 7	140
4.51	Ammonia concentrations in mg/L NH ₄ -N for experiment 7	141

4.52	Nitrate concentrations in mg/L NO ₃ -N for experiment 7	142
4.53	Nitrite concentrations in mg/L NO ₂ -N for experiment 7	143
4.54	Denitrification rates for experiment 7	146
4.55	Total nitrate removed and removal rates for experiment 7	146
4.56	Carbon to nitrogen ratios for experiment 7	147
4.57	Labeling for experiment 8	149
4.58	pH measurements for experiment 8.....	149
4.59	Temperature measurements for experiment 8	150
4.60	Ammonia concentrations in mg/L NH ₄ -N for experiment 8.....	150
4.61	Nitrate concentrations in mg/L NO ₃ -N for experiment 8.....	151
4.62	Nitrite concentrations in mg/L NO ₂ -N for experiment 8	152
4.63	Denitrification rates for experiment 8	155
4.64	Total nitrate removed and removal rates for experiment 8	155
4.65	Carbon to nitrogen ratios for experiment 8	156
4.66	Labeling for experiment 9	158
4.67	pH measurements for experiment 9.....	158
4.68	Temperature measurements for experiment 9	159
4.69	Ammonia concentrations in mg/L NH ₄ -N for experiment 9.....	160
4.70	Nitrate concentrations in mg/L NO ₃ -N for experiment 9.....	161
4.71	Nitrite concentrations in mg/L NO ₂ -N for experiment 9	162
4.72	Carbon to nitrogen ratios for experiment 9	164
4.73	Labeling for experiment 10	166
4.74	pH measurements for experiment 10.....	166

4.75	Temperature measurements for experiment 10	167
4.76	Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 10	167
4.77	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 10	168
4.78	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 10	169
4.79	Carbon to nitrogen ratios for experiment 10	172
4.80	Labeling for experiment 11	174
4.81	pH measurements for experiment 11	174
4.82	Temperature measurements for experiment 11	175
4.83	Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 11	175
4.84	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 11	176
4.85	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 11	177
4.86	Carbon to nitrogen ratios for experiment 11	180
4.87	Labeling for experiment 12	182
4.88	pH measurements for experiment 12	183
4.89	Temperature measurements for experiment 12	184
4.90	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 12	185
4.91	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 12	187
4.92	Denitrification rates for experiment 12	189
4.93	Total nitrate removed and removal rates for experiment 12	190
4.94	Carbon to nitrogen ratios for experiment 12	191
4.95	Labeling for experiment 13	194
4.96	pH measurements for experiment 13	194
4.97	Temperature measurements for experiment 13	195

4.98	Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 13	195
4.99	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 13	196
4.100	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 13	197
4.101	Carbon to nitrogen ratios for experiment 13	200
4.102	Labeling for experiment 14	203
4.103	pH measurements for experiment 14.....	203
4.104	Temperature measurements for experiment 14	204
4.105	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 14.....	205
4.106	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 14	206
4.107	Denitrification rates for experiment 14	208
4.108	Total nitrate removed and removal rates for experiment 14	209
4.109	Carbon to nitrogen ratios for experiment 14	210
4.110	Labeling for experiment 15	212
4.111	pH measurements for experiment 15.....	212
4.112	Temperature measurements for experiment 15	213
4.113	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 15.....	213
4.114	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 15	214
4.115	Denitrification rates for experiment 15	216
4.116	Total nitrate removed and removal rates for experiment 15	217
4.117	Carbon to nitrogen ratios for experiment 15	218
4.118	Labeling for experiment 16	220
4.119	pH measurements for experiment 16.....	221
4.120	Temperature measurements for experiment 16.....	222

4.121	Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 16.....	223
4.122	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 16.....	224
4.123	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 16	225
4.124	Carbon to nitrogen ratios for experiment 16	228
4.125	Labeling for experiment 17	230
4.126	pH measurements for experiment 17.....	230
4.127	Temperature measurements for experiment 17	231
4.128	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 17.....	231
4.129	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 17	232
4.130	Carbon to nitrogen ratios for experiment 17	234
4.131	Labeling for experiment 18	236
4.132	pH measurements for experiment 18.....	237
4.133	Temperature measurements for experiment 18.....	238
4.134	DO for growth media with Aqua 1 in mg/L for Experiment 18.....	238
4.135	DO for growth media with Aqua 2 in mg/L for Experiment 18.....	239
4.136	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 18.....	240
4.137	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment 18	241
A.1a	Weights and concentrations for experiment A.1	261
A.1b	pH measurements for experiment A.1	262
A.1c	Temperature measurements for experiment A.1	263
A.1d	Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment A.1	264
A.1e	Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment A.1	266
A.1f	Nitrite concentrations in mg/L $\text{NO}_2\text{-N}$ for experiment A.1.....	268

A.2a	Weights and concentrations for experiment A.2	271
A.2b	pH measurements for experiment A.2.....	272
A.2c	Temperature measurements for experiment A.2	273
A.2d	Ammonia concentrations in mg/L NH ₄ -N for experiment A.2	274
A.2e	Nitrate concentrations in mg/L NO ₃ -N for experiment A.2	275
A.2f	Nitrite concentrations in mg/L NO ₂ -N for experiment A.2.....	277

LIST OF FIGURES

Figure	Page
2.1 The natural nitrogen cycle (Evans & Perlman).....	5
2.2 Ion diagram of nitrogen cycle (Lunquist, 2017)	11
2.3 Modified Ludzack-Ettinger preanoxic system (Metcalf & Eddy, 2003)	14
2.4 Captor ® and Limpor ® suspended growth fixed-film packing process (Metcalf & Eddy, 2003)	15
2.5 Downflow packed bed reactor (Metcalf & Eddy, 2003).....	16
3.1 Examples of 1L bottles for solution storage	27
3.2 Wastewater collection at the SLO WRRF (HDR, 2014)	28
3.3 Existing process schematic at SLO WRRF (HDR, 2014)	39
3.4 Bioreactor fill line	40
3.5 Bioreactor set-up	41
3.6 Total organic carbon vials used to collect samples.....	42
3.7 Bioreactor set-up at the SLO WRRF (HDR, 2014)	44
4.1 Effect of <i>Bacillus</i> strains and 50 ppm Aqua on nitrate over time	71
4.2 Effect of <i>Bacillus</i> strains and 50 ppm Aqua on nitrite over time.....	72
4.3 Effect of <i>Bacillus</i> strains and 50 ppm Aqua on total nitrogen over time.....	76
4.4 Effect of 50 ppm Aqua on ammonia in DAFT and primary, secondary, and final clarifier wastewater over time	79
4.5 Effect of 50 ppm Aqua on nitrate in DAFT and primary, secondary, and final clarifier wastewater over time.....	83

4.6	Effect of 50 ppm Aqua on nitrite in DAFT and primary, secondary, and final clarifier wastewater over time.....	84
4.7	Effect of 50 ppm Aqua on total nitrogen in DAFT and primary, secondary, and final clarifier wastewater over time.....	90
4.8	Effect of 50 ppm Aqua on ammonia in DAFT, primary clarifier, and secondary clarifier wastewater over time	95
4.9	Effect of 50 ppm Aqua on ammonia in sludgewash wastewater over time	95
4.10	Effect of 50 ppm Aqua on nitrate in DAFT, sludgewash, primary clarifier, and secondary clarifier wastewater over time.....	97
4.11	Effect of 50 ppm Aqua on nitrite in DAFT, sludgewash, primary clarifier, and secondary clarifier wastewater over time.....	99
4.12	Effect of 50 ppm Aqua on total nitrogen in DAFT, primary clarifier, and secondary clarifier wastewater over time	106
4.13	Effect of 50 ppm Aqua on total nitrogen in sludgewash wastewater over time	107
4.14	Effect of 500 ppm Aqua with new inoculation method on nitrate in secondary clarifier wastewater	112
4.15	Effect of 500 ppm Aqua with new inoculation method on nitrite in secondary clarifier wastewater	114
4.16	Effect of 5 ppm Aqua on ammonia in secondary clarifier wastewater.....	123
4.17	Effect of 5 ppm Aqua on nitrate in secondary clarifier wastewater	124
4.18	Effect of 5 ppm Aqua on nitrite in secondary clarifier wastewater	125
4.19	Effect of 5 ppm Aqua on total nitrogen in secondary clarifier wastewater	129

4.20	Effect of 2.5 ppm Aqua on ammonia in secondary clarifier wastewater	132
4.21	Effect of 2.5 ppm Aqua on nitrate in secondary clarifier wastewater	133
4.22	Effect of 2.5 ppm Aqua on nitrite in secondary clarifier wastewater	134
4.23	Effect of 2.5 ppm Aqua on total nitrogen in secondary clarifier wastewater	138
4.24	Effect of 50 ppm Aqua on ammonia in secondary clarifier wastewater	142
4.25	Effect of 50 ppm Aqua on nitrate in secondary clarifier wastewater	143
4.26	Effect of 50 ppm Aqua on nitrite in secondary clarifier wastewater	144
4.27	Effect of 50 ppm Aqua on total nitrogen in secondary clarifier wastewater	148
4.28	Effect of 25 ppm Aqua on ammonia in sludgewash wastewater	151
4.29	Effect of 25 ppm Aqua on nitrate in sludgewash wastewater.....	152
4.30	Effect of 25 ppm Aqua on nitrite in sludgewash wastewater	153
4.31	Effect of 25 ppm Aqua on total nitrogen in sludgewash wastewater	157
4.32	Effect of 10 ppm Aqua on ammonia in sludgewash wastewater	160
4.33	Effect of 10 ppm Aqua on nitrate in sludgewash wastewater.....	161
4.34	Effect of 10 ppm Aqua on nitrite in sludgewash wastewater	162
4.35	Effect of 10 ppm Aqua on total nitrogen in sludgewash wastewater	164
4.36	Effect of 5 ppm Aqua on ammonia in sludgewash wastewater	168
4.37	Effect of 5 ppm Aqua on nitrate in sludgewash wastewater.....	169
4.38	Effect of 5 ppm Aqua on nitrite in sludgewash wastewater	170
4.39	Effect of 5 ppm Aqua on total nitrogen in sludgewash wastewater	172
4.40	Effect of 2.5 ppm Aqua on ammonia in sludgewash wastewater	176
4.41	Effect of 2.5 ppm Aqua on nitrate in sludgewash wastewater.....	177
4.42	Effect of 2.5 ppm Aqua on nitrite in sludgewash wastewater	178

4.43	Effect of 2.5 ppm Aqua on total nitrogen in sludgewash wastewater	180
4.44	Effect of 25 ppm activated Aqua on nitrate in secondary clarifier wastewater.....	186
4.45	Effect of 25 ppm activated Aqua on nitrite in secondary clarifier wastewater.....	187
4.46	Effect of 25 ppm activated Aqua on total nitrogen in secondary clarifier wastewater.....	192
4.47	Effect of 25 ppm activated Aqua under partial aeration on ammonia in secondary clarifier wastewater.....	196
4.48	Effect of 25 ppm activated Aqua under partial aeration on nitrate in secondary clarifier wastewater	197
4.49	Effect of 25 ppm activated Aqua under partial aeration on nitrite in secondary clarifier wastewater	198
4.50	Effect of 25 ppm activated Aqua under partial aeration on total nitrogen in secondary clarifier wastewater.....	201
4.51	Effect of 25 ppm Biogenesis on nitrate in secondary clarifier wastewater.....	206
4.52	Effect of 25 ppm Biogenesis on nitrite in secondary clarifier wastewater	207
4.53	Effect of 25 ppm Biogenesis on total nitrogen in secondary clarifier wastewater.....	210
4.54	Effect of 25 ppm Biogenesis on nitrate in secondary clarifier wastewater in lab.....	214
4.55	Effect of 25 ppm Biogenesis on nitrite in secondary clarifier wastewater in lab.....	215

4.56 Effect of 25 ppm Biogenesis on total nitrogen in secondary clarifier wastewater in lab.....	219
4.57 Effect of 25 ppm activated Aqua on ammonia in final clarifier wastewater	223
4.58 Effect of 25 ppm activated Aqua on nitrate in final clarifier wastewater	225
4.59 Effect of 25 ppm activated Aqua on nitrite in final clarifier wastewater.....	226
4.60 Effect of 25 ppm activated Aqua on total nitrogen in final clarifier wastewater.....	228
4.61 Effect of 25 ppm Aqua on nitrate in final clarifier plus 5% primary clarifier wastewater.....	232
4.62 Effect of 25 ppm Aqua on nitrite in final clarifier plus 5% primary clarifier wastewater.....	233
4.63 Effect of 25 ppm Aqua on total nitrogen in final clarifier plus 5% primary clarifier wastewater	235
4.64 Example of beaker with distances where dissolved oxygen was measured	239
4.65 Effect of 500 ppm activated Aqua under high aeration on nitrate in growth media.....	240
4.66 Effect of 500 ppm activated Aqua under high aeration on nitrite in growth media.....	242
4.67 Effect of 500 ppm activated Aqua under high aeration on total nitrogen in growth media	244
A.1a Effect of 25 ppm Aqua on ammonia in secondary clarifier wastewater	265
A.1b Effect of 25 ppm Aqua on nitrate in secondary clarifier wastewater	267
A.1c Effect of 25 ppm Aqua on nitrite in secondary clarifier wastewater	269

A.2a Effect of 10 ppm Aqua on ammonia in secondary clarifier wastewater	274
A.2b Effect of 10 ppm Aqua on nitrate in secondary clarifier wastewater	276
A.2c Effect of 10 ppm Aqua on nitrite in secondary clarifier wastewater	278
B.1 Example of points omitted for first order kinetics determination.....	279
B.2 Example of first order kinetics determined once points were omitted	280
B.3 Example of points omitted for zero order kinetics determination	280
B.4 Example of zero order kinetics determined once points were omitted.....	281

LIST OF ABBREVIATIONS

BOD	Biological Oxygen Demand
BR	Bioreactor
COD	Chemical Oxygen Demand
Conc	Concentration
C:N	Carbon to Nitrogen
DAFT	Dissolved Air Flotation Thickener
DI	Deionized
Dist.....	Distance
DO.....	Dissolved Oxygen
GM	Growth Media
IC.....	Ion Chromatograph
NPDES	National Pollutants Discharge Elimination System
MC	Microbial Cultures
RAS.....	Return Activated Sludge
SBR.....	Sequencing Batch Reactor
SLO WRRF.....	San Luis Obispo Water Resource Recovery Facility
SN	Serial Number
SW.....	Sludgewash
TM.....	Trace Minerals
TSB	Trypticase Soy Broth
TSS.....	Total Suspended Solids
Vol.....	Volume

WW Wastewater
WWTP Wastewater Treatment Plant

CHAPTER 1: INTRODUCTION

Water is an essential part of life on this earth, which is why treatment and reuse of water is so important. Water bodies are used for recreation, irrigation, and drinking water. Treated wastewater is discharged into these water bodies. The wastewater must be treated or it detrimentally impacts wildlife and humans. Currently, wastewater is treated through biological, physical, or chemical processes. Nature can treat wastewater, however it can only handle so much (Perlman, 2016). Therefore, wastewater must be treated in wastewater treatment plants (WWTPs) before being discharged. All wastewater is required to meet national pollutant discharge elimination system (NPDES) standards before being discharged back into the environment. Regulations are becoming increasingly strict. Therefore, treatment plants can have a difficult time meeting these standards. Research into different treatment processes is necessary for treatment plants to meet these limits.

Regulations are becoming stricter due to harmful effects discharged chemicals have on the environment. A major water quality problem in the United States is nutrient pollution. One of the main sources of nutrient pollution is excess nitrogen (EPA, Nutrient Pollution: The Problem, 2017). Nitrogen maintains life on earth (Harrison, 2003). However, too much nitrogen causes adverse effects on aquatic and human life. The main concerns for aquatic and human life are eutrophication, algal growth, ammonia, nitrate, and nitrite.

1.1 Sources and Transport of Nitrogen

Many anthropogenic sources cause excess nitrogen, including agriculture, urban storm water, and wastewater. The fertilizer used in the agriculture industry contains large

amounts of nitrogen. When rainstorms occur, the fertilizer creates high nitrogen levels in the storm water. This storm water can runoff into nearby streams and lakes or seep into the groundwater. Storm water runoff in urbanized areas carries pollutants from the impervious ground surface, including nitrogen, into storm water pipes. The storm water is discharged to the water bodies, creating high levels of nitrogen in rivers, lakes, streams, oceans, and seas. Decomposition of organic nitrogen compounds in wastewater, such as urea, causes an increase in ammonia. Ammonia contributes to excess nitrogen if left untreated. (EPA, Nutrient Pollution: Sources and Solutions, 2017).

1.2 Impacts on Aquatic Life

Excess nitrogen causes eutrophication in water bodies (NOAA, 2008). Eutrophication increases growth of plant life and algae. When the plants and algae decompose, water quality and dissolved oxygen (DO) levels decrease in the water. Dissolved oxygen is essential for aquatic life. Significant removal of dissolved oxygen causes the water to become hypoxic and create dead zones, which stress and harm the aquatic life (NOAA, 2008). Decrease in water quality can cause changes in the ecosystem and negatively impact the existing habitat. Low water quality results in a loss of submerged aquatic vegetation, loss of biodiversity, and shifts in the food web (Rabalais, 2002). Algae growth can also harm aquatic life. Algae block out the sunlight and clog fish gills, resulting in death of the fish (EPA, Nutrient Pollution: The Effects: Environment, 2017). Also, harmful algal blooms create toxins that harm or kill fish and other animals (EPA, Nutrient Pollution: The Effects: Environment, 2017).

Excess nitrogen can cause high levels of ammonia in the water bodies. If high levels of ammonia are present, then fish have difficulty excreting their own ammonia into the water. The ammonia builds up in their tissues and blood and leads to death (EPA, Aquatic Life Criteria - Ammonia, 2017). Nitrification of ammonia consumes oxygen and alkalinity. Incomplete nitrification occurs if not enough oxygen or alkalinity is supplied. Incomplete nitrification means ammonia does not convert to nitrate. Instead, ammonia converts to nitrite, which is toxic to fish at levels as low as 0.1 mg/L (Francis-Floyd, Watson, Petty, & Pouder, 2009).

1.3 Impacts on Human Health

Toxic algal blooms have detrimental effects on human health. People become sick if they eat the infected fish, drink the contaminated water, or come in contact with the polluted water (EPA, Nutrient Pollution: The Problem, 2017). Health problems include rashes, stomach or liver illness, respiratory problems, and neurological affects (EPA, Nutrient Pollution: The Effects: Human Health, 2017).

Nitrates and nitrites have a detrimental effect on human health. Nitrite changes the hemoglobin in the body to a form called methemoglobin, which no longer carries oxygen in the blood to the rest of the body. High concentrations of nitrate cause methemoglobinemia in infants, also known as blue baby syndrome. Methemoglobinemia reduces the carrying capacity of oxygen in the blood. Lack of oxygen in the blood results in brain damage or death from suffocation (New Hampshire Department of Environmental Services, 2006).

1.4 Objective

Conventional wastewater treatment technologies cannot remove nitrate to stricter permit levels before discharge. Advanced wastewater treatment technologies can be implemented to decrease nitrogen. However, they can be costly when combining with conventional treatment (Hartmand & Cleland, 2007). This thesis will focus on researching a more efficient and low-cost nitrogen removal product called BiOWiSH™ Aqua.

BiOWiSH™ Aqua is a bacterial consortium comprised mainly of *Bacillus* and *Lactobacillus* bacteria. BiOWiSH™ bacteria have been proven to reduce excess nitrogen in a laboratory setting through nitrification and denitrification (Gorsuch, Roberts, Lenhoff, & Showell). However, laboratory results often differ from field implementation (Herrero & Stuckey, 2014). The purpose of this study is to assess how Aqua bacteria remove ammonia and nitrate from wastewater. The potential bacterial metabolic pathways and rate of removal will be determined in laboratory and field settings.

CHAPTER 2: LITERATURE REVIEW

Conventional WWTPs use biological, chemical, or physical processes to remove nitrogen from wastewater. Background into the mechanics of nitrogen removal will be discussed. Conventional and advanced nitrogen removal processes will be analyzed as well.

2.1 Mechanisms of Nitrogen Removal

The main processes of the nitrogen cycle are nitrogen fixation, assimilation, ammonification, nitrification, and denitrification (Figure 2.1).

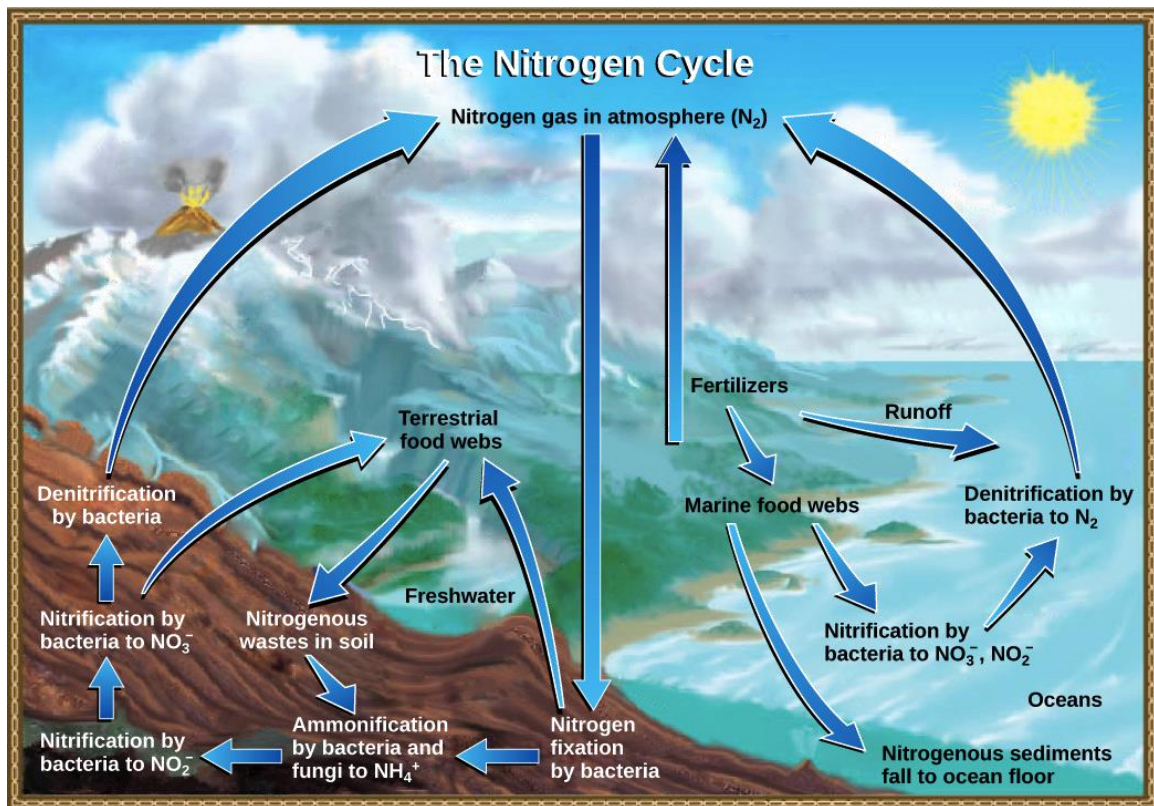
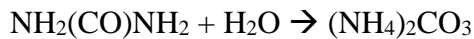


Figure 2.1: The natural nitrogen cycle (Evans & Perlman)

Nitrogen fixation is a process that converts nitrogen gas in the atmosphere to ammonium. Bacteria that exist within the soil or in root nodules of legumes are capable of achieving

nitrogen fixation (EPA, The Nitrogen Cycle, 2017). The bacteria use enzymes called nitrogenase to carry out nitrogen fixation (Postgate, 1998).

Ammonification is a process that converts organic nitrogen into ammonium. Humans and animals produce organic nitrogen in their wastes. These wastes serve as a substrate for the ammonification process. Urea is an organic nitrogen waste produced from humans that is typically found in wastewater (Power & Prasad, 1997). Urea hydrolysis converts urea into ammonium carbonate via the microbial enzyme urease (Ehrlich & Newman, 2009):



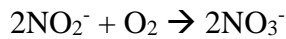
The ammonium carbonate eventually becomes ammonium, carbon dioxide, and water. For every one unit of urea converted, two units of ammonium are produced (DeCoste & Zumdahl, 2010).

Assimilation is a process that converts ammonium into organic nitrogen. Ammonium originates from either nitrogen fixation or ammonification. The ammonium that is assimilated serves as a nutrient in microbial metabolic processes responsible for protein production (Metcalf & Eddy, 2003). The typical metabolic processes for ammonium assimilation in bacteria is the glutamine synthetase-glutamate synthase pathway and the glutamate dehydrogenase pathway. These pathways are inhibited by low carbon sources. Therefore, carbon is essential for assimilation to occur (van Heeswijk, Westerhoff, & Boogerd, 2013).

Nitrification is the process of biological conversion of ammonium into nitrate. In typical wastewater, aerobic autotrophic bacteria are responsible for nitrification. The carbon source, electron donor (substrate oxidized), and the electron acceptor are CO_2 , NH_4^+ , and O_2 respectively. Nitrification is carried out by two different groups of aerobic autotrophic bacteria called Nitrosomonas and Nitrobacter. Nitrosomonas convert the ammonium into nitrite in the presence of oxygen:



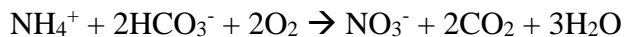
Nitrobacter convert the nitrite into nitrate in the presence of oxygen:



The total oxidation reaction of nitrification is:



Oxygen is an essential reactant in this process. The oxygen required for complete oxidation of ammonia is 4.57 g O_2 /g N oxidized (Appendix C). Nitrosomonas bacteria require 3.43 g O_2 /g N oxidized (Appendix C) and Nitrobacter bacteria require 1.14 g O_2 /g N oxidized (Appendix C). Alkalinity is also consumed within this nitrification process (Appendix C), which results in a lower pH environment. The equation above shows that hydrogen is produced, which consumes alkalinity. The equation can be re-written to show the alkalinity consumed during nitrification:



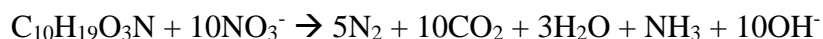
For each gram of ammonium as nitrogen converted, 7.14 grams of alkalinity as CaCO_3 is consumed (Metcalf & Eddy, 2003). The nitrates produced from nitrification can either be assimilated or denitrified (EPA, The Nitrogen Cycle, 2017).

The two types of nitrate removal processes are known as assimilating and dissimilating nitrate removal. Denitrification is the dissimilating process that converts nitrate into nitrogen gas. This process is connected with the respiratory electron transport chain, which uses nitrate or nitrite as an electron acceptor for oxidation of organic and inorganic electron donors (Zhu & Getting, 2012). Denitrification can occur with heterotrophic and autotrophic bacteria. Heterotrophic bacteria use organic materials as a carbon source. Autotrophic bacteria use inorganic materials, typically carbon dioxide, as a carbon source. Denitrification can occur in aerobic conditions by nitrifying bacteria, allowing simultaneous nitrification and denitrification to occur. The outside portion of the floc achieves nitrification, while the inside achieves denitrification. However, nitrification is limited due to low DO levels, and denitrification is limited due to a low carbon source. The outside portion of the floc uses the carbon source for nitrification, leaving very little for denitrification to occur (Metcalf & Eddy, 2003).

For typical anoxic heterotrophic denitrification, the steps are nitrate to nitrite, followed by nitric oxide, to nitrous oxide, to nitrogen gas:



Organic compounds that serve as the electron donor are typically organic materials in wastewater, organic materials produced from decay, or an exogenous source such as methanol or acetate. When wastewater is the electron donor, the stoichiometric reaction is:



Oxygen is a rate limiter for the denitrification process. The bacteria will choose to use oxygen before using nitrate as an electron acceptor because using oxygen will yield more

energy (ATP). Denitrification produces alkalinity, which results in a higher pH environment (Metcalf & Eddy, 2003).

Assimilating nitrate removal is a process that converts nitrate into ammonia for use in cell synthesis. Essentially, nitrate is converted into organic nitrogen (cell synthesis). Cell synthesis creates new cells, therefore increasing the mass of bacteria (Metcalf & Eddy, 2003).

Anaerobic ammonium oxidation (Anammox) is an autotrophic oxidation process that converts ammonium into nitrogen gas using nitrite as an electron acceptor. Anammox occurs in the absence of oxygen and does not require external carbon sources. Anammox bacteria have a very slow growth rate. Therefore, using systems with good biomass retention, like SBR, are crucial (Hu, Lotti, Loosdrecht, & Kartal, 2013).

The Anammox process can be coupled with ammonia-oxidizing bacteria. This creates a processes called completely autotrophic nitrogen removal over nitrate (CANON). The ammonium oxidizing bacteria convert half of the ammonium into nitrite. The Anammox bacteria then convert the ammonium and nitrite into nitrogen gas (Pynaert, Smets, Wyffels, Beheydt, Siciliano, & Verstraete, 2003; Winkler, et al., 2012). The CANON process reduces the oxygen required for ammonia removal and reduces the biological organic carbon demand. However, the growth rate of CANON bacteria is slow. Therefore, use of biofilm technologies is beneficial (Wang, Liu, Xu, Zhao, Yang, & Wang, 2017).

Factors, such as pH, temperature, COD, and salinity, affect the Anammox process (Hu, Lotti, Loosdrecht, & Kartal, 2013). High amounts of COD inhibit the Anammox process. This can be problematic because wastewater treatment requires the removal of COD. The Anammox process can also release nitrate, which could cause WWTPs to violate discharge standards (Winkler, et al., 2012). Simultaneous Anammox and denitrification could solve the COD and nitrate problem. However, an optimal COD concentration must be found in order to balance the amount of Anammox and denitrifying bacteria. Denitrifying bacteria dominate at higher COD concentrations. More denitrifying bacteria cause large nitrate and nitrite removal, but little to no ammonia removal. Anammox bacteria dominate at lower COD concentrations. More Anammox bacteria cause large ammonia removal, but little to no nitrate removal (Li, Qiang, Yu, Wang, Zhang, & Li, 2016).

Heterotrophic bacteria are capable of simultaneous aerobic nitrification and denitrification (Gorsuch, Roberts, Lenhoff, & Showell). Therefore, heterotrophic bacteria allow denitrification to occur in an oxygen rich environment (Choi, Zhang, Song, & Hwang, 2016). Heterotrophic bacteria are more desirable than typical nitrifying and denitrifying bacteria because they can handle higher concentrations of ammonia and low C:N ratios (Chen, Gu, Hao, & Chen, 2016). Use of heterotrophic bacteria could also reduce costs of typical nitrification and denitrification systems. Heterotrophic bacteria only require one aerobic tank and use minimal external carbon sources for denitrification (Choi, Zhang, Song, & Hwang, 2016). *Alcaligenes* is a bacteria species that can perform heterotrophic nitrification and aerobic denitrification. It has two metabolic pathways, including an entire synchronous process, and a shortcut process. The entire synchronous heterotrophic

nitrification-aerobic denitrification (HN-AD) process can be described as $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$. The shortcut HN-AD process can be described as $\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$. The shortcut process does not convert nitrate in an intermediate step. This improves the total nitrogen removal rate by 13% and simultaneous nitrification and denitrification efficiency by 11% (Chen, Gu, Hao, & Chen, 2016).

The specifics of nitrogen cycle ion exchanges are depicted in Figure 2.2 (Lunquist, 2017).

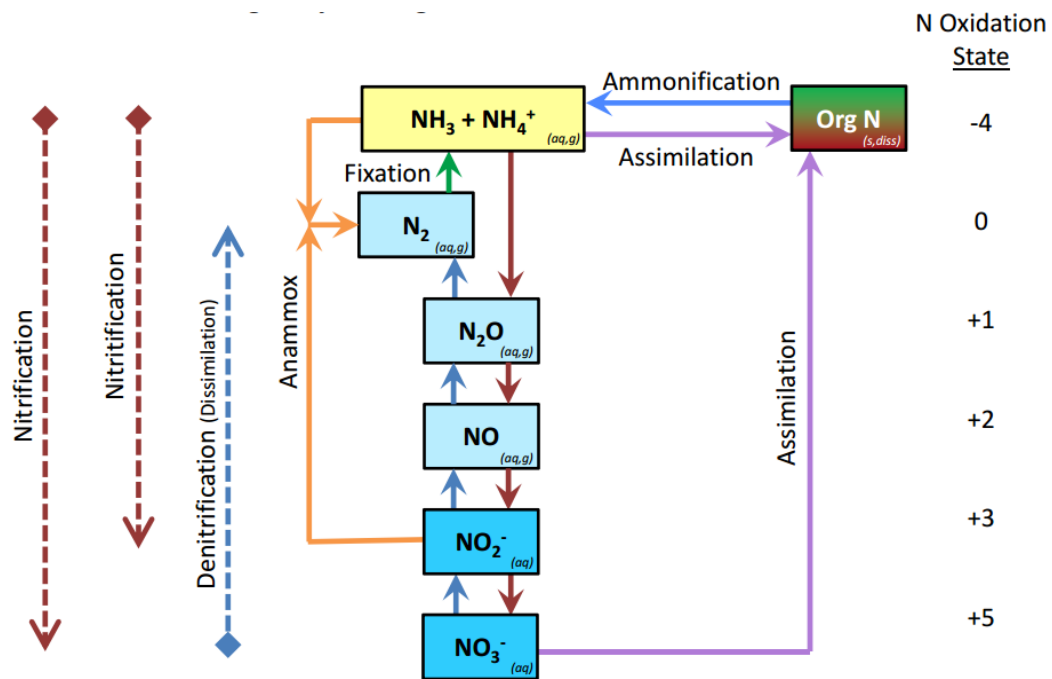


Figure 2.2: Ion diagram of nitrogen cycle (Lunquist, 2017)

Other environmental factors can influence nitrification and denitrification. According to Metcalf and Eddy, nitrification rates decline for pH values less than 6.8. The optimal pH for nitrification is 7.5 to 8.0. Reasonable nitrification rates occur in a pH range of 7.0 to

7.2. Nitrification consumes 7.14 grams of alkalinity per gram of nitrogen removed, which is why larger pH's are desirable. However, most bacteria cannot survive in a pH larger than 9.5. Denitrification rates are not as influenced by pH change compared to nitrification. No significant impact on the denitrification rate occurs between a pH of 7 and 8 (Metcalf & Eddy, 2003). Dawson and Murphy did discover a decrease in the denitrification rate when the pH is decreased from 7 to 6 in batch unacclimated tests (Dawson & Murphy, 1972). However, pH is not as much of an issue in denitrification because alkalinity is produced from the reaction. About 3.57 grams of alkalinity is produced per gram of nitrate as nitrogen removed. Increases in temperature improve both nitrification and denitrification rates (Metcalf & Eddy, 2003).

2.2 Typical Industrial Nitrogen Removal Processes

Wastewater is treated by ammonification, nitrification, and denitrification at WWTPs. Ammonification of urea and other proteins in the influent produces ammonia. Nitrification and denitrification occur to convert the toxic ammonia into nitrogen gas. These nitrogen removal processes are typically achieved through suspended growth, attached growth, and other physio-chemical treatment processes at WWTPs (Metcalf & Eddy, 2003).

2.2.1 Biological Suspended Growth Processes

Suspended growth processes ensure biological oxygen demand (BOD) removal and nitrification. The processes have an aerobic zone where suspension of solids and aeration occur. Solids separation occurs in a secondary clarifier. The solids are recycled back into the aeration tank to maintain a sufficient concentration of bacteria. The typical suspended

growth aeration tank designs are complete mix activated sludge, plug flow, extended aeration, and sequentially operated systems (Metcalf & Eddy, 2003; EPA, Nutrient Control Design Manual, 2010).

Anoxic tanks are added to suspended growth processes to ensure BOD removal, nitrification, and denitrification. To achieve denitrification, the anoxic tank must have a lack of oxygen and an abundance of nitrate. However, the amount of denitrification that occurs depends on the carbon source and the hydraulic retention time. The designs for denitrification include preanoxic, postanoxic, and simultaneous nitrification/denitrification (Metcalf & Eddy, 2003).

In postanoxic designs, the carbon source concentration is low because most of the BOD is removed in the aerobic tank. Therefore, an external carbon source, such as methanol, may be required for denitrification to occur (Metcalf & Eddy, 2003).

Preanoxic design processes include Ludzack-Ettinger, modified Ludzack-Ettinger, step feed, sequencing batch reactor, Bio-denitroTM, and NitroxTM. The modified Ludzack-Ettinger (MLE) is the most common nitrogen removal process that includes an anoxic tank, aerobic tank, and secondary clarifier (Figure 2.3). It uses return activated sludge (RAS) and an internal recycle for a more efficient nitrogen removal process. The RAS provides the bacteria needed for denitrification. The internal recycle feeds the high nitrate wastewater produced from the aerobic zone back into the anoxic tank. Placing the anoxic tank before the aerobic tank is advantageous because the influent wastewater provides

enough organic materials for denitrification (EPA, Nutrient Control Design Manual, 2010). The MLE process is adaptable to existing suspended growth processes and can achieve a total nitrogen concentration of less than 10 mg/L. However, a disadvantage of the MLE process is that the nitrogen removal capability depends on the internal recycle (Metcalf & Eddy, 2003).

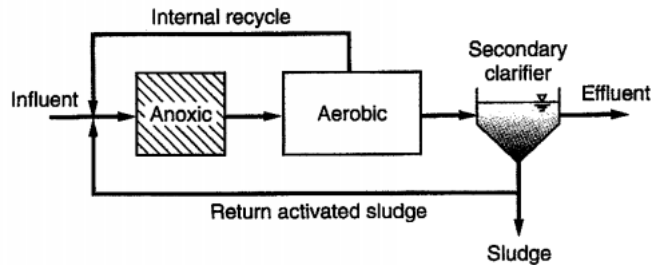


Figure 2.3: Modified Ludzack-Ettinger preanoxic system (Metcalf & Eddy, 2003)

2.2.2 Biological Attached Growth Processes

Attached growth processes achieve BOD removal and nitrification. These processes use packing material to grow biofilm that can achieve nitrogen removal. Attached growth biofilm has zones of aerobic, anoxic, and anaerobic conditions. Attached growth processes can be grouped into nonsubmerged, suspended growth with fixed-film packing, and submerged processes (Metcalf & Eddy, 2003; EPA, Nutrient Control Design Manual, 2010).

Suspended growth with fixed-film packing occurs when packing material is placed within the activated sludge tank (Figure 2.4). The packing material can be suspended or fixed in the tank. Packing material enhances the activated sludge process by increasing the biomass concentration in the tank. A larger biomass concentration reduces basin size during the

design process. Fixed film processes increase treatment capacity, reduce sludge production, and enhance sludge settling ability. However, fixed film processes have many of the same problems that suspended growth processes have, such as high energy and maintenance costs. If fine bubbling aeration equipment is used in fixed film processes, the packing material can discourage the efficient mass transfer of the fine bubbles (Metcalf & Eddy, 2003). Also, solids retention times cannot be controlled in the tank (EPA, Nutrient Control Design Manual, 2010).

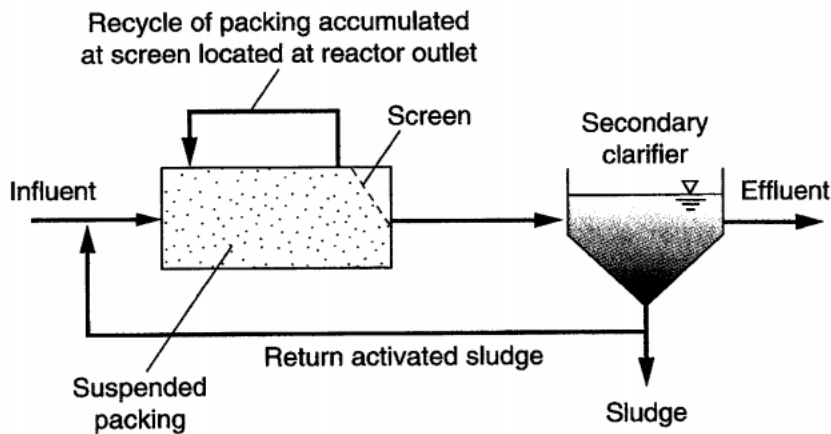


Figure 2.4: Captor ® and Limpor ® suspended growth fixed-film packing process (Metcalf & Eddy, 2003)

Attached growth denitrification can be achieved using different postanoxic or preanoxic processes. Processes include downflow/upflow packed bed reactors, upflow fluidized bed reactors, and submerged rotating biological contactors (Metcalf & Eddy, 2003).

Upflow/downflow packed bed reactors provide filtration and denitrification (Figure 2.5). A small sized sand is used as a packing material. The sand captures the solids and provides enough surface area for biological growth to occur without excessive headloss. Packed bed

reactors produce low total suspended solids (TSS) and nitrogen concentrations. Downflow packed beds experience increased headloss from solids and nitrogen gas accumulation. Air scour is needed to remove the nitrogen gas. Air or water backwash is needed to remove the solids. Upflow packed beds do not experience nitrogen gas accumulation, but they still require backwashing (Metcalf & Eddy, 2003; EPA, Nutrient Control Design Manual, 2010).

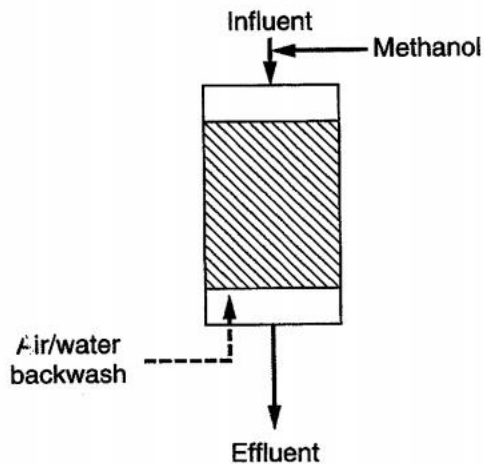


Figure 2.5: Downflow packed bed reactor (Metcalf & Eddy, 2003)

2.2.3. Physio-Chemical Treatment Processes

Many processes achieve physical and chemical total nitrogen removal, including air stripping, distillation, and ion exchange (Capodaglio, Hlavinek, & Raboni, 2015). These treatment processes can be used as advanced treatment after secondary treatment. Physical and chemical treatment processes tend to be more expensive processes because they produce very low nitrogen effluent. WWTPs typically rely on biological treatment due to the high cost of physical and chemical treatment. However, wastewater regulations are becoming increasingly stringent. WWTPs will need to implement these advanced

treatment processes unless more efficient biological treatment processes are discovered (Metcalf & Eddy, 2003).

2.3 Bioaugmentation

Many of the wastewater treatment processes described in Section 2.2 have low removal efficiencies or are expensive to install. Bioaugmentation is a great way to selectively implement bacteria with ideal nitrification and denitrification rates. Bioaugmentation will enhance removal efficiencies and is a low-cost alternative to upgrading or completely remodeling the conventional treatment systems (Kim, Park, Cho, Nam, Park, & Bajpai, 2005).

Numerous studies have been conducted to determine the effects of *Bacillus* bacteria on simultaneous aerobic nitrification and denitrification. A laboratory study was conducted to determine the metabolic pathways of heterotrophic *Bacillus* bacteria. The study concluded that *Bacillus* bacteria undergo aerobic nitrification and aerobic denitrification. *Bacillus subtilis* is largely involved in nitrification. *Bacillus cereus* and *Bacillus licheniformis* are largely involved in nitrogen production during simultaneous nitrification and denitrification (Kim, Park, Cho, Nam, Park, & Bajpai, 2005).

Further research into different conditions affecting *Bacillus* bacteria have been analyzed. A laboratory and pilot-scale study was conducted to determine the total nitrogen removed via aerobic nitrification and denitrification using *Bacillus subtilis*. Aerobic conditions in lab were achieved via shaking. Aerobic conditions in field were achieved with a controlled

air supply system. *Bacillus subtilis* was tested in an inorganic carbon source medium (basal inorganic medium) with various concentrations of ammonium. In medium with 100 mg/L $\text{NH}_4\text{-N}$, about 20% of $\text{NH}_4\text{-N}$ was removed as gaseous nitrogen. Higher initial concentrations of $\text{NH}_4\text{-N}$ resulted in decreased total nitrogen removal. *Bacillus subtilis* was also tested in basal medium with different organic carbon sources added. In medium with 100 mg/L $\text{NH}_4\text{-N}$ and acetate added at a C:N ratio of 11:1, about 52% of $\text{NH}_4\text{-N}$ was removed as gaseous nitrogen. *Bacillus subtilis* was also tested in acetate basal medium with different C:N ratios. In medium with 100 mg/L $\text{NH}_4\text{-N}$, a C:N ratio of 6:1 achieved 58% of $\text{NH}_4\text{-N}$ removal as gaseous nitrogen. Increasing the C:N ratio to 26:1 decreased the $\text{NH}_4\text{-N}$ removed to 44%. Decreasing the C:N ratio to 2:1 decreased the $\text{NH}_4\text{-N}$ removed to 22%. Therefore, a lower C:N ratio affects nitrogen removal more than higher C:N ratios. An SBR pilot study was performed using high strength wastewater. At steady state, the SBR without *Bacillus subtilis* removed 61% of total nitrogen. The SBR with *Bacillus subtilis* removed 81% of total nitrogen. Therefore, *Bacillus subtilis* achieved greater nitrogen removal compared to the natural bacteria (Yang, Wang, Zhang, & Zhou, 2010).

Bioaugmentation bacteria can also be grown using controlled dissolved oxygen concentrations. A study conducted in China determined the success of nitrification and denitrification from bioaugmentation under low temperatures of about 10°C. A SBR system was created with mineral media and secondary clarifier sludge. The best bacterial consortium was selected by using different DO rates. Steadily increasing the DO to 6 mg/L, rather than starting at 6 mg/L, increased the nitrate removal efficiency. The selected aerobic denitrification bacteria were analyzed against the original secondary clarifier feed for

nitrification and denitrification in lab. Both sets of bacteria were inoculated in mineral medium at 10°C under aerobic conditions via shaking. The nitrate concentration decreased only for the selected bacteria. Also, the nitrite increased and decreased over time, signifying denitrification. The ammonium decreased at a faster rate for the selected bacteria compared to the original bacteria. Therefore, successful heterotrophic nitrification and simultaneous aerobic denitrification occurred using the selected bacteria in low temperature conditions (Yao, Ni, Chen, & Borthwick, 2012).

2.4 BiOWiSH™ Bioaugmentation

Bioaugmentation of aerobic nitrification and denitrification bacteria was successful using mineral and complex media in pilot scale studies (Yao, Ni, Chen, & Borthwick, 2012; Yang, Wang, Zhang, & Zhou, 2010). BiOWiSH™ created a bioaugmentation product called Aqua, which includes *Bacillus*, *Pediococcus*, and *Lactobacillus* bacteria (Gorsuch, Roberts, Lenhoff, & Showell). Aqua consists of active microbial cultures, dextrose, and salt in a powder form (BiOWiSH, 2016). Approximately 95% of Aqua is dextrose.

Eva Lee studied the metabolic processes of Aqua in growth media (Lee, 2012). Ideal C:N ratios were determined for ammonia removal, nitrate removal, and simultaneous nitrification and denitrification. In the ammonia removal experiment, Aqua showed assimilation of ammonia rather than nitrification. A carbon to nitrogen (C:N) ratio of 6:1 provided the least ammonia removal, which was 1.6 ppm (0.23 ppm/day). Higher C:N ratios inhibit the enzymatic activity involved with ammonia assimilation. A ratio of 2:1 removed 6.2 ppm (3.06 ppm/day) ammonia and a C:N ratio of 4:1 removed 8.1 ppm (1.9

ppm/day) ammonia. Therefore, a C:N ratio of 2:1 to 4:1 is optimal. In the nitrate removal experiment, Aqua exhibited denitrification. A ratio of 2:1 removed 10 ppm/day of nitrate and a C:N ratio of 6:1 removed 7 ppm/day of nitrate. A C:N ratio of 2:1 to 6:1 is optimal for denitrification. A ratio of 2:1 is best because not only does it have a better nitrate removal rate, but higher C:N ratios allow growth of other organisms that compete with Aqua. In the simultaneous aerobic nitrification and denitrification experiment, no ammonia removal occurred at a C:N ratio of 1:1. A ratio of 2:1 gave an ammonia removal rate of 1.59 ppm/day and a nitrate removal rate of 5.86 ppm/day. A ratio of 3:1 gave an ammonia removal rate of 1.62 ppm/day and a nitrate removal rate of 4.71 ppm/day. A ratio of 2:1 is ideal because it had high removal rates and it completely eliminated the nitrate (Lee, 2012).

BiOWiSHTM studied aerobic heterotrophic nitrification and denitrification. Although autotrophs have a better nitrification and denitrification rate, heterotrophic bacteria can perform simultaneous aerobic nitrification and denitrification. Twelve heterotrophic bacterial species belonging to *Bacillus*, *Pediococcus*, and *Lactobacillus* were isolated from Aqua. The isolated bacteria species were tested for aerobic nitrification and aerobic denitrification in mineral media and sterilized wastewater. The nitrification study showed that as ammonia levels decreased, nitrite levels increased and total nitrogen levels decreased. Nitrate was not produced, which means the bacteria species tested did not follow typical chemolithoautotrophic aerobic nitrification. The metabolic process of the bacteria instead converted ammonia into nitrite and then immediately into nitrogen gas. The denitrification study showed nitrate removal followed by nitrite production. Total nitrogen analysis was not completed in this study, so conversions of nitrite into nitrogen gas cannot

be concluded. However, since the metabolic process of these isolated bacterial species follows typical denitrification, nitrite was likely converted into nitrogen gas (Gorsuch, Roberts, Lenhoff, & Showell).

CHAPTER 3: METHODS AND MATERIALS

In all experiments, there was preparation of the solutions and preparation of the samples. The solutions are prepared before the run occurs. The samples are prepared during the run. The solutions are set up initially and the samples are taken from the solutions over time.

3.1 Recipes

Growth media and TSB had the same recipe for all experiments. To prepare growth media, 1000 mg/L Dextrose, 250 mg/L K_2HPO_4 , 250 mg/L KH_2PO_4 , 6 $\mu\text{L/L}$ $FeCl_3$, and 2.3 mg/L $MnCl_2$ were added to deionized (DI) water. To prepare the TSB, 30 g/L of TSB powder was dissolved in DI water.

If Aqua in a liquid form was added to growth media solutions, the amounts measured would reflect the total volume of the solution. For instance, if 10 mL of liquid Aqua was added to 990 mL of growth media, then the growth media would be measured out for 1L.

3.2 Growth Media and Control Solution Preparation

All solutions that did not contain wastewater were always autoclaved in a Lancer UE650 to help prevent contamination. Typical solutions that were autoclaved were growth media, DI water, or TSB.

3.3 Aqua Activation Method

Activated Aqua was used in experiments 12, 13, 16, and 18. Activating Aqua allowed the bacteria to reach maximum cell count before being added to each solution bottle. The process of activating Aqua started with preparing growth media as stated in Section 3.1. The TSB was 10% of the total volume, so 3 g/L of TSB was added to the growth media mixture. The growth media and TSB were prepared as stated in Section 3.2. Dry Aqua was also added to the growth media and TSB mixture. The growth media, TSB, and Aqua was placed in either the ThermoForma Orbital Shaker Model 480 (SN: 100652), Bew Brunswick Scientific incubator shaker series 25, or Lab Line Instruments Model 3630 (SN: 0401-0024) for incubation at 25 to 30°C. The mixture was aerated using the Yu Ting Aquarium air pump YT-302C (SN: X0016XI34L) or the Hagen Elite802 air pump. The Yu Ting Aquarium air pump had adjustable flow rates, but the maximum was 48 gallons per hour. The Hagen Elite802 had a flow rate of 2500 cubic centimeters per minute. Beakers of water were placed in the incubator to minimize evaporation. Activation was usually conducted overnight (around 30-40 hours).

3.4 Inoculation Methods

Liquids were dosed into solutions using a calibrated pipette. Solutions were inoculated with activated Aqua using this technique.

Dry powders were weighed using a calibrated scale. Weigh paper and weigh boats were rinsed with DI to ensure all the product got into the solution. The amount of DI rinse added to the solution was negligible.

3.5 Incubation Methods

After solutions had all the necessary constituents added, they were placed in an incubator. Either the ThermoForma Orbital Shaker Model 480 (SN: 100652), Bew Brunswick Scientific incubator shaker series 25, or Lab Line Instruments Model 3630 (SN: 0401-0024) was used for incubation. Incubation occurred at 25 to 30°C. The incubators were also used to shake the solutions. Shaking speed could be adjusted on each incubator used. If solutions were open to the air, then beakers of water were added into the incubator to impede evaporation.

3.6 Sample Set-Up

Sampling was conducted after each solution in an experiment was set up. Temperature and pH were measured for each sample using a calibrated Oakton Acorn series meter (SN: 347237).

After pH and temperature were measured, all samples were acidified and filtered. Samples were acidified by adding 4-5 drops of sulfuric acid. This ensured the ammonia was in its non-gaseous form (NH_4^+), which prevented volatilization of ammonia. All samples were also filtered through 0.22 micrometer filters before analysis. A Millipore pump (SN: 123909) was used for filtration. Filtration prevented clogging of analyzer tubes. Acidification was always conducted before filtration to prevent volatilization of ammonia that could occur from filtering (Blackwell, Bowen, Parker, & Crowe, 2015).

3.7 Sample Analysis

After samples were acidified and filtered, they were analyzed with a Timberline and ion chromatograph.

Ammonia was measured using the Timberline Instruments TL-2800 Ammonia Analyzer (SN: 030911002). The Timberline was connected to a Cetac ASX-260 AutoSampler. A caustic and buffer solution were required for Timberline operation. The caustic solution was 5% potassium hydroxide (KOH) in DI water. The buffer solution was 500 mg/L boric acid adjusted to a pH of 6.9 by adding 4% 1M ammonium hydroxide (NH₄OH) drop wise. Calibration standards were measured before every set of samples run through the Timberline. Calibration standards were diluted from a concentrated standard of 100 mg/L NH₄-N. The concentrated standard was made by weighing out 95.5 mg of NH₄Cl and dissolving it in 250 mL of DI water (Appendix D). The calibration standards measured were 0, 1, 5, 10, 25, 50, and 75 ppm NH₄-N. (Blackwell, Bowen, Parker, & Crowe, 2015)

Nitrate and nitrite concentrations were measured using an ion chromatograph (IC). A Thermo Scientific Dionex ICS-1600 IC (SN: 15022638) was coupled with a Thermo Scientific Dionex AS-DV (SN: 15022516) carousel to allow for easier sample injection. The IC has a guard column, separator column, suppressor, and conductivity cell specifically for anion analysis. The guard column was a Thermo Scientific Dionex IonPac AS9-HC (SN: 025322). The separator column was a Thermo Scientific Dionex IonPac AS9-HC (SN: 023252). The suppressor was a Thermo Scientific Dionex AERS 500 (SN: 082540). The conductivity cell was a Thermo Scientific DS6 Heated Conductivity Cell

(SN: 16022686). Eluent was required for IC operation. The eluent solution was 953.9 mg/L Na_2CO_3 dissolved in DI water to produce a 9mM carbonate solution (Appendix D). Calibration standards were measured approximately once every month. Calibration standards were diluted from a concentrated standard of 100 mg/L $\text{NO}_3\text{-N}$. The concentrated standard was made by weighing out 151.79 mg of NaNO_3 and dissolving it in 250 mL of DI water (Appendix D). The calibration standards measured varied between months. In total, calibrations included 0, 0.25, 0.5, 1, 5, 10, 25, 50, 75 and 100 ppm $\text{NO}_3\text{-N}$ (Thermo Scientific, 2012).

DO was measured using a calibrated YSI Pro 20 (SN: 13E101525) with an YSI 1234 PRO-BOD probe (SN: 13E100187).

3.8 Laboratory Method

Laboratory experiments were conducted to determine the metabolic rates and processes of different bacteria strains isolated from Aqua. Experiments also determined the ideal location at the SLO WRRF for ammonia and nitrate removal using Aqua. Wastewater from the SLO WRRF for laboratory experiments was obtained from the primary clarifier, secondary clarifier, final clarifier, supernatant storage lagoon (sludgewash), and DAFT (Figure 3.2). Average characteristics of the wastewater were obtained from the SLO WRRF (Table 3.1). Solutions were stored in 250 mL to 1L bottles (Figure 3.1).

Laboratory solutions were incubated at different temperatures for the experiments. Initial laboratory experiments were conducted in warmer temperatures to ensure that Aqua growth

occurred. The laboratory solutions that corresponded with field solutions were conducted in temperatures similar to the ambient temperature of the week to ensure more comparable data. Laboratory experiments conducted after field experiments were incubated in temperatures similar to ambient temperatures in the summer to simulate how the experiment would work if conducted in field.

Table 3.1: Characteristics of SLO WRRF wastewater

Wastewater	NH ₃ -N (mg/L)	Alkalinity as CaCO ₃ (mg/L)	BOD (mg/L)	TSS (mg/L)	TKN (mg/L)	Total Phosphorus (mg/L)	NO ₃ -N (mg/L)
Primary Clarifier	36.35	-	181.6	69.67	46.48	6.5	-
Secondary Clarifier	16.08	-	70.48	36.09	20.82	5.97	-
Final Clarifier	1.29	1.29	7.1	14.46	-	5.43	28.83
DAFT	25.42	323.71	190.8	167.04	40.46	7.89	3.94
Sludgewash	575.62	1631.91	177.5	120.5	527.48	15.76	-



Figure 3.1: Examples of 1L bottles for solution storage

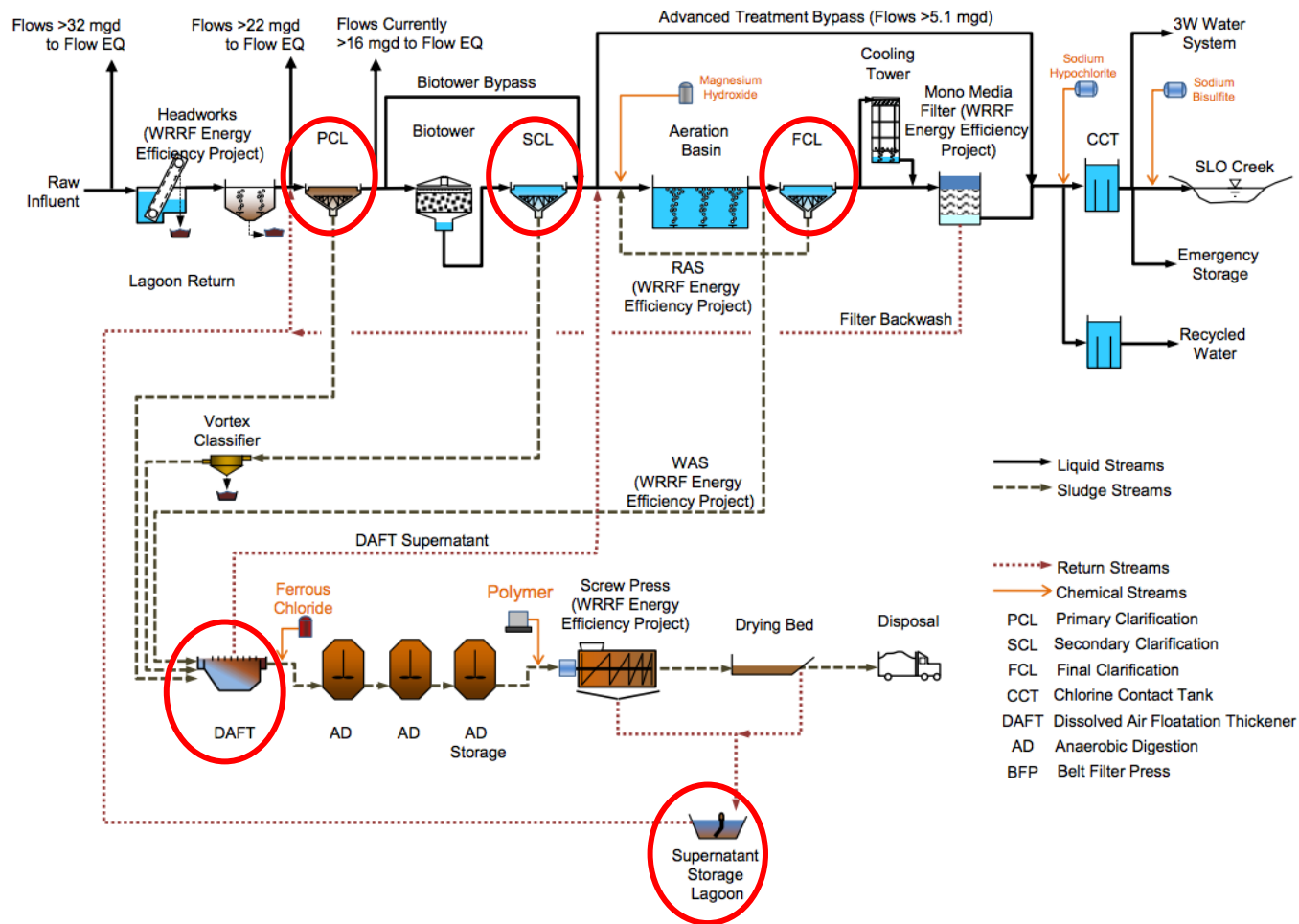


Figure 3.2: Wastewater collection at the SLO WRRF (HDR, 2014)

3.8.1 Experiment 1 – Effect of *Bacillus* Strains and Aqua on Denitrification

Solutions were prepared by first incubating TSB and Aqua bacteria mixtures. Then, the mixtures were added to growth media solutions. Samples were taken from the solutions to measure nitrate and nitrite.

To create the TSB and Aqua mixture, five flasks of 70 mL TSB were prepared, as described in Section 3.1 and 3.2. After the TSB cooled, four different *Bacillus* strains found in Aqua were swabbed from streak plates (provided by BiOWiSH™) and inoculated in four different TSB flasks. The *Bacillus* strains were *Bacillus subtilis mojavensis*, *amyloliquefaciens*, *pumilus*, and *licheniformis*. About 3.5 mg of Aqua was weighed out and dosed into the fifth TSB flask, as described in Section 3.4. The mixtures needed to be in a slightly open air and warm environment for optimal growth. Therefore, the five flasks of inoculated TSB were capped with a breathable top and were stored in the incubator at 30°C, as described in Section 3.5. Incubation lasted for 32 hours. A concentration of 50 ppm Aqua was needed in this experiment. Only 5 ppm was added before incubation due to a math error. Therefore, another 31.5 mg of Aqua was added to the TSB with Aqua sample after incubation to produce 50 ppm Aqua.

To create the solutions for the experiment, five bottles of growth media were prepared, as described in Section 3.1 and 3.2. All solutions had a total volume of 700 mL. Each 70 mL TSB mixture was dosed into the five different bottles containing 630 mL of growth media.

Therefore, the TSB was 10% of the total solution. About 212.5 mg of NaNO_3 was added to all solution bottles to produce 50 ppm $\text{NO}_3\text{-N}$, as described in Section 3.4.

During the experiment, solutions needed to be in an anoxic environment so nitrate removal could be studied. Solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the solutions in an anoxic and warm environment, laboratory solutions were capped with a solid lid and kept in the incubator at 30°C , as described in Section 3.5. Sampling was conducted every 8 hours for 2.5 days. Samples were analyzed according to Section 3.7. Samples were not set up according to Section 3.6 because it was unknown at the time that filtration needed to occur for the IC. Temperature and pH were also not measured.

3.8.2 Experiment 2a & 2b – Effect of 50 ppm Aqua on Wastewater Collected from DAFT and Primary, Secondary, and Final Clarifiers

Wastewater was taken from the DAFT, primary clarifier, secondary clarifier, and final clarifier for Experiments 2a and 2b. The wastewater was partially sterile for all solutions. Partial sterilization was used to determine how Aqua worked in wastewater with little to no natural bacteria. Experiment 2a only analyzed nitrate and nitrite removal. Experiment 2b only analyzed ammonia removal.

Experiment 2a – Nitrate and nitrite analysis

Solutions were prepared by first incubating a TSB and Aqua mixture. Then, the mixture was added to different wastewater solutions. Samples were taken from the solutions to measure nitrate and nitrite.

To create the TSB and Aqua mixture, 25 mL of TSB was prepared, as described in Section 3.1 and 3.2. After the TSB cooled, about 125 mg of Aqua was weighed out and dosed into the TSB, as described in Section 3.4. This created a concentration of 5000 mg/L Aqua. The mixture needed to be in a slightly open air and warm environment for optimal growth. Therefore, the mixture of inoculated TSB was capped with a breathable top and was stored in the incubator at 35°C, as described in Section 3.5. Incubation lasted for 15 hours.

To create the solutions for the experiment, wastewater was collected from the treatment plant according to Section 3.10, growth media was prepared according to Section 3.1 and 3.2, and DI was prepared according to Section 3.2. All solutions had a total volume of 500 mL. All solutions were filtered through the 1.2 micrometer filter before any additions. About 5 mL of the TSB and Aqua mixture was added to 495 mL of DAFT, primary clarifier, secondary clarifier, final clarifier, and growth media solutions. The TSB was only 1% of the total volume because it increases the initial nitrogen concentration. Controls for each wastewater were prepared. A DI control was prepared as well. Aqua was not added to the controls. About 75.89 mg of NaNO_3 was added to all solution bottles to produce a concentration of at least 25 mg/L $\text{NO}_3\text{-N}$, as described in Section 3.4. Some wastewater already contained nitrate, therefore some solutions were over 25 mg/L $\text{NO}_3\text{-N}$.

During the experiment, solutions needed to be in an anoxic environment so nitrate removal could be studied. Solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the solutions in an anoxic and warm environment, the laboratory solutions were capped with a solid lid and incubated at 35°C, as described in Section 3.5. Sampling was conducted twice a day for three days. Samples were analyzed using the IC (Section 3.7). Samples were not set up according to Section 3.6 because wastewater was partially sterile and didn't need further filtration. Temperature and pH were also not measured.

Experiment 2b – Ammonia analysis

Solutions were prepared by first incubating a TSB and Aqua mixture. Then, the mixture was added to different wastewater solutions. Samples were taken from the solutions to measure ammonia.

To create the TSB and Aqua mixture, 45 mL of TSB was prepared, as described in Section 3.1 and 3.2. After the TSB cooled, about 225 mg of Aqua was weighed out and dosed into the TSB, as described in Section 3.4. This created a concentration of 5000 mg/L Aqua. The mixture needed to be in a slightly open air and warm environment for optimal growth. Therefore, the mixture of inoculated TSB was capped with a breathable top and was stored in the incubator at 35°C, as described in Section 3.5. Incubation lasted for 30 hours.

To create the solutions for the experiment, wastewater was collected from the treatment plant according to Section 3.10, growth media was prepared according to Section 3.1 and 3.2, and DI was prepared according to Section 3.2. All solutions had a total volume of 800 mL. All solutions were acidified to a pH of around 2 and filtered through 1.2 micrometer filter before any additions. After acidification and filtration, the pH was increased to 6 - 6.2 so the Aqua could survive in solution. About 8 mL of the TSB and Aqua mixture was added to 792 mL of DAFT, primary clarifier, secondary clarifier, final clarifier, and growth media solutions. The TSB was only 1% of the total volume because it increases the initial nitrogen concentration. Controls for each wastewater were prepared. A DI control was prepared as well. Aqua was not added to the controls. About 76.42 mg of NH_4Cl was added to all solution bottles to produce a concentration of at least 25 mg/L $\text{NH}_4\text{-N}$, as described in Section 3.4. Some wastewater already contained ammonia, therefore some solutions were over 25 mg/L $\text{NH}_4\text{-N}$.

During the experiment, solutions needed to be in an aerobic environment so ammonia removal could be studied. Solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an aerobic and warm environment, solutions were open to the air, shaken at 100 rpm, and incubated at 30°C, as described in Section 3.5. Sampling was conducted once a day for five days. Samples were analyzed using the Timberline (Section 3.7). Samples were not set up according to Section 3.6 because wastewater was partially sterile and didn't need further filtration. Temperature and pH were also not measured.

3.8.3 Experiment 3 – Effect of 50 ppm Aqua on Wastewater Collected from DAFT, Sludgewash, Primary Clarifier, and Secondary Clarifier

Solutions were prepared by first incubating a TSB and Aqua mixture. Then, the mixture was added to different wastewater solutions. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, and pH.

To create the TSB and Aqua mixture, 45 mL of TSB was prepared, as described in Section 3.1 and 3.2. After the TSB cooled, about 225 mg of Aqua was weighed out and dosed into the TSB, as described in Section 3.4. This created a concentration of 5000 mg/L Aqua. The mixture needed to be in a slightly open air and warm environment for optimal growth. Therefore, the mixture of inoculated TSB was capped with a breathable top and was stored in the incubator at 30°C, as described in Section 3.5. Incubation lasted for 18.5 hours.

To create the solutions for the experiment, wastewater was collected from the treatment plant according to Section 3.10, growth media was prepared according to Section 3.1 and 3.2, and DI was prepared according to Section 3.2. All solutions had a total volume of 800 mL. About 8 mL of the TSB and Aqua mixture was added to 792 mL of DAFT, primary clarifier, secondary clarifier, and sludgewash solutions. The TSB was only 1% of the total volume because it increases the initial nitrogen concentration. Controls for each wastewater were prepared. A DI and growth media control were prepared as well. Aqua was not added to the controls. About 76.42 mg of NH_4Cl and 121.43 mg NaNO_3 were added to all solution bottles, except sludgewash, to produce a concentration of at least 25 mg/L $\text{NH}_4\text{-N}$ and 25 mg/L $\text{NO}_3\text{-N}$. These powders were dosed into solutions according to Section 3.4. Some

wastewater already contained ammonia and/or nitrate, therefore some solutions were over 25 mg/L $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$. Ammonia and nitrate were not added to sludgewash because high concentrations of both already existed in the wastewater.

During the experiment, solutions needed to be in an aerobic environment so ammonia removal could be studied. Solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an aerobic and warm environment, solutions were open to the air, shaken at 110 rpm, and kept in the incubator at 30°C, as described in Section 3.5. Sampling was conducted once to twice a day for eleven days. The samples were prepared and analyzed according to Section 3.6 and 3.7.

3.8.4 Experiment 4 – Effect of 500 ppm Aqua with New Inoculation Method on Secondary Clarifier Wastewater

Solutions were prepared by adding liquid or dry Aqua, liquid or dry microbial cultures, trace minerals, and nitrate to the secondary clarifier wastewater solutions (Table 3.2). The microbial cultures have the same recipe as Aqua, except 70% of the powder is freeze dried microbes, instead of the 1% normally in Aqua. Samples were taken from the solutions to measure nitrate, nitrite, pH, and temperature

To create the liquid Aqua and microbial cultures, 10 grams of Aqua (or microbial cultures) was mixed with 100 mL DI using a stir plate to create a concentration of 100,000 ppm Aqua. About 0.5 μL was added to wastewater solutions to obtain a concentration of 500 ppm Aqua, as described in Section 3.4. Trace minerals were added to half of the solutions

to determine if wastewater had enough trace minerals for Aqua to use. Trace minerals were dosed according to Section 3.4. About 1% of the total volume was mineral solution.

To create the solutions for the experiment, secondary clarifier wastewater was collected from the treatment plant according to Section 3.10, and DI was prepared according to Section 3.2. Dry and liquid Aqua or microbial cultures were dosed into the solutions as described in Section 3.4. Trace minerals were dosed into solutions as described in Section 3.4. Nitrate was dosed into solutions as described in Section 3.4. A control of secondary clarifier wastewater was prepared. A DI control was prepared as well. Aqua was not added to the controls.

During the experiment, solutions needed to be in an anoxic environment so nitrate removal could be studied. Solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. Sampling was conducted every two hours for ten hours. The samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.2: Weights and concentrations for experiment 4

Label	BiOWiSH™			Nitrate (NaNO ₃)		Trace Minerals Amount (mL)	Total Vol (L)
	Type	Amount	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)		
Secondary with dry Aqua and TM	Aqua	50 mg	500	15.18	25	1	0.1
Secondary with liquid Aqua and TM	Liquid Aqua	0.5 mL	500	15.18	25	1	0.1
Secondary with dry MC and TM	Microbial culture	50 mg	500	15.18	25	1	0.1
Secondary with liquid MC and TM	Liquid Microbial culture	0.5 mL	500	15.18	25	1	0.1
Secondary with dry Aqua	Aqua	50 mg	500	15.18	25	-	0.1
Secondary with liquid Aqua	Liquid Aqua	0.5 mL	500	15.18	25	-	0.1
Secondary with dry MC	Microbial culture	50 mg	500	15.18	25	-	0.1
Secondary with liquid MC	Liquid Microbial culture	0.5 mL	500	15.18	25	-	0.1
Secondary without Aqua	-	-	-	15.18	25	-	0.1
Secondary with 50 ppm Aqua	Aqua	25 mg	50	75.89	25	-	0.5
Control	-	-	-	151.79	25	-	1

3.9 San Luis Obispo Water Reclamation Facility Description

The San Luis Obispo Water Resource and Reclamation Facility (SLO WRRF) uses a combined aerobic treatment process. The system includes headworks, primary clarifier, trickling filter, secondary clarifier, activated sludge, final clarifier, filtration, and chlorine contact tank (Figure 3.3). The solids produced from the primary, the waste activated sludge, and the scum from the primary and secondary clarifier are sent to the dissolved air flotation thickeners (DAFT) so they can be thickened before going into the anaerobic digesters. After the anaerobic digesters, the sludge is dewatered with a belt filter press and sludge drying beds. The supernatant from the dewatering processes, also called sludgewash, is

sent to a lagoon that is aerated by a surface mixer. Water from this lagoon is high strength and is slowly sent back into the headworks during low flows and loads (HDR, 2014).

The SLO WRRF's NPDES permit was recently updated with more stringent disinfection byproduct and nitrate limits. The SLO WRRF discharge permit requires that effluent must not exceed 0.025 mg/L for ammonia as nitrogen and 10 mg/L for nitrate as nitrogen.

The SLO WRRF currently cannot meet those limits, so upgrades are necessary (HDR, 2014).

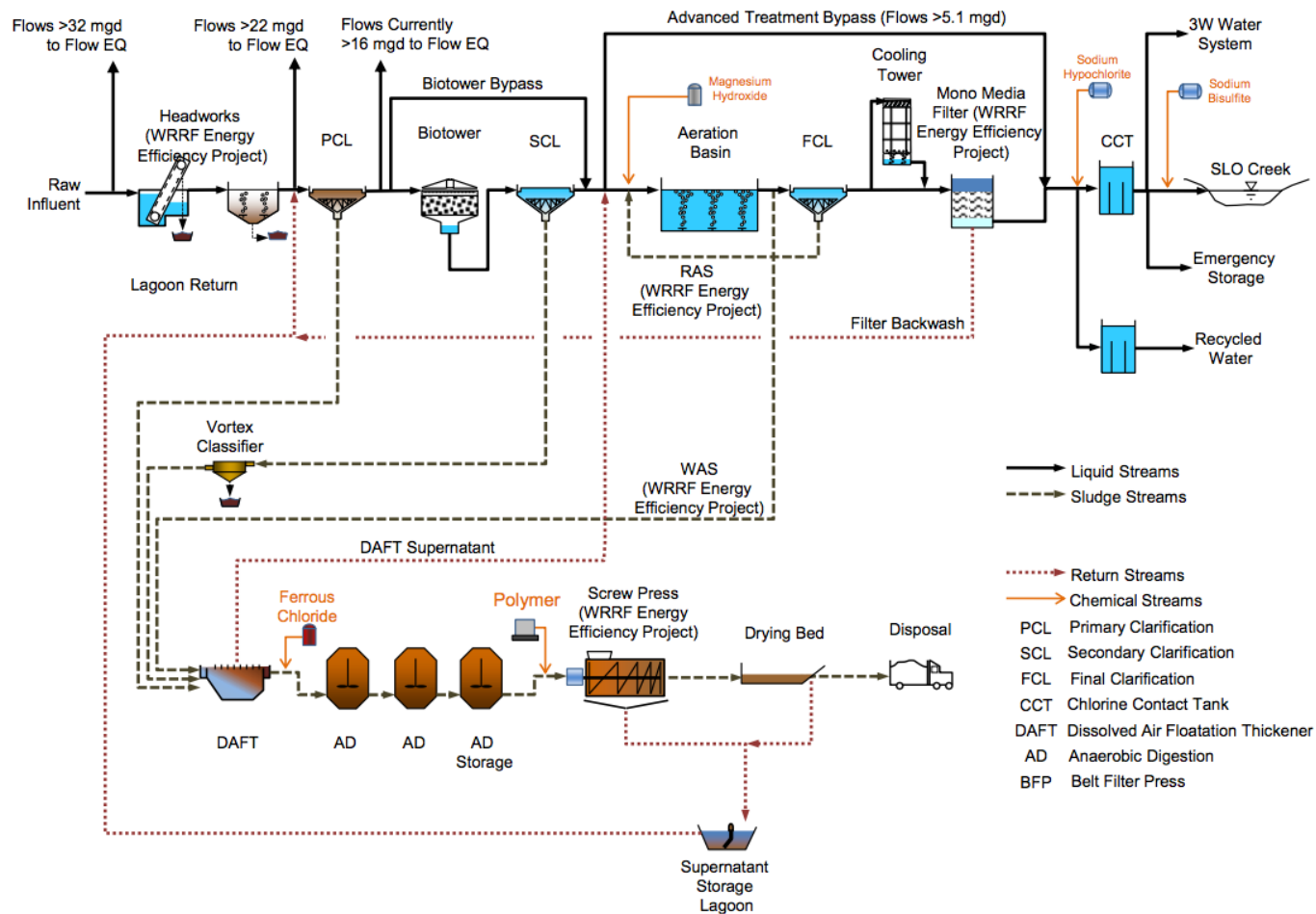


Figure 3.3: Existing process schematic at SLO WRRF (HDR, 2014)

3.10 Bioreactor Set-Up

Field method includes set-up of batch bioreactors at the SLO WRRF. The reactors are 55-gallon trash cans filled with approximately 40 gallons (151 liters) of wastewater. Pumps were used to fill the trash cans with wastewater to the inner handle line (Figure 3.4). The water pumps used were Shurflo (Model: 2088-594-154) and Aleko Multi-Function pump (Model: G2949). The trash cans were placed in a wooden holder and attached to railings at the treatment plant to prevent any tipping (Figure 3.5).



Figure 3.4: Bioreactor fill line



Figure 3.5: Bioreactor set-up

Any dry material dosed into the bioreactors was weighed out in lab and brought on-site in either sterile Falcon tubes or total organic carbon vials (Figure 3.6). If the material was dry, DI water was used to rinse the vials to prevent loss of the material. Liquid material was measured into a sterilized bottle either by weight or pipetting. The liquid material was brought on-site and rinsed with DI to obtain all material from the bottle. The amount of DI water to wastewater in the bioreactor was assumed to be negligible.



Figure 3.6: Total organic carbon vials used to collect samples

Lids on trashcans were attached completely for runs under anoxic conditions. Lids had holes at the top and were loosely attached for runs under aerobic conditions. Topfin battery powered air pumps (SN: 2047, 2125) were also used to aerate for aerobic conditions. The flowrate was 1.8 L/min.

Samples were taken using a disposable pipette. Samples were generally taken near the top of the water surface since the disposable pipettes could only reach that far down. Wastewater samples from the bioreactors were collected in clean total organic carbon vials. Temperature and pH were taken in each bioreactor on-site. The SLO WRRF was about 5 minutes away, so any reactions occurring in the samples during that time were considered negligible.

Clean 2 liter bottles were used to collect wastewater for laboratory experiments. Wastewater was dispensed into the bottles two different ways. Pumps used to fill the bioreactors were also used to fill the 2 liter bottles. Sampling sticks already existing at the

SLO WRRF were also used to pour wastewater into bottles. If the same sampler had to be used for different wastewater, then the sampler would be rinsed with the wastewater before dispensing.

3.11 Field Method

All bioreactor solutions had corresponding laboratory solutions. Lab solutions served as controls for the experiments. The lab sample concentrations were compared to the field sample concentrations to determine if there was a significant difference between the two. Large differences mean that field conditions potentially affected ammonia and nitrate removal. The lab solutions were kept in a more controlled and ideal environment compared to the bioreactor samples. Lab solutions were kept in an incubator to provide a constant temperature for optimal bacterial growth. The bioreactors, however, were kept outside where temperature fluctuates constantly. The lab solutions were also set up in sterilized and clean bottles. However, the bioreactors could only be cleaned, not sterilized, since plastic was used for experiment preparation. Therefore, bioreactor solutions were more likely to become contaminated compared to laboratory samples. Bioreactors were set up at the supernatant storage lagoon (sludgewash) and secondary clarifier (Figure 3.7).

At times, the secondary clarifier would not have nitrate. Typical wastewater treatment plants have nitrate production after aerobic treatment. Therefore, secondary clarifier wastewater was spiked with nitrate to create similar characteristics to typical wastewater and to better determine the effects of denitrification.

3.11.1 Experiment 5 – Effect of 5 ppm Aqua on Secondary Clarifier Wastewater

Solutions were prepared by adding dry Aqua and nitrate to the secondary clarifier wastewater solutions (Table 3.3). Ammonia was not added because the wastewater contained 25 ppm of ammonia. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, secondary clarifier wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. Dry Aqua and nitrate were dosed into the lab solutions as described in Section 3.4. Two lab controls of secondary clarifier wastewater were prepared. A DI lab control was prepared as well. Aqua was not added to the controls. The bioreactor was set up according to Section 3.10. Dry Aqua and nitrate were dosed into the bioreactor solutions, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as described in Section 3.10. Sampling was conducted twice a day for six days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.3: Weights and concentrations for experiment 5

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Total Vol (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Aqua	Field	755	5	23000	25	151
Lab Secondary with Aqua	Lab	5	5	151.8	25	1
Lab Secondary without Aqua or nitrate	Lab	-	-	-	-	1
Lab Secondary without Aqua	Lab	-	-	151.8	25	1
Control	Lab	-	-	151.8	25	1

3.11.2 Experiment 6 – Effect of 2.5 ppm Aqua on Secondary Clarifier Wastewater

Solutions were prepared by adding dry Aqua and nitrate to the secondary clarifier wastewater solutions (Table 3.4). Ammonia was not added because the wastewater contained 25 ppm of ammonia. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, secondary clarifier wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. Dry Aqua and nitrate were dosed into the lab solutions as described in Section 3.4. Two lab controls of secondary clarifier wastewater were prepared. A DI lab control was prepared as well. Aqua was not added to the controls. The bioreactor was set up according to Section 3.10. Dry Aqua and nitrate were dosed into the bioreactor solutions, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as described in Section 3.10. Sampling was conducted twice a day for five days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.4: Weights and concentrations for experiment 6

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Total Volume (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Aqua	Field	378	2.5	23000	25	151
Lab Secondary with Aqua	Lab	2.5	2.5	151.8	25	1
Lab Secondary without Aqua or nitrate	Lab	-	-	-	-	1
Lab Secondary without Aqua	Lab	-	-	151.8	25	1
Control	Lab	-	-	151.8	25	1

3.11.3 Experiment 7 – Effect of 50 ppm Aqua on Secondary Clarifier Wastewater

Solutions were prepared by adding dry Aqua and nitrate to the secondary clarifier wastewater solutions (Table 3.5). Ammonia was not added because the wastewater contained 25 ppm of ammonia. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, secondary clarifier wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. Dry Aqua and nitrate were dosed into the lab solutions as described in Section 3.4. Two lab controls of secondary clarifier wastewater were prepared. A DI lab control was prepared as well. Aqua was not added to the controls. The bioreactor was set up according to Section 3.10. Dry Aqua and nitrate were dosed into the bioreactor solutions, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as described in Section 3.10. Sampling was conducted twice a day for five days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.5: Weights and concentrations for experiment 7

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Total Vol (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Aqua	Field	7550	50	23000	25	151
Lab Secondary with Aqua	Lab	50	50	151.8	25	1
Lab Secondary without Aqua	Lab	-	-	151.8	25	1
Lab Secondary without Aqua or nitrate	Lab	-	-	-	-	1
Control	Lab	-	-	151.8	25	1

3.11.4 Experiment 8 – Effect of 25 ppm Aqua on Sludgewash Wastewater

Solutions were prepared by adding dry Aqua to the sludgewash wastewater solutions (Table 3.6). Ammonia and nitrate were not added because the wastewater contained about 700 ppm ammonia and 60 ppm nitrate. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, sludgewash wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. The lab samples had a total volume of 1L. Dry Aqua was dosed into one lab solution, as described in Section 3.4. For this sample, 10 mg of Aqua was accidentally added instead of 25 mg of Aqua, which resulted in a concentration of 10 ppm Aqua. A lab control of sludgewash wastewater was prepared. A DI lab control was prepared as well. About 151.8 mg of NaNO_3 and 95.5 mg of NH_4Cl were added to the DI lab control to produce 25 ppm $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, as described in Section 3.4. Aqua was not added to the controls. The bioreactor was set up according to Section 3.10. The bioreactor solution had a total volume of 151L. About 3800 mg of Aqua was dosed into the bioreactor solution to produce 25 ppm Aqua, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as

described in Section 3.10. Sampling was conducted twice a day for six days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.6: Weights and concentrations for experiment 8

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Ammonia (NH ₄ Cl)		Total Vol (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Sludgewash with Aqua	Field	3800	25	-	-	-	-	151
Lab Sludgewash with Aqua	Lab	10	10	-	-	-	-	1
Lab Sludgewash without Aqua	Lab	-	-	-	-	-	-	1
Control	Lab	-	-	151.8	25	95.5	25	1

3.11.5 Experiment 9 – Effect of 10 ppm Aqua on Sludgewash Wastewater

Solutions were prepared by adding dry Aqua to the sludgewash wastewater solutions (Table 3.7). Ammonia and nitrate were not added because the wastewater contained about 700 ppm ammonia and 60 ppm nitrate. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, sludgewash wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. The lab samples had a total volume of 1L. About 10 mg of Aqua was dosed into one lab solution to produce 10 ppm Aqua, as described in Section 3.4. A lab control of

sludgewash wastewater was prepared. A DI lab control was prepared as well. About 151.8 mg of NaNO_3 and 95.5 mg of NH_4Cl were added to the DI lab control to produce 25 ppm $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, as described in Section 3.4. Aqua was not added to the controls. The bioreactor was set up according to Section 3.10. The bioreactor solution had a total volume of 151L. About 1500 mg of Aqua was dosed into the bioreactor solution to produce 10 ppm Aqua, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C , as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as described in Section 3.10. Sampling was conducted twice a day for six days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.7: Weights and concentrations for experiment 9

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Ammonia (NH ₄ Cl)		Total Vol (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Sludgewash with Aqua	Field	1500	10	-	-	-	-	151
Lab Sludgewash with Aqua	Lab	10	10	-	-	-	-	1
Lab Sludgewash without Aqua	Lab	-	-	-	-	-	-	1
Control	Lab	-	-	151.8	25	95.5	25	1

3.11.6 Experiment 10 – Effect of 5 ppm Aqua on Sludgewash Wastewater

Solutions were prepared by adding dry Aqua to the sludgewash wastewater solutions (Table 3.8). Ammonia and nitrate were not added because the wastewater contained about 700 ppm ammonia and 60 ppm nitrate. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, sludgewash wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. The lab samples had a total volume of 1L. About 5 mg of Aqua was dosed into one lab solution to produce 5 ppm Aqua, as described in Section 3.4. A lab control of sludgewash wastewater was prepared. A DI lab control was prepared as well. About 151.8 mg of NaNO₃ and 95.5 mg of NH₄Cl were added to the DI lab control to produce 25 ppm NO₃-N and NH₄-N, as described in Section 3.4. Aqua was not added to the controls. The

bioreactor was set up according to Section 3.10. The bioreactor solution had a total volume of 151L. About 755 mg of Aqua was dosed into the bioreactor solution to produce 5 ppm Aqua, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as described in Section 3.10. Sampling was conducted twice a day for five days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.8: Weights and concentrations for experiment 10

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Ammonia (NH ₄ Cl)		Total Vol (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Sludgewash with Aqua	Field	755	5	-	-	-	-	151
Lab Sludgewash with Aqua	Lab	5	5	-	-	-	-	1
Lab Sludgewash without Aqua	Lab	-	-	-	-	-	-	1
Control	Lab	-	-	151.8	25	95.5	25	1

3.11.7 Experiment 11 – Effect of 2.5 ppm Aqua on Sludgewash Wastewater

Solutions were prepared by adding dry Aqua to the sludgewash wastewater solutions (Table 3.9). Ammonia and nitrate were not added because the wastewater contained about 700 ppm ammonia and 60 ppm nitrate. Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, sludgewash wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. The lab samples had a total volume of 1L. About 2.5 mg of Aqua was dosed into one lab solution to produce 2.5 ppm Aqua, as described in Section 3.4. A lab control of sludgewash wastewater was prepared. A DI lab control was prepared as well. About 151.8 mg of NaNO_3 and 95.5 mg of NH_4Cl were added to the DI lab control to produce 25 ppm $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, as described in Section 3.4. Aqua was not added to the controls. The bioreactor was set up according to Section 3.10. The bioreactor solution had a total volume of 151L. About 378 mg of Aqua was dosed into the bioreactor solution to produce 5 ppm Aqua, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as

described in Section 3.10. Sampling was conducted twice a day for five days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.9: Weights and concentrations for experiment 11

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Ammonia (NH ₄ Cl)		Total Vol (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Sludgewash with Aqua	Field	378	2.5	-	-	-	-	151
Lab Sludgewash with Aqua	Lab	2.5	2.5	-	-	-	-	1
Lab Sludgewash without Aqua	Lab	-	-	-	-	-	-	1
Control	Lab	-	-	151.8	25	95.5	25	1

3.11.8 Experiment 12 – Effect of 25 ppm Activated Aqua on Secondary Clarifier

Wastewater

Solutions were prepared by adding activated and dry Aqua, and nitrate to the secondary clarifier wastewater solutions (Table 3.10). Samples were taken from the solutions to measure nitrate, nitrite, pH, and temperature. Ammonia was not measured because Aqua did not have any effect on ammonia for the past anoxic experiments with secondary clarifier wastewater.

Activated Aqua was prepared as described in Section 3.3. About 1L of growth media plus TSB was made, however the total volume was about 821 mL due to evaporation. About

6362.7 mg Aqua was weighed out and dosed into the growth media and TSB mixture, as described in Section 3.4. This resulted in a concentration of 7750 mg/L Aqua. The mixture needed to be in an aerated and warm environment for optimal growth. Therefore, the activated Aqua mixture was aerated and stored in the incubator at 27°C, as described in Section 3.5. Incubation and aeration lasted for 21 hours.

To create the solutions for the lab experiment, secondary clarifier wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. Dry and activated Aqua were dosed into the lab solutions as described in Section 3.4. Nitrate was dosed into lab solutions as described in Section 3.4. Two lab controls of secondary clarifier wastewater were prepared. A DI lab control was prepared as well. Aqua was not added to the controls. The bioreactor was set up according to Section 3.10. Dry and activated Aqua were dosed into the bioreactor solutions as described in Section 3.10. Nitrate was dosed into bioreactor solutions as described in Section 3.10. A bioreactor control of secondary clarifier wastewater was prepared. Aqua was not added to the control.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as

described in Section 3.10. Sampling was conducted twice a day for six days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.10: Weights and concentrations for experiment 12

Label	Sample in Field or Lab	BiOWiSH™			Nitrate (NaNO ₃)		Total Vol (L)
		Weight Dry Aqua (mg)	Volume Activated Aqua (mL)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Activated Aqua	Field	-	500	25	23500	25	155
BR Secondary with Dry Aqua	Field	3785	-	25	23500	25	155
BR Secondary without Aqua	Field	-	-	-	23500	25	155
Lab Secondary with Activated Aqua	Lab	-	3.2	25	151.8	25	1
Lab Secondary with Dry Aqua	Lab	25	-	25	151.8	25	1
Lab Secondary without Aqua	Lab	-	-	-	151.8	25	1
Lab Secondary without Aqua or nitrate	Lab	-	-	-	-	-	1
Control	Lab	-	-	-	151.8	25	1

3.11.9 Experiment 13 – Effect of 25 ppm Activated Aqua under Partial Aeration on Secondary Clarifier Wastewater

Solutions were prepared by adding activated and dry Aqua, and nitrate to the secondary clarifier wastewater solutions (Table 3.11). Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

Activated Aqua was prepared as described in Section 3.3. About 1L of growth media plus TSB was made, however the total volume was about 799 mL due to evaporation. About 6192.3 mg Aqua was weighed out and dosed into the growth media and TSB mixture, as described in Section 3.4. This resulted in a concentration of 7750 mg/L Aqua. The mixture needed to be in an aerated and warm environment for optimal growth. Therefore, the activated Aqua mixture was aerated and stored in the incubator at 26°C, as described in Section 3.5. Incubation and aeration lasted for 40 hours.

The bioreactor was set up according to Section 3.10. The bioreactor solutions had a total volume of about 155L. About 500 mL of activated Aqua was added to the one bioreactor solution to produce 25 ppm Aqua, as described in Section 3.10. About 3785 mg of dry Aqua was added to the one bioreactor solution to produce 25 ppm Aqua, as described in Section 3.10. A bioreactor control of secondary clarifier wastewater was prepared. Aqua was not added to the control. About 23500 mg of NaNO_3 and 14800 mg of NH_4Cl were added to all bioreactor solutions to produce 25 ppm $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, as described in Section 3.10. To create the control solution for the lab experiment, DI was prepared according to Section 3.2. The DI lab solution had a total volume of 1L. About 151.8 mg of NaNO_3 and 95.5 mg of NH_4Cl were added to the DI lab solution to produce 25 ppm $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$, as described in Section 3.4. Aqua was not added to the DI lab solution. Lab solutions were not prepared for wastewater because there were not enough aerators to be used both in lab and in field.

During the experiment, the lab solution was kept in anoxic conditions. In order to keep the lab solution in an anoxic and warm environment, the solution was capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. During the experiment, the bioreactor solutions needed to be in an aerobic and warm environment so bacterial growth could occur. In order to keep the bioreactor solutions in an aerobic and warm environment, the bioreactor solutions were set up according to section 3.10. Sampling was conducted twice a day for seven days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.11: Weights and concentrations for experiment 13

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Ammonia (NH ₄ Cl)		Total Vol (L)
		Weight or Volume Aqua	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Activated Aqua	Field	500 mL Activated	25	23500	25	14800	25	155
BR Secondary with Dry Aqua	Field	3785 mg Dry	25	23500	25	14800	25	155
BR Secondary without Aqua	Field	-	-	23500	25	14800	25	155
Control	Lab	-	-	151.8	25	95.5	25	1

3.11.10 Experiment 14 – Effect of 25 ppm Biogenesis on Secondary Clarifier

Wastewater

Solutions were prepared by adding Biogenesis, Aqua, and nitrate to the secondary clarifier wastewater solutions (Table 3.12). Samples were taken from the solutions to measure nitrate, nitrite, pH, and temperature. Ammonia was not measured because Aqua did not

have any effect on ammonia for the past anoxic experiments with secondary clarifier wastewater.

To create the solutions for the lab experiment, secondary clarifier wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. Aqua and Biogenesis were dosed into the lab solutions, as described in Section 3.4. A lab control of secondary clarifier wastewater was prepared. A DI lab control was prepared as well. Aqua and Biogenesis were not added to the controls. Nitrate was dosed into lab solutions, as described in Section 3.4. The bioreactor was set up according to Section 3.10. Aqua and Biogenesis were dosed into the bioreactor solutions, as described in Section 3.10. A bioreactor control of secondary clarifier wastewater was prepared. Aqua and Biogenesis were not added to the control. Nitrate was dosed into bioreactor solutions, as described in Section 3.10.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 26°C as described in Section 3.5. The bioreactor solutions were also kept in anoxic conditions, as described in Section 3.10. Sampling was conducted twice a day for five days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.12: Weights and concentrations for experiment 14

Label	Sample in Field or Lab	Bacterial Product		Nitrate (NaNO ₃)		Total Vol (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Biogenesis	Field	23500 Biogenesis	25	3875	25	155
BR Secondary with Aqua	Field	23500 Aqua	25	3875	25	155
BR Secondary without Aqua	Field	-	-	3875	25	155
Lab Secondary with Biogenesis	Lab	25 Biogenesis	25	151.8	25	1
Lab Secondary with Aqua	Lab	25 Aqua	25	151.8	25	1
Lab Secondary without Aqua	Lab	-	-	151.8	25	1
Control	Lab	-	-	151.8	25	1

3.12 Laboratory Method on Field Wastewater during Cold Weather

Laboratory methods were conducted during colder weather conditions. If bioreactor experiments were conducted during the winter, degradation rates would be slower. Due to limited time, experiments were conducted in lab only.

3.12.1 Experiment 15 – Effect of 25 ppm Biogenesis on Secondary Clarifier

Wastewater Conducted in Lab

Solutions were prepared by adding Biogenesis, Aqua, and nitrate to the secondary clarifier wastewater solutions (Table 3.13). Samples were taken from the solutions to measure nitrate, nitrite, pH, and temperature. Ammonia was not measured because Aqua did not have any effect on ammonia for the past anoxic experiments with secondary clarifier wastewater.

To create the solutions for the lab experiment, secondary clarifier wastewater was collected from the treatment plant as described in Section 3.10 and DI was prepared according to Section 3.2. All solutions had a total volume of 1L. About 25 mg of Biogenesis was dosed into one wastewater solution, as described in Section 3.4. This created a concentration of 25 mg/L of Biogenesis. About 25 mg of Aqua was dosed into one wastewater solution, as described in Section 3.4. This created a concentration of 25 mg/L of Aqua. A lab control of secondary clarifier wastewater was prepared. A DI lab control was prepared as well. Aqua and Biogenesis were not added to the controls. About 151.8 mg of NaNO_3 was added to all solution bottles to produce a concentration of 25 mg/L $\text{NO}_3\text{-N}$, as described in Section 3.4.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 25°C , as described in Section 3.5. Sampling was conducted twice a day for six days. Samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.13: Weights and concentrations for experiment 15

Label	Bacterial Product		Nitrate (NaNO ₃)		Total Vol (L)
	Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
Secondary with Biogenesis	25 Biogenesis	25	151.8	25	1
Secondary with Aqua	25 Aqua	25	151.8	25	1
Secondary without Aqua	-	-	151.8	25	1
Control	-	-	139.7	25	0.92

3.12.2 Experiment 16 – Effect of 25 ppm Activated Aqua on Final Clarifier**Wastewater**

Solutions were prepared by adding activated and dry Aqua, ammonia, and nitrate to the final clarifier wastewater solutions (Table 3.14). Samples were taken from the solutions to measure ammonia, nitrate, nitrite, pH, and temperature.

Activated Aqua was prepared as described in Section 3.3. About 1L of growth media plus TSB was made, however the total volume was about 799 mL due to evaporation. About 6192.3 mg Aqua was weighed out and dosed into the growth media and TSB mixture, as described in Section 3.4. This resulted in a concentration of 7750 mg/L Aqua. The mixture needed to be in an aerated and warm environment for optimal growth. Therefore, the activated Aqua mixture was aerated and stored in the incubator at 26°C, as described in Section 3.5. Incubation and aeration lasted for 45 hours.

To create the solutions for the lab experiment, final clarifier wastewater was collected from the treatment plant according to Section 3.10 and DI was prepared according to Section 3.2. All solutions had a total volume of 1L. About 25 mg of dry Aqua was dosed into one

lab solution to produce 25 ppm Aqua, as described in Section 3.4. About 3.22 mL of activated Aqua was dosed into one lab solution to produce 25 ppm activated Aqua, as described in Section 3.4. Two lab controls of final clarifier wastewater were prepared. One lab wastewater control had ammonia and nitrate additions, and the other had no additions. A DI lab control was prepared as well. Aqua was not added to the controls. Nitrate and ammonia were dosed into lab solutions as described in Section 3.4. About 95.5 mg of NH_4Cl and 151.8 mg of NaNO_3 was added to all solution bottles, except one wastewater control solution. This produced a concentration of 25 mg/L $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$.

During the experiment, lab solutions needed to be in an anoxic and warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 26°C, as described in Section 3.5. Sampling was conducted twice a day for seven days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.14: Weights and concentrations for experiment 16

Label	BiOWiSH™		Nitrate (NaNO ₃)		Ammonia (NH ₄ Cl)		Total Vol (L)
	Weight or Volume Aqua	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	Weight (mg)	Conc as N (mg/L)	
Final with Activated Aqua	3.22 mL Activated Aqua	25	151.8	25	95.5	25	1
Final with dry Aqua	25 mg Dry	25	151.8	25	95.5	25	1
Final without Aqua	-	-	151.8	25	95.5	25	1
Final without Aqua or nitrate	-	-	-	-	-	-	1
Control	--	--	151.8	25	95.5	25	1

3.12.3 Experiment 17 – Effect of 25 ppm Aqua on Final Clarifier Plus 5% Primary Clarifier Wastewater

Solutions were prepared by adding Aqua, nitrate, and 5% primary clarifier wastewater to the final clarifier wastewater solutions (Table 3.15). Samples were taken from the solutions to measure nitrate, nitrite, pH, and temperature.

To create the solutions for the lab experiment, final and primary clarifier wastewater was collected from the treatment plant as described in Section 3.10 and DI was prepared according to Section 3.2. All solutions had a total volume of 1L. About 50 mL of primary clarifier wastewater was added to 950 mL of final clarifier wastewater. Therefore, 5% of the total volume was primary clarifier wastewater. There were two solutions with Aqua where one acted as a duplicate. About 25 mg dry Aqua was dosed into the two lab solutions,

as described in Section 3.4. This created a concentration of 25 mg/L Aqua. A lab control of final clarifier with primary clarifier wastewater was prepared. A DI lab control was prepared as well. Aqua was not added to the controls. About 151.8 mg of NaNO_3 was added to all solution bottles to produce a concentration of at least 25 mg/L $\text{NO}_3\text{-N}$, as described in Section 3.4. The primary and final clarifier wastewater solution already contained nitrate, therefore the wastewater solution ended up totaling around 60 mg/L $\text{NO}_3\text{-N}$.

During the experiment, lab solutions needed to be in an anoxic environment so nitrate removal could be studied. Lab solutions also needed to be in a needed to be in a warm environment so bacterial growth could occur. In order to keep the lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 26°C, as described in Section 3.5. Sampling was conducted twice a day for five days. Laboratory and bioreactor samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.15: Weights and concentrations for experiment 17

Label	BiOWiSH™		Nitrate (NaNO_3)		Total Volume (L)
	Weight (mg)	Concentration (mg/L)	Weight (mg)	Concentration as N (mg/L)	
Final + 5% Primary with Aqua 1	25	25	151.8	25	1
Final + 5% Primary with Aqua 2	25	25	151.8	25	1
Final + 5% Primary without Aqua	-	-	151.8	25	1
Control	-	-	151.8	25	1

3.12.4 Experiment 18 – Effect of 500 ppm Activated Aqua under High Aeration on Growth Media

Solutions were prepared by adding activated and dry Aqua, ammonia, and nitrate to the growth media solutions (Table 3.16). Samples were taken from the solutions to measure nitrate, nitrite, pH, temperature, and DO.

Activated Aqua was prepared as described in Section 3.3. About 2L of growth media plus TSB was made according to the Section 3.1 and 3.2. About 1.5L of this mixture was set aside for creating the solutions. About 400 mL of the mixture was used for activated Aqua. About 1000 mg Aqua was weighed out and dosed into the growth media and TSB mixture, as described in Section 3.4. This resulted in a concentration of 2500 mg/L Aqua. The mixture needed to be in an aerated and warm environment for optimal growth. Therefore, the activated Aqua mixture was aerated and stored in the incubator at 25°C, as described in Section 3.5. Incubation and aeration lasted for 18.5 hours.

To create the solutions for the lab experiment, the growth media plus TSB made earlier was collected and DI was prepared according to Section 3.2. All solutions had a total volume of 500 mL. Activated Aqua was dosed into the lab solutions as described in Section 3.4. About 100 mL of activated Aqua was dosed into each growth media solution. A DI lab control was prepared as described in Section 3.2. Aqua was not added to the control. Nitrate was dosed into lab solutions as described in Section 3.4. About 151.8 mg of NaNO_3 was added to all solution bottles to produce a concentration of 50 mg/L $\text{NO}_3\text{-N}$. There were

two growth medium solutions that were aerated where one acted as a duplicate. One growth medium solution was anoxic.

During the experiment, one growth medium solution and the DI control solution needed to be in an anoxic environment so nitrate removal could be studied. The two solutions also needed to be in a warm environment so bacterial growth could occur. In order to keep the two lab solutions in an anoxic and warm environment, solutions were capped with a solid lid and kept in the incubator at 25°C. During the experiment, lab samples needed to be in an aerobic and warm environment so bacterial growth could occur. In order to keep the lab samples in an aerobic and warm environment, samples were bubbled on high with the aerators, listed in Section 3.3, and incubated at 25°C, as described in Section 3.5. Sampling was conducted five times a day for three days. Laboratory samples were prepared and analyzed according to Section 3.6 and 3.7.

Table 3.16: Weights and concentrations for experiment 18

Label	BiOWiSH™		Nitrate (NaNO ₃)		Total Volume (L)
	Volume (mL)	Concentration (mg/L)	Weight (mg)	Concentration as N (mg/L)	
Growth Media with Aqua 1	100	500	151.8	50	0.5
Growth Media with Aqua 2	100	500	151.8	50	0.5
Growth Media without air	100	500	151.8	50	0.5
Control	-	-	151.8	50	0.5

CHAPTER 4: RESULTS AND DISCUSSION

Ammonia, nitrate, nitrite, pH, and temperature were measured for laboratory and field data. The method detection limit (MDL) for the Timberline was 6 mg/L. The MDL for the Ion Chromatograph was 1 mg/L. Any concentrations below these limits cannot be accurately detected and will be labelled as non-detect (ND) in tables. The lower concentrations will be shown in graphs for easier analysis. Since the discharge limit for ammonia is 0.025 mg/L as nitrogen, it will be impossible to make conclusions on whether ammonia removal below the discharge limit occurred.

Control verification standards, spikes, and splits were tested for each experiment. Different times between the pH, temperature, ammonia concentrations, and nitrate and nitrite concentrations occurred because data that did not pass these quality assurance and quality control tests were discarded.

Degradation rate constants were calculated using trend lines on graphs. Sample calculations were shown in appendix B.

Sample preparation and procedural error can cause contamination. Errors included improperly cleaned IC and timberline tubes and caps, improperly cleaned and sterilized TOC tubes and caps, improperly cleaned and sterilized experiment bottles and caps, improperly rinsed pH and temperature probe, and improperly rinsed filter materials. Filtering can cause a lower concentration in samples because undried filtering materials release drops of DI into the sample. The time to run through samples in the IC and

timberline can be long. This can increase the temperature of the samples and potentially cause the bacteria to continue their metabolic processes. The metabolic processes can decrease the concentrations in the sample. The acidification and filtration should help prevent bacteria from continuing their metabolic processes. However, it could still happen. Volatilization of ammonia can occur before the samples were acidified. Samples were poured out into TOC vials and stay open to the air while pH and temperature were read. If experiment bottles were open to the air, then evaporation or volatilization could occur. Evaporation would result in increased concentrations of nitrogen, while volatilization would result in decreased concentrations of ammonia. Beakers of water were added to open air runs to prevent evaporation. However, some evaporation of solutions can still occur.

4.1 Laboratory Results

Preliminary laboratory experiments analyzed the metabolisms of Aqua and of different bacterial species within Aqua. Analysis of nitrification and denitrification in wastewater at different treatment processes at the SLO WRRF was conducted. The impact of different inoculation methods on nitrification and denitrification was analyzed. The effects of more concentrated Aqua on nitrification and denitrification were also analyzed. The effects of trace minerals on nitrate removal were also analyzed.

4.1.1 Experiment 1 – Effect of *Bacillus* Strains and Aqua on Denitrification

Nitrate removal for Aqua and other *Bacillus* strains were tested in growth media. This experiment determined whether Aqua denitrifies in growth media, similar to the research study conducted by BiOWiSH™ (Gorsuch, Roberts, Lenhoff, & Showell). It also

determined which strains were responsible for denitrification and if combining all *Bacillus* strains created a symbiotic or antibiotic relationship. *Bacillus* bacteria were assumed to follow typical anoxic heterotrophic metabolic processes for denitrification. Nitrate and nitrite were measured over a span of 3 days (Table 4.1, Table 4.2, Figure 4.1, and Figure 4.2).

Table 4.1: Nitrate concentrations in mg/L NO₃-N for experiment 1

Time (hours)	0	8	16	23	33	58
GM with Aqua	53.28	ND	ND	ND	ND	ND
GM with <i>B. Amyloliquefaciens</i>	53.47	28.25	2.11	3.90	2.97	ND
GM with <i>B. Licheniformis</i>	53.18	ND	ND	ND	ND	ND
GM with <i>B. Subtilis Mojavensis</i>	58.36	39.14	12.99	2.87	ND	ND
GM with <i>B. Pumilus</i>	54.42	55.28	55.40	55.67	55.80	55.71

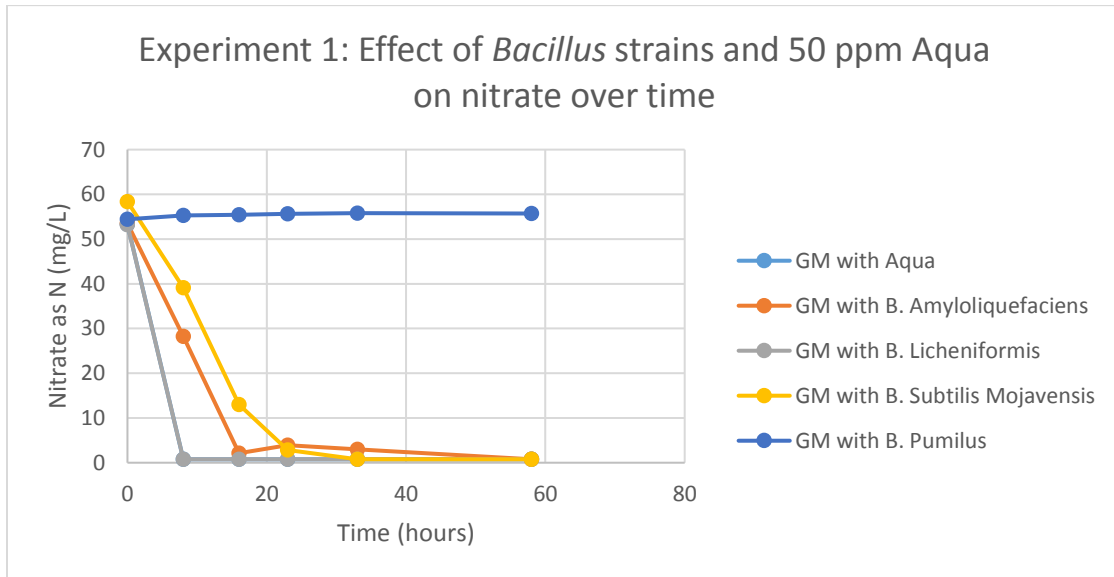


Figure 4.1: Effect of *Bacillus* strains and 50 ppm Aqua on nitrate over time

Table 4.2: Nitrite concentrations in mg/L NO₂-N for experiment 1

Time (hours)	0	8	16	23	33	58
GM with Aqua	ND	49.46	48.98	47.81	44.73	39.01
GM with <i>B. Amyloliquefaciens</i>	ND	ND	ND	ND	1.57	ND
GM with <i>B. Licheniformis</i>	ND	41.18	35.14	25.87	10.33	3.13
GM with <i>B. Subtilis Mojavensis</i>	ND	15.07	35.72	45.26	45.10	33.52
GM with <i>B. Pumilus</i>	ND	ND	ND	ND	ND	ND

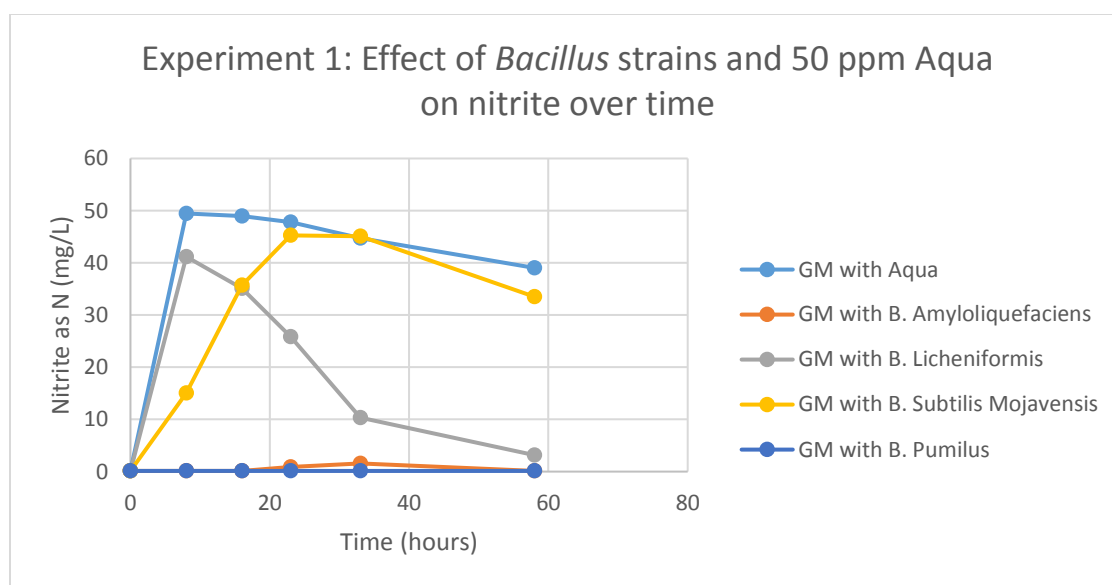


Figure 4.2: Effect of *Bacillus* strains and 50 ppm Aqua on nitrite over time

Denitrification occurred for Aqua and all *Bacillus* species, except *Bacillus pumilus*. The Aqua and *Bacillus licheniformis* achieved the greatest nitrate removal rates. However, *Bacillus licheniformis* achieved a better nitrite removal rate when compared to Aqua. A slower nitrite removal rate in Aqua was likely because bacterial competition occurred in the Aqua mixture. The *Bacillus licheniformis* prevailed in nitrate removal in the Aqua mixture. However, a different bacterium in the mixture had a more efficient metabolic process for using nitrite, so that bacteria prevailed in nitrite removal. A slower nitrite

removal rate for Aqua could be because Aqua did not incubate for 32 hours, while the other *Bacillus* bacteria did. Therefore, incubation of Aqua could make a difference in nitrite removal.

Bacillus amyloliquefaciens had the next best nitrate removal rate. The metabolic process for this specific bacteria was unique since it did not produce any nitrite. Nitrate removal likely occurred via assimilation or denitrification without production of intermediate nitrite. Nitrogen gas and organic nitrogen content would need to be measured to know the exact process.

Bacillus subtilis mojavensis had the slowest nitrate removal rate. Excluding the *Bacillus pumilus* strain, which experienced no nitrate removal. In previous studies, *Bacillus subtilis mojavensis* performed nitrification more readily (Section 2.3). Therefore, this bacterium could achieve nitrification and denitrification. However, denitrification would occur at a slower rate. *Bacillus subtilis mojavensis* follows the typical denitrification process where a decrease in nitrate results in an increase in nitrite. However, the decrease in nitrite from denitrification was slow compared to the other bacteria. Aqua more closely followed *Bacillus subtilis mojavensis* nitrite removal rates.

Zero and first order degradation rates were calculated for nitrate removal. Samples followed zero order degradation for nitrate removal. Therefore, zero order kinetics values were compared to other literature values (Table 4.3). The total amount of nitrate removed was calculated by subtracting the initial value from the first value to reach zero (Table 4.4).

Aqua achieved a specific and volumetric denitrification rate in the middle range of reported literature values. The other *Bacillus* bacteria, except for *Bacillus pumilus*, also achieved a volumetric denitrification rate in the middle range of the reported literature values. Over the course of 1 day, the nitrate levels reached below the discharge permit level of 10 mg/L for the SLO WRRF. Some reached this level in as little as 8 hours.

Table 4.3: Denitrification rates for experiment 1

Source	System	Specific Denitrification Rate (mg NO ₃ -N/mg MLVSS/d)	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temp
This Study	Aqua	3.15*	157.54	~30°C
	<i>Bacillus Amyloliquefaciens</i>	N/A	77.05	
	<i>Bacillus Licheniformis</i>	N/A	157.23	
	<i>Bacillus Subtilis Mojavensis</i>	N/A	60.26	
(Metcalf & Eddy, 2003)	Preanoxic Tanks	0.04 – 0.42	95 – 995 ¹	N/A
	Postanoxic Tanks	0.01 – 0.04	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	N/A	240	10°C
		N/A	730	20°C
(Reardon, Kolby, & Odo, 1996)	Batch Tests	0.032 – 0.07	N/A	N/A
(Lee, 2012)	Column Tests with Mineral Media	0.192	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	N/A	10-30	N/A

¹Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

*Calculated by dividing the volumetric denitrification rate by initial concentration of Aqua added

Table 4.4: Total nitrate removed and removal rates for experiment 1

Sample	Amount Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
GM with Aqua	52.28	8	6.5643	0.5297
GM with <i>B. Amyloliuefaciens</i>	51.36	16	3.2103	0.0682
GM with <i>B. Licheniformis</i>	52.18	8	6.5512	0.5295
GM with <i>B. Subtilis Mojavensis</i>	55.49	23	2.5107	0.139
GM with <i>B. Pumilus</i>	-	-	-	-

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

According to research done by Eva Lee for BiOWiSH™, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. In this experiment, carbon was added from TSB, growth media, and Aqua. Growth media added 1000 mg/L dextrose. TSB added 250 mg/L dextrose because only 10% of the TSB was added to the solution. Aqua added 47.5 mg/L dextrose because about 95% of Aqua is dextrose. The solution contained a total of 1297.5 mg/L dextrose, which is 518.5 mg/L dextrose as carbon. The initial nitrate concentration ranged from 53 to 58 ppm. This created a carbon to nitrogen ratio of 9.4:1, which was above the ideal nitrate removal rate. Therefore, the C:N ratio could have decreased the nitrate removal rates.

The total nitrogen was calculated by adding the nitrate and nitrite (Figure 4.3). The *Bacillus Amyloliuefaciens* had the best total nitrogen removal because it had the second best nitrate removal rate and did not produce any nitrite. The *Bacillus Licheniformis* also had good total nitrogen removal because it had the fastest nitrate removal rate and it removed the nitrite that spiked from denitrification. Although Aqua and *Bacillus Subtilis Mojavensis*

had good nitrate removal, they were not able to remove nitrite. Bacteria competition was likely occurring between the different bacterial species in Aqua. The bacteria dominant in nitrate removal was likely *Bacillus Licheniformis*. The bacteria dominant in nitrite removal was likely *Bacillus Subtilis Mojavensis*.

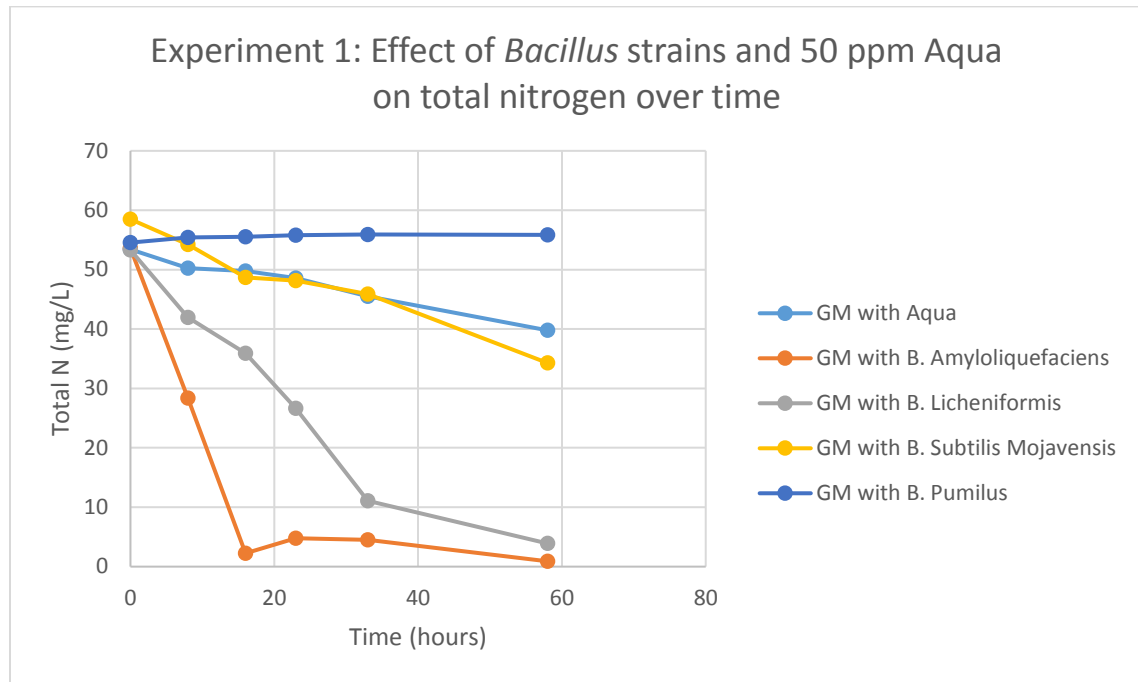


Figure 4.3: Effect of *Bacillus* strains and 50 ppm Aqua on total nitrogen over time

Aqua was proven to undergo denitrification from this experiment, experiments performed by Eva Lee, and experiments performed by BiOWiSHTM (Lee, 2012; Gorsuch, Roberts, Lenhoff, & Showell). Next, nitrate removal was analyzed in sterilized wastewater obtained from different industrial treatment processes at the SLO WRRF to determine if the same processes and rates occur. Different treatment process wastewaters were analyzed to determine which wastewater Aqua performs best in. Ammonia removal was also analyzed to determine whether nitrogen can be removed in an earlier treatment process at the SLO WRRF.

4.1.2 Experiment 2a & 2b – Effect of 50 ppm Aqua on Wastewater Collected from DAFT and Primary, Secondary, and Final Clarifiers

Ammonia and nitrate removal were analyzed for Aqua in partially sterilized wastewater from the primary clarifier, secondary clarifier, final clarifier, and DAFT. Effects of different wastewater types on ammonia and nitrate removal were analyzed. Ammonia removal and nitrate removal were tested separately for this experiment. The ammonia run only had $\text{NH}_4\text{-N}$ added and nitrate/nitrite run only had $\text{NO}_3\text{-N}$ added.

Table 4.5: Labeling for experiment 2

Description	Label
In-lab DI with 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	Control
In-lab Growth Media with 25ppm Aqua and 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	GM with Aqua
In-lab Growth Media with 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	GM without Aqua
In-lab Secondary Clarifier with 25ppm Aqua and 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	Secondary with Aqua
In-lab Secondary Clarifier with 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	Secondary without Aqua
In-lab Primary Clarifier with 25ppm Aqua and 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	Primary with Aqua
In-lab Primary Clarifier with 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	Primary without Aqua
In-lab Final Clarifier with 25ppm Aqua and 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	Final with Aqua
In-lab Final Clarifier with 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	Final without Aqua
In-lab Dissolved Air Flotation Thickener with 25ppm Aqua and 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	DAFT with Aqua
In-lab Dissolved Air Flotation Thickener with 25ppm $\text{NO}_3\text{-N}$ or 25ppm $\text{NH}_4\text{-N}$	DAFT without Aqua

Ammonia removal was observed (Table 4.6 and Figure 4.4). Filtration and acidification were conducted before the experiment was started. The filtration removed some bacteria, since bacteria range from 0.2 to 3 micrometers in size (MWH, 2005). The acidification likely killed a lot of the natural bacteria as well.

Table 4.6: Ammonia concentrations in mg/L NH₄-N for experiment 2

Time (hours)	0	24	72	96	Amount Degraded
Control	28.28	27.90	31.65	32.48	-4.2
GM with Aqua	24.84	24.50	29.30	30.19	-5.35
GM without Aqua	28.17	28.29	31.57	29.51	-1.34
Secondary with Aqua	24.28	16.13	7.50	ND	18.28
Secondary without Aqua	17.70	10.94	ND	ND	11.7
Primary with Aqua	45.62	32.36	12.13	7.24	38.38
Primary without Aqua	35.63	23.02	9.05	ND	29.63
Final with Aqua	40.76	30.79	12.94	7.35	33.41
Final without Aqua	33.61	22.33	8.48	ND	27.61
DAFT with Aqua	48.39	35.32	13.14	6.06	42.33
DAFT without Aqua	40.81	20.27	4.93	2.99	37.82

*Amount degraded assumes the non-detect values are 6 mg/L, which is the detection limit. This gives a conservative estimate of the amount of ammonia removed.

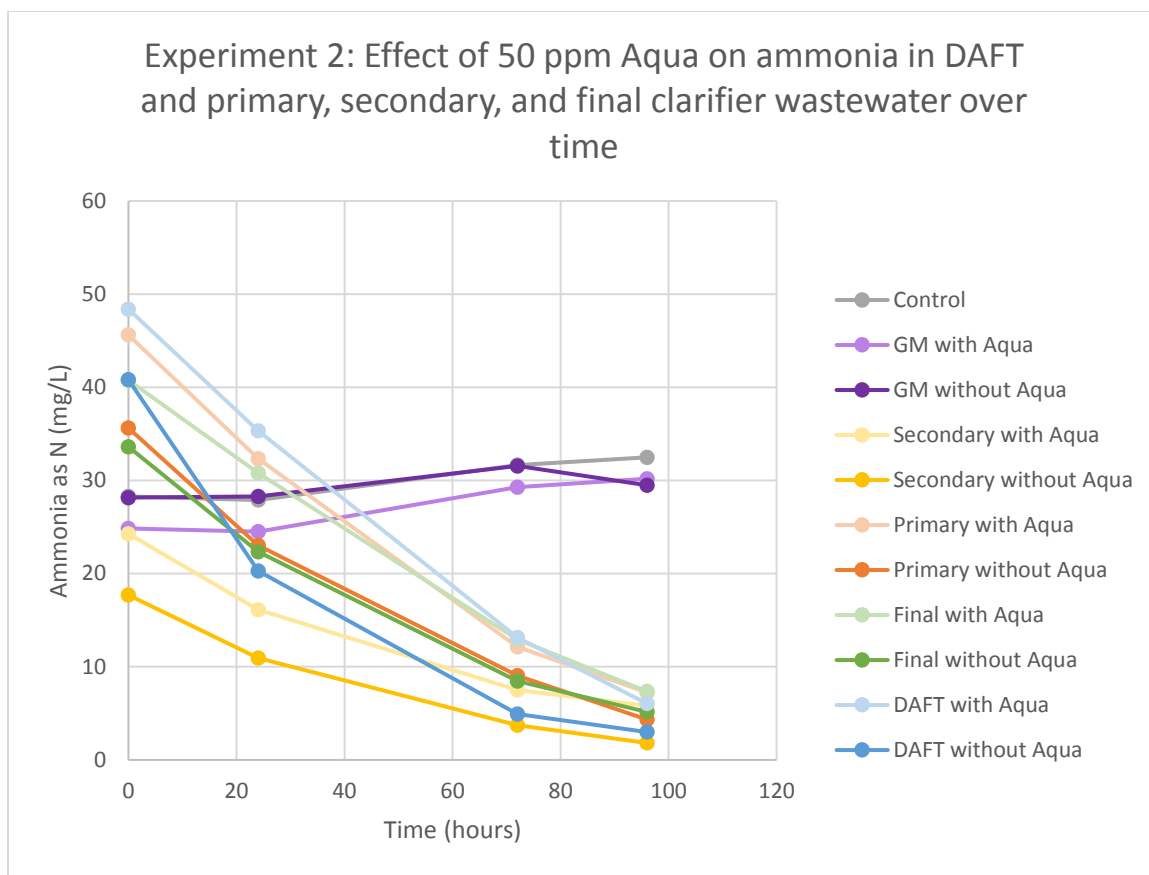


Figure 4.4: Effect of 50 ppm Aqua on ammonia in DAFT and primary, secondary, and final clarifier wastewater over time

Ammonia removal occurred for both the natural bacteria and Aqua. Although the starting points of the samples were not the same, the amount degraded over time can be analyzed to determine whether Aqua made a difference in the removal of ammonia. Aqua generally started at a higher concentration than the natural bacteria because the TSB adds ammonia to solution. Natural bacteria were assumed to follow the typical chemolithoautotrophic metabolic processes and the Aqua was assumed to follow the typical heterotrophic metabolic processes known for Aqua bacteria. The nitrate, nitrite, organic nitrogen, and nitrogen gas data needs to be observed to determine if assimilation or nitrification occurred.

For now, nitrification will be assumed as the only source of ammonia removal so nitrification removal rates can be compared to typical literature values.

The growth media and DI control samples experienced an increase in ammonia due to sample preparation error. The error was likely due to evaporation from the open air environment, which increased the concentration of nitrogen in the water. Growth media with Aqua did not experience nitrification because the growth media recipe was not ideal for nitrification to occur (e.g. not enough trace minerals were provided). The amount of ammonia removed using Aqua was not much higher than the natural bacteria. The increase was likely due to higher concentrations of ammonia to start in Aqua solutions. The removal rate will increase with a larger starting concentration in solution, as long as the larger starting concentration is not toxic to the bacteria (Zhang, Liu, Ai, Miao, Zheng, & Liu, 2012). Therefore, the Aqua did not prevail in ammonia removal. The natural bacteria were responsible for the ammonia removal. Therefore, autotrophic ammonia removal rates of the natural bacteria were preferable to the heterotrophic ammonia removal rates of the Aqua bacteria. Ammonia removal may not have occurred for Aqua because the pH was too low for Aqua to perform nitrification.

Zero and first order degradation rates were calculated for ammonia removal. Samples followed a zero order degradation rate for ammonia removal. Therefore, zero order kinetics values were compared to other literature values (Table 4.7). The amount of ammonia removed was calculated by subtracting the initial value from the final value (Table 4.8). Since the solutions with Aqua and without Aqua were dominated by the natural bacteria,

the nitrification rates will be a range between the two. All wastewater solutions achieved a volumetric nitrification rate lower than reported literature values. Low nitrification rates were likely due to low oxygen concentrations from slightly aerobic conditions and partially sterile conditions.

Table 4.7: Nitrification rates for experiment 2

Source	System	Volumetric Nitrification Rate (mg NH ₄ -N/L/d)	Temp
This Study	Secondary Wastewater without Aqua	3.9 – 4.6	~30°C
	DAFT Wastewater without Aqua	9.10 – 10.68	
	Final Wastewater without Aqua	7.08 – 8.47	
	Primary Wastewater without Aqua	7.66 – 9.7	
(Tarre & Green, 2004)	Attached Biomass Reactor	2000 – 5600	N/A
	Suspended Biomass Reactor	1100	N/A
(Tijhuris, Van Loorsdrecht, & Heijnen, 1992)	Biofilm Airlift Suspension Reactor	6000	N/A
(Choubert, Racault, Grasmick, Beck, & Hedit, 2005)	Extended Aeration Activated Sludge Pilot Plant	79.2 – 220.8	10°C
(Li & Wu, 2014)	Laboratory Sequencing Batch Reactors	122.4 – 254.4	25°C

Table 4.8: Total ammonia removed and removal rates for experiment 2

Sample	Amount Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
Final with Aqua	33.14	96	0.3528	0.0179
Final without Aqua	27.61	96	0.2949	0.0197
Secondary with Aqua	18.28	96	0.1898	0.0151
Secondary without Aqua	11.70	96	0.1624	0.0194
DAFT with Aqua	42.33	96	0.4452	0.0214
DAFT without Aqua	37.82	96	0.3791	0.0277
Primary with Aqua	38.38	96	0.4041	0.0235
Primary without Aqua	29.63	96	0.319	0.0214

*Amount degraded assumes the non-detect values are 6 mg/L, which is the detection limit. This gives a conservative estimate of the amount of ammonia removed.

Nitrate was observed (Table 4.9, Table 4.10, Figure 4.5, and Figure 4.6). Filtration through 1.2 micrometer filters was conducted before the experiment was started. The filtration removed some bacteria, since bacteria range from 0.2 to 3 micrometers in size (MWH, 2005).

Table 4.9: Nitrate concentrations in mg/L NO₃-N for experiment 2

Time (hours)	0	7	12	28.5	37	47	53	Amount Degraded
DAFT without Aqua	25.53	26.49	28.78	28.89	27.09	27.09	24.76	0.77
DAFT with Aqua	25.29	26.04	28.34	20.50	3.22	ND	ND	24.29
Primary without Aqua	25.66	26.37	28.61	28.78	27.03	27.05	26.38	-0.72
Primary with Aqua	25.82	26.43	28.67	26.46	17.62	ND	ND	24.82
Final without Aqua	34.68	35.34	37.97	37.93	36.08	35.94	36.20	-1.52
Final with Aqua	34.75	35.34	37.40	ND	ND	ND	ND	33.75
Secondary without Aqua	69.12	70.30	73.82	74.15	71.52	71.65	72.05	-2.94
Secondary with Aqua	69.45	14.50	9.30	1.14	1.07	1.21	1.16	68.29
GM without Aqua	26.33	26.71	28.84	28.95	27.23	27.09	26.54	-0.20
GM with Aqua	26.17	ND	ND	ND	ND	ND	ND	25.17
Control	26.36	26.64	28.80	28.88	27.18	27.21	27.32	-0.96

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

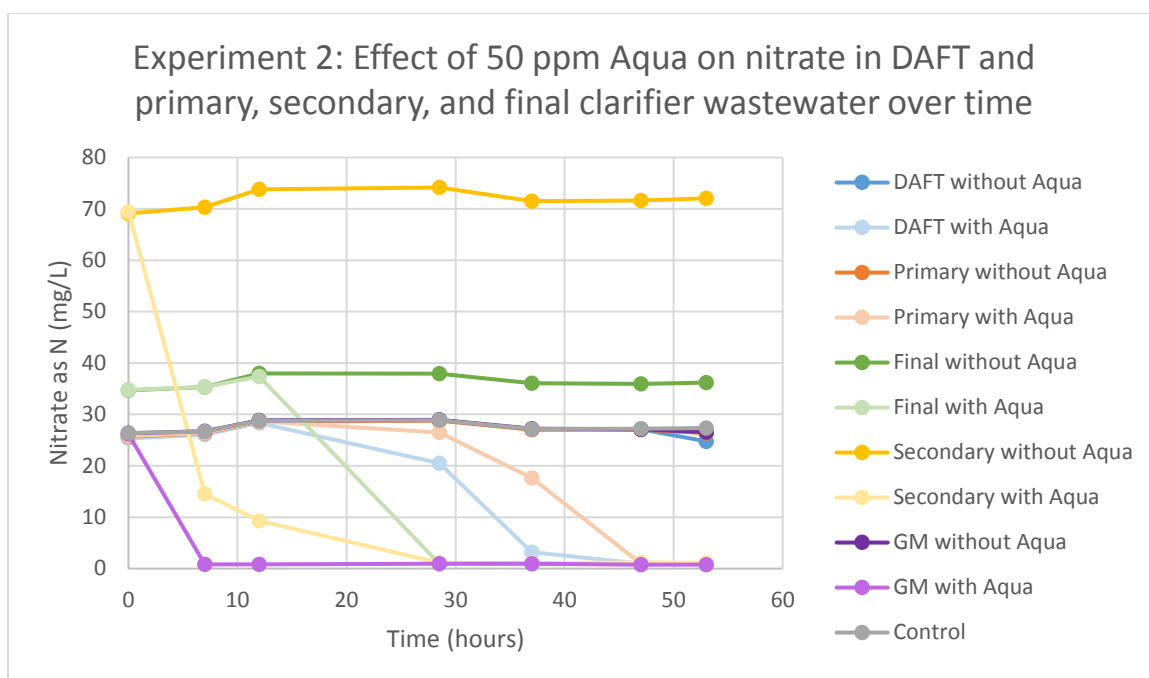


Figure 4.5: Effect of 50 ppm Aqua on nitrate in DAFT and primary, secondary, and final clarifier wastewater over time

Table 4.10: Nitrite concentrations in mg/L NO₂-N for experiment 2

Time (hours)	0	7	12	28.5	37	47	53	Amount Degraded
DAFT without Aqua	ND	ND	ND	ND	ND	ND	2.78	-1.78
DAFT with Aqua	ND	ND	ND	7.56	23.40	24.08	ND	0
Primary without Aqua	ND	ND	ND	ND	ND	ND	1.47	-0.47
Primary with Aqua	ND	ND	ND	2.50	8.88	25.56	15.33	-14.33
Final without Aqua	2.31	2.14	2.38	2.61	2.24	2.58	2.58	-0.27
Final with Aqua	2.24	2.26	2.90	37.58	35.58	35.11	33.98	-31.74
Secondary without Aqua	ND	ND	ND	ND	ND	ND	ND	0
Secondary with Aqua	ND	48.90	57.00	64.85	63.45	63.31	63.52	-62.52
GM without Aqua	ND	ND	ND	ND	ND	ND	ND	0
GM with Aqua	ND	24.68	25.79	26.04	24.90	20.21	10.16	-9.16
Control	ND	ND	ND	ND	ND	ND	ND	0

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrite removed.

*1st with increased to 14.33 ppm, then decreased. DAFT with increased to 23.08 ppm, then decreased.

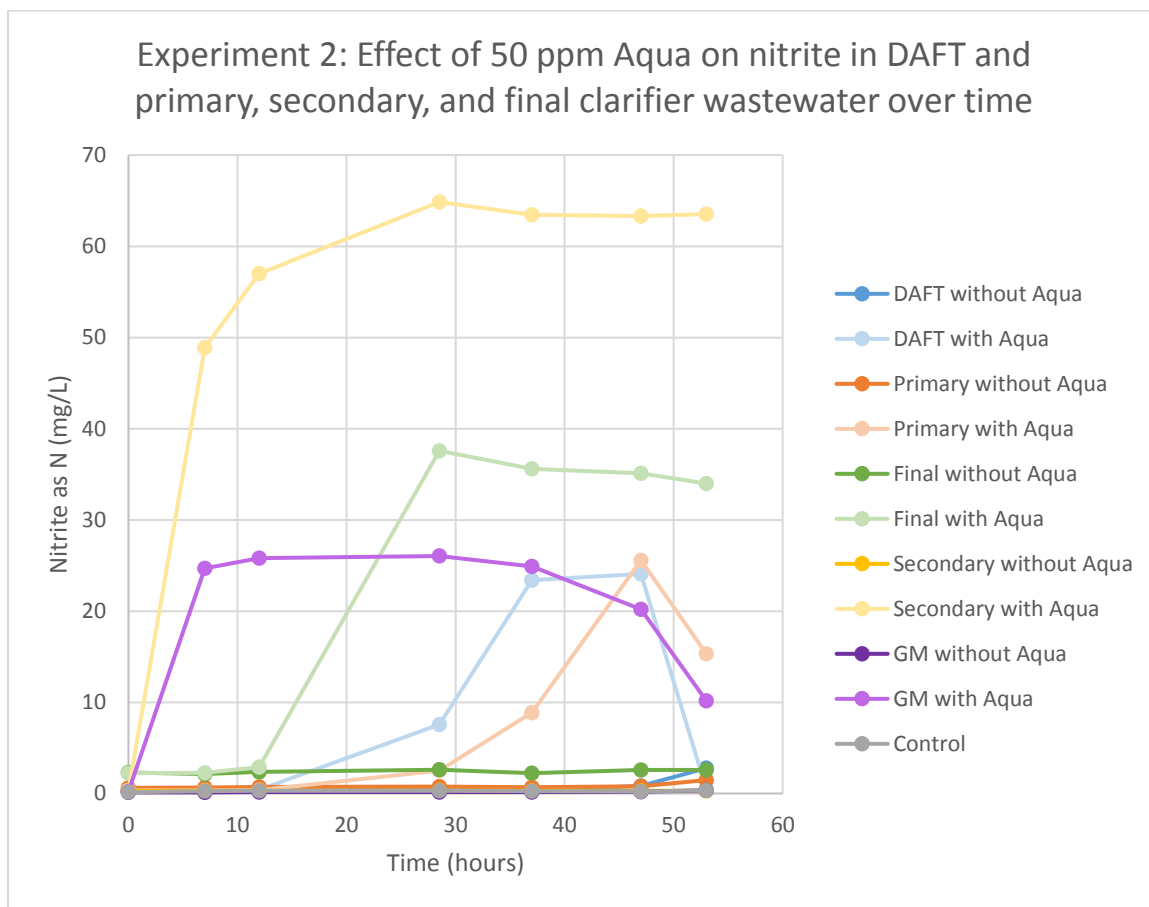


Figure 4.6: Effect of 50 ppm Aqua on nitrite in DAFT and primary, secondary, and final clarifier wastewater over time

Denitrification was observed in the anoxic environment for Aqua bacteria because as nitrate decreased, nitrite increased. Wastewater without Aqua maintained a steady amount of nitrate and nitrite because filtration likely got rid of many of the natural bacteria. Aqua was assumed to follow the typical heterotrophic metabolic processes known for Aqua bacteria.

The amounts of nitrate removed and nitrite produced were calculated. The secondary clarifier removed the most nitrate likely because it had a higher nitrate concentration to start (Zhang, Liu, Ai, Miao, Zheng, & Liu, 2012). However, there was no lag phase for nitrate removal in secondary clarifier wastewater. All samples achieved complete nitrate removal, but only DAFT, primary clarifier, and growth media achieved some nitrite removal as well. Secondary and final clarifier could likely remove nitrite with more time. They also have higher nitrite concentrations, which could potentially be toxic to nitrite removal bacteria.

Rapid nitrate removal with slow nitrite removal in GM corresponds with what was found in experiment 1. The different constituents in each WW change removal rates for Aqua. Secondary and final clarifier WW have quick nitrate removal rates with little to no nitrite removal. DAFT and primary WW had lagged and slower nitrate removal rates, but experienced nitrite removal. This could be because C:N ratios for DAFT and primary clarifier were higher than secondary and final clarifier WW.

Zero and first order degradation rates were calculated for nitrate removal. Samples followed a zero order degradation rate for nitrate removal. Therefore, zero order kinetics values were compared to other literature values (Table 4.11). The amount of nitrate removed was calculated by subtracting the initial value from the first value to reach zero (Table 4.12). Natural bacteria samples do not have degradation equations because nitrate removal did not occur. Aqua achieved a specific and volumetric denitrification rate in the middle range of reported literature values. Over the course of 2 days, the nitrate levels

reached below the discharge permit level of 10 mg/L for the SLO WRRF. Some reached this level in as little as 12 hours.

Table 4.11: Denitrification rates for experiment 2

Source	System	Specific Denitrification Rate (mg NO ₃ -N/mg MLVSS/d)	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temp
This Study	Secondary Wastewater with Aqua	N/A	124.66	~30°C
	DAFT Wastewater with Aqua	N/A	22.38	
	Final Wastewater with Aqua	N/A	53	
	Primary Wastewater with Aqua	N/A	33.29	
	Growth Media with Aqua	1.74*	86.97	
(Metcalf & Eddy, 2003)	Preanoxic Tanks	0.04 – 0.42	95 – 995 ¹	N/A
	Postanoxic Tanks	0.01 – 0.04	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	N/A	240	10°C
		N/A	730	20°C
(Reardon, Kolby, & Odo, 1996)	Batch Tests	0.032 – 0.07	N/A	N/A
(Lee, 2012)	Column Tests with Mineral Media	0.192	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	N/A	10-30	N/A

¹Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

*Calculated by dividing the volumetric denitrification rate obtained from graph by initial concentration of Aqua added

Table 4.12: Total nitrate removed and removal rates for experiment 2

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Amount Nitrite Increased (mg N/L)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
Final with Aqua	33.75	28.5	35.34	2.2083	0.2217
Secondary with Aqua	68.31	28.5	63.85	5.1943	0.1382
DAFT with Aqua	22.07	37	22.40	0.9326	0.1634
Primary with Aqua	24.82	47	24.56	1.3870	0.1828
GM with Aqua	25.17	7	23.68	3.6236	0.4974

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed. The same is assumed for the nitrite increased so that uniformity between nitrate removal and nitrite production is ensured.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts for each treatment process at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The final clarifier did not have carbon data. Therefore, carbon amounts were assumed to match with the monthly high effluent BOD value of 7.1 mg/L. The growth media has 1000 mg/L dextrose, which equates to 399.6 mg/L dextrose as carbon. A solution of TSB and Aqua was added to the different wastewaters. The TSB added 25 mg/L dextrose because only 1% of the TSB was added to each solution. The Aqua added 47.5 mg/L dextrose because 95% of Aqua is dextrose. The total carbon from TSB and Aqua was 29 mg/L dextrose as carbon. The C:N ratios were different for each wastewater (Table 4.13). For C:N ratios higher than 4:1, ammonia removal rates were inhibited. For C:N ratios less than 2:1, ammonia removal rates were lower. For C:N ratios higher than 6:1, nitrate removal rates were still high, but

not as high as a C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were lower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler M. , 2005). The C:N ratios for wastewater without Aqua were not included because the natural bacteria likely experience autotrophic metabolic rates. Therefore, BOD (organic carbon) cannot be used to find C:N ratios for the autotrophic bacteria. The C:N ratio for secondary clarifier solutions did not impact the ammonia removal rate. The high C:N ratios for primary clarifier, DAFT, and the growth media solutions could have inhibited the ammonia removal rates because higher carbon content inhibits enzymatic activity involved with ammonia assimilation (Lee, 2012). The low C:N ratios for final clarifier solutions could have decreased the ammonia removal rates. The high C:N ratios for growth media, DAFT, and primary clarifier with and without Aqua could have decreased the nitrate removal rates. The low C:N ratios for final clarifier and secondary clarifier with and without Aqua could have decreased the nitrate removal rates.

Table 4.13: Carbon to nitrogen ratios for experiment 2

WW	Carbon in WW ¹	Total NH ₄ -N ²	Total NO ₃ -N ²	Total C	C:N for NH ₄ -N	C:N for NO ₃ -N
Primary with Aqua	181.6	46	25	210.6	4.6:1	8.4:1
Primary without Aqua		36	25	181.6	-	7.3:1
Secondary with Aqua	70.48	24	70	99.48	3.9:1	1.4:1
Secondary without Aqua		18	70	70.48	-	1:1
Final with Aqua	7.1	41	35	36.1	0.9:1	1:1
Final without Aqua		34	35	7.1	-	0.2:1
DAFT with Aqua	190.8	48	25	219.8	4.6:1	8.8:1
DAFT without Aqua		41	25	190.8	-	7.6:1
GM with Aqua	399.6	25	25	428.6	17.1:1	17.1:1
GM without Aqua		25	25	399.6	16:1	16:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial, TSB, and Aqua. Total N includes initial, TSB, and spiked 25 mg/L

Total nitrogen removal was calculated by adding the nitrate and nitrite (Figure 4.7). Ammonia was not added because it was tested at a different time and under different conditions than the nitrate test. Total nitrogen stayed constant for all samples for about 45 hours. Near the end of the experiment, total nitrogen started to drop for primary clarifier and DAFT wastewater. This was because the nitrite concentrations started to decrease. Running the experiment for a longer time period may have showed further total nitrogen removal for the samples. The steady total nitrogen concentration over time follows the Aqua denitrification trend seen in Experiment 1.

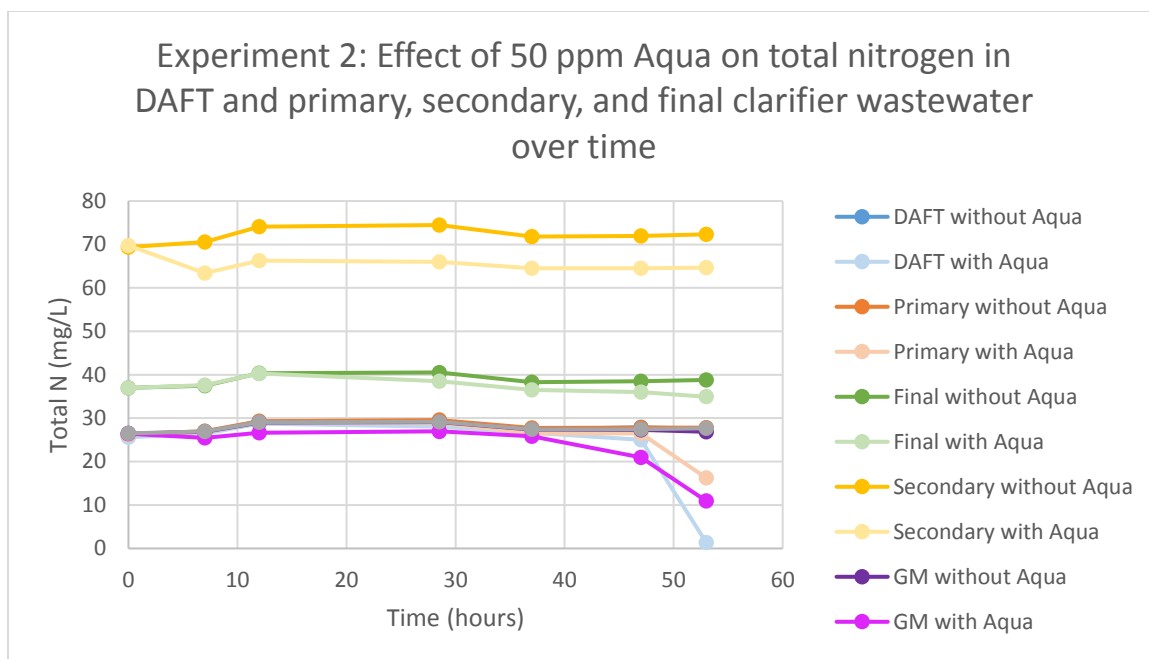


Figure 4.7: Effect of 50 ppm Aqua on total nitrogen in DAFT and primary, secondary, and final clarifier wastewater over time

Aqua achieved denitrification in this experiment, experiments performed by Eva Lee, and experiments performed by BiOWiSHTM (Gorsuch, Roberts, Lenhoff, & Showell; Lee, 2012). In experiments performed by Eva Lee and BiOWiSHTM, Aqua achieved ammonia removal from assimilation and intermediate nitrification in growth media and sterile wastewater. However, ammonia removal rates were similar between Aqua and natural bacteria in this experiment. Therefore, Aqua ammonia removal in partially sterile wastewater was not proven. In Experiment 3, unsterilized wastewater (i.e. no sterilization or filtration before the start of the experiment) was analyzed to determine if it has an impact on removal rates and bacterial metabolic processes of Aqua. Simultaneous partially aerobic nitrification and denitrification of Aqua was analyzed in unsterilized wastewater because Aqua bacteria are known to follow this process (Section 2.3 and 2.4).

If removal rates are improved with Aqua, then Aqua could be added to aerobic processes, such as activated sludge, to remove nitrogen.

4.1.3 Experiment 3 – Effect of 50 ppm Aqua on Wastewater Collected from DAFT, Sludgewash, Primary Clarifier, and Secondary Clarifier

Ammonia and nitrate removal were analyzed for Aqua in wastewater from the secondary clarifier, final clarifier, sludgewash, and DAFT (Table 4.16, Table 4.17, Table 4.18, Figure 4.8, Figure 4.9, Figure 4.10, and Figure 4.11). Effects of different wastewater types on ammonia and nitrate removal were analyzed. Ammonia and nitrate were tested simultaneously to determine if Aqua achieves aerobic nitrification and denitrification in unsterilized wastewater, similar to studies conducted by other researchers (Section 2.3 and 2.4). The pH was measured to determine its potential effects on ammonia and nitrate removal (Table 4.15). Different times between the pH, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is medium because less than half the data points did not pass QA/QC. Starting concentrations for ammonia, nitrate, and nitrite analysis are unknown because data was lost.

Table 4.14: Labeling for experiment 3

Description	Label
In-lab DI with 25ppm NO ₃ -N and 25ppm NH ₄ -N	Control
In-lab Growth Media with 25ppm NO ₃ -N and 25ppm NH ₄ -N	GM without Aqua
In-lab Secondary Clarifier with 25ppm Aqua, 25ppm NO ₃ -N, and 25ppm NH ₄ -N	Secondary with Aqua
In-lab Secondary Clarifier with 25ppm NO ₃ -N and 25ppm NH ₄ -N	Secondary without Aqua
In-lab Sludgewash with 25ppm Aqua	SW with Aqua
In-lab Sludgewash	SW without Aqua
In-lab Final Clarifier with 25ppm Aqua, 25ppm NO ₃ -N, and 25ppm NH ₄ -N	Final with Aqua
In-lab Final Clarifier with 25ppm NO ₃ -N and 25ppm NH ₄ -N	Final without Aqua
In-lab Dissolved Air Flotation Thickener with 25ppm Aqua, 25ppm NO ₃ -N, and 25ppm NH ₄ -N	DAFT with Aqua
In-lab Dissolved Air Flotation Thickener with 25ppm NO ₃ -N and 25ppm NH ₄ -N	DAFT without Aqua

Table 4.15: pH measurements for experiment 3

Time (hours)	0	12	29	35	50	109	123	150.5	175.5	191	215	239
SW without Aqua	7.9	8.48	9.32	9.31	9.32	9.31	9.4	9	8.94	8.64	7.87	7.05
SW with Aqua	7.87	8.44	9.33	9.34	9.46	9.09	9.18	8.9	8.79	8.61	7.4	6.66
DAFT without Aqua	7.65	7.96	9.09	9.07	9.17	9.12	8.67	6.86	6.67	6.65	6.69	6.66
DAFT with Aqua	7.48	7.85	8.82	8.82	8.92	9	8.82	7.56	6.59	6.6	6.62	6.64
Secondary without Aqua	7.42	8.09	8.99	8.96	9.01	8.46	8.44	8.31	8.38	8.5	8.35	8.4
Secondary with Aqua	7.04	7.68	8.84	8.86	8.99	8.08	7.88	8.02	8.01	7.97	7.86	7.86
Final without Aqua	6.74	7.92	9.16	9.16	9.16	9.04	8.93	8.16	7.39	6.82	6.35	6.22
Final with Aqua	6.69	7.22	8.98	8.87	9.06	9.09	9.1	9.07	8.56	8.29	8.21	8.22
Control	6.92	5.36	5.86	3.63	7.19	7.46	6.85	7.77	7.82	7.85	7.72	7.44
GM without Aqua	6.46	6.43	7.17	7.09	7.19	7	6.9	6.6	6.1	5.4	4.18	3.7

The pH for 14% of all samples was within the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). About 19% of all samples were below a pH of 6.8, which is where nitrification rates decline significantly. Most of the samples below 6.8 were DI control and growth media samples. Therefore, pH did not have a significant impact on the nitrification rate of all wastewater samples. The pH for 25% of all samples was within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). Only 5% of all samples were below a pH of 6, which inhibits the denitrification rate. Most of the

samples below 6 were DI control and growth media samples. Therefore, pH did not have a significant impact on the denitrification rate of all wastewater samples.

Table 4.16: Ammonia concentrations in mg/L NH₄-N for experiment 3

Time (hours)	34	150.5	175.5	215	239	Total Amount Degraded	Normalized Amount Degraded
Final with Aqua	39.34	30.68	20.86	ND	ND	33.34	8.66
Final without Aqua	21.77	9.97	ND	ND	ND	15.77	11.80
Secondary with Aqua	54.72	ND	ND	ND	ND	48.72	53.72
Secondary without Aqua	11.91	ND	ND	ND	ND	5.91	10.91
DAFT with Aqua	100.92	22.73	17.34	17.92	19.36	81.56	78.18
DAFT without Aqua	70.50	14.12	15.17	14.29	15.00	55.51	56.39
Control	22.01	30.12	31.36	31.82	32.80	-10.79	-8.12
GM without Aqua	26.47	34.83	35.50	44.08	47.23	-20.76	-8.36
SW with Aqua	835.75	268.50	211.71	169.46	143.50	692.25	567.25
SW without Aqua	836.52	312.46	246.89	194.33	180.74	655.78	524.06

*Normalized degraded amount is the difference between 34 and 150.5 hours only

*Amount degraded assumes the non-detect values are 6 mg/L, which is the detection limit. This gives a conservative estimate of the amount of ammonia removed.

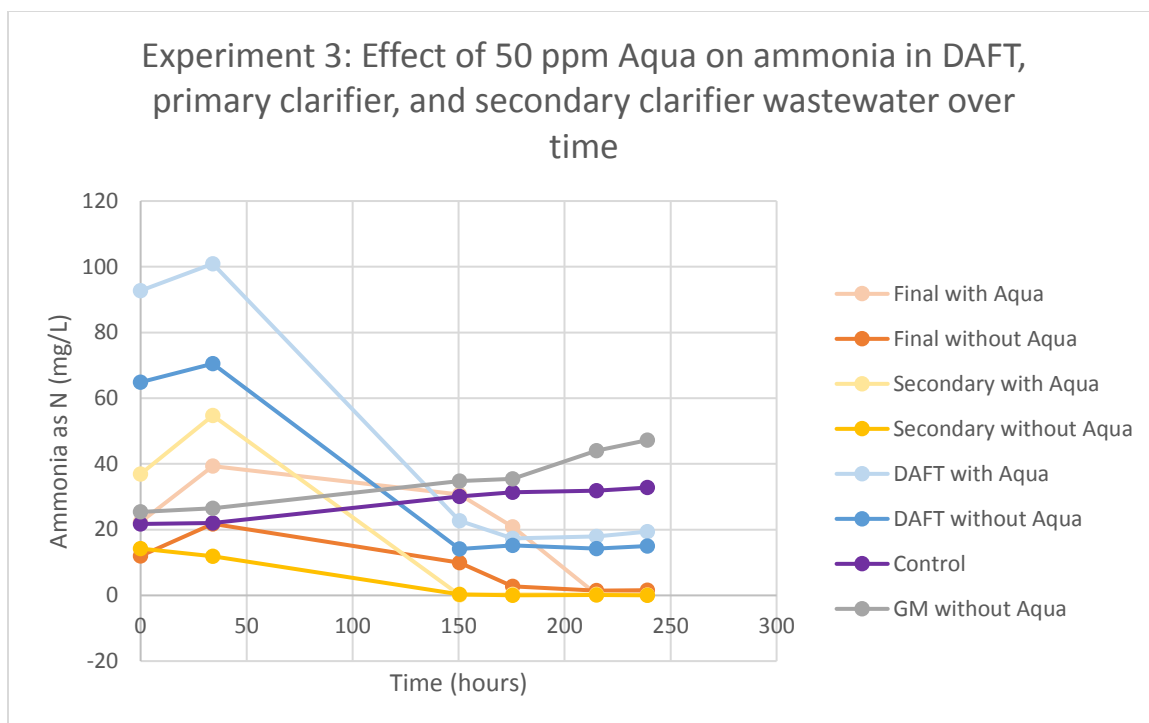


Figure 4.8: Effect of 50 ppm Aqua on ammonia in DAFT, primary clarifier, and secondary clarifier wastewater over time

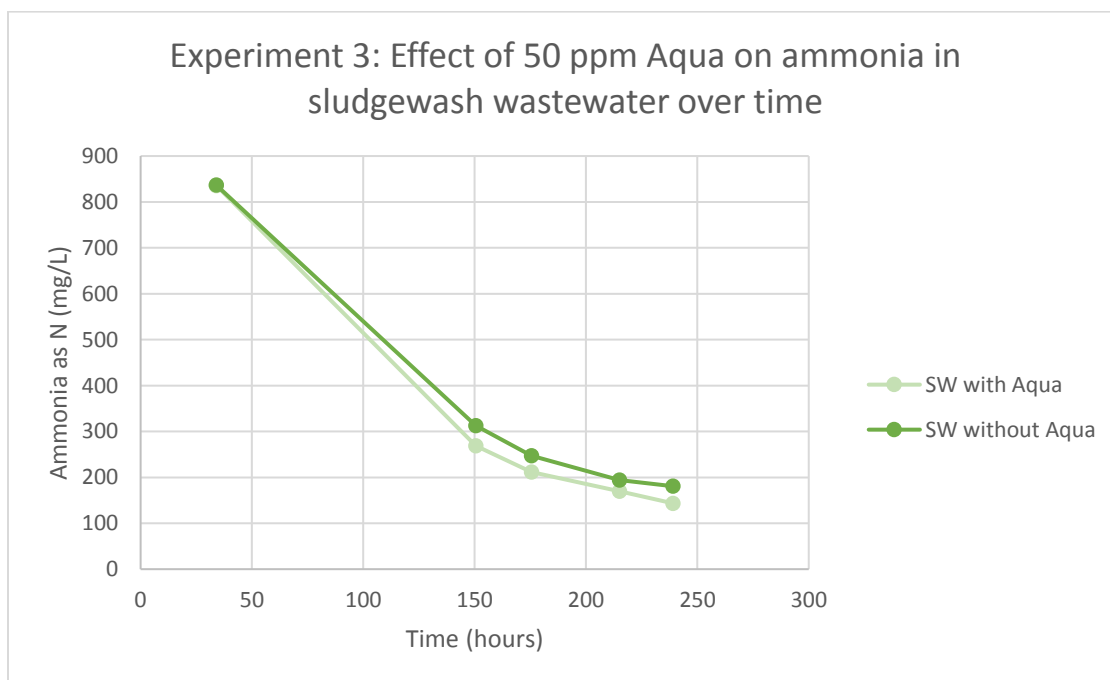


Figure 4.9: Effect of 50 ppm Aqua on ammonia in sludgewash wastewater over time

Table 4.17: Nitrate concentrations in mg/L NO₃-N for experiment 3

Time (hours)	4.5	50	109	123	150.5	174.5	Amount Degraded	Difference Between With and Without Aqua
DAFT without Aqua	6.05	2.74	4.72	13.40	47.50	52.98	-46.93	14.7
DAFT with Aqua	1.54	ND	ND	2.15	27.23	33.77	-32.23	
Final without Aqua	52.54	49.95	53.85	58.05	68.94	78.81	-26.27	3.09
Final with Aqua	20.85	23.36	25.49	33.01	31.44	44.03	-23.18	
SW without Aqua	4.04	2.66	2.82	2.95	4.44	7.28	-3.24	0.6
SW with Aqua	ND	ND	ND	1.14	1.23	3.44	-2.67	
Secondary without Aqua	26.10	28.75	61.85	64.50	83.26	83.45	-57.35	14.58
Secondary with Aqua	3.91	1.86	22.88	37.72	45.35	46.68	-42.77	
GM without Aqua	23.61	26.94	29.70	30.44	32.20	30.42	-6.82	-
Control	25.38	26.87	28.66	29.44	30.34	31.11	-5.73	-

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit.

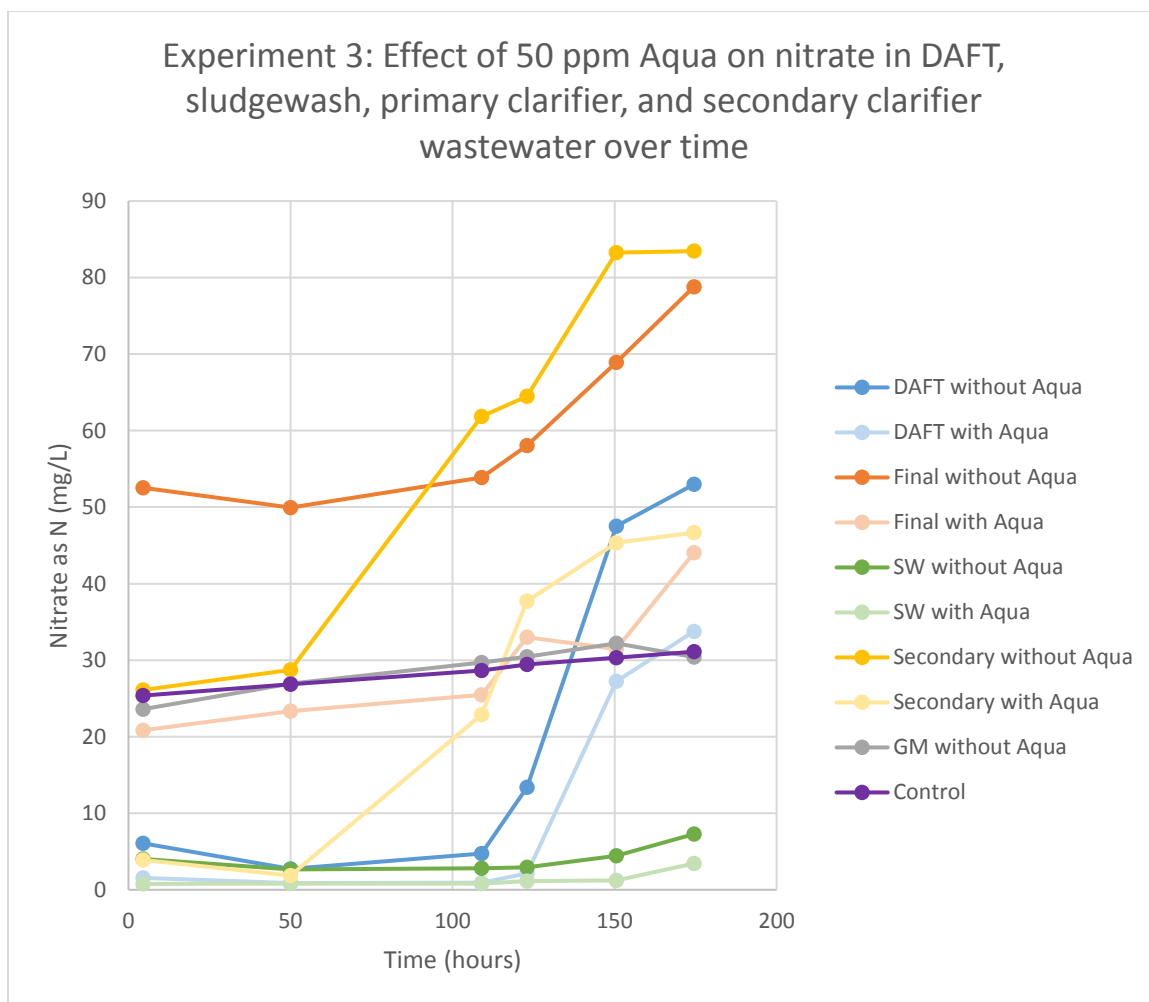


Figure 4.10: Effect of 50 ppm Aqua on nitrate in DAFT, sludgewash, primary clarifier, and secondary clarifier wastewater over time

Table 4.18: Nitrite concentrations in mg/L NO₂-N for experiment 3

Time (hours)	4.5	50	109	123	150.5	174.5	Amount Degraded
DAFT without Aqua	12.31	1.27	2.72	2.28	3.96	3.01	9.29
DAFT with Aqua	8.80	ND	1.38	2.16	2.90	3.16	5.64
Final without Aqua	6.68	2.30	ND	1.67	2.55	2.84	3.84
Final with Aqua	8.86	ND	1.84	4.08	2.98	3.15	5.71
SW without Aqua	8.99	ND	ND	ND	3.51	3.94	5.05
SW with Aqua	24.30	ND	ND	ND	2.90	3.69	20.61
Secondary without Aqua	9.14	1.29	1.22	1.99	2.33	2.21	6.93
Secondary with Aqua	13.66	1.64	2.76	2.44	3.68	2.69	10.97
GM without Aqua	8.40	ND	ND	ND	1.10	1.61	6.79
Control	4.01	ND	ND	ND	ND	1.85	2.16

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrite removed.

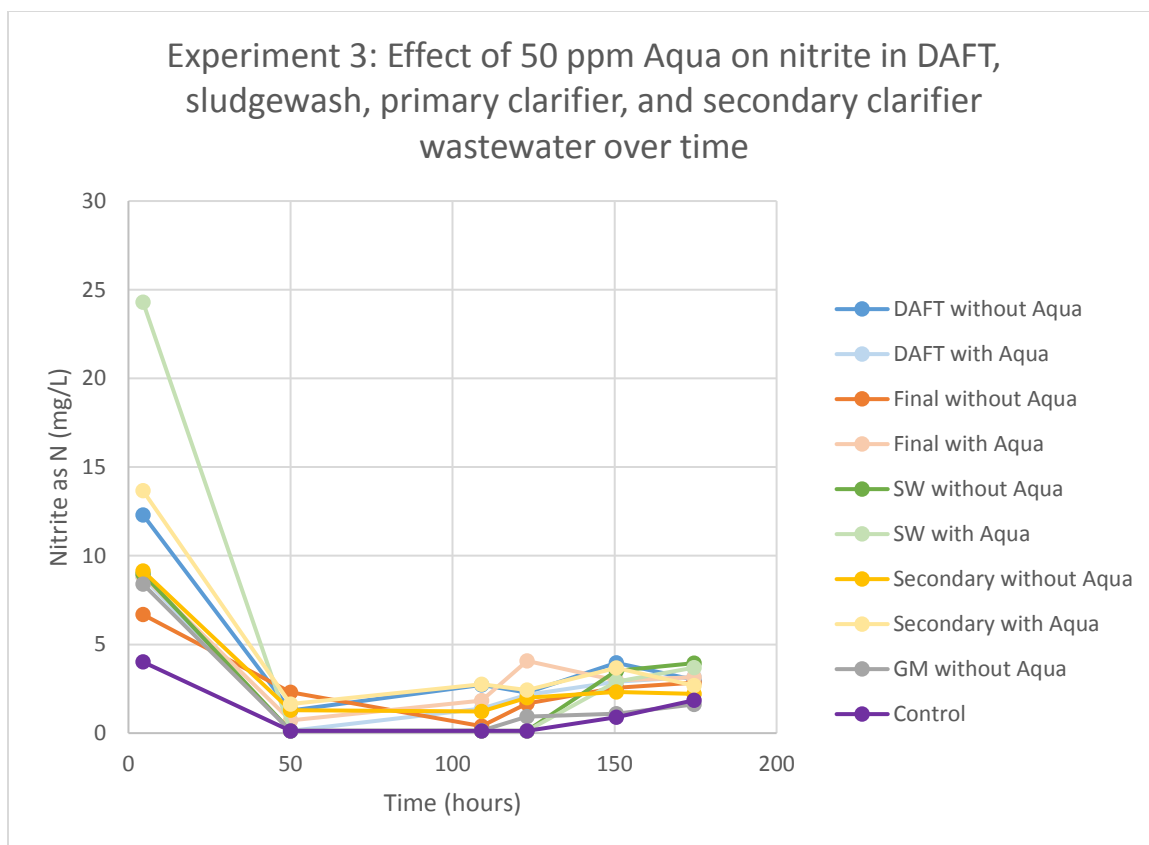


Figure 4.11: Effect of 50 ppm Aqua on nitrite in DAFT, sludgewash, primary clarifier, and secondary clarifier wastewater over time

All samples containing wastewater exhibited ammonia removal over a period of 4 days in a partially aerated environment. Ammonia concentrations for sludgewash started at hour 49 because of Timberline complications. The sludgewash was at a very high concentration. A setting on the Timberline needed to be changed to read the high concentrations. However, it was unknown that this setting needed to be changed until hour 49. Natural bacteria were assumed to follow the typical chemoautotrophic metabolic processes and the Aqua was assumed to follow the typical heterotrophic metabolic processes known for Aqua bacteria. The ammonia removal was due to nitrification because as ammonia decreased, nitrate increased.

The starting concentrations for the secondary clarifier, DAFT, and final clarifier were different between the natural bacteria wastewater and Aqua inoculated wastewater. The difference was likely due to the TSB increasing the ammonia concentration. The amount of ammonia removed using Aqua was higher at times than the natural bacteria. The increase was likely due to higher concentrations of ammonia to start in Aqua solutions. The removal rate will increase with a larger starting concentration in solution, as long as the larger starting concentration is not toxic to the bacteria (Zhang, Liu, Ai, Miao, Zheng, & Liu, 2012). If natural bacteria wastewater started at the same ammonia concentration as the Aqua inoculated wastewater, the two samples would likely have the same ammonia removal rate. Therefore, the natural bacteria were in charge of nitrification and the Aqua did not make a difference in ammonia removal. The chemoautotrophic metabolic process was more favorable than the heterotrophic metabolic process.

The sludgewash samples had the same starting concentration between the natural and Aqua inoculated wastewater. The sludgewash with Aqua removed 36.47 ppm $\text{NH}_4\text{-N}$ more than the natural bacteria sample. However, the natural bacteria nitrified about 650 ppm $\text{NH}_4\text{-N}$, so most of the ammonia removal was due to the natural bacteria. The 36.47 ppm $\text{NH}_4\text{-N}$ difference was could be due to sample preparation error including contamination, dilution from glassware rinses, evaporation, or volatilization. Assimilation from the Aqua could have occurred to result in this difference as well. However, adding Aqua to assimilate 36 ppm more ammonia is likely not worth the cost. Sludgewash did not experience typical

nitrification because the nitrate concentration did not increase as ammonia was removed. Likely assimilation occurred or a long lag time for nitrate production occurred.

Zero and first order degradation rates were calculated for ammonia removal. Since the solutions with Aqua and without Aqua were dominated by the natural bacteria, the nitrification rates will be a range between the two. Samples followed a zero order degradation rate for ammonia removal. Therefore, zero order kinetics values were compared to other literature values (Table 4.19). The amount of ammonia removed was calculated by subtracting the initial value from the lowest end value (Table 4.20). Aqua achieved a volumetric nitrification rate lower than reported literature values. The reason was likely because the experiment occurred under slightly aerobic conditions. Low oxygen concentrations could have caused the nitrification rate to be much slower.

Table 4.19: Nitrification rates for experiment 3

Source	System	Volumetric Nitrification Rate (mg NH ₄ -N/L/d)	Temp
This Study	Secondary Wastewater	2.40 – 11.25	~30°C
	DAFT Wastewater	11.62 – 14.78	
	Final Wastewater	2.98 – 4.52	
	Sludgewash Wastewater	79.77	
	Sludgewash Wastewater with Aqua	83.49	
(Tarre & Green, 2004)	Attached Biomass Reactor	2000 – 5600	N/A
	Suspended Biomass Reactor	1100	N/A
(Tijhuris, Van Loorsdrecht, & Heijnen, 1992)	Biofilm Airlift Suspension Reactor	6000	N/A
(Choubert, Racault, Grasmick, Beck, & Heduit, 2005)	Extended Aeration Activated Sludge Pilot Plant	79.2 – 220.8	10°C
(Li & Wu, 2014)	Laboratory Sequencing Batch Reactors	122.4 – 254.4	25°C

Table 4.20: Total ammonia removed and removal rates for experiment 3

Sample	Amount Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
Final without Aqua	15.77 – 18.48	141.5	0.1242 – 0.1884	0.0122 – 0.0198
Secondary without Aqua	5.91 – 48.72	116.5	0.0999 – 0.4686	0.0325 – 0.0516
DAFT without Aqua	56.39 – 78.18	116.5	0.4840 – 0.6157	0.0126 – 0.0138
SW with Aqua	692.25	205	3.4788	0.0087
SW without Aqua	655.78	205	3.3238	0.0077

*Amount degraded assumes the non-detect values are 6 mg/L, which is the detection limit. This gives a conservative estimate of the amount of ammonia removed.

*The removal time period starts with 34 hours because that is where lag time stops

Ammonia and nitrate were measured for the same run. Since the conditions were partially aerobic, nitrification was expected to occur and cause an increase in nitrate levels. However, since simultaneous nitrification and denitrification was predicted for Aqua bacteria, there should be either a decrease or a less drastic increase in nitrate levels for Aqua.

Slower nitrate production was observed in samples with Aqua compared to samples without Aqua. Natural bacteria and Aqua bacteria were assumed to follow the typical heterotrophic metabolic processes. The natural bacteria samples were a baseline to determine the amount of nitrate produced from nitrification. The difference between the amount of nitrate produced over time for samples with and without Aqua was determined. This difference determined whether Aqua achieved aerobic nitrate removal.

The DAFT and secondary clarifier wastewater samples with Aqua produced less nitrate than samples without Aqua. Therefore, the Aqua achieved aerobic nitrate removal for these wastewaters. When nitrate was removed for Aqua samples, nitrite was not produced. Therefore, nitrate removal likely occurred via assimilation or denitrification without production of intermediate nitrite. Nitrogen gas and organic nitrogen content would need to be measured to know the exact process. The slight increase in nitrite over time was due to procedural error because the control sample increased in nitrite as well. The secondary wastewater with Aqua was 14.58 ppm lower in nitrate compared to the natural bacteria. The DAFT wastewater with Aqua was 14.7 ppm lower in nitrate compared to the natural bacteria. The final clarifier wastewater experienced similar nitrate increase rates between

samples with and without Aqua. The sludgewash wastewater did not show any nitrate increase over time. This meant that Aqua did not help remove nitrate for final clarifier and sludgewash wastewater. Over the course of 2 days, the nitrate levels failed to reach below the discharge permit level of 10 mg/L nitrate as nitrogen for the SLO WRRF. However, this may have been achievable over a longer period of time.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts of each treatment process at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The final clarifier did not have carbon data. Therefore, the carbon concentration was assumed to match with the monthly high effluent BOD value of 7.1 mg/L. The growth media has 1000 mg/L dextrose, which equates to 399.6 mg/L dextrose as carbon. A solution of TSB and Aqua was added to the different wastewaters. The TSB added 25 mg/L dextrose because only 1% of the TSB was added to each solution. The Aqua added 47.5 mg/L dextrose because 95% of Aqua is dextrose. The total carbon from TSB and Aqua was 29 mg/L dextrose as carbon. The C:N ratios were different for each wastewater (Table 4.21). For C:N ratios higher than 4:1, ammonia removal rates were inhibited. For C:N ratios less than 2:1, ammonia removal rates were decreased. For C:N ratios higher than 6:1, nitrate removal rates were still be high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were decreased. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency. (Winkler M. , 2005). The

C:N ratios for wastewater without Aqua were not included because the natural bacteria likely experience autotrophic metabolic rates. Therefore, BOD (organic carbon) cannot be used to find C:N ratios for the autotrophic bacteria. The C:N ratios for DAFT samples did not impact the ammonia removal rate. The low C:N ratios for sludgewash, final clarifier, and secondary clarifier with Aqua samples could have decreased the ammonia removal rates. The C:N ratio for secondary clarifier without Aqua did not impact the nitrate removal rate. The high C:N ratios for sludgewash, DAFT, and secondary with Aqua samples could have slightly decreased the nitrate removal rates. The low C:N ratios for final clarifier samples could have decreased the nitrate removal rates.

Table 4.21: Carbon to nitrogen ratios for experiment 3

WW	Carbon in WW ¹	Total NH ₄ -N ²	Total NO ₃ -N ²	Total C	C:N for NH ₄ -N	C:N for NO ₃ -N
Sludgewash with Aqua	177.5	835.8	0.8	206.5	0.2:1	258.1:1
Sludgewash without Aqua		836.5	4.0	177.5	-	44.4:1
Secondary with Aqua	70.48	54.7	3.9	99.48	1.8:1	25.5:1
Secondary without Aqua		11.9	26.1	70.48	-	2.7:1
Final with Aqua	7.1	39.3	52.5	36.1	0.9:1	0.7:1
Final without Aqua		21.8	20.8	7.1	-	0.3:1
DAFT with Aqua	190.8	100.9	1.5	219.8	2.2:1	146.5:1
DAFT without Aqua		70.5	6	190.8	-	31.8:1

¹Values obtained from the SLO WRRF data. Assumed final clarifier was same as monthly high BOD effluent from plant

²Obtained from initial points from data collected

*Total C includes initial, TSB, and Aqua. Total N includes initial, TSB, and spiked 25 mg/L

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.12 and Figure 4.13). Interpolation of some data points occurred to have enough for a total nitrogen

curve. Total nitrogen removal occurred for sludgewash. However, the addition of Aqua only removed 40 ppm more nitrogen, which is small compared to the total amount of nitrogen in sludgewash. Total nitrogen removal occurred for DAFT wastewater as well. However, production of nitrate from nitrification was lagged, so there is an increase of total nitrogen near the end of the run for DAFT wastewater without Aqua. The addition of Aqua in DAFT wastewater resulted in a lower total nitrogen concentration compared to the natural bacteria. Total nitrogen increased for secondary and final clarifier wastewater without Aqua solutions. The increase could be due to other bacteria metabolic processes that produce nitrogen. The addition of Aqua to secondary and final clarifier wastewater caused relatively steady total nitrogen concentrations over time. Therefore, addition of Aqua in secondary and final clarifier wastewater resulted in a lower total nitrogen concentration compared to the natural bacteria.

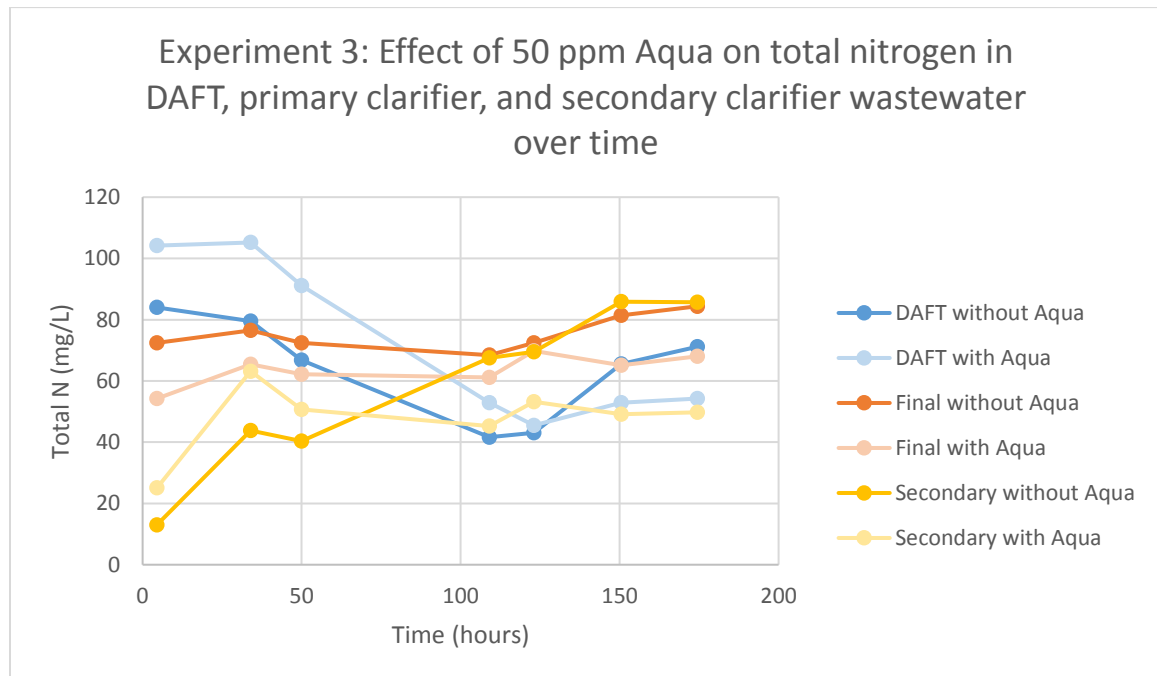


Figure 4.12: Effect of 50 ppm Aqua on total nitrogen in DAFT, primary clarifier, and secondary clarifier wastewater over time

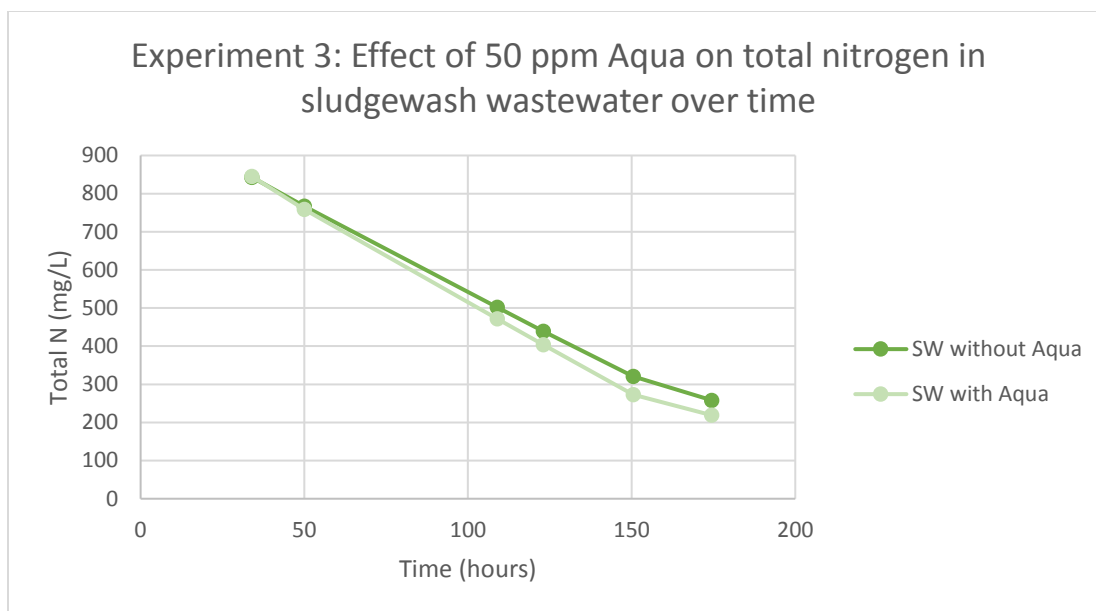


Figure 4.13: Effect of 50 ppm Aqua on total nitrogen in sludgewash wastewater over time

Experiment 2 and 3 showed that adding Aqua does not help with ammonia removal. Therefore, the rest of the experiments will focus on nitrate removal. Some experiments will look at the ammonia levels to ensure that ammonia does not change much for the natural versus Aqua inoculated wastewater. The wastewater that will be analyzed more in depth is the secondary wastewater. It achieved the quickest denitrification rate in Experiment 2 and it achieved aerobic denitrification in Experiment 3. This was likely due to the fact that the secondary clarifier wastewater had enough nitrate and BOD to denitrify. It also had better C:N ratios than the other wastewaters, which could contribute to the decreased lag time of nitrate removal for Aqua. Sludgewash will also be analyzed in depth because the nitrate concentration did not change in Experiment 3. Therefore, nitrate removal in high nitrogen concentrated wastewater was not determined. Observing denitrification in these conditions would be beneficial in determining if the sludgewash wastewater is toxic to Aqua.

Determining if Aqua can reduce these nitrogen levels would be extremely beneficial for the SLO WRRF. The sludgewash wastewater is introduced in small doses at the beginning of the treatment train due to the high nitrogen levels. Therefore, decreasing the nitrogen levels in sludgewash means more of the sludgewash wastewater could be added at the beginning of the treatment train. Final clarifier wastewater will also be analyzed to see how carbon amounts affect the nitrate removal.

4.1.4 Experiment 4 - Effect of 500 ppm Aqua with New Inoculation Method on Secondary Clarifier Wastewater

Nitrate removal was analyzed for Aqua in secondary clarifier wastewater under anoxic conditions in lab (Table 4.25, Table 4.26, Figure 4.14, and Figure 4.15). Temperature and pH were measured to determine their potential effects on nitrate removal (Table 4.23 and Table 4.24). Different times between the pH, temperature, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to no data points did not pass QA/QC.

This experiment determined whether the dry inoculation method can accurately represent Aqua in a laboratory setting. Ideally, Aqua would be dosed into laboratory and bioreactor solutions dry. The product only contains 1% of active microbial cultures (BiOWiSH Technologies, 2016). Less than 100 mg of Aqua would be weighed out and dosed into the solutions. Therefore, active microbial cultures may not get into the dose weighed out. This experiment tested a weight of 50 mg and 5 mg to see if Aqua was accurately represented.

This experiment also determined if using 70% microbial cultures in a powder (instead of Aqua's 1%) would make a difference in nitrate removal. This experiment also determined if adding trace minerals would improve nitrate removal.

Table 4.22: Labeling for experiment 4

Description	Label
In-lab secondary wastewater with 25 ppm NO ₃ -N	Secondary without Aqua
In-lab secondary wastewater with 50 ppm Aqua and 25 ppm NO ₃ -N	Secondary with 50 ppm Aqua
In-lab secondary wastewater with 500 ppm Aqua and 25 ppm NO ₃ -N and 1% trace minerals	Secondary with dry Aqua and TM
In-lab secondary wastewater with 500 ppm microbial powder 25 ppm NO ₃ -N and 1% trace minerals	Secondary with dry MC and TM
In-lab secondary wastewater with 500 ppm liquid Aqua and 25 ppm NO ₃ -N and 1% trace minerals	Secondary with liquid Aqua and TM
In-lab secondary wastewater with 500 ppm liquid microbial powder 25 ppm NO ₃ -N and 1% trace minerals	Secondary with liquid MC and TM
In-lab secondary wastewater with 500 ppm Aqua and 25 ppm NO ₃ -N	Secondary with dry Aqua
In-lab secondary wastewater with 500 ppm microbial powder 25 ppm NO ₃ -N	Secondary with dry MC
In-lab secondary wastewater with 500 ppm liquid Aqua and 25 ppm NO ₃ -N	Secondary with liquid Aqua
In-lab secondary wastewater with 500 ppm liquid microbial powder 25 ppm NO ₃ -N	Secondary with liquid MC
In-lab DI water with no Aqua and 25 ppm NO ₃ -N	Control

Table 4.23: pH measurements for experiment 4

Time (hours)	0	2	4	6.5	8	10
Control	7.01	5.23	6.56	6.82	6.95	6.88
Secondary without Aqua	7.19	7.12	7.13	7.27	7.32	7.48
Secondary with dry Aqua and TM	7.16	7.04	6.96	6.57	6.19	6.13
Secondary with dry MC and TM	7.1	7	6.75	6.51	6.39	6.66
Secondary with liquid Aqua and TM	7.2	7.08	6.92	6.44	6.18	5.96
Secondary with liquid MC and TM	7.14	6.91	6.76	6.51	6.27	6.59
Secondary with dry Aqua	7.22	7.25	7.08	6.63	6.34	6.24
Secondary with dry MC	7.21	7.11	6.91	6.6	6.62	6.95
Secondary with liquid Aqua	7.26	7.18	7.11	6.7	6.29	6.23
Secondary with liquid MC	7.18	7.02	6.85	6.6	6.44	6.86
Secondary with 50 ppm Aqua	7.06	6.86	6.83	6.71	6.76	6.79

The pH for 36% of all samples were within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). About 62% of all samples were below a pH of 7 and none are above a pH of 8. About 3% of the samples are below a pH of 6, which is where denitrification is inhibited. Therefore, pH did not impact the denitrification rate.

Table 4.24: Temperature measurements for experiment 4

Time (hours)	0	2	4	6.5	8	10
Control	20.9	24.2	24.6	25.8	26.9	25.1
Secondary without Aqua	21.1	26.4	26.8	27.1	27.4	27
Secondary with dry Aqua and TM	21.2	26.2	27	26.9	27.2	26.7
Secondary with dry MC and TM	21.1	26.7	26.4	26.8	27.3	26.5
Secondary with liquid Aqua and TM	21	26.9	27.3	27.1	26.9	26.4
Secondary with liquid MC and TM	20.8	26.7	26.8	26.7	26.8	25.9
Secondary with dry Aqua	21.2	25.5	27.2	26	26.4	25.5
Secondary with dry MC	21.1	26.2	27	26.6	26.5	25.7
Secondary with liquid Aqua	21	26.3	26.8	26.5	26.5	25.5
Secondary with liquid MC	20.9	26.5	26.9	27.1	26.3	25.3
Secondary with 50 ppm Aqua	21.5	26.5	27.4	27	27	26.3

Laboratory temperatures were around 25°C to ensure good bacterial growth. The samples fluctuated between 21°C to 27°C. The fluctuations in temperature at the beginning could impact denitrification rates. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973).

Table 4.25: Nitrate concentrations in mg/L NO₃-N for experiment 4

Time (hours)	0	2	4	6.5	8	10	Amount Degraded
Secondary without Aqua	17.35	18.53	18.17	14.51	19.19	19.73	-2.38
Secondary with 50 ppm Aqua	17.78	20.05	18.11	11.66	12.92	12.18	5.61
Secondary with dry Aqua and TM	16.47	18.38	17.58	10.36	5.49	N/A	15.47
Secondary with dry MC and TM	19.49	20.17	14.46	3.51	2.17	N/A	18.49
Secondary with liquid Aqua and TM	18.49	18.71	20.67	11.40	6.71	N/A	17.49
Secondary with liquid MC and TM	17.03	17.46	12.82	2.77	N/A	N/A	16.03
Secondary with dry Aqua	18.47	18.04	19.05	11.60	7.53	N/A	17.47
Secondary with dry MC	18.66	17.56	16.69	6.01	N/A	N/A	17.66
Secondary with liquid Aqua	21.64	17.21	18.86	11.73	8.42	N/A	20.64
Secondary with liquid MC	16.90	19.90	14.28	4.82	N/A	N/A	15.90
Control	23.19	25.21	24.88	24.65	24.83	24.80	-1.61

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

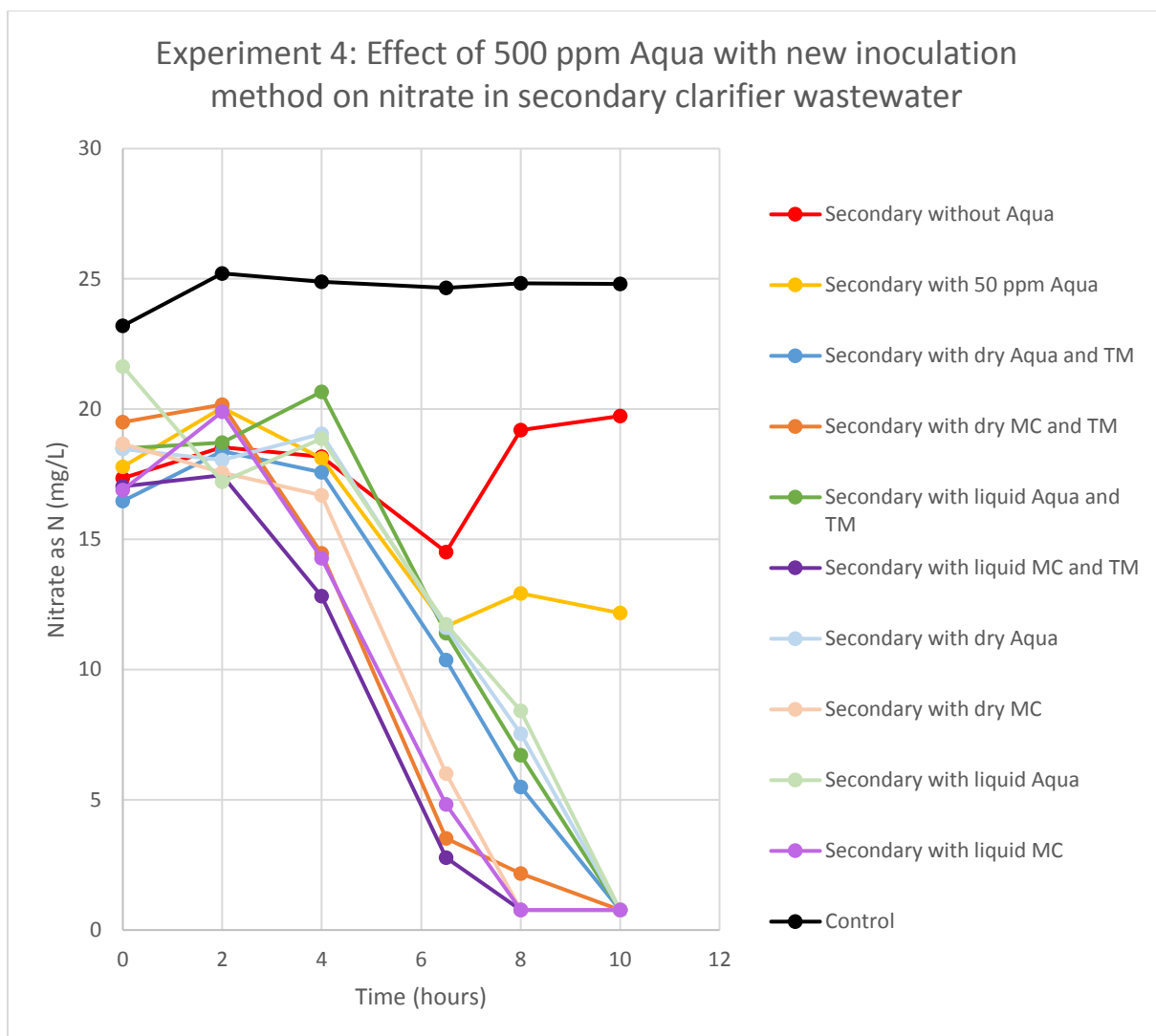


Figure 4.14: Effect of 500 ppm Aqua with new inoculation method on nitrate in secondary clarifier wastewater

Table 4.26: Nitrite concentrations in mg/L NO₂-N for experiment 4

Time (hours)	0	2	4	6.5	8	10
Secondary without Aqua	11.30	5.25	9.32	5.24	6.98	7.58
Secondary with 50 ppm Aqua	4.09	10.67	8.46	7.18	11.04	7.97
Secondary with dry Aqua and TM	6.35	5.29	8.44	7.08	6.72	5.57
Secondary with dry MC and TM	5.93	8.86	12.26	7.32	9.48	11.08
Secondary with liquid Aqua and TM	5.84	10.32	7.15	7.24	7.10	5.54
Secondary with liquid MC and TM	5.69	11.38	11.71	8.04	6.35	10.89
Secondary with dry Aqua	5.44	N/A	12.64	6.75	6.67	5.83
Secondary with dry MC	7.72	9.64	12.32	9.48	6.98	7.38
Secondary with liquid Aqua	5.63	4.89	11.01	6.64	6.51	7.28
Secondary with liquid MC	5.45	5.58	10.46	9.32	6.07	7.22
Control	N/A	N/A	4.85	6.35	5.78	5.39

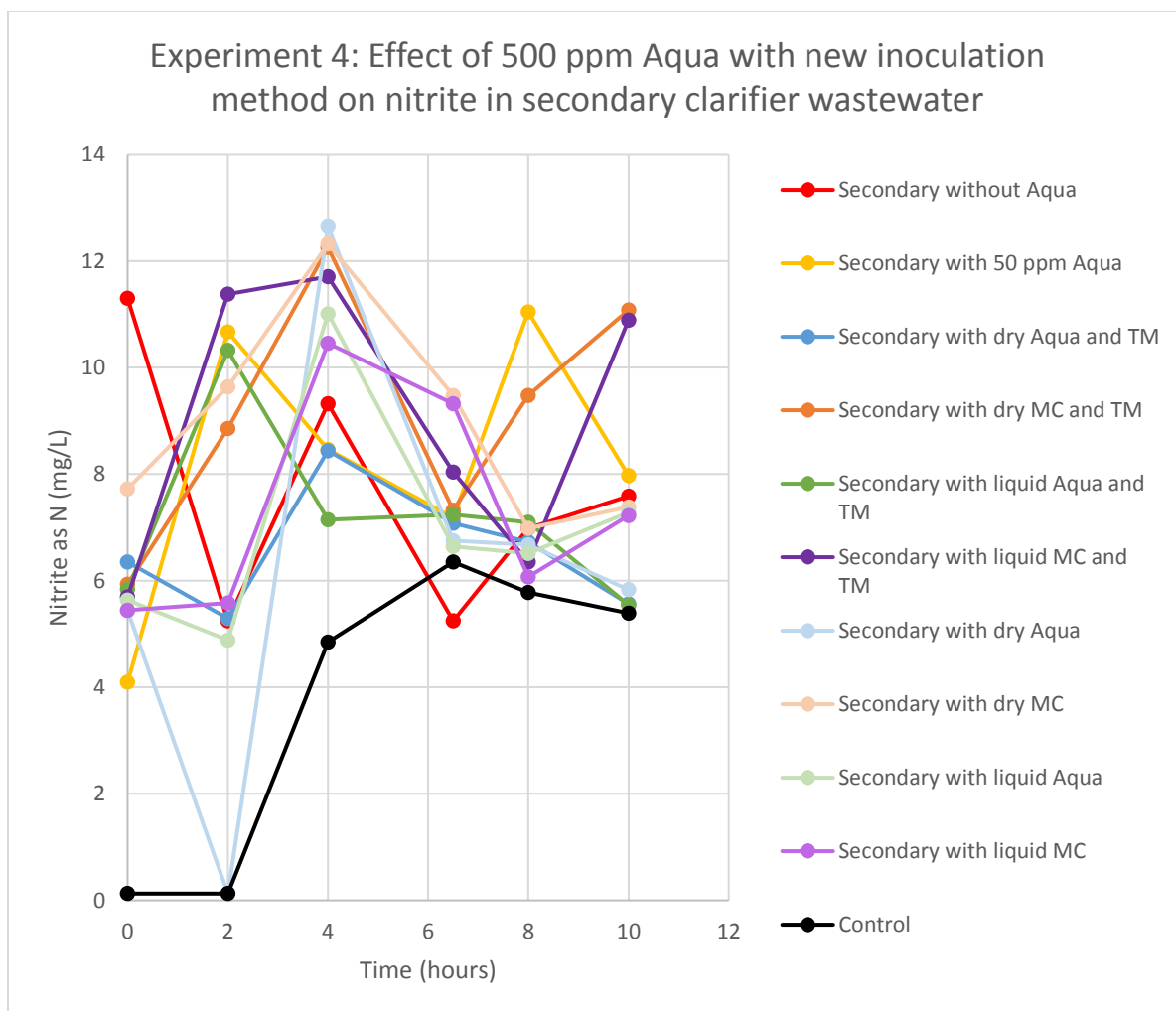


Figure 4.15: Effect of 500 ppm Aqua with new inoculation method on nitrite in secondary clarifier wastewater

All samples containing Aqua and microbial cultures exhibited nitrate removal over a period of about 10 hours. Determining whether bacteria achieved denitrification or assimilation for nitrate removal cannot be concluded because the DI control experienced an increase in nitrite over time. The increase in nitrite was likely due to contamination within the solution bottle itself. Therefore, nitrite measurements cannot be used for analysis. However, the nitrate measurements for the DI control were steady, which means nitrate can still be

analyzed. The microbial cultures degraded nitrate quicker than Aqua. The increased removal was likely because the amount of microbes within the powder was higher. The microbial cultures started removing nitrate after 2 hours, rather than after 4 hours. The addition of trace minerals did not make a difference in the rate of degradation. Therefore, the minerals already existing in the wastewater were enough for the growth of Aqua bacteria. The inoculation methods resulted in removal amounts that were, at most, 2 ppm different. Therefore, the inoculating as a liquid or as a dry powder does not affect the amount of nitrate degraded.

Samples containing Aqua or microbial cultures degraded more nitrate than the natural bacteria. The 500 ppm concentration samples worked best. All 500 ppm concentration samples degraded about 18 ppm nitrate in 10 hours. The 50 ppm concentration worked as well. It degraded about 5 ppm nitrate in 10 hours. The natural bacteria were not able to degrade any nitrate in 10 hours. Therefore, a weight as low as 5 mg provides enough microbes in Aqua to accurately represent Aqua's capability of removing more nitrate than natural bacteria.

Zero and first order degradation rates were calculated for nitrate removal. The samples followed zero order kinetics. Therefore, zero order kinetic values were compared to other literature values (Table 4.27). The amount of nitrate removed was calculated by subtracting the initial value from the lowest end value (Table 4.28). Aqua achieved a volumetric denitrification rate in the middle range of reported literature values. Over the course of ten hours, the samples containing 500 ppm Aqua were below the discharge permit level of 10

mg/L nitrate as nitrogen for the SLO WRRF. The natural bacteria wastewater failed to reach below the permit value of 10 mg/L nitrate as nitrogen. The 50 ppm Aqua concentration got close to 10 mg/L, but it likely needed more time to get below 10 mg/L.

Table 4.27: Denitrification rates for experiment 4

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Secondary Clarifier Wastewater with 500 ppm Aqua	68 - 80	~25°C
	Secondary Clarifier Wastewater with 500 ppm cultures	71.3 - 96.2	~25°C
	Secondary Clarifier Wastewater with 50 ppm Aqua	45.5	~25°C
	Secondary Clarifier Wastewater	~0	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹ Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.28: Total nitrate removed and removal rates for experiment 4

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
Secondary with 50 ppm Aqua	4.87	8	1.8943	0.0479
Secondary with dry Aqua and TM	10.98	8	2.8344	0.5047
Secondary with dry MC and TM	17.33	8	3.206	0.4223
Secondary with liquid Aqua and TM	11.78	8	3.3159	0.525
Secondary with liquid MC and TM	16.03	8	2.9715	0.5266
Secondary with dry Aqua	10.94	8	3.0221	0.5071
Secondary with dry MC	17.66	8	4.009	0.7324
Secondary with liquid Aqua	13.22	8	2.9546	0.5007
Secondary with liquid MC	15.90	8	3.2751	0.5157

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added a total of 47.5 or 475 mg/L of dextrose to the lab samples, since about 95% of Aqua is dextrose. This equates to 19 or 190 mg/L as carbon. About 70% of the microbial cultures powder was the cultures and 1% of the microbial cultures powder, like in Aqua, was assumed to be salt. Therefore, 29% was dextrose. The microbial cultures added 145 mg/L dextrose to the lab samples, which equates to 57.95 mg/L as carbon. The C:N ratios were different for the samples (Table

4.29). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The high C:N ratios for samples with and without 500 ppm Aqua could have decreased the nitrate removal rates. The C:N ratio for 50 ppm Aqua was ideal. Therefore, the C:N ratio did not affect the nitrate removal rate.

Table 4.29: Carbon to nitrogen ratios for experiment 4

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Secondary with 500 ppm Aqua	70.48	16.5 – 18.7	260.48	15.3:1
Secondary with 500 ppm Microbial Cultures		16.9 – 19.5	128.43	7:1
Secondary with 50 ppm Aqua		17.8	89.48	5:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C for activated Aqua includes initial, Aqua, and TSB. Total C for Aqua includes initial and Aqua

Over 10 hours, all samples with Aqua or microbial cultures experienced nitrate removal. The 500 ppm concentration of Aqua had a higher nitrate removal rate than the 50 ppm Aqua concentration. The microbial cultures had a quicker nitrate removal rate than Aqua. The addition of trace minerals did not impact the rate of nitrate removal. Using dry versus liquid inoculation for Aqua or microbial cultures did not impact the nitrate removal rate. In this experiment, 50 mg and 5 mg of Aqua weights were used. Both showed increased nitrate removal compared to the natural bacteria. This means dry Aqua inoculation with weights from 5 to 50 mg do contain enough bacteria in them to make a difference in nitrate removal.

4.2 Field Results

Field experiments analyzed the effect of different Aqua doses on nitrification and denitrification in wastewater at different treatment processes at the SLO WRRF. Analysis of high nitrate and ammonia wastewater on Aqua were also conducted. Experiments also analyzed the effect of activating Aqua and providing partial aeration on nitrification and denitrification. Comparison of Aqua with a competitor bacterial mixture was also analyzed.

Field experiments were conducted during the summer when temperatures were ideal. Bioreactors were set up concurrently at the sludgewash lagoon and secondary clarifier to maximize the number of experiments conducted under ideal temperatures. Occasionally, material needed for sample analysis was backlogged. Multiple experiments would need to be stored in the fridge at about 4°C. Therefore, results from some experiments were unknown when another experiment had started. For instance, the experiment with 5 ppm Aqua in secondary clarifier wastewater was in the fridge while the experiment with 2.5 ppm Aqua in secondary clarifier wastewater was conducted.

4.2.1 Experiment 5 – Effect of 5 ppm Aqua on Secondary Clarifier Wastewater

Ammonia and nitrate removal was analyzed for Aqua in secondary clarifier wastewater under anoxic conditions in lab and in field (Table 4.33, Table 4.34, Table 4.35, Figure 4.16, Figure 4.17, and Figure 4.18). This experiment was one of a series to determine the best Aqua dosage to achieve nitrate removal in the secondary clarifier. The timeline of different dosages tested was first 25 ppm Aqua, then 10 ppm, 5 ppm, 2.5 ppm, and 50 ppm. Preliminary tests suggested that secondary clarifier wastewater had nitrate in it originally.

However, that changed when the bioreactor experiments started. The 25 ppm and 10 ppm Aqua dose data had to be discarded because the starting nitrate concentrations were around 5 ppm. Both of these experiments can be found in appendix B.

Temperature and pH were measured to determine their potential effects on ammonia and nitrate removal (Table 4.31 and Table 4.32). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.30: Labeling for experiment 5

Description	Label
In-field bioreactor secondary clarifier wastewater with 5 ppm Aqua and 25 ppm NO ₃ -N	BR Secondary with Aqua
In-lab secondary clarifier wastewater with 5 ppm Aqua and 25 ppm NO ₃ -N	Lab Secondary with Aqua
In-lab secondary clarifier wastewater with no Aqua and 25 ppm NO ₃ -N	Lab Secondary without Aqua
In-lab secondary clarifier wastewater with no Aqua or NO ₃ -N	Lab Secondary without Aqua or nitrate
In-lab DI water with no Aqua and 25 ppm NO ₃ -N	Control

Table 4.31: pH measurements for experiment 5

Time (hours)	0	17	24	41	48	65	72	89	96	113	120
BR Secondary with Aqua	7.79	7.85	8.02	7.8	7.91	7.99	7.98	7.99	8.14	7.89	8.17
Lab Secondary with Aqua	7.82	7.65	7.7	7.44	7.53	7.42	7.47	7.5	7.45	7.17	7.22
Lab Secondary without Aqua or nitrate	7.76	7.69	7.83	7.43	7.51	7.4	7.4	7.44	7.47	7.13	7.19
Control	6.02	6.4	6.84	6.44	7.09	6.81	6.45	6.36	6.76	6.05	6.21
Lab Secondary without Aqua	7.7	7.69	7.83	7.34	7.54	7.45	7.51	7.55	7.55	7.26	7.31

The pH for 42% of all samples were within the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). About 15% of all samples were below a pH of 6.8, which is where nitrification rates decline significantly. Therefore, pH could have an impact on the nitrification rate. The pH for 76% of all samples was within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). None of all samples were below a pH of 6, which inhibits the denitrification rate. Therefore, pH did not have a significant impact on the denitrification rate. None of the bioreactor samples were below a pH of 6.8. Therefore, the pH conditions for the bioreactor are more favorable than the laboratory samples for nitrification and denitrification.

Table 4.32: Temperature measurements for experiment 5

Time (hours)	0	17	24	41	48	65	72	89	96	113	120
BR Secondary with Aqua	25.7	22.9	25.7	23.2	23.2	25	26.2	23.2	27.3	23.3	29.1
Lab Secondary with Aqua	25.1	25.4	26.3	25.7	25.7	26.9	27.7	25.7	27.3	26	27.6
Lab Secondary without Aqua or nitrate	24.9	25.6	26.3	25.8	25.8	27.1	27.5	25.5	27.3	25.9	27.8
Control	22.8	25.3	26.4	25.7	25.7	27.1	27.5	25.3	27.4	26	27.8
Lab Secondary without Aqua	24.9	25.2	26.3	25.6	25.6	27	27.7	25.5	27.5	25.5	27.6

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 23°C and 29°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.33: Ammonia concentrations in mg/L NH₄-N for experiment 5

Time (hours)	0	17	24	41	48	65	72
Control	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua	32.24	29.14	30.43	31.99	33.24	33.82	35.51
Lab Secondary without Aqua or nitrate	30.00	27.58	30.95	33.03	33.00	33.25	32.29
Lab Secondary with Aqua	34.37	27.45	32.47	32.17	32.49	33.52	33.44
BR Secondary with Aqua	30.40	29.30	31.79	33.36	31.95	32.57	33.04

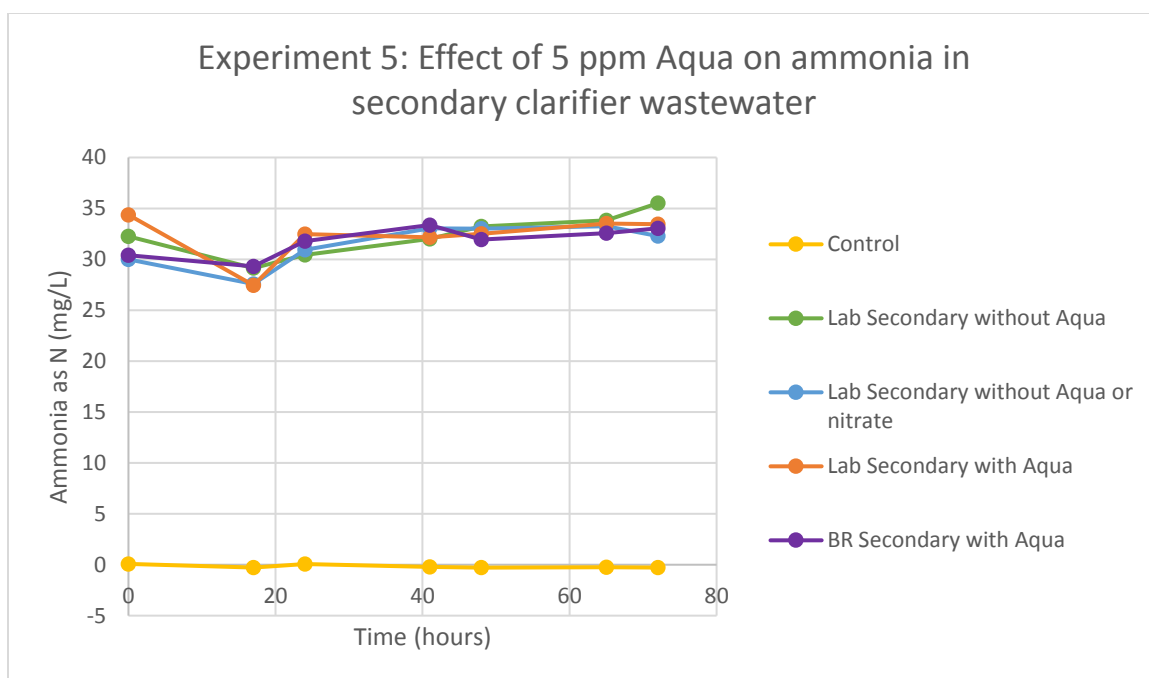


Figure 4.16: Effect of 5 ppm Aqua on ammonia in secondary clarifier wastewater

Table 4.34: Nitrate concentrations in mg/L NO₃-N for experiment 5

Time (hours)	0	17	24	41	65	72	89	113	120	Amount Degraded
BR Secondary with Aqua	27.48	19.36	19.95	16.88	13.90	13.70	12.45	11.11	10.91	16.57
Lab Secondary with Aqua	29.54	20.44	22.90	18.22	16.64	16.16	15.52	15.21	15.36	14.18
Lab Secondary without Aqua or nitrate	6.83	1.77	ND	ND	ND	ND	ND	1.00	1.08	5.75
Lab Secondary without Aqua	32.60	21.30	21.04	18.40	16.78	17.79	15.95	15.53	15.82	16.78
Control	23.15	24.59	24.55	24.66	24.32	24.42	24.37	24.29	24.36	-1.21

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

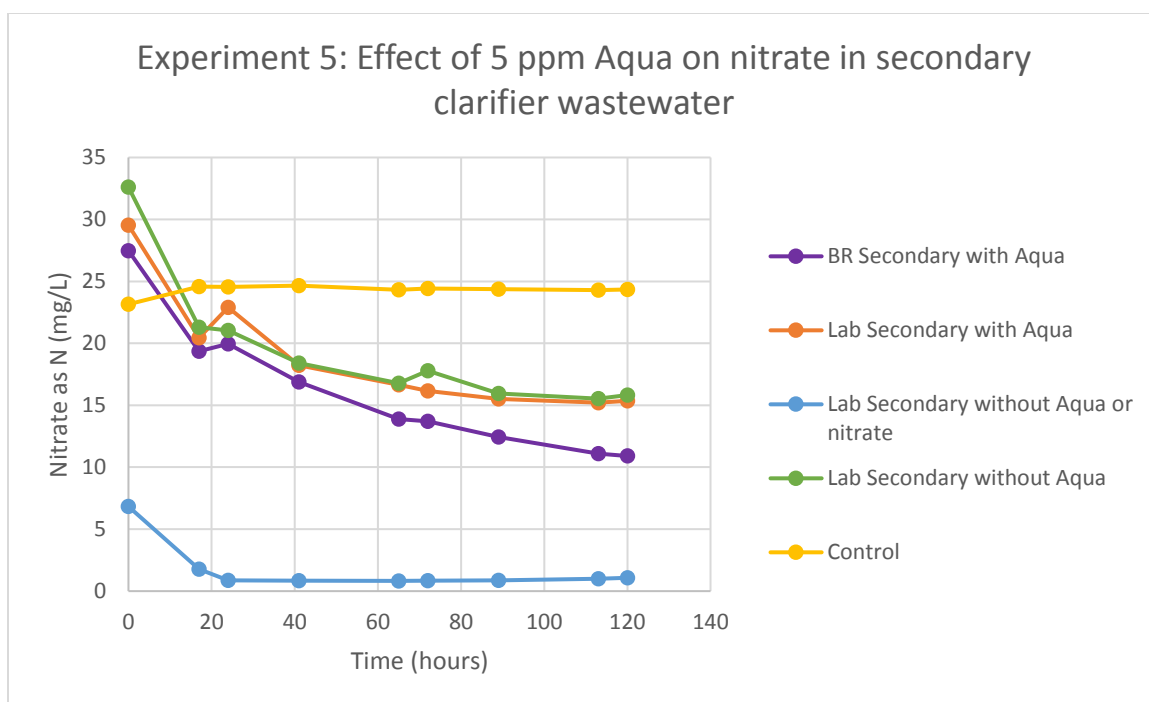


Figure 4.17: Effect of 5 ppm Aqua on nitrate in secondary clarifier wastewater

Table 4.35: Nitrite concentrations in mg/L NO₂-N for experiment 5

Time (hours)	0	17	24	41	65	72	89	113	120
BR Secondary with Aqua	1.01	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary with Aqua	1.05	ND	ND	ND	ND	ND	ND	1.11	1.01
Lab Secondary without Aqua or nitrate	1.09	ND	ND	ND	ND	ND	ND	1.01	ND
Lab Secondary without Aqua	1.15	ND	ND	ND	ND	ND	ND	1.25	1.11
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND

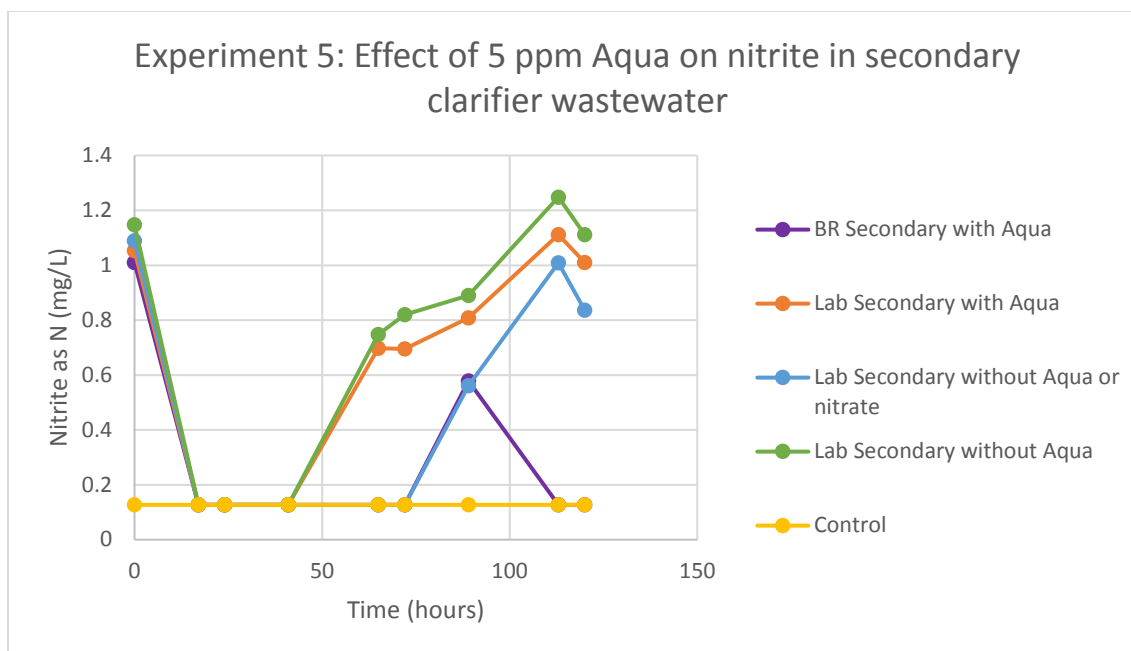


Figure 4.18: Effect of 5 ppm Aqua on nitrite in secondary clarifier wastewater

The ammonium concentration fluctuated between 30 and 35 ppm ammonia. Therefore, bacteria did not process ammonia. The anoxic conditions inhibited nitrification and assimilation of ammonia. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration.

The bioreactor sample was a 5 ppm different from the laboratory sample with Aqua for nitrate removal. The laboratory and bioreactor samples did not follow the same nitrate removal rates, so the laboratory sample without Aqua cannot be compared to bioreactor data. Therefore, outside factors did affect bioreactor samples.

All samples containing nitrate and wastewater exhibited nitrate removal over a period of about 3 days. As nitrate decreased, nitrite only increased by 1 ppm. Therefore, nitrate removal likely occurred via assimilation or denitrification without production of intermediate nitrite. Nitrogen gas and organic nitrogen content would need to be measured to know the exact process. The nitrite samples were below the 1 ppm MDL, which means that the sample points could be inaccurate. Therefore, the nitrite increase could be due to instrumental error. A longer run time may show an increase of nitrite above 1 ppm, which would mean the bacteria denitrified the nitrate. The natural bacteria wastewater behaved the same as the wastewater with Aqua. They all achieved a nitrate removal of 14 to 16 ppm nitrate. Therefore, an addition of 5 ppm Aqua did not increase the nitrate removal rate.

Zero and first order degradation rates were calculated for nitrate removal. Since the lab solutions with Aqua and without Aqua were dominated by the natural bacteria, the denitrification rates will be a range between the two. Typically denitrification follows zero order kinetics (Lee, 2012). However, all samples followed a first order degradation rate. Zero order kinetic values were compared to other literature values (Table 4.36). The amount of nitrate removed was calculated by subtracting the initial value from the final value (Table 4.37). Natural bacteria achieved a volumetric denitrification rate in the lower range of reported literature values. The clarifier was not designed for denitrification, which explains why the denitrification rates of the natural bacteria are low. Over the course of five days, all wastewater samples failed to reach below the permit value of 10 mg/L nitrate as nitrogen.

Table 4.36: Denitrification rates for experiment 5

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Secondary Clarifier Wastewater	2.28 - 2.81	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹ Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.37: Total nitrate removed and removal rates for experiment 5

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
BR Secondary with Aqua	16.57	120	0.1169	0.007
Lab Secondary with Aqua	14.18	120	0.0954	0.0047
Lab Secondary without Aqua	16.78	120	0.1009	0.0047

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

According to research done by Eva Lee for BiOWiSH™, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF shows the average carbon amounts in 2014 for

the different wastewaters. The Aqua added 4.75 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 1.9 mg/L as carbon. The C:N ratios were different for the samples (Table 4.38). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratios for samples with Aqua did not impact the nitrate removal rate. The sample without Aqua fell below 2.5:1, which could have slowed the nitrate removal rate. However, the C:N ratio was only 0.4 off from the ideal C:N ratio. Therefore, the C:N ratio did not impact nitrate removal for all samples.

Table 4.38: Carbon to nitrogen ratios for experiment 5

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Secondary with Aqua (lab and bioreactor)	70.48	27-30	72.38	2.5:1
Secondary without Aqua		33	70.48	2.1:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.19). The total nitrogen decreased by about 15 ppm for all samples. Therefore, the addition of Aqua did not improve total nitrogen removal.

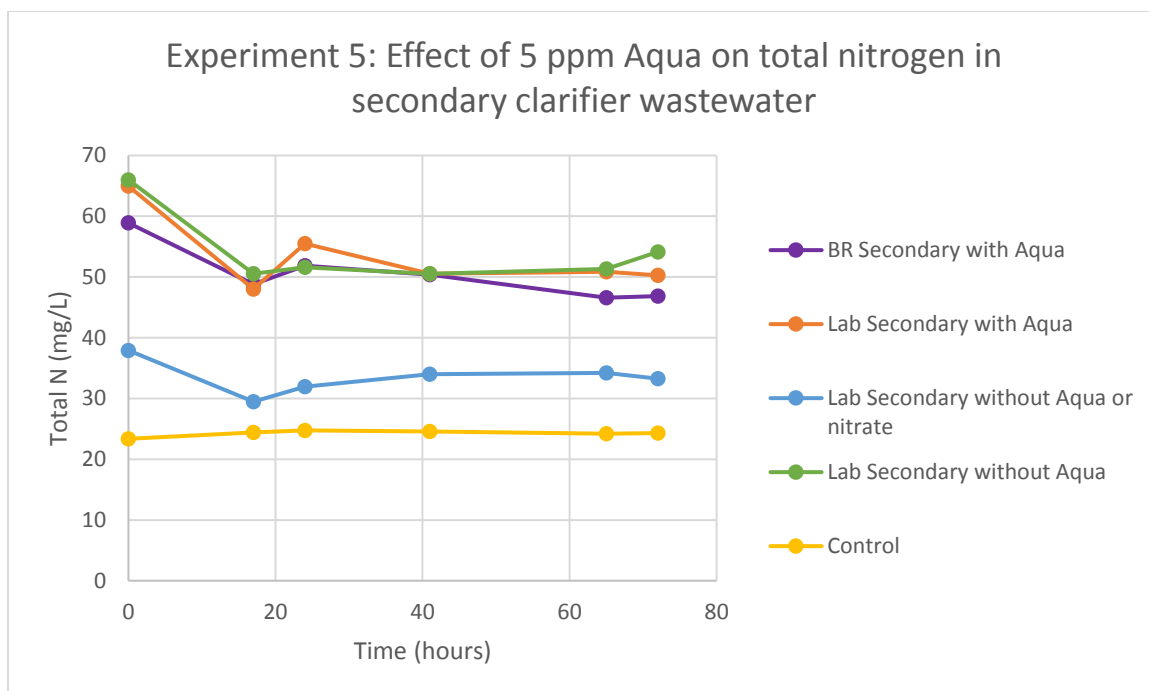


Figure 4.19: Effect of 5 ppm Aqua on total nitrogen in secondary clarifier wastewater

Nitrate removal rates for Aqua were the same as natural bacteria when 5 ppm of Aqua was used. Another low dose of Aqua was tested because the results of the 5 ppm dose experiment were not obtained before the next run was started. The 2.5 ppm dose test served as a check that lower doses cannot increase nitrate removal in secondary clarifier wastewater.

4.2.2 Experiment 6 – Effect of 2.5 ppm Aqua on Secondary Clarifier Wastewater

Ammonia and nitrate removal were analyzed for Aqua in secondary clarifier wastewater under anoxic conditions in lab and in field (Table 4.42, Table 4.43, Table 4.44, Figure 4.20, Figure 4.21, and Figure 4.22). This experiment was one of a series to determine the best Aqua dosage to achieve nitrate removal in the secondary clarifier. Temperature and pH

were measured to determine their potential effects on ammonia and nitrate removal (Table 4.40 and Table 4.41). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.39: Labeling for experiment 6

Description	Label
In-field bioreactor secondary clarifier wastewater with 2.5 ppm Aqua and 25 ppm NO ₃ -N	BR Secondary with Aqua
In-lab secondary clarifier wastewater with 2.5 ppm Aqua and 25 ppm NO ₃ -N	Lab Secondary with Aqua
In-lab secondary clarifier wastewater with no Aqua and 25 ppm NO ₃ -N	Lab Secondary without Aqua
In-lab secondary clarifier wastewater with no Aqua or NO ₃ -N	Lab Secondary without Aqua or nitrate
In-lab DI water with no Aqua and 25 ppm NO ₃ -N	Control

Table 4.40: pH measurements for experiment 6

Time (hours)	0	18	25	42	49	66	73	91	97
BR Secondary with Aqua	7.83	7.95	8.08	8.05	7.62	7.5	7.32	7.31	7.43
Lab Secondary with Aqua	7.63	7.57	7.45	7.28	7	6.71	6.62	6.45	6.57
Lab Secondary without Aqua or nitrate	7.66	7.62	7.51	7.35	6.99	6.73	6.61	6.49	6.52
Lab Secondary without Aqua	7.65	7.62	7.45	7.3	7.02	6.82	6.61	6.49	6.48
Control	6.79	6.57	5.86	6.87	7.14	6.81	6.54	6.35	6.38

The pH for 22% of all samples were within the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). About 38% of all samples were below a pH of 6.8,

which is where nitrification rates decline significantly. Therefore, pH could have an impact on the nitrification rate. The pH for 49% of all samples was within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). One sample was below a pH of 6, which inhibits the denitrification rate. Therefore, pH did not have a significant impact on the denitrification rate. All of the bioreactor samples were above a pH of 6.8. Therefore, the pH conditions for the bioreactor are more favorable than the laboratory samples for nitrification and denitrification.

Table 4.41: Temperature measurements for experiment 6

Time (hours)	0	18	25	42	49	66	73	91	97
BR Secondary with Aqua	24.2	19	25.6	19.7	26.3	17.6	23.8	17.7	25.9
Lab Secondary with Aqua	24.3	24.8	27.5	26.7	26.1	26	26.2	26.3	27.2
Lab Secondary without Aqua or nitrate	24.4	24.4	27.5	27.2	26	26	26.6	26.1	26.9
Lab Secondary without Aqua	24.5	24.6	27.8	26.6	26	25.6	26.4	26.2	26.9
Control	23.5	24.6	27.4	26.5	26.1	23.7	24.7	22.2	27.1

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 17°C and 26°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.42: Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 6

Time (hours)	0	19	25	42.5	49	66.5	73	97
Control	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua	25.33	24.96	24.05	24.37	26.31	25.70	25.07	25.42
Lab Secondary without Aqua or nitrate	23.60	25.03	25.46	25.05	26.11	27.31	25.66	25.27
Lab Secondary with Aqua	22.95	23.99	25.13	28.93	25.42	24.79	26.46	25.00
BR Secondary with Aqua	19.56	22.34	23.16	23.63	23.19	22.39	22.61	24.52

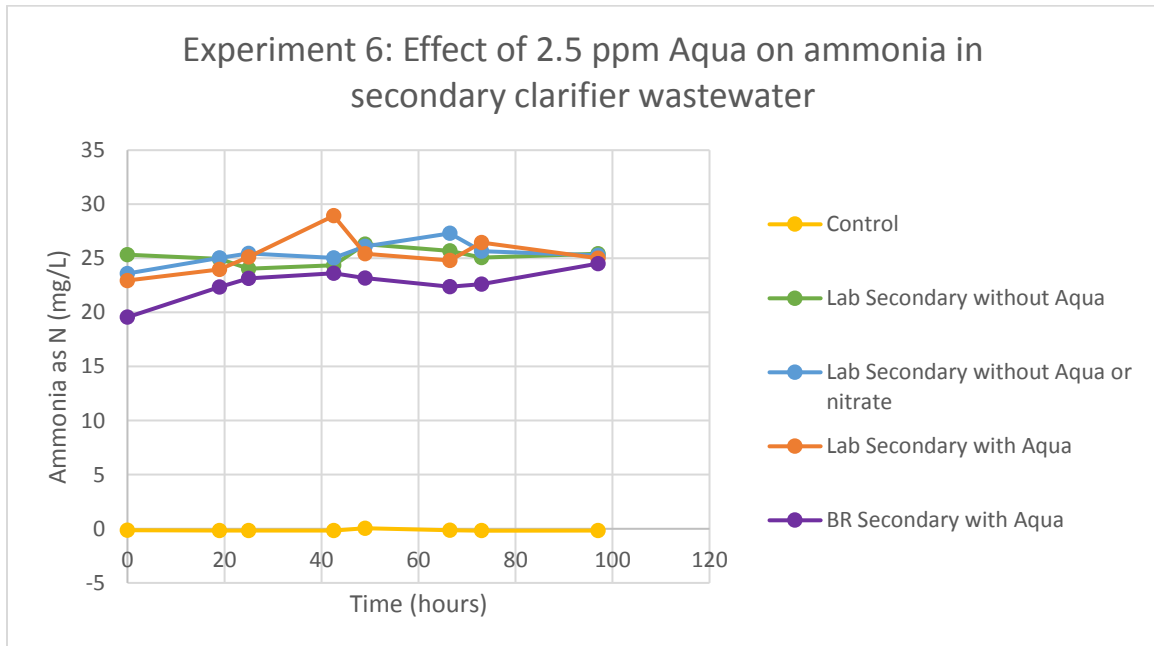


Figure 4.20: Effect of 2.5 ppm Aqua on ammonia in secondary clarifier wastewater

Table 4.43: Nitrate concentrations in mg/L NO₃-N for experiment 6

Time (hours)	0	19	25	42.5	49	66.5	73	97	Amount Degraded
BR Secondary with Aqua	25.24	21.79	18.03	20.56	19.89	17.41	16.67	17.65	7.59
Lab Secondary with Aqua	28.91	23.15	22.90	20.89	20.29	19.45	20.06	18.58	10.33
Lab Secondary without Aqua or nitrate	6.45	2.16	1.22	ND	ND	ND	ND	ND	5.45
Lab Secondary without Aqua	32.27	24.20	22.81	20.69	21.26	19.94	19.76	19.60	12.67
Control	24.90	24.84	25.05	25.12	24.42	24.73	24.62	24.44	0.47

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

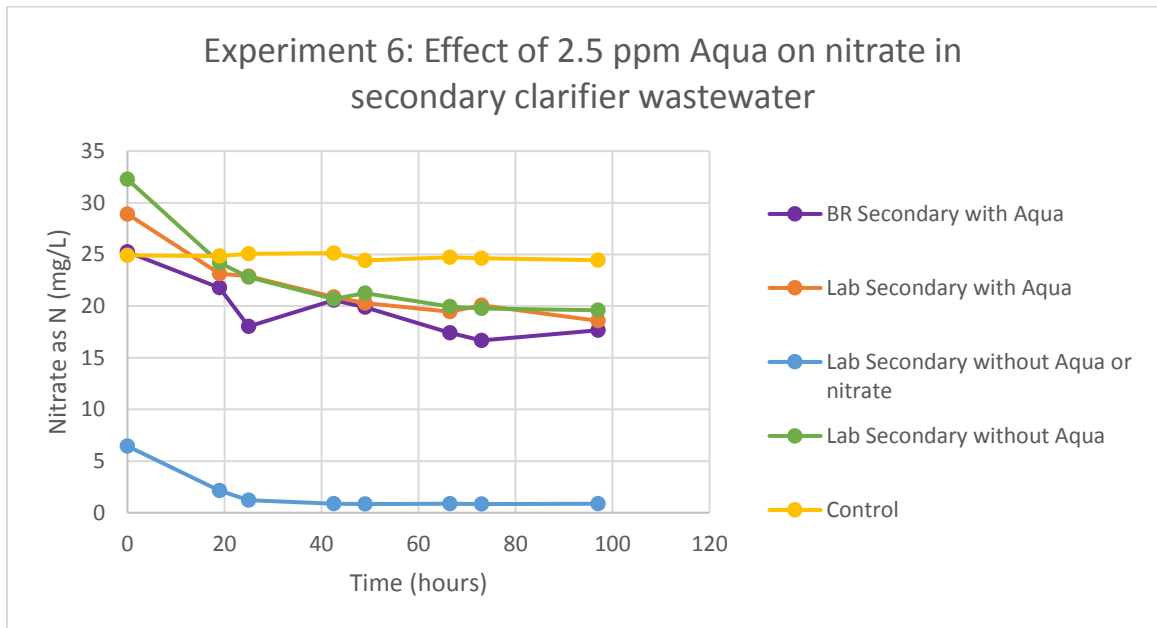
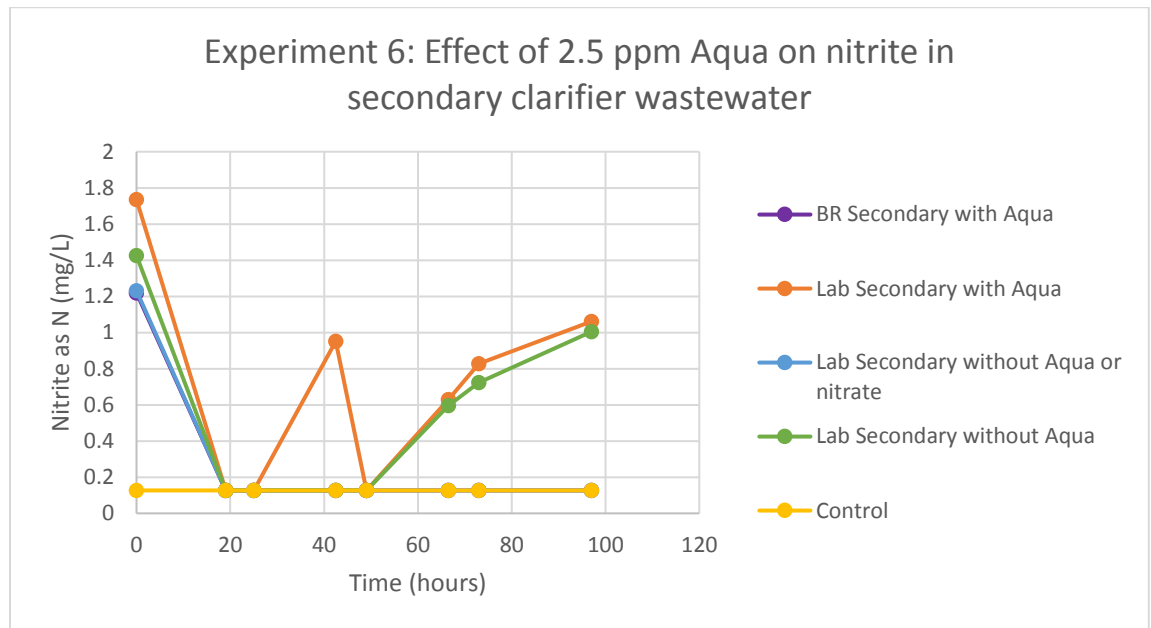


Figure 4.21: Effect of 2.5 ppm Aqua on nitrate in secondary clarifier wastewater

Table 4.44: Nitrite concentrations in mg/L NO₂-N for experiment 6

Time (hours)	0	19	25	42.5	49	66.5	73	97
BR Secondary with Aqua	1.22	ND	ND	ND	ND	ND	ND	ND
Lab Secondary with Aqua	1.73	ND	ND	ND	ND	ND	ND	1.06
Lab Secondary without Aqua or nitrate	1.23	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua	1.43	ND	ND	ND	ND	ND	ND	1.00
Control	ND	ND	ND	ND	ND	ND	ND	ND

**Figure 4.22: Effect of 2.5 ppm Aqua on nitrite in secondary clarifier wastewater**

The ammonium concentration fluctuated between 20 and 30 ppm ammonia. Therefore, bacteria did not process ammonia. The anoxic conditions inhibited nitrification and assimilation of ammonia. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. The samples containing Aqua behaved the same as

natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration.

All samples containing nitrate and wastewater exhibited nitrate removal over a period of about 4 days. As nitrate decreased, nitrite only increased by 1 ppm. Therefore, nitrate removal likely occurred via assimilation or denitrification without production of intermediate nitrite. Nitrogen gas and organic nitrogen content would need to be measured to know the exact process. The nitrite samples were below the 1 ppm MDL, which means that the sample points could be inaccurate. Therefore, the nitrite increase could be due to instrumental error. A longer run time may show an increase of nitrite above 1 ppm, which would mean the bacteria denitrified the nitrate. The natural bacteria wastewater behaved the same as the wastewater with Aqua. They all achieved a nitrate removal of 8 to 12 ppm nitrate. The small difference in removal amounts was due to different starting concentrations. Therefore, an addition of 2.5 ppm Aqua did not increase the nitrate removal rate.

The bioreactor sample was only about 3 ppm different than the laboratory sample. This was likely due to sample preparation error, since the difference is so small. The laboratory and bioreactor samples followed the same nitrate removal rates, so the laboratory sample without Aqua can be compared to bioreactor data. Also, outside factors did not affect bioreactor samples.

Zero and first order degradation rates were calculated for nitrate removal. Since the solutions with Aqua and without Aqua were dominated by the natural bacteria, the denitrification rates will be a range between the two. Both natural bacteria and Aqua followed zero order degradation for nitrate removal. Therefore, zero order kinetic values were compared to other literature values (Table 4.45). The amount of nitrate removed was calculated by subtracting the initial value from the final value (Table 4.46). Natural bacteria achieved a volumetric denitrification rate in the lower range of reported literature values. The clarifier was not designed for denitrification, which explains why the denitrification rates of the natural bacteria are low. Over the course of five days, all wastewater samples failed to reach below the permit value of 10 mg/L nitrate as nitrogen.

Table 4.45: Denitrification rates for experiment 6

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Secondary Clarifier Wastewater	1.81 - 2.74	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹ Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.46: Total nitrate removed and removal rates for experiment 6

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
BR Secondary with Aqua	7.59	97	0.0725	0.0035
Lab Secondary with Aqua	10.33	97	0.0917	0.004
Lab Secondary without Aqua	12.67	97	0.1097	0.0045

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

*Removal rates should all be the same. They are not completely the same because of different starting concentrations.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 2.38 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 0.95 mg/L as carbon. The C:N ratios were different for the samples (Table 4.47). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratios for samples with Aqua did not impact the nitrate removal rate. The sample without Aqua fell below 2.5:1, which could have slowed the nitrate removal rate. However, the C:N ratio was only 0.3 off from the ideal C:N ratio. Therefore, the C:N ratio did not impact nitrate removal for all samples.

Table 4.47: Carbon to nitrogen ratios for experiment 6

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Secondary with Aqua (lab and bioreactor)	70.48	25-29	71.43	2.6:1
Secondary without Aqua		32	70.48	2.2:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.23). The total nitrogen decreased by about 10 ppm for the lab samples with and without Aqua. The bioreactor sample did not experience any total nitrogen removal, likely because it started at a slightly lower total nitrogen concentration than the laboratory samples. If all samples started at the same concentration, it is likely that they would all have similar total nitrogen removal. The addition of Aqua did not improve total nitrogen removal.

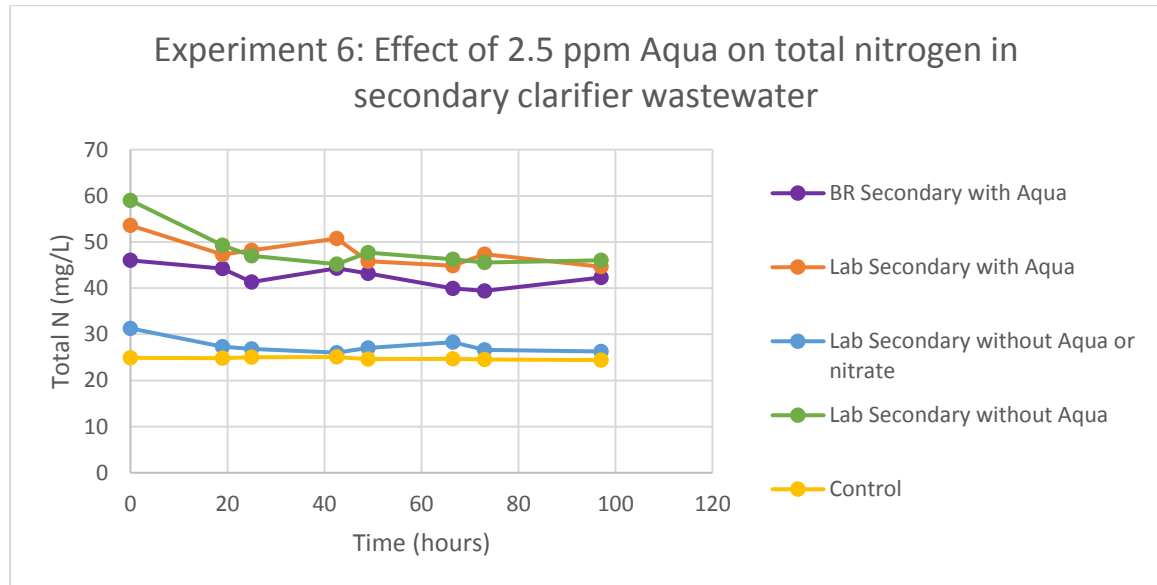


Figure 4.23: Effect of 2.5 ppm Aqua on total nitrogen in secondary clarifier wastewater

Nitrate removal rates for Aqua were the same as natural bacteria when 2.5 ppm of Aqua was used. Therefore, lower doses cannot accelerate nitrate removal. An Aqua dose of 50 ppm was tested to determine whether a higher dose will increase the nitrate removal rate.

4.2.3 Experiment 7 – Effect of 50 ppm Aqua on Secondary Clarifier Wastewater

Ammonia and nitrate removal were analyzed for Aqua in secondary clarifier wastewater under anoxic conditions in lab and in field (Table 4.51, Table 4.52, Table 4.53, Figure 4.24, Figure 4.25, and Figure 4.26). This experiment was one of a series to determine the best Aqua dosage to achieve nitrate removal in the secondary clarifier. Temperature and pH were measured to determine their potential effects on ammonia and nitrate removal (Table 4.49 and Table 4.50). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.48: Labeling for experiment 7

Description	Label
In-field bioreactor secondary clarifier wastewater with 50 ppm Aqua and 25 ppm NO ₃ -N	BR Secondary with Aqua
In-lab secondary clarifier wastewater with 50 ppm Aqua and 25 ppm NO ₃ -N	Lab Secondary with Aqua
In-lab secondary clarifier wastewater with no Aqua and 25 ppm NO ₃ -N	Lab Secondary without Aqua
In-lab secondary clarifier wastewater with no Aqua or NO ₃ -N	Lab Secondary without Aqua or nitrate
In-lab DI water with no Aqua and 25 ppm NO ₃ -N	Control

Table 4.49: pH measurements for experiment 7

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
BR Secondary with Aqua	7.13	7.14	7.29	7.08	7.23	7.21	7.31	7.22	7.28
Lab Secondary with Aqua	7.06	6.68	6.73	6.61	6.71	6.65	6.59	6.55	6.63
Lab Secondary without Aqua or nitrate	7.09	6.84	6.82	6.68	6.66	6.62	6.56	6.50	6.55
Lab Secondary without Aqua	7.17	6.91	6.93	6.86	6.85	6.82	6.76	6.65	6.73
Control	6.64	7.45	6.93	6.91	7.09	6.79	6.68	6.52	7.00

None of the samples were within the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). About 46% of all samples were below a pH of 6.8, which is where nitrification rates decline significantly. Therefore, pH could have an impact on the nitrification rate. The pH for 33% of all samples was within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). None of all samples were below a pH of 6, which inhibits the denitrification rate. Therefore, pH did not have a significant impact on the denitrification rate. None of the bioreactor samples were below a pH of 6.8. Therefore, the pH conditions for the bioreactor are more favorable than the laboratory samples for nitrification and denitrification.

Table 4.50: Temperature measurements for experiment 7

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
BR Secondary with Aqua	26.1	22.4	26.9	21.2	26.2	20.2	26.9	22.2	26.7
Lab Secondary with Aqua	27.0	27.3	26.6	26.4	26.7	26.6	26.7	27.1	27.6
Lab Secondary without Aqua or nitrate	27.1	27.3	26.5	26.5	26.9	26.9	27.2	27.3	27.6
Lab Secondary without Aqua	26.9	27.2	26.5	26.4	26.8	27.0	27.1	27.0	27.9
Control	35.1	27.1	26.2	24.8	26.5	26.9	26.0	26.4	27.8

Laboratory temperatures were around 27°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 20°C and 27°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.51: Ammonia concentrations in mg/L NH₄-N for experiment 7

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
BR Secondary with Aqua	26.09	30.23	26.47	30.32	28.13	29.18	29.36	30.08	30.72
Lab Secondary with Aqua	25.08	25.79	26.48	28.43	28.06	31.12	30.16	30.05	29.60
Lab Secondary without Aqua or nitrate	26.11	26.74	28.86	27.36	28.35	30.06	28.78	29.66	30.54
Lab Secondary without Aqua	26.38	27.52	28.33	28.68	28.64	29.37	29.65	29.85	30.07
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND

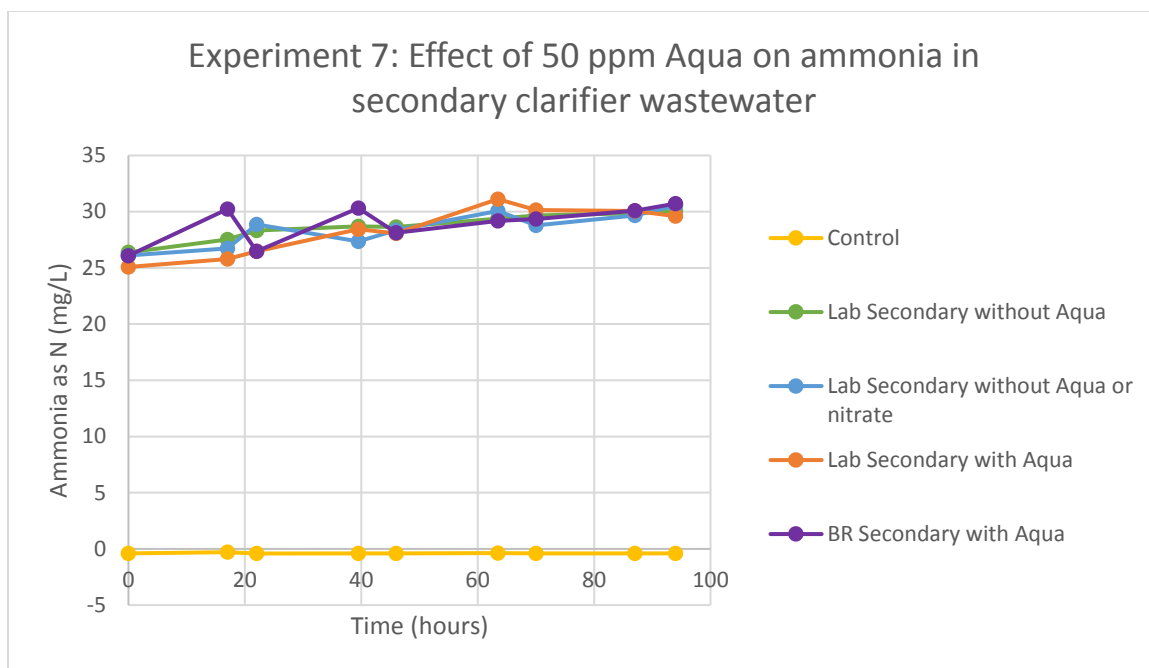


Figure 4.24: Effect of 50 ppm Aqua on ammonia in secondary clarifier wastewater

Table 4.52: Nitrate concentrations in mg/L NO₃-N for experiment 7

Time (hours)	0	17	22	63.5	70	87	94	Amount Degraded
BR Secondary with Aqua	22.77	8.53	6.99	2.98	2.62	2.17	1.91	20.86
Lab Secondary with Aqua	21.66	10.72	9.34	5.00	4.52	4.01	3.82	17.83
Lab Secondary without Aqua or nitrate	ND	ND	ND	ND	ND	ND	ND	0
Control	27.96	24.42	21.57	24.61	24.44	24.60	25.15	2.81
Lab Secondary without Aqua	21.54	19.12	17.72	13.86	14.25	13.72	13.42	8.13

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

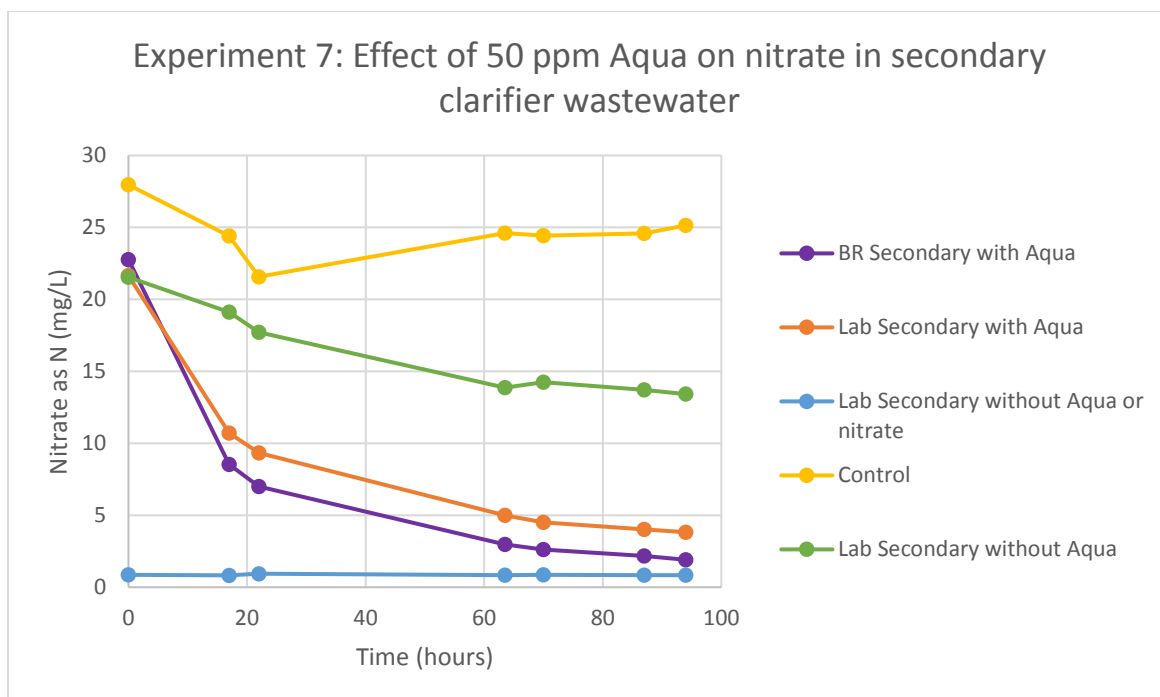


Figure 4.25: Effect of 50 ppm Aqua on nitrate in secondary clarifier wastewater

Table 4.53: Nitrite concentrations in mg/L NO₂-N for experiment 7

Time (hours)	0	17	22	63.5	70	87	94
BR Secondary with Aqua	ND	ND	ND	ND	ND	ND	ND
Lab Secondary with Aqua	ND	ND	ND	ND	ND	ND	1.07
Lab Secondary without Aqua or nitrate	ND	ND	ND	ND	ND	ND	ND
Control	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua	ND	ND	ND	ND	ND	1.16	1.15

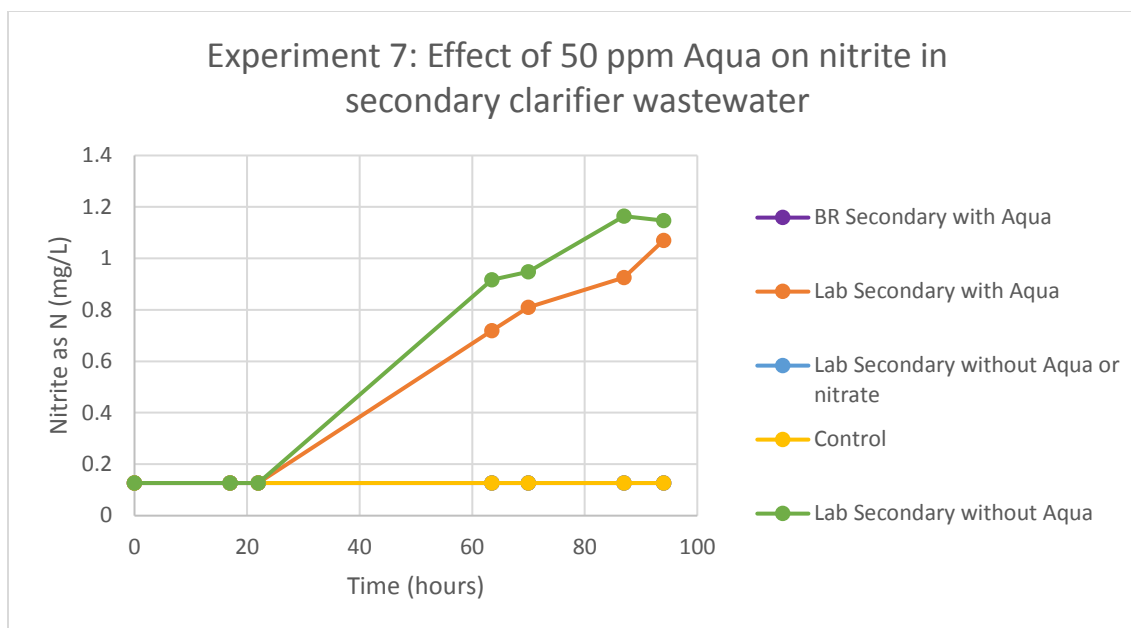


Figure 4.26: Effect of 50 ppm Aqua on nitrite in secondary clarifier wastewater

The ammonium concentration fluctuated between 25 and 30 ppm. The anoxic conditions inhibited nitrification and assimilation of ammonia. Instead, ammonia increased by about 5 ppm $\text{NH}_4\text{-N}$. Ammonification may have occurred to create this concentration increase. The small increase could also be due to sample preparation error including contamination or dilution from glassware rinses. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration.

All samples containing nitrate and wastewater exhibited nitrate removal over a period of about 4 days. As nitrate decreased, nitrite only increased by 1 ppm. Therefore, nitrate removal likely occurred via assimilation or denitrification without production of

intermediate nitrite. Nitrogen gas and organic nitrogen content would need to be measured to know the exact process. The nitrite samples were below the 1 ppm MDL, which means that the sample points could be inaccurate. Therefore, the nitrite increase could be due to instrumental error. A longer run time may show an increase of nitrite above 1 ppm, which would mean the bacteria denitrified the nitrate. Both samples containing Aqua removed nitrate at a faster rate than the natural bacteria in the wastewater. Aqua achieved 10 to 12 ppm more nitrate removal than the natural bacteria. Therefore, using 50 ppm of Aqua would be beneficial for decreasing nitrate in secondary clarifier wastewater.

The bioreactor sample was only 3 ppm different from the laboratory sample. This could be due to the difference in pH or procedural error. The bioreactor samples fell within the recommended pH range for denitrification. Only one laboratory samples fell within the recommended pH range for denitrification. However, the difference between the two is so small that the laboratory sample without Aqua can be compared to bioreactor data.

Zero and first order degradation rates were calculated for nitrate removal. The natural bacteria wastewater followed zero order kinetics. However, Aqua followed first order kinetics. Zero order kinetic values were compared to other literature values (Table 4.54). The amount of nitrate removed was calculated by subtracting the initial value from the final value (Table 4.55). Samples achieved a volumetric denitrification rate lower than reported literature values. Over the course of one day, the nitrate levels of samples containing Aqua reached below the discharge permit level of 10 mg/L for the SLO WRRF. The natural bacteria wastewater failed to reach below the permit value of 10 mg/L nitrate as nitrogen.

Table 4.54: Denitrification rates for experiment 7

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Secondary Clarifier Wastewater with Aqua	3.64 – 4.02	~25°C
This Study	Secondary Clarifier Wastewater	1.77	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹ Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.55: Total nitrate removed and removal rates for experiment 7

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
BR Secondary with Aqua	20.86	94	0.1675	0.0237
Lab Secondary with Aqua	17.83	94	0.1517	0.0168
Lab Secondary without Aqua	7.29	94	0.0739	0.005

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To

determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 47.5 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 19 mg/L as carbon. The C:N ratios were different for the samples (Table 4.56). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.56: Carbon to nitrogen ratios for experiment 7

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Secondary with Aqua (lab and bioreactor)	70.48	22-23	89.48	4:1
Secondary without Aqua		22	70.48	3.2:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.27). About 3 ppm total nitrogen was removed for samples without Aqua. The total nitrogen decreased by about 15 ppm for laboratory and bioreactor samples with Aqua. Therefore, the addition of Aqua did improve total nitrogen removal.

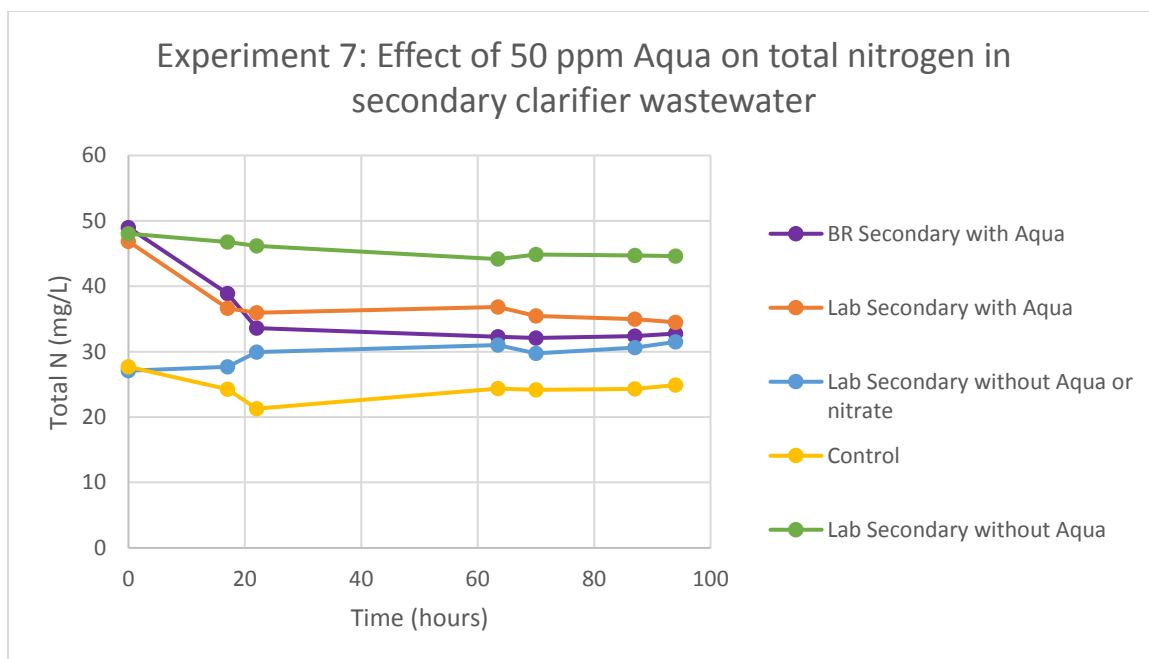


Figure 4.27: Effect of 50 ppm Aqua on total nitrogen in secondary clarifier wastewater

Nitrate removal rates for Aqua were higher than natural bacteria when 50 ppm of Aqua was used. This was the lowest concentration of Aqua in this series of experiments that could be used to achieve increased nitrate removal rates. However, in later experiments, 25 ppm Aqua was found to remove more nitrate than natural bacteria as well.

4.2.4 Experiment 8 – Effect of 25 ppm Aqua on Sludgewash Wastewater

Ammonia and nitrate removal were analyzed for Aqua in sludgewash wastewater under anoxic conditions in lab and in field (Table 4.60, Table 4.61, Table 4.62, Figure 4.28, Figure 4.29, and Figure 4.30). This experiment was one of a series to determine the best Aqua dosage to achieve nitrate removal in the sludgewash storage lagoon. Temperature and pH were measured to determine their potential effects on ammonia and nitrate removal

(Table 4.58 and Table 4.59). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.57: Labeling for experiment 8

Description	Label
In-field bioreactor sludgewash wastewater with 25 ppm Aqua	BR Sludgewash with Aqua
In-lab sludgewash wastewater with 10 ppm Aqua	Lab Sludgewash with Aqua
In-lab sludgewash wastewater with no Aqua or NO ₃ -N or NH ₄ -N	Lab Sludgewash without Aqua
In-lab DI water with no Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	Control

Table 4.58: pH measurements for experiment 8

Time (hours)	0	19	41	48	65	72	89	96	113	120
BR Sludgewash with Aqua	8.09	8.26	8.26	8.36	8.37	8.37	8.33	8.36	8.56	8.38
Lab Sludgewash with Aqua	8.03	8.23	8.2	8.27	8.24	8.12	8.18	8.18	8.2	8.15
Lab Sludgewash without Aqua	8.02	8.19	8.17	8.23	8.24	8.15	8.19	8.22	8.24	8.13
Control	5.31	6.3	6.8	6.77	6.8	6.6	6.14	6.57	6.3	6.2

The pH for all samples except the DI control was above the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). Samples were only slightly above 8, so the nitrification rate should not be affected. All of the DI control samples were at or below a pH of 6.8, which is where nitrification rates decline significantly (Metcalf & Eddy, 2003). None of the samples were within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). Samples were only slightly above 8, so the denitrification rate

should not be affected. Only one sample (0.02%) was below a pH of 6, which is where denitrification is inhibited.

Table 4.59: Temperature measurements for experiment 8

Time (hours)	0	19	41	48	65	72	89	96	113	120
BR Sludgewash with Aqua	25.6	22.3	21.9	26.1	22.1	26.2	23	26.1	20.1	28.8
Lab Sludgewash with Aqua	27.4	26.4	24.4	25.5	26.8	27	26	27.3	26.4	27.2
Lab Sludgewash without Aqua	27.4	26.3	24.7	25.6	26.7	27.1	25.6	27.3	26.4	27.7
Control	26.9	25.8	24.4	25.6	26.8	27	25.4	27.3	26.7	27.3

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 20°C and 29°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.60: Ammonia concentrations in mg/L NH₄-N for experiment 8

Time (hours)	0	41	48	65	72	89	96
Control	26.36	26.15	29.66	23.70	25.62	24.25	25.00
Lab Sludgewash without Aqua	625.85	649.13	710.84	567.48	655.30	588.95	612.44
Lab Sludgewash with Aqua	695.21	614.08	603.93	560.51	648.53	617.63	588.81
BR Sludgewash with Aqua	612.29	591.05	604.92	534.58	687.57	621.64	601.31

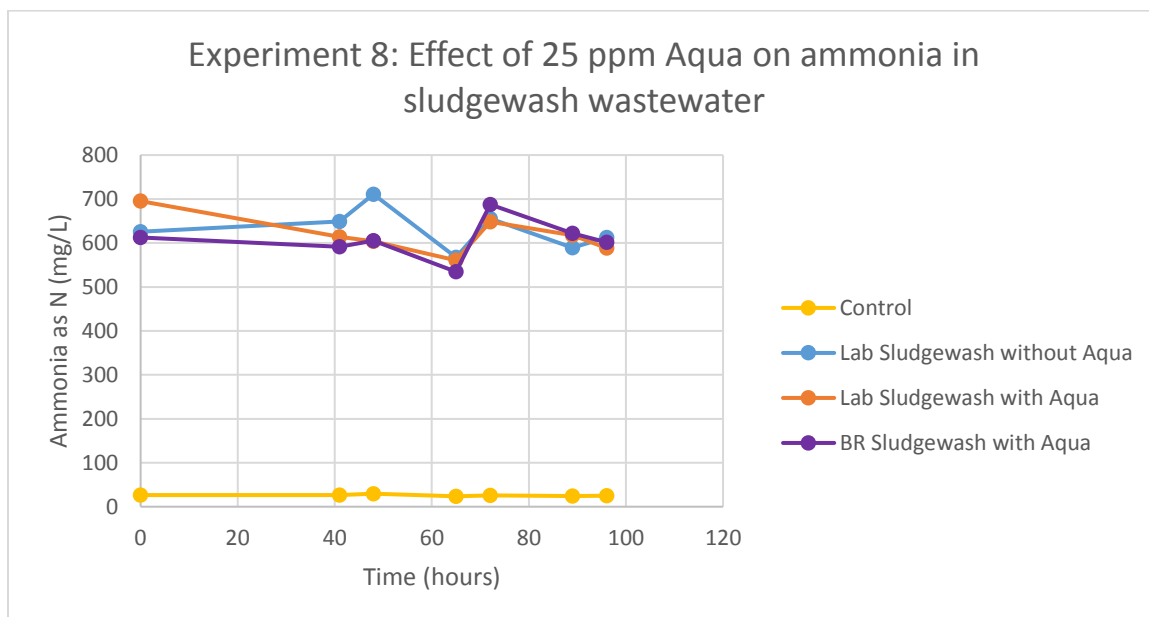


Figure 4.28: Effect of 25 ppm Aqua on ammonia in sludgewash wastewater

Table 4.61: Nitrate concentrations in mg/L NO₃-N for experiment 8

Time (hours)	0	41	65	72	89	120	Amount Degraded
BR Sludgewash with Aqua	66.30	39.90	41.44	39.02	36.06	39.90	30.24
Lab Sludgewash with Aqua	74.53	42.75	43.94	33.49	38.59	42.75	35.94
Lab Sludgewash without Aqua	69.47	56.07	42.19	37.79	38.15	47.00	31.32
Control	23.85	23.35	21.09	22.41	21.19	21.52	2.65

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

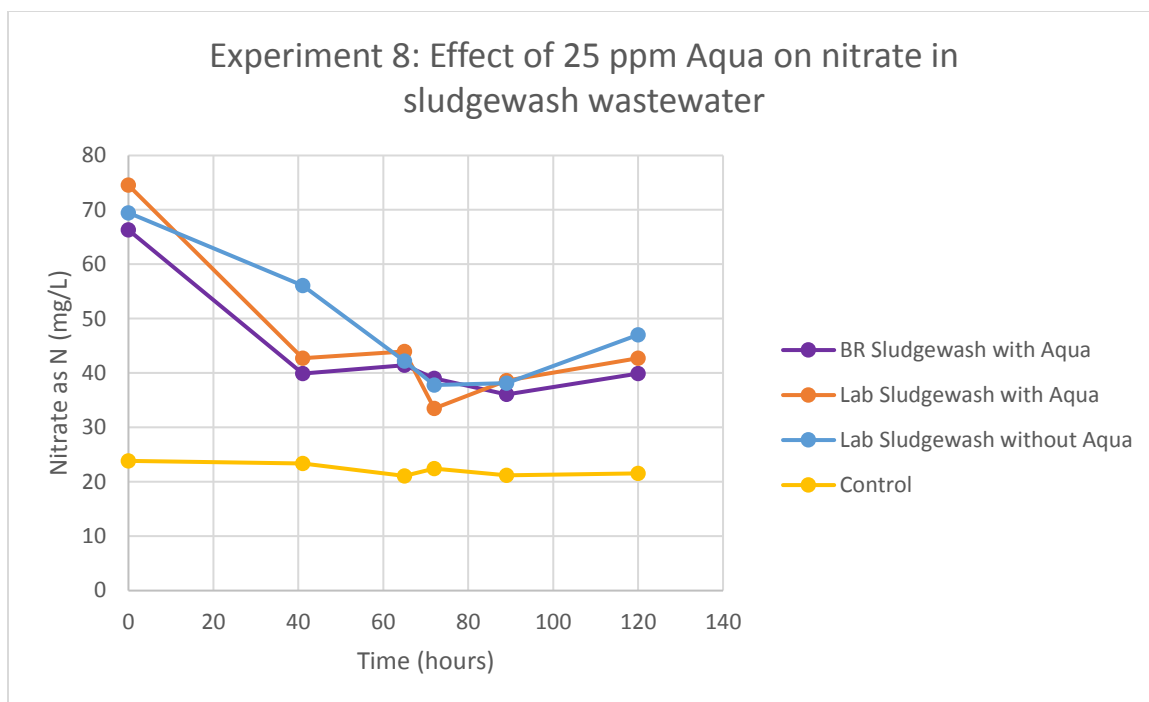


Figure 4.29: Effect of 25 ppm Aqua on nitrate in sludgewash wastewater

Table 4.62: Nitrite concentrations in mg/L NO₂-N for experiment 8

Time (hours)	0	41	65	72	89	120
BR Sludgewash with Aqua	5.29	8.79	6.14	7.12	7.62	8.79
Lab Sludgewash with Aqua	5.93	7.88	8.73	11.72	7.98	7.88
Lab Sludgewash without Aqua	5.11	7.46	7.04	7.71	7.91	9.46
Control	ND	ND	ND	ND	ND	ND

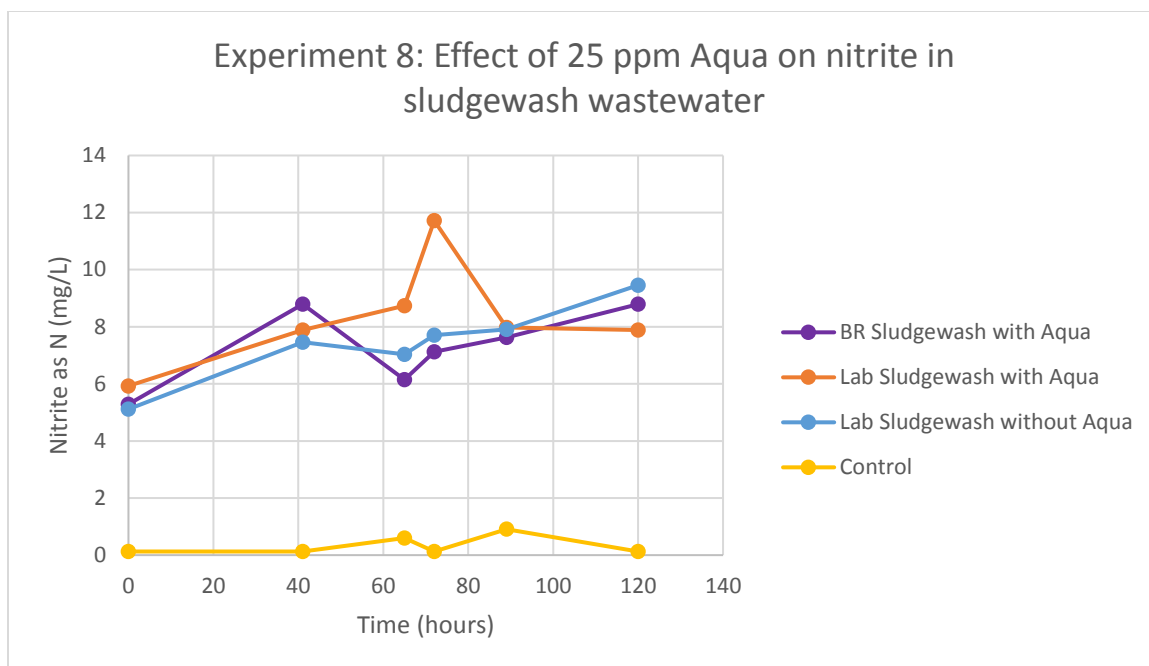


Figure 4.30: Effect of 25 ppm Aqua on nitrite in sludgewash wastewater

The ammonium concentration fluctuated between 600 and 700 ppm. Therefore, bacteria did not process ammonia. The anoxic conditions likely inhibited nitrification and assimilation of ammonia. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration.

All samples containing nitrate and wastewater exhibited nitrate removal over a period of about 5 days. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. The nitrate removal was due to denitrification. As nitrate decreased, nitrite

increased by about 3 ppm. However, nitrite never decreased, which means complete denitrification did not occur. The natural bacteria wastewater behaved the same as the wastewater with Aqua. They all achieved a nitrate removal of 30 to 35 ppm nitrate. The small difference in removal amounts was due to different starting concentrations. Therefore, an addition of 25 ppm Aqua did not increase the nitrate removal rate.

Zero and first order degradation rates were calculated for nitrate removal. Since the solutions with Aqua and without Aqua were dominated by the natural bacteria, the denitrification rates will be a range between the two. Samples followed a zero order degradation for nitrate removal. Therefore, zero order kinetic values were compared to other literature values (Table 4.63). The amount of nitrate removed was calculated by subtracting the initial value from the final value (Table 4.64). Natural bacteria achieved a volumetric denitrification rate in the low range of the reported literature values. The storage lagoon was not designed for denitrification, which explains why the denitrification rates of the natural bacteria are low. Over the course of five days, all wastewater samples failed to reach below the permit value of 10 mg/L nitrate as nitrogen.

Table 4.63: Denitrification rates for experiment 8

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Sludgewash Wastewater	5.12 – 6.3	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.64: Total nitrate removed and removal rates for experiment 8

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
BR Sludgewash with Aqua	30.24	120	0.2134	0.0042
Lab Sludgewash with Aqua	35.94	120	0.2628	0.0048
Lab Sludgewash without Aqua	31.32	120	0.2309	0.0043

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

*Removal rates should all be the same. They are not completely the same because of different starting concentrations.

According to research done by Eva Lee for BiOWiSH™, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon

source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 23.75 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 9.5 mg/L as carbon. The C:N ratios were different for the samples (Table 4.65). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.65: Carbon to nitrogen ratios for experiment 8

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Sludgewash with Aqua (lab and bioreactor)	177.5	66-75	187	2.7:1
Sludgewash without Aqua		70	177.5	2.5:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.31). The total nitrogen was steady around 700 ppm for all samples. Therefore, no total nitrogen removal occurred. Also, the addition of Aqua did not improve total nitrogen removal.

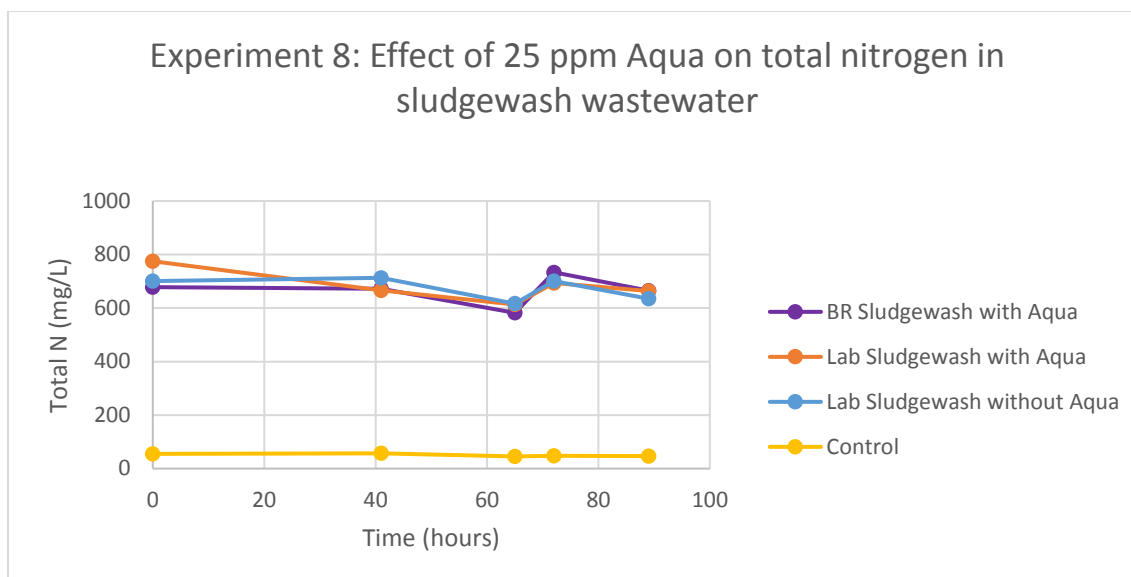


Figure 4.31: Effect of 25 ppm Aqua on total nitrogen in sludgewash wastewater

Nitrate removal rates for Aqua were the same as natural bacteria when 25 ppm of Aqua was used. Therefore, Aqua doses lower than 25 ppm were unlikely to achieve nitrogen removal. This was probably due to the high starting concentrations of ammonia and nitrate in the wastewater, which could inhibit Aqua growth. Sludgewash could also have high salinity, deficient minerals or trace elements, and high concentration of metals or other toxic chemicals that could inhibit Aqua growth. Another low dose of Aqua was tested because the results of this 25 ppm dose experiment were not obtained before the next run was started. This was due to backlog of needed supplies and limited spaces in the IC. The 10 ppm dose was tested to verify that Aqua doses lower than 25 ppm do not accelerate nitrate removal.

4.2.5 Experiment 9 – Effect of 10 ppm Aqua on Sludgewash Wastewater

Ammonia and nitrate removal were analyzed for Aqua in sludgewash wastewater under anoxic conditions in lab and in field (Table 4.69, Table 4.70, Table 4.71, Figure 4.32,

Figure 4.33, and Figure 4.34). This experiment was one of a series to determine the best Aqua dosage to achieve nitrate removal in the sludgewash storage lagoon. Temperature and pH were measured to determine their potential effects on ammonia and nitrate removal (Table 4.67 and Table 4.68). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.66: Labeling for experiment 9

Description	Label
In-field bioreactor sludgewash wastewater with 10 ppm Aqua	BR Sludgewash with Aqua
In-lab sludgewash wastewater with 10 ppm Aqua	Lab Sludgewash with Aqua
In-lab sludgewash wastewater with no Aqua or NO ₃ -N or NH ₄ -N	Lab Sludgewash without Aqua
In-lab DI water with no Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	Control

Table 4.67: pH measurements for experiment 9

Time (hours)	0	17	24	41	48	65	71	89	96	113	120
BR Sludgewash with Aqua	8.04	8.18	8.21	8.3	8.23	8.25	8.28	8.37	8.18	8.41	8.35
Lab Sludgewash with Aqua	8.01	8.06	8.09	8.12	8.09	8.07	2.09	8.12	8	8.12	8.1
Lab Sludgewash without Aqua	8.01	8.05	8.11	8.14	8.11	8.1	8.11	8.13	7.99	8.11	8.09
Control	6.6	6.58	6.24	6.58	6.24	6.77	6.15	6.35	6	6.18	6.07

The pH for all samples except the DI control samples was above the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). Samples were only slightly above 8, so the nitrification rate should not be affected. All of the DI control samples were below a pH of 6.8, which is where nitrification rates decline significantly (Metcalf & Eddy, 2003). The pH for all samples except the DI control samples was above the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). Samples were only slightly above 8, so the denitrification rate should not be affected. None of the samples were below a pH of 6, which is where denitrification is inhibited.

Table 4.68: Temperature measurements for experiment 9

Time (hours)	0	17	24	41	48	65	71	89	96	113	120
BR Sludgewash with Aqua	25.4	24.2	28.4	24.5	26.1	23.5	26.9	23.1	28.1	21.8	27.1
Lab Sludgewash with Aqua	25.5	26.4	27.7	26.8	26.8	24.9	27.3	26.6	27.5	25.8	26.9
Lab Sludgewash without Aqua	25.1	26.7	27.4	26.8	27	25.1	27.4	26.1	27.5	25.8	26.7
Control	30.1	26.3	27.3	26.9	27	25.1	27.6	26.6	27.6	25.4	26.5

Laboratory temperatures were around 27°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 22°C and 28°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.69: Ammonia concentrations in mg/L $\text{NH}_4\text{-N}$ for experiment 9

Time (hours)	0	17	24	41	48	65	71	89	96	113	120
Control	27.99	28.43	26.39	26.76	29.50	29.79	29.98	30.64	30.26	29.65	28.59
Lab Sludgewash without Aqua	672.74	693.44	651.42	690.46	680.77	655.85	695.07	696.54	684.58	687.09	668.68
Lab Sludgewash with Aqua	662.82	636.14	725.44	711.47	698.24	681.87	659.22	694.64	717.59	667.18	639.70
BR Sludgewash with Aqua	703.09	636.15	688.10	608.78	669.43	698.07	649.46	661.35	671.79	672.63	630.66

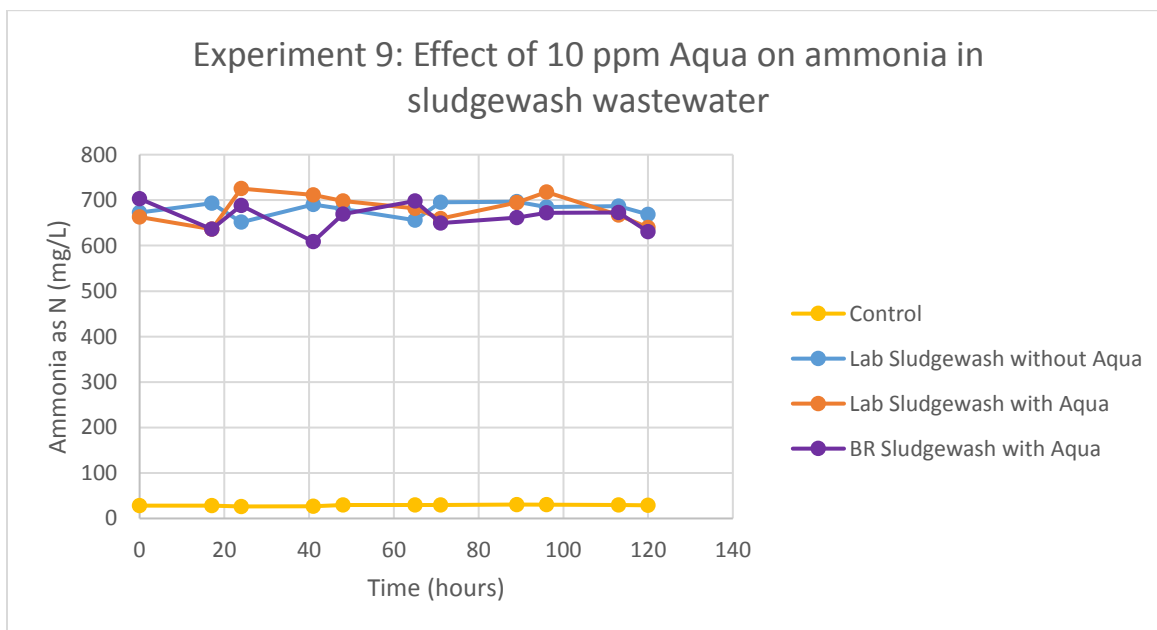


Figure 4.32: Effect of 10 ppm Aqua on ammonia in sludgewash wastewater

Table 4.70: Nitrate concentrations in mg/L NO₃-N for experiment 9

Time (hours)	0	17	41	65	89	96	113	120
BR Sludgewash with Aqua	54.00	45.63	38.56	35.61	35.94	40.92	32.32	41.57
Lab Sludgewash with Aqua	46.81	44.82	42.48	36.54	32.15	37.96	28.56	43.32
Lab Sludgewash without Aqua	51.57	41.84	56.51	37.24	35.72	39.94	36.17	39.90
Control	25.25	25.44	23.81	25.48	26.08	26.12	26.06	25.87

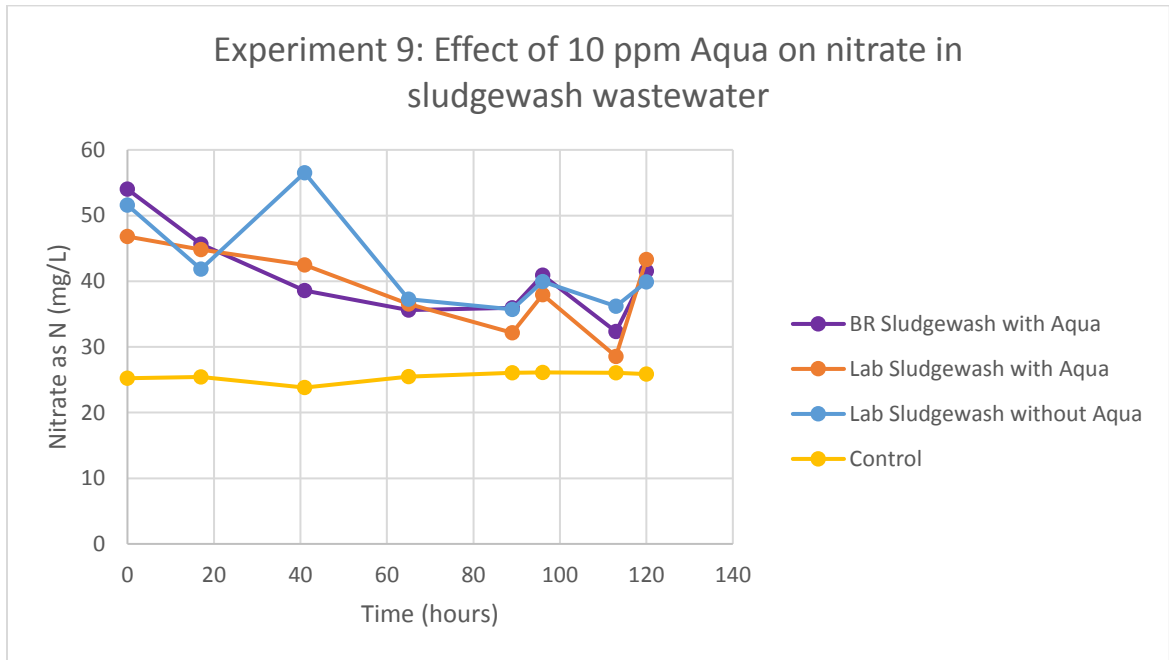
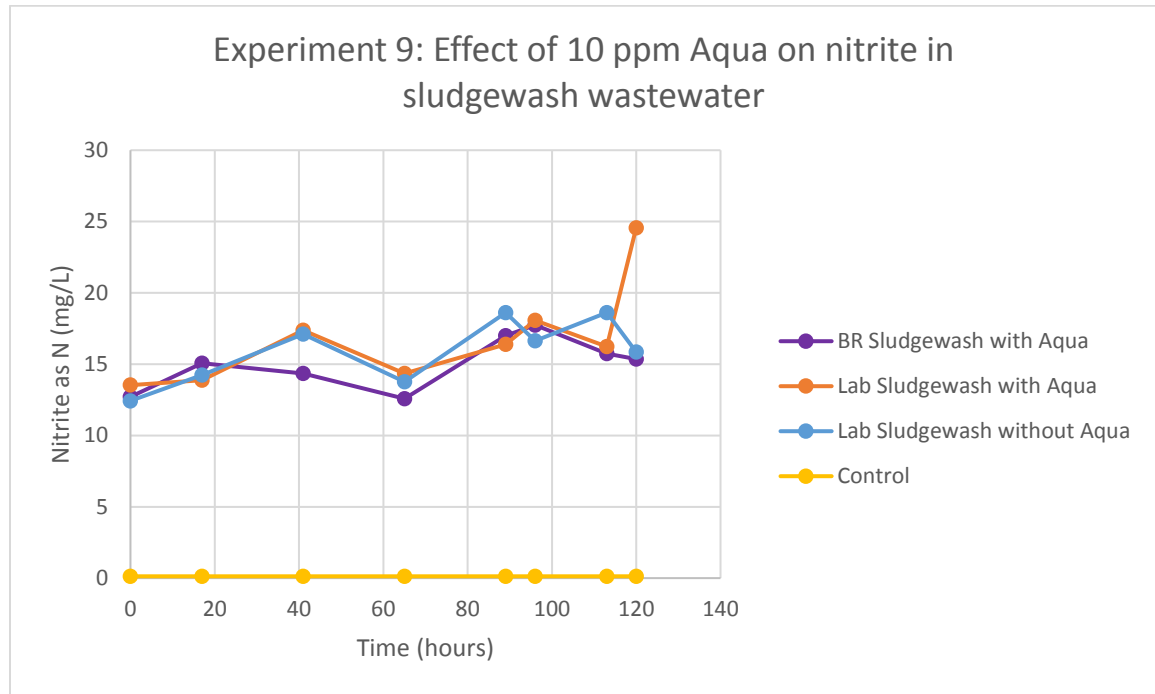


Figure 4.33: Effect of 10 ppm Aqua on nitrate in sludgewash wastewater

Table 4.71: Nitrite concentrations in mg/L NO₂-N for experiment 9

Time (hours)	0	17	41	65	89	96	113	120
BR Sludgewash with Aqua	12.70	15.07	14.34	12.58	16.99	17.74	15.75	15.35
Lab Sludgewash with Aqua	13.53	13.87	17.38	14.35	16.40	18.07	16.23	24.57
Lab Sludgewash without Aqua	12.43	14.23	17.11	13.77	18.61	16.64	18.62	15.86
Control	ND	ND	ND	ND	ND	ND	ND	ND

**Figure 4.34: Effect of 10 ppm Aqua on nitrite in sludgewash wastewater**

The ammonium concentration fluctuated between 600 and 700 ppm. Therefore, bacteria did not process ammonia. The anoxic conditions likely inhibited nitrification and assimilation of ammonia. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and

bioreactor samples could be compared. The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration.

All samples containing nitrate and wastewater exhibited about 10 ppm nitrate removal over a period of about 5 days. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. As nitrate decreased, nitrite increased. Therefore, nitrate removal was due to denitrification. The wastewater with Aqua exhibited about the same nitrate removal as the wastewater without Aqua. Therefore, the addition of 10 ppm Aqua did not increase the nitrate removal rate.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 9.5 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 3.8 mg/L as carbon. The C:N ratios were different for the samples (Table 4.72). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency

(Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.72: Carbon to nitrogen ratios for experiment 9

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Sludgewash with Aqua (lab and bioreactor)	177.5	46.8 - 54	181.4	3.6:1
Sludgewash without Aqua		52	177.5	3.4:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.35).

The total nitrogen was steady around 750 ppm for all samples. Therefore, no total nitrogen removal occurred. Also, the addition of Aqua did not improve total nitrogen removal.

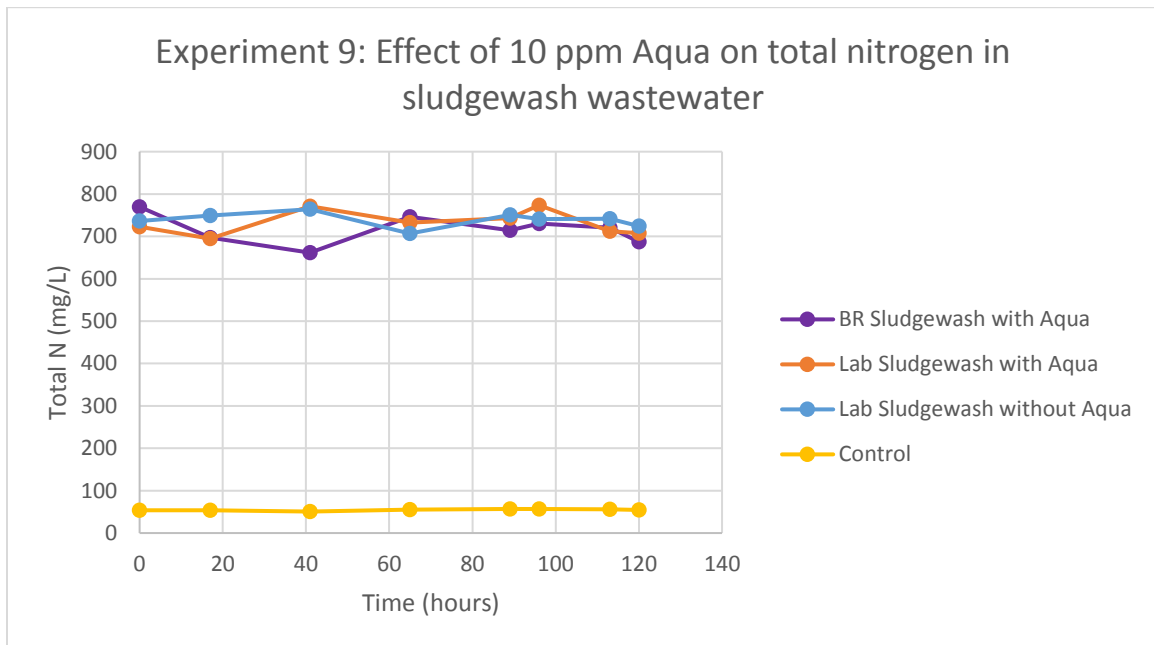


Figure 4.35: Effect of 10 ppm Aqua on total nitrogen in sludgewash wastewater

Ammonia and nitrate removal were not observed when 10 ppm of Aqua was used. Therefore, Aqua doses lower than 10 ppm were unlikely to achieve nitrogen removal. This was probably due to the high starting concentrations of ammonia and nitrate in the wastewater, which could inhibit Aqua growth. Sludgewash could also have high salinity, deficient minerals or trace elements, and high concentration of metals or other toxic chemicals that could inhibit Aqua growth. Another low dose of Aqua was tested because the results of this 10 ppm dose experiment were not obtained before the next run was started. This was due to backlog of needed supplies and limited spaces in the IC. The 5 ppm dose was tested to verify that Aqua doses lower than 25 ppm do not accelerate nitrate removal.

4.2.6 Experiment 10 – Effect of 5 ppm Aqua on Sludgewash Wastewater

Ammonia and nitrate removal were analyzed for Aqua in sludgewash wastewater under anoxic conditions in lab and in field (Table 4.76, Table 4.77, Table 4.78, Figure 4.36, Figure 4.37, and Figure 4.38). This experiment was one of a series to determine the best Aqua dosage to achieve nitrate removal in the sludgewash storage lagoon. Temperature and pH were measured to determine their potential effects on ammonia and nitrate removal (Table 4.74 and Table 4.75). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.73: Labeling for experiment 10

Description	Label
In-field bioreactor sludgewash wastewater with 5 ppm Aqua	BR Sludgewash with Aqua
In-lab sludgewash wastewater with 5 ppm Aqua	Lab Sludgewash with Aqua
In-lab sludgewash wastewater with no Aqua or NO ₃ -N or NH ₄ -N	Lab Sludgewash without Aqua
In-lab DI water with no Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	Control

Table 4.74: pH measurements for experiment 10

Time (hours)	0	19	25	42.5	49	66.5	73	91	97
BR Sludgewash with Aqua	8.13	8.36	8.19	8.36	7.95	8.08	7.89	7.88	7.88
Lab Sludgewash with Aqua	7.87	8.16	8.06	7.97	7.67	7.6	7.48	7.39	7.49
Lab Sludgewash without Aqua	7.87	8.17	8.09	7.98	7.7	7.63	7.48	7.44	7.49
Control	6.43	6.79	6.54	6.08	6.04	5.67	5.98	5.71	5.81

The pH for 22% of all samples was within the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). All of the wastewater samples were within or above the recommended range. None of the wastewater samples and all of the DI control samples are below a pH of 6.8, which is where nitrification rates decline significantly (Metcalf & Eddy, 2003). Therefore, pH could have an impact on the nitrification rate of the DI control samples, but not on the nitrification rate of the wastewater samples. The pH for 39% of all samples was within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). All of the wastewater samples were within or above the recommended range. None of the wastewater samples and 44% of the DI control samples were below a pH of 6,

which is where denitrification is inhibited. Therefore, pH could have an impact on the denitrification rate of the DI control samples, but not on the denitrification rate of the wastewater samples.

Table 4.75: Temperature measurements for experiment 10

Time (hours)	0	19	25	42.5	49	66.5	73	91	97
BR Sludgewash with Aqua	24.1	18.8	25.4	18.8	25.3	17.1	23.4	17.8	25.2
Lab Sludgewash with Aqua	24.1	25	27.6	26.6	25.8	26.6	27.2	27	26.3
Lab Sludgewash without Aqua	24.1	25	27.6	27.1	25.6	26.8	27.2	26.9	26.6
Control	23.6	24.3	27.7	26.5	25.9	26.6	27.1	27.2	25.9

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 17°C and 25°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.76: Ammonia concentrations in mg/L NH₄-N for experiment 10

Time (hours)	0	19	25	42.5	49	66.5	73	91	97
Control	26.00	26.67	26.33	26.53	26.70	26.19	26.21	26.28	26.21
Lab Sludgewash without Aqua	634.95	629.73	629.62	620.50	606.72	590.38	608.78	638.51	771.20
Lab Sludgewash with Aqua	605.11	628.13	654.76	640.60	618.97	552.87	604.82	634.30	579.62
BR Sludgewash with Aqua	635.89	612.66	624.17	592.02	645.58	621.36	617.98	624.76	617.79

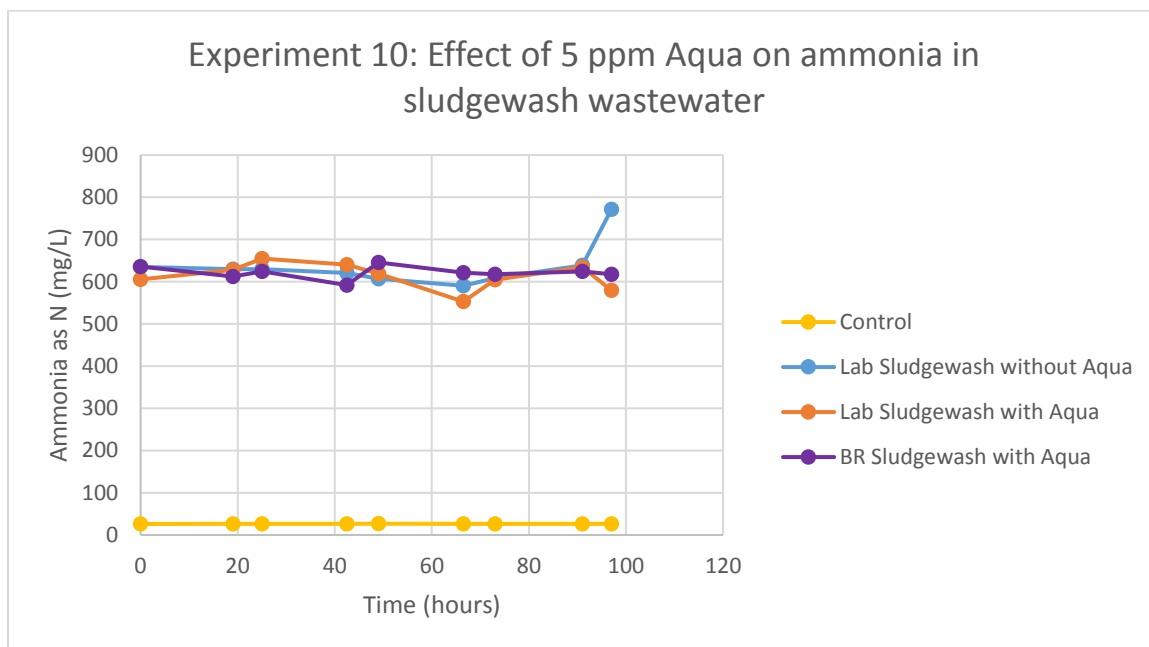


Figure 4.36: Effect of 5 ppm Aqua on ammonia in sludgewash wastewater

Table 4.77: Nitrate concentrations in mg/L NO₃-N for experiment 10

Time (hours)	0	19	25	42.5	49	66.5	73	91	97
BR Sludgewash with Aqua	58.70	46.45	56.36	42.01	58.26	51.73	45.86	55.74	48.42
Lab Sludgewash with Aqua	54.06	42.04	48.06	35.56	49.19	39.65	44.51	44.74	49.43
Lab Sludgewash without Aqua	58.03	43.45	49.60	37.92	50.81	42.07	46.08	53.47	45.29
Control	24.35	24.86	24.96	24.65	24.80	24.67	24.68	24.55	24.75

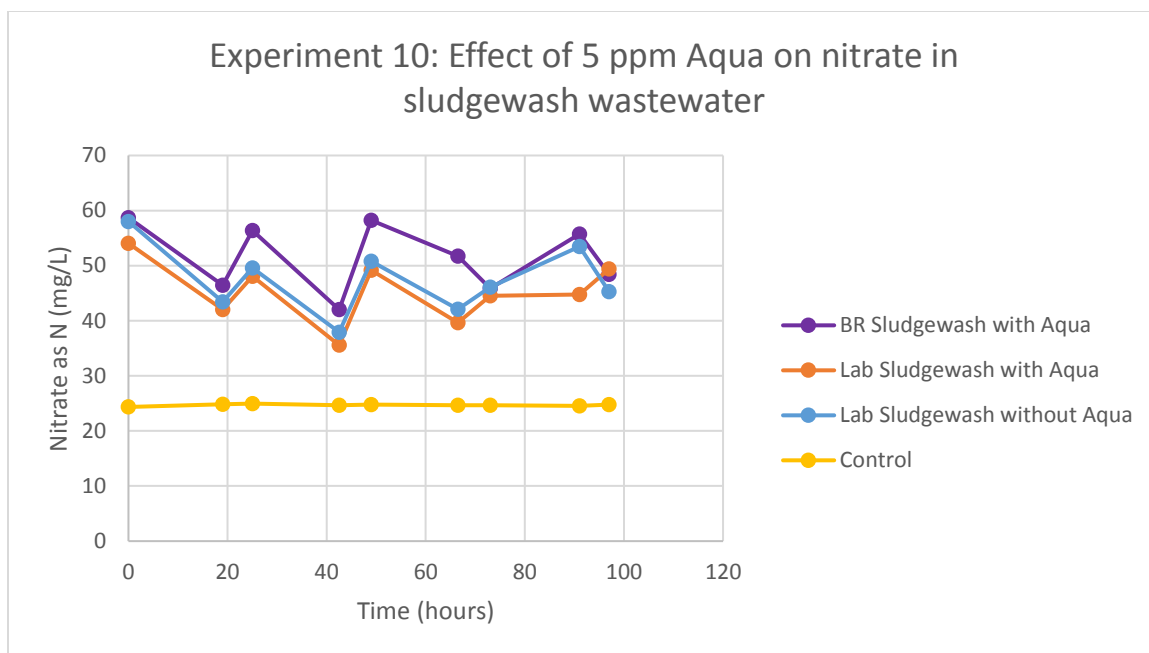


Figure 4.37: Effect of 5 ppm Aqua on nitrate in sludgewash wastewater

Table 4.78: Nitrite concentrations in mg/L NO₂-N for experiment 10

Time (hours)	0	19	25	42.5	49	66.5	73	91	97
BR Sludgewash with Aqua	16.47	13.85	16.31	15.23	15.13	15.54	15.27	3.97	6.12
Lab Sludgewash with Aqua	14.60	15.78	16.80	13.66	15.20	14.19	15.47	4.13	5.45
Lab Sludgewash without Aqua	16.15	13.07	17.37	15.21	16.37	12.42	13.68	4.53	5.73
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND

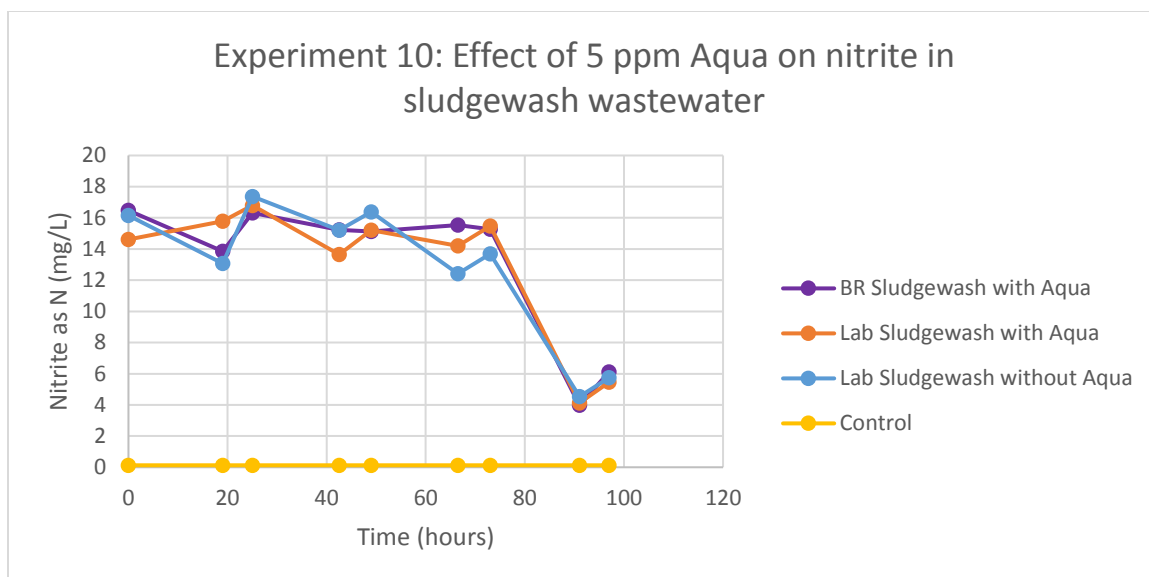


Figure 4.38: Effect of 5 ppm Aqua on nitrite in sludgewash wastewater

The ammonium concentration fluctuated between 600 and 650 ppm. Therefore, bacteria did not process ammonia. Ammonia increased at the end for the natural bacteria sample. The ammonia increase could be due to ammonification or sample preparation error. The anoxic conditions likely inhibited nitrification and assimilation of ammonia. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration.

All samples containing nitrate and wastewater fluctuated around 50 ppm nitrate. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. Nitrite decreased by about 10 ppm at the end of the run for all samples.

Therefore, the natural bacteria in the wastewater could remove the nitrite, likely by denitrification. Samples with and without Aqua resulted in very similar concentrations throughout the experiment. Therefore, the addition of 5 ppm Aqua did not increase the nitrate removal rate. The large concentrations in nitrate and ammonia were likely too toxic for any Aqua nitrate degrading bacteria to thrive in.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 4.75 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 1.9 mg/L as carbon. The C:N ratios were different for the samples (Table 4.79). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.79: Carbon to nitrogen ratios for experiment 10

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Sludgewash with Aqua (lab and bioreactor)	177.5	54 – 58.7	179.4	3.2:1
Sludgewash without Aqua		58	177.5	3.1:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.39).

The total nitrogen was steady around 700 ppm for all samples. Therefore, no total nitrogen removal occurred for all samples. Also, the addition of Aqua did not improve total nitrogen removal.

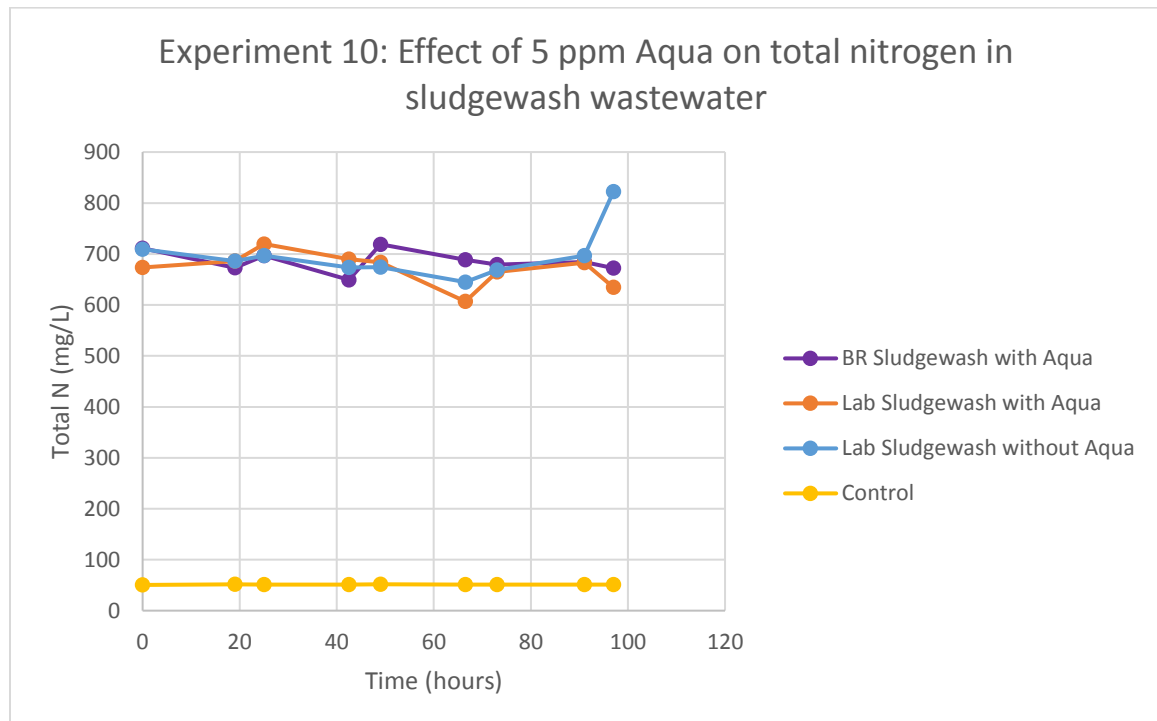


Figure 4.39: Effect of 5 ppm Aqua on total nitrogen in sludgewash wastewater

Ammonia and nitrate removal were not observed when 5 ppm of Aqua was used. Therefore, Aqua doses lower than 5 ppm were unlikely to achieve nitrogen removal. This was probably due to the high starting concentrations of ammonia and nitrate in the wastewater, which could inhibit Aqua growth. Sludgewash could also have high salinity, deficient minerals or trace elements, and high concentration of metals or other toxic chemicals that could inhibit Aqua growth. Another low dose of Aqua was tested because the results of this 5 ppm dose experiment were not obtained before the next run was started. This was due to backlog of needed supplies and limited spaces in the IC. The 2.5 ppm dose was tested to verify that Aqua doses lower than 25 ppm do not accelerate nitrate removal.

4.2.7 Experiment 11 – Effect of 2.5 ppm Aqua on Sludgewash Wastewater

Ammonia and nitrate removal were analyzed for Aqua in sludgewash wastewater under anoxic conditions in lab and in field (Table 4.83, Table 4.84, Table 4.85, Figure 4.40, Figure 4.41, and Figure 4.42). This experiment was one of a series to determine the best Aqua dosage to achieve nitrate removal in the sludgewash storage lagoon. Temperature and pH were measured to determine their potential effects on ammonia and nitrate removal (Table 4.81 and Table 4.82). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.80: Labeling for experiment 11

Description	Label
In-field bioreactor sludgewash wastewater with 2.5 ppm Aqua	BR Sludgewash with Aqua
In-lab sludgewash wastewater with 2.5 ppm Aqua	Lab Sludgewash with Aqua
In-lab sludgewash wastewater with no Aqua or NO ₃ -N or NH ₄ -N	Lab Sludgewash without Aqua
In-lab DI water with no Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	Control

Table 4.81: pH measurements for experiment 11

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
BR Sludgewash with Aqua	7.62	7.92	7.94	8.14	7.79	7.93	7.82	7.89	7.84
Lab Sludgewash with Aqua	7.6	7.55	7.42	7.49	7.62	7.54	7.46	7.5	7.49
Lab Sludgewash without Aqua	7.56	7.56	7.42	7.48	7.64	7.56	7.48	7.5	7.52
Control	6.44	6.71	6.42	6.44	6.66	6.38	6.62	6.54	6.65

The pH for 47% of all samples was within the recommended 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). None of the wastewater samples and all of the DI control samples are below a pH of 6.8, which is where nitrification rates decline significantly (Metcalf & Eddy, 2003). Therefore, pH could have an impact on the nitrification rate of the DI control samples, but not on the nitrification rate of the wastewater samples. The pH for 72% of all samples was within the recommended 7 to 8 for denitrification (Metcalf & Eddy, 2003). All of the wastewater samples were within or above the recommended range. None of the DI control samples were within the recommended range. None of the samples were below

a pH of 6, which is where denitrification is inhibited. Therefore, pH did not have an impact on the denitrification rate.

Table 4.82: Temperature measurements for experiment 11

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
BR Sludgewash with Aqua	24	21	26.2	19.7	24.7	19.4	26.6	21.5	26.6
Lab Sludgewash with Aqua	24.6	26.5	26.4	24.8	27.1	26.8	27.2	26.9	27.9
Lab Sludgewash without Aqua	24.8	26.7	26.4	24.8	26.9	26.7	27.2	26.8	27.6
Control	35	26.8	27	25	26.9	25.6	26.9	26.9	27.1

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 19°C and 27°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.83: Ammonia concentrations in mg/L NH₄-N for experiment 11

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
Control	21.24	20.21	21.10	24.16	20.08	19.73	20.75	21.37	21.00
Lab Sludgewash without Aqua	632.11	610.75	633.38	654.50	592.43	632.79	637.93	688.80	654.04
Lab Sludgewash with Aqua	614.45	604.21	609.27	618.57	611.76	664.01	673.10	653.10	671.01
BR Sludgewash with Aqua	602.53	671.80	580.77	631.83	604.42	608.88	636.87	605.95	642.32

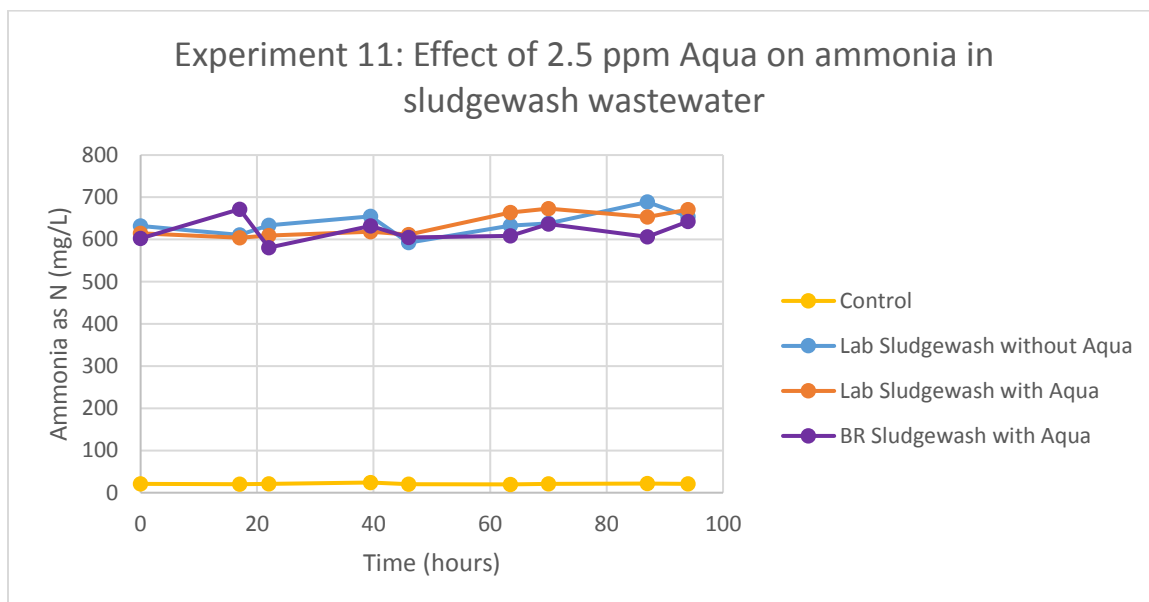


Figure 4.40: Effect of 2.5 ppm Aqua on ammonia in sludgewash wastewater

Table 4.84: Nitrate concentrations in mg/L NO₃-N for experiment 11

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
BR Sludgewash with Aqua	62.77	61.86	53.35	54.66	52.66	60.15	71.16	59.52	60.47
Lab Sludgewash with Aqua	61.48	62.21	53.02	49.78	51.07	55.90	60.33	54.51	55.21
Lab Sludgewash without Aqua	58.82	62.24	51.24	49.05	46.66	54.29	53.54	56.38	54.01
Control	26.42	26.00	26.85	28.51	23.99	23.80	24.89	25.35	25.73

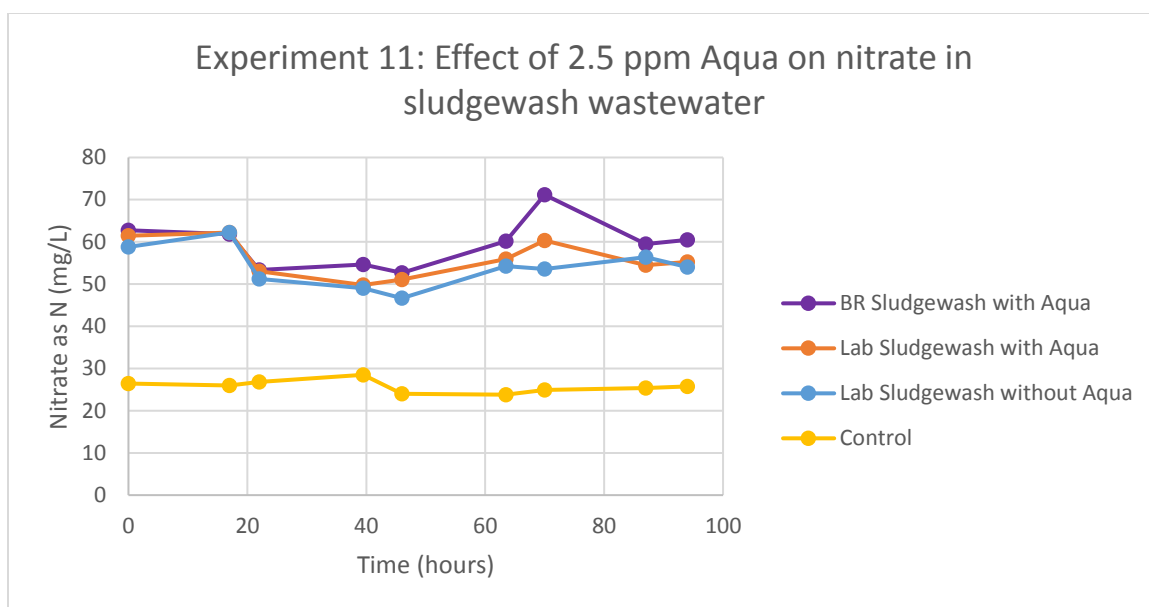


Figure 4.41: Effect of 2.5 ppm Aqua on nitrate in sludgewash wastewater

Table 4.85: Nitrite concentrations in mg/L NO₂-N for experiment 11

Time (hours)	0	17	22	39.5	46	63.5	70	87	94
BR Sludgewash with Aqua	7.07	4.67	8.00	4.49	8.55	4.62	4.13	5.06	4.38
Lab Sludgewash with Aqua	6.47	4.34	8.85	5.06	9.29	4.77	5.35	5.53	5.40
Lab Sludgewash without Aqua	7.76	4.67	8.28	4.64	7.05	5.26	5.34	5.09	4.49
Control	ND	ND	ND	1.00	ND	ND	ND	ND	ND

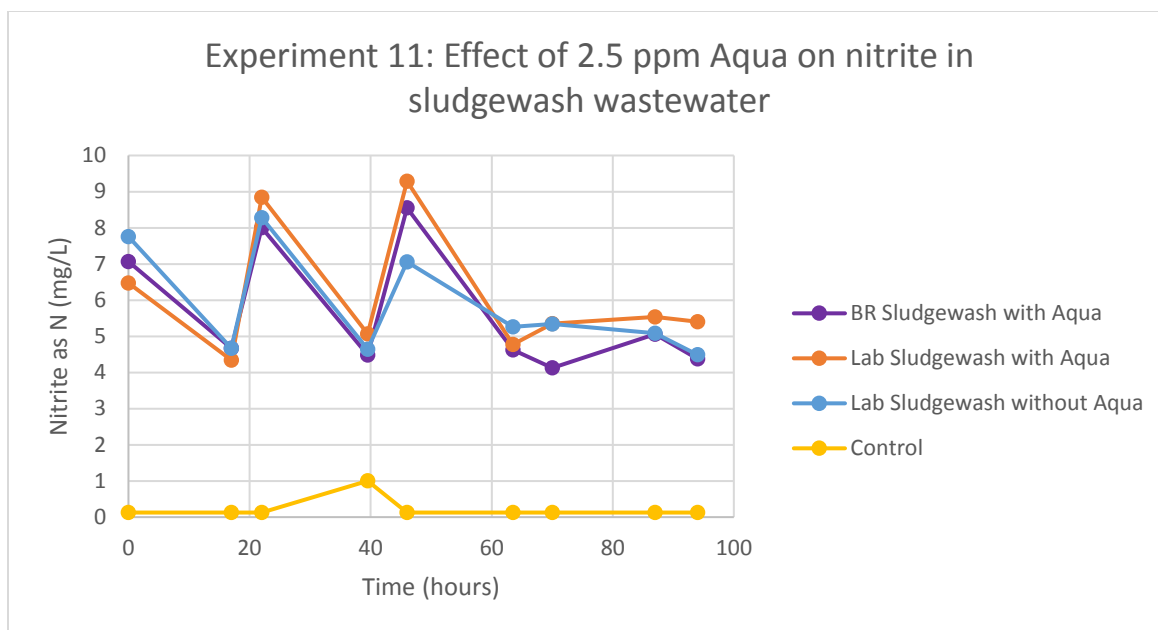


Figure 4.42: Effect of 2.5 ppm Aqua on nitrite in sludgewash wastewater

The ammonium concentration fluctuated between 600 and 650 ppm. Therefore, bacteria did not process ammonia. The anoxic conditions also likely inhibit nitrification and assimilation of ammonia. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration.

All samples containing nitrate and wastewater fluctuated around 50 ppm nitrate. Laboratory and bioreactor samples did not differ in concentration. Therefore, outside factors did not affect bioreactor samples, and lab and bioreactor samples could be compared. Fluctuations in nitrite occurred. Overall, nitrite decreased by about 3 ppm for

all samples, signifying possible nitrite reduction by denitrifying bacteria. Samples with and without Aqua resulted in very similar concentrations throughout the experiment. Therefore, the addition of 2.5 ppm Aqua did not increase the nitrate removal rate. The large concentrations in nitrate and ammonia were likely too toxic for any Aqua nitrate degrading bacteria to thrive in. Fluctuations were likely due to sample preparation error including contamination from improperly cleaned glassware or dilution from glassware rinses.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 2.375 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 0.95 mg/L as carbon. The C:N ratios will be different for the samples (Table 4.86). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.86: Carbon to nitrogen ratios for experiment 11

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Sludgewash with Aqua (lab and bioreactor)	177.5	61.5 – 62.8	178.45	2.9:1
Sludgewash without Aqua		58.8	177.5	3:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.43).

The total nitrogen was steady around 700 ppm for all samples. Therefore, no total nitrogen removal occurred. Also, the addition of Aqua did not improve total nitrogen removal.

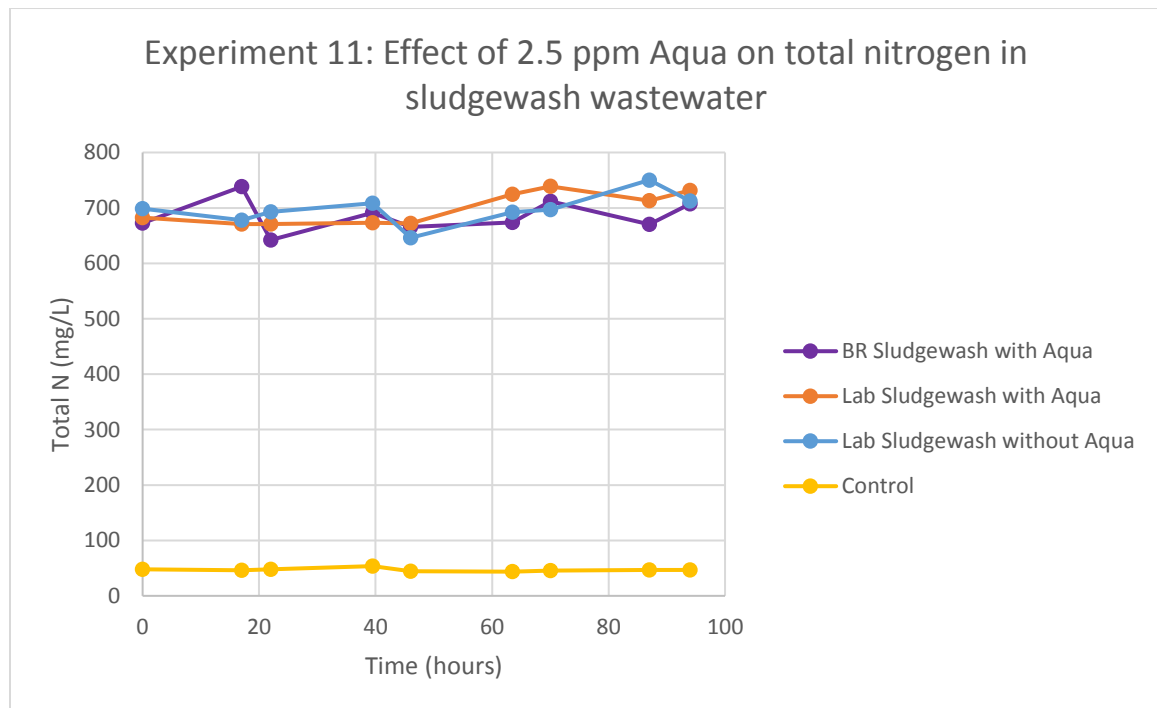


Figure 4.43: Effect of 2.5 ppm Aqua on total nitrogen in sludgewash wastewater

Ammonia and nitrate removal were not observed when 2.5 ppm of Aqua was used. Therefore, Aqua doses lower than 2.5 ppm were unlikely to achieve nitrogen removal. This was probably due to the high starting concentrations of ammonia and nitrate in the wastewater, which could inhibit Aqua growth. Sludgewash could also have high salinity, deficient minerals or trace elements, and high concentration of metals or other toxic chemicals that could inhibit Aqua growth.

Nitrate removal did not occur for Experiment 10 and 11, even though it did in Experiment 8 and 9. This could be due to no solids recycle occurring for the sludgewash. Therefore, natural bacteria in the sludgewash will be in a lag, log, stationary, or death phase during different times. For Experiment 8 and 9, the wastewater was likely collected in the log or stationary phase. For Experiment 10 and 11, the wastewater was likely collected in the death phase, which would explain the lack of nitrate removal. Experiment 10 and 11 also had lower pH's than Experiment 8 and 9, which could slow denitrification rates for Experiment 10 and 11.

4.2.8 Experiment 12 – Effect of 25 ppm Activated Aqua on Secondary Clarifier

Wastewater

Nitrate removal was analyzed for Aqua in secondary clarifier wastewater under anoxic conditions in lab and in field (Table 4.90, Table 4.91, Figure 4.44, and Figure 4.45). This experiment determined whether activating the Aqua first before inoculation resulted in better denitrification rates. Ideally activating the Aqua would produce a large cell count, therefore skipping the lag time in the beginning of bacterial growth. Temperature and pH

were measured to determine their potential effects on nitrate removal (Table 4.88 and Table 4.89). Different times between the pH, temperature, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.87: Labeling for experiment 12

Description	Label
In-field bioreactor secondary wastewater with 25 ppm Activated Aqua and 25 ppm NO ₃ -N	BR Secondary with Activated Aqua
In-field bioreactor secondary wastewater with 25 ppm Dry Aqua and 25 ppm NO ₃ -N	BR Secondary with Dry Aqua
In-field bioreactor secondary wastewater with no Aqua and 25 ppm NO ₃ -N	BR Secondary without Aqua
In-lab secondary wastewater with 25 ppm Activated Aqua and 25 ppm NO ₃ -N	Lab Secondary with Activated Aqua
In-lab secondary wastewater with 25 ppm Dry Aqua and 25 ppm NO ₃ -N	Lab Secondary with Dry Aqua
In-lab secondary wastewater with no Aqua and 25 ppm NO ₃ -N	Lab Secondary without Aqua
In-lab secondary wastewater with 25 ppm Aqua and no NO ₃ -N	Lab Secondary with Aqua but without nitrate
In-lab DI water with no Aqua and 25 ppm NO ₃ -N	Control

Table 4.88: pH measurements for experiment 12

Time (hours)	0	18	25	42.5	50	66.5	73	92	97	114	121
BR Secondary with Activated Aqua	6.82	6.93	7.06	7.07	7.1	7.1	7.17	7.14	7.16	7.25	7.16
BR Secondary with Dry Aqua	6.9	6.95	6.99	7.01	7.02	7.12	7.1	7.12	7.1	7.17	7.12
BR Secondary without Aqua	6.88	7.06	7.2	7.11	7.09	7.16	7.16	7.14	7.16	7.2	7.13
Lab Secondary with Aqua but without nitrate	6.88	6.61	6.57	6.41	6.46	6.39	6.39	6.41	6.36	6.36	6.27
Lab Secondary with Activated Aqua	6.91	6.64	6.58	6.51	6.5	6.5	6.5	6.55	6.38	6.45	6.41
Lab Secondary with Dry Aqua	6.88	6.78	6.71	6.4	6.59	6.53	6.53	6.56	6.56	6.42	6.44
Lab Secondary without Aqua	6.92	6.83	6.83	6.68	6.67	6.62	6.62	6.64	6.56	6.47	6.5
Control	7.34	6.57	7.01	6.52	6.63	6.52	6.52	6.49	6.53	6.5	6.27

The pH for 33% of all samples was within the recommended 7 to 8 for denitrification (Metcalf & Eddy, 2003). About 82% of the bioreactor samples were within the 7 to 8 range, but only 4% of the laboratory samples are within the range. Therefore, the pH conditions for the bioreactor were more favorable than for the laboratory samples. None of the samples are below a pH of 6, which is where denitrification is inhibited. Therefore, pH did not have a significant impact on the denitrification rate.

Table 4.89: Temperature measurements for experiment 12

Time (hours)	0	18	25	42.5	50	66.5	73	92	97	114	121
BR Secondary with Activated Aqua	25.2	18.9	25.8	20.2	26.8	18.9	24	21.1	25	18.2	24.4
BR Secondary with Dry Aqua	26.5	19.2	25.8	22.5	28.4	21	25.1	23.4	25.9	20.6	26.8
BR Secondary without Aqua	25.5	18.6	24	22	25.7	20.6	22.7	22.5	23.6	20.3	24
Lab Secondary with Aqua but without nitrate	26.9	27	26.8	26.2	27.2	24.8	27.1	26.8	26.6	25.9	26.8
Lab Secondary with Activated Aqua	27	26.7	27.2	26.4	27.1	25.5	27.1	26.1	26.4	26.4	26.6
Lab Secondary with Dry Aqua	26.9	26.4	26.6	26.1	26.9	25.9	26.9	26.2	26.5	26.1	26.6
Lab Secondary without Aqua	27	25.9	27	26.3	27.1	26.5	27	24.3	26.3	26.2	26.3
Control	25.3	25.5	26.7	25	26.7	24.4	27.2	23.7	25.8	25.2	25

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 18°C and 27°C, which has a significant impact on the rate of denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.90: Nitrate concentrations in mg/L NO₃-N for experiment 12

Time (hours)	0	18	25	50	66.5	73	92	97	114	121	Amount Degraded
BR Secondary with Activated Aqua	23.91	15.86	14.76	11.42	10.74	11.76	9.59	9.37	9.13	9.02	14.78
BR Secondary with Dry Aqua	23.88	13.19	13.08	9.73	9.46	8.08	7.93	7.87	7.14	7.60	16.74
BR Secondary without Aqua	22.96	22.36	18.09	17.02	17.36	15.05	14.63	14.22	15.59	13.77	7.37
Control	24.20	26.84	24.32	24.11	24.67	24.10	24.62	24.65	24.38	24.40	-0.17
Lab Secondary with Aqua but without nitrate	3.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.12
Lab Secondary with Activated Aqua	22.21	17.65	16.33	13.18	12.84	11.78	11.50	11.05	10.82	10.26	11.39
Lab Secondary with Dry Aqua	23.66	15.92	14.59	13.50	12.69	12.34	11.73	12.87	11.41	11.01	12.24
Lab Secondary without Aqua	22.24	20.49	19.77	17.77	17.68	17.15	17.21	17.39	16.19	16.30	6.05

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

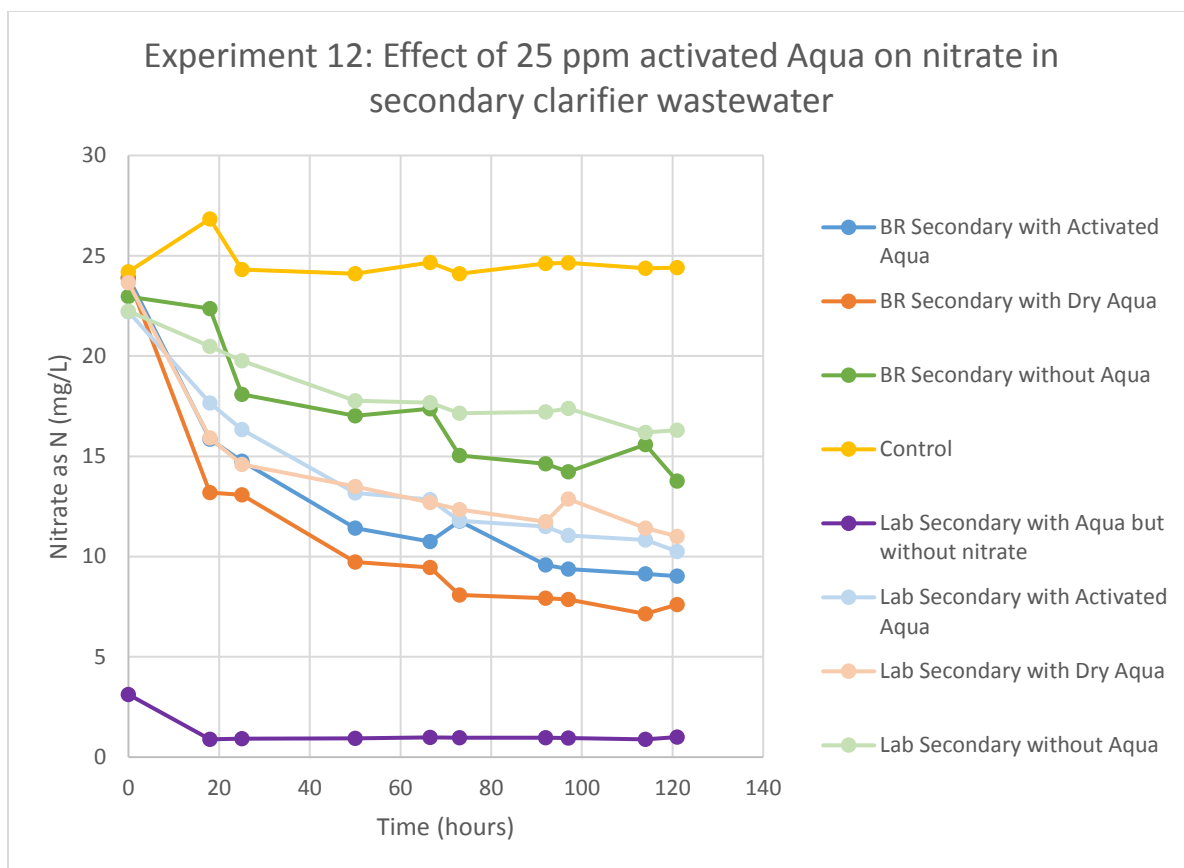


Figure 4.44: Effect of 25 ppm activated Aqua on nitrate in secondary clarifier wastewater

Table 4.91: Nitrite concentrations in mg/L NO₂-N for experiment 12

Time (hours)	0	18	25	50	66.5	73	92	97	114	121
BR Secondary with Activated Aqua	1.30	6.59	ND	ND	ND	ND	1.34	ND	1.15	ND
BR Secondary with Dry Aqua	1.09	10.59	ND	ND	1.06	1.10	6.41	1.45	1.05	2.02
BR Secondary without Aqua	1.38	7.66	ND	ND	1.24	1.23	7.22	1.10	1.30	ND
Control	ND	ND	ND	ND	ND	ND	ND	1.50	ND	ND
Lab Secondary with Aqua but without nitrate	1.61	ND	ND	ND	ND	ND	ND	ND	ND	8.19
Lab Secondary with Activated Aqua	1.41	ND	ND	ND	ND	ND	6.56	1.03	1.37	1.45
Lab Secondary with Dry Aqua	1.45	ND	ND	ND	ND	1.12	1.27	1.15	1.80	2.19
Lab Secondary without Aqua	1.44	ND	ND	ND	0.98	1.13	1.28	7.28	1.31	3.83

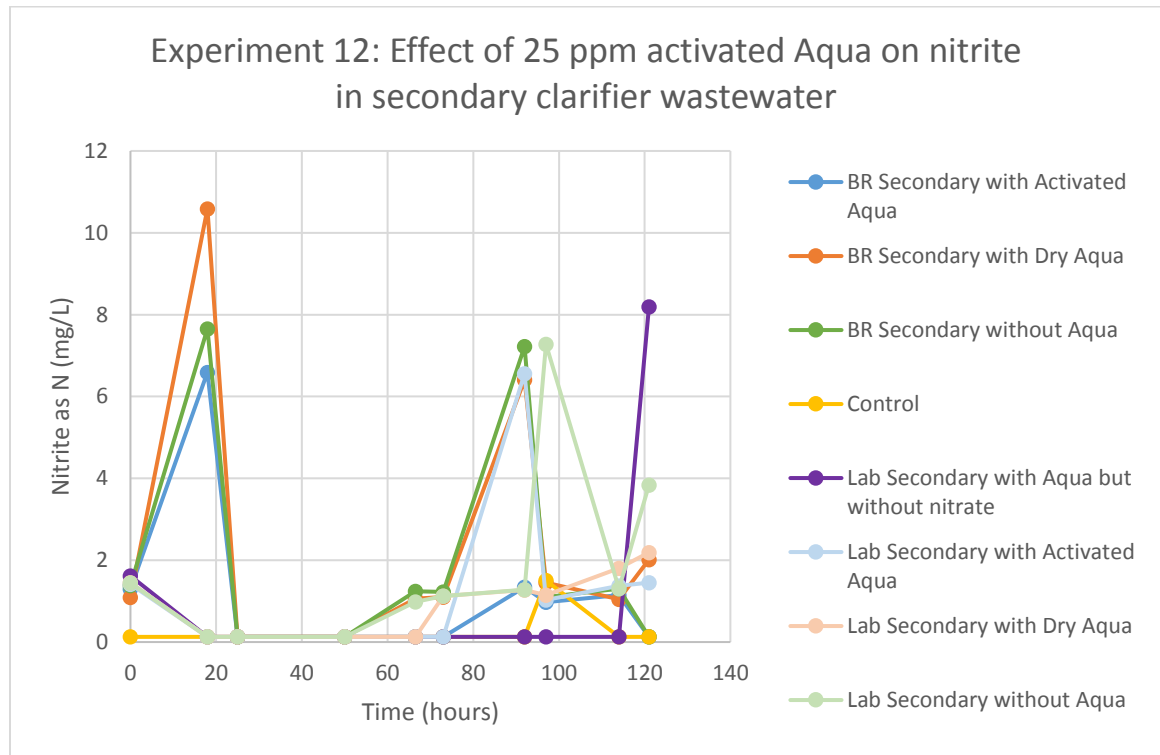


Figure 4.45: Effect of 25 ppm activated Aqua on nitrite in secondary clarifier wastewater

All samples containing nitrate and wastewater exhibited nitrate removal over a period of about 4 days. The bioreactor sample was only 3 ppm different from the laboratory sample. Since the difference is small, the laboratory samples can be compared to bioreactor data. Nitrate removal likely occurred via assimilation or denitrification. At the beginning, bioreactor samples experienced increased nitrite when nitrate decreased. Nitrite concentrations spiked throughout the experiment as well. This nitrite increases could have been caused by denitrification, procedural error, or other metabolic processes. All samples containing Aqua removed nitrate at a faster rate than the natural bacteria in the wastewater. The activated Aqua was only a maximum of 2 ppm different than non-activated Aqua. Therefore, activated Aqua did not produce better results for nitrate removal. All Aqua samples achieved 5 to 10 ppm more nitrate removal than the natural bacteria. Therefore, using 25 ppm of Aqua could be beneficial for decreasing nitrate in secondary clarifier wastewater. A cost to benefit analysis should be conducted to determine whether using Aqua would be beneficial.

Zero and first order degradation rates were calculated for nitrate removal. The natural bacteria wastewater samples followed zero order kinetics, but the Aqua inoculated wastewater followed first order kinetics. Zero order kinetic values were compared to other literature values (Table 4.92). The amount of nitrate removed was calculated by subtracting the initial value from the final value (Table 4.93). Over the course of five days, the samples containing Aqua were at or below the discharge permit level of 10 mg/L nitrate as nitrogen for the SLO WRRF. The natural bacteria wastewater failed to reach below the permit value

of 10 mg/L nitrate as nitrogen. Both Aqua and natural bacteria achieved a volumetric denitrification rate in the lower range of the literature values.

Table 4.92: Denitrification rates for experiment 12

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Secondary Clarifier Wastewater with Aqua	12– 16.3	~25°C
This Study	Secondary Clarifier Wastewater without Aqua	6 - 9	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.93: Total nitrate removed and removal rates for experiment 12

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
BR Secondary with Activated Aqua	14.89	121	0.1061	0.0076
BR Secondary with Dry Aqua	16.28	121	0.1115	0.0089
BR Secondary without Aqua	9.19	121	0.0787	0.0044
Lab Secondary with Activated Aqua	11.95	121	0.0936	0.0064
Lab Secondary with Dry Aqua	12.64	121	0.0797	0.0051
Lab Secondary without Aqua	5.93	121	0.0483	0.0026

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. Activated Aqua consists of TSB, growth media, and Aqua. Carbon was added from TSB, growth media, and Aqua. Growth media added 1000 mg/L dextrose. TSB added 250 mg/L dextrose because only 10% of the TSB was added to the activated Aqua solution. Aqua added 7362.5 mg/L dextrose because about 95% of Aqua is dextrose. The activated Aqua solution contained a total of 8612.5 mg/L dextrose. About 0.32% of the activated Aqua was added to the wastewater samples. Therefore, a total of 27.56 mg/L dextrose was added. This equates to 11 mg/L dextrose as carbon. The dry Aqua added 23.75 mg/L dextrose, which equates to 9.5 mg/L dextrose as carbon. The C:N ratios were different for the samples (Table 4.94). For C:N ratios higher than 6:1, nitrate removal

rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.94: Carbon to nitrogen ratios for experiment 12

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Secondary with Activated Aqua (lab and bioreactor)	70.48	22.2 – 23.9	81.48	3.5:1
Secondary with Aqua (lab and bioreactor)		23.7 – 23.9	79.98	3.3:1
Secondary without Aqua		22.4 - 23	70.48	3.1:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C for activated Aqua includes initial, Aqua, and TSB. Total C for Aqua includes initial and Aqua

Total nitrogen was calculated by adding the nitrate and nitrite (Figure 4.46). The total nitrogen decreased for all wastewater samples. The total nitrogen decreased by about 15 ppm for bioreactor samples with activated Aqua and dry Aqua. The total nitrogen decreased by about 12 ppm for laboratory samples with activated Aqua and dry Aqua. Total nitrogen decreased by about 7 ppm for bioreactor and lab samples without Aqua. Both lab and bioreactor samples with Aqua had better total nitrogen removal than samples without Aqua. Therefore, Aqua did improve total nitrogen removal.

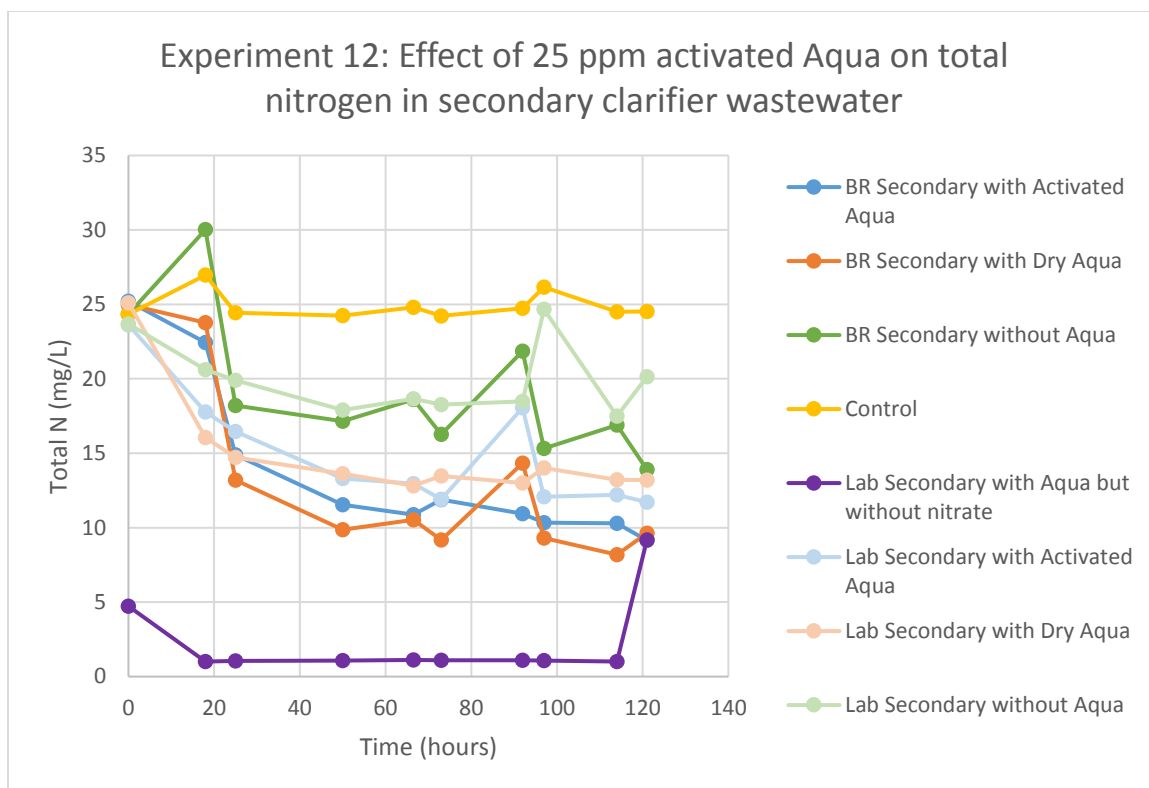


Figure 4.46: Effect of 25 ppm activated Aqua on total nitrogen in secondary clarifier wastewater

Nitrate removal rates for Aqua and Activated Aqua were higher than natural bacteria when 25 ppm of Aqua was used. However, activated Aqua did not make a difference in the removal rate compared to dosing with dry Aqua. This experiment was performed again, but in aerobic conditions to determine how activation affects aerobic nitrification and denitrification.

4.2.9 Experiment 13 – Effect of 25 ppm Activated Aqua under Partial Aeration on Secondary Clarifier Wastewater

Ammonia and nitrate removal were analyzed for Aqua in secondary clarifier wastewater under partially aerobic conditions in field (Table 4.98, Table 4.99, Table 4.100, Figure 4.47, Figure 4.48, and Figure 4.49). Bubblers would aerate during the day only because the batteries would die overnight, which is why it is only partial aeration. Laboratory solutions could not be run at the same time because there were not enough bubblers. It was assumed that natural bacteria followed typical chemoautotrophic metabolic processes and Aqua followed typical heterotrophic metabolic processes for nitrification. This experiment determined whether activating the Aqua first before inoculation will achieve better aerobic nitrification and denitrification rates. Ideally activating the Aqua would produce a large cell count, therefore skipping the lag time in the beginning of bacterial growth. Temperature and pH were measured to determine their potential effects on ammonia and nitrate removal (Table 4.96 and Table 4.97). Different times between the pH, temperature, ammonia, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.95: Labeling for experiment 13

Description	Label
In-field bioreactor secondary wastewater with 25 ppm Activated Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	BR Secondary with Activated Aqua
In-field bioreactor secondary wastewater with 25 ppm Dry Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	BR Secondary with Dry Aqua
In-field bioreactor secondary wastewater with no Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	BR Secondary without Aqua
In-lab DI water with no Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	Control

Table 4.96: pH measurements for experiment 13

Time (hours)	0	18	25	44	49	66	72	92	96.5	114	120	139	144
BR Secondary with Activated Aqua	7.13	7.3	7.42	7.52	7.49	7.73	7.43	7.52	7.51	7.63	7.51	7.43	7.45
BR Secondary with Dry Aqua	7.17	7.47	7.39	7.47	7.41	7.7	7.55	7.44	7.45	7.53	7.37	7.38	7.26
BR Secondary without Aqua	7.15	7.73	7.59	7.62	7.57	7.7	7.52	7.54	7.54	7.69	7.54	7.54	7.38
Control	6.16	6.59	6.84	6.44	6.62	6.78	6.85	6.8	6.4	6.3	6.51	6.92	5.23

The pH for 38.5% of all samples was within the recommended 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). About 51.3% of all wastewater samples were within the recommended range. None of the wastewater samples and 16.3% of the DI control samples were below a pH of 6.8, which is where nitrification rates decline significantly (Metcalf & Eddy, 2003). Therefore, pH could have an impact on the nitrification rate of the DI control samples, but not on the nitrification rate of the wastewater samples. The pH for 75% of all samples was within the recommended 7 to 8 for denitrification (Metcalf & Eddy, 2003). However, none of the DI control samples were within that range. About 7.6% of DI control

samples were below a pH of 6, which is where denitrification is inhibited. Therefore, pH did not have a significant impact on the denitrification rate.

Table 4.97: Temperature measurements for experiment 13

Time (hours)	0	18	25	44	49	66	72	92	96.5	114	120	139	144
BR Secondary with Activated Aqua	24.8	18.1	23.7	21.8	27.4	24	31.7	29.8	29.8	18	24.4	16.7	21.2
BR Secondary with Dry Aqua	25.2	19	23.5	22.2	27.4	23.8	28.9	28.5	28.5	17.9	24.2	16.4	20.3
BR Secondary without Aqua	24.4	19	24.4	23.5	28.2	25.6	31.5	29.8	29.8	18.7	25.1	17	21.3
Control	27.6	25.8	25.7	25.3	26.9	26.5	27.2	27.1	27.1	27	26.4	25	26.6

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 17°C and 29°C, which has a significant impact on the rate of nitrification and denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.98: Ammonia concentrations in mg/L NH₄-N for experiment 13

Time (hours)	0	18	44	49	66	72	92	96.5	114	120	139	144
Control	28.42	27.46	28.48	26.72	27.81	27.23	26.81	25.86	27.64	26.12	25.79	25.79
BR Secondary without Aqua	59.71	59.53	54.72	57.46	59.48	54.67	60.13	61.85	56.12	54.17	52.10	50.64
BR Secondary with Dry Aqua	56.43	60.64	56.70	58.57	57.81	58.31	56.42	55.41	55.98	51.46	49.35	47.40
BR Secondary with Activated Aqua	58.87	59.84	59.81	58.26	58.86	68.66	61.51	65.64	64.03	52.09	52.59	53.97

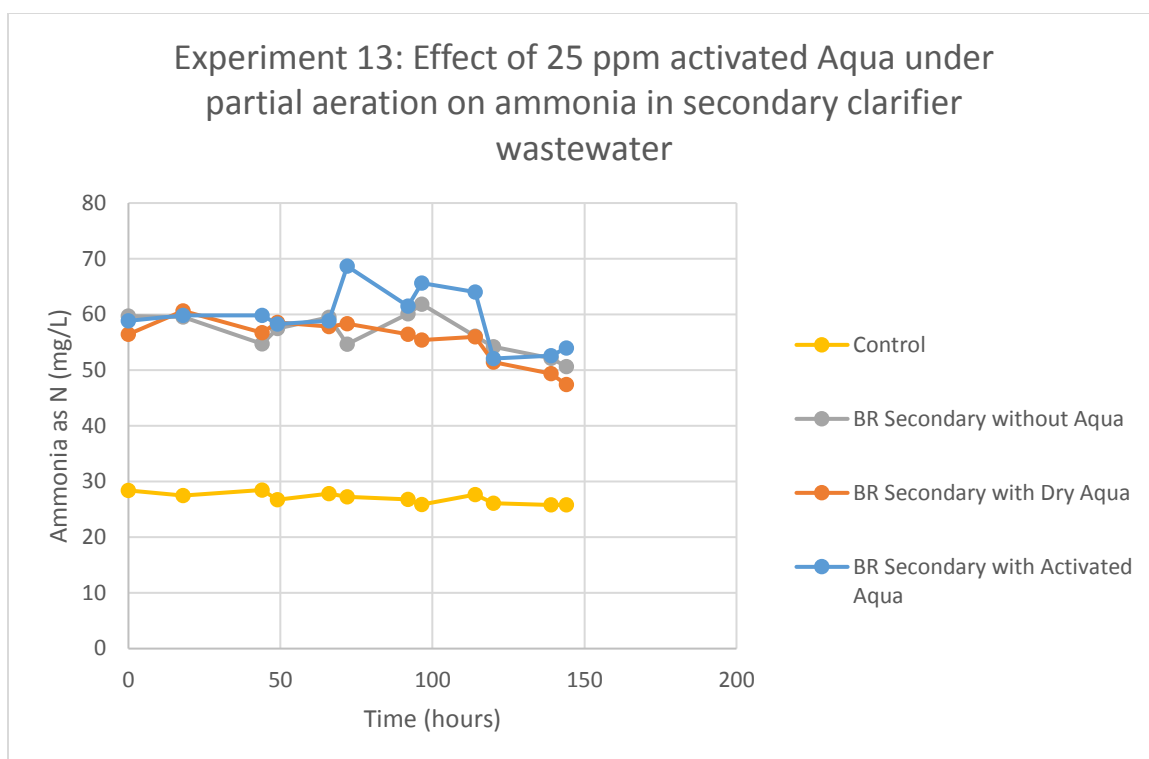


Figure 4.47: Effect of 25 ppm activated Aqua under partial aeration on ammonia in secondary clarifier wastewater

Table 4.99: Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment 13

Time (hours)	0	18	44	49	66	72	92	96.5	114	139	144
BR Secondary with Activated Aqua	25.72	24.98	24.39	23.62	23.44	26.94	22.57	24.69	24.97	23.03	24.02
BR Secondary with Dry Aqua	25.72	27.11	25.05	25.06	25.18	24.83	23.93	22.91	24.19	28.65	29.23
BR Secondary without Aqua	26.86	27.00	23.97	26.16	26.76	24.51	25.20	25.39	24.94	30.46	32.07
Control	27.33	24.09	24.60	26.20	24.15	24.20	23.78	25.35	23.99	26.73	23.82

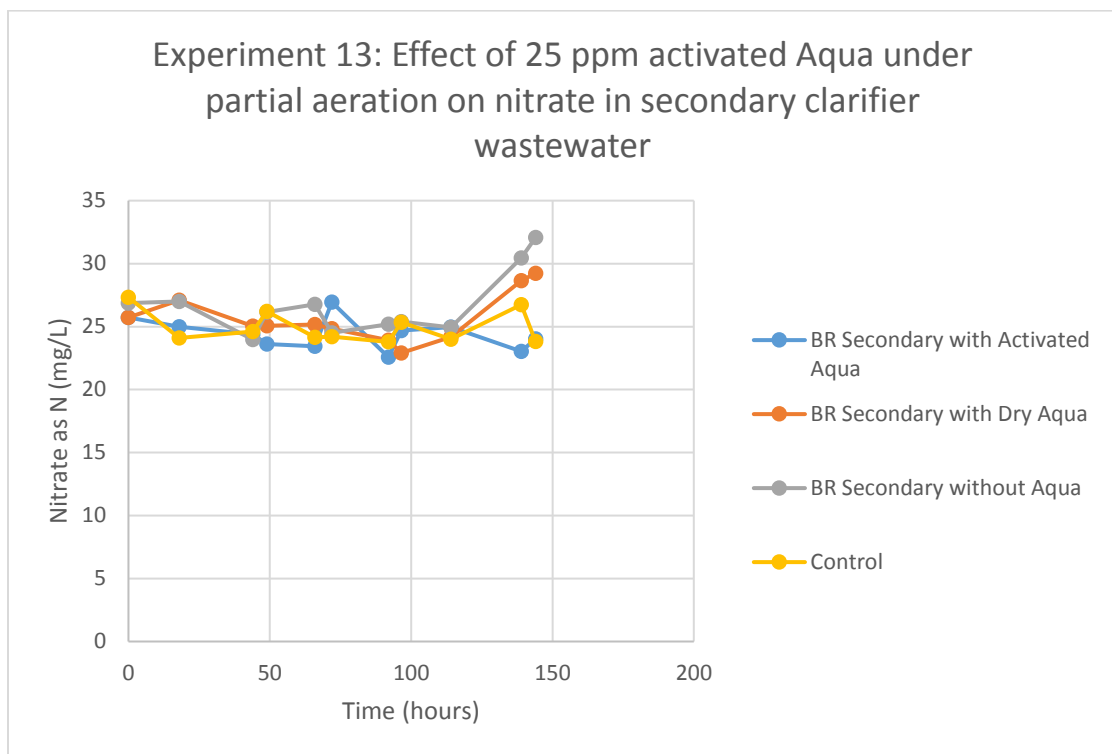


Figure 4.48: Effect of 25 ppm activated Aqua under partial aeration on nitrate in secondary clarifier wastewater

Table 4.100: Nitrite concentrations in mg/L NO₂-N for experiment 13

Time (hours)	0	18	44	49	66	72	92	96.5	114	139	144
BR Secondary with Activated Aqua	1.39	1.57	1.40	1.44	1.19	1.25	1.31	1.66	1.76	1.73	6.74
BR Secondary with Dry Aqua	1.55	1.57	1.25	1.33	1.26	1.33	1.72	1.57	2.07	1.77	1.93
BR Secondary without Aqua	1.44	1.66	1.24	1.48	1.31	1.27	1.51	1.53	1.91	1.65	1.92
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

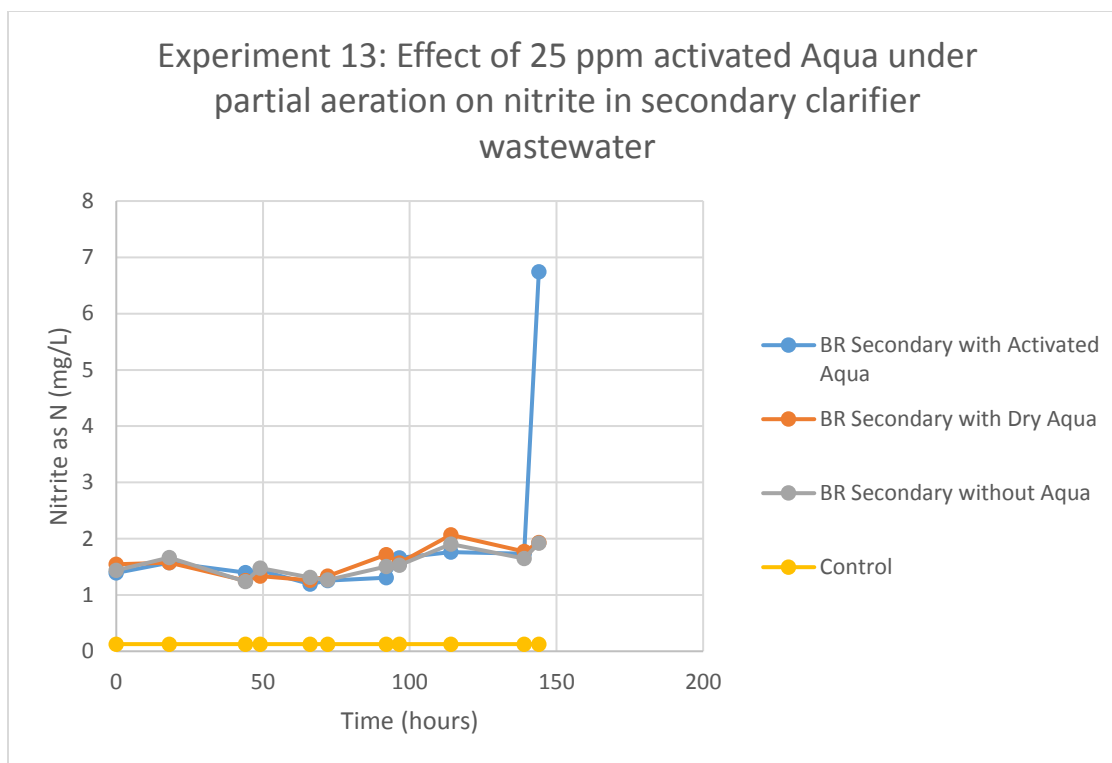


Figure 4.49: Effect of 25 ppm activated Aqua under partial aeration on nitrite in secondary clarifier wastewater

The ammonium concentration fluctuated around 60 ppm. At the end of the experiment, the ammonium concentration decreased 10 ppm. This decrease in ammonium was due to nitrification. When 10 ppm ammonia was removed, 5 ppm nitrate was produced and about 0.5 ppm nitrite was produced (besides one outlier). The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration, so chemoautotrophs had the preferred metabolic process. A longer experiment time would have been ideal because a better trend could be seen and degradation rates could be calculated and compared to literature values. Only a few data points showed this ammonium degradation, which was not enough to calculate degradation rates from.

The nitrate concentration fluctuated around 25 ppm. At the end of the experiment, the nitrate concentration increased by about 5 ppm. This was likely due to nitrification. The nitrite fluctuated around 1.5 ppm. At the end of the experiment, the nitrite increased to about 2 ppm. One outlier increased 6 ppm at the end of the experiment. The increases could be from the intermediate stage in nitrification where the ammonium is converted into nitrite. Using 25 ppm of Aqua under partially aerobic conditions would not be beneficial for decreasing nitrate because nitrate removal did not occur. A longer experiment run time may be needed to see if aerobic denitrification is possible for Aqua. Aeration could have been too high or too low for simultaneous nitrification and denitrification to occur. The activated Aqua followed the same pattern as dry Aqua. Therefore, activated Aqua did not produce better results for nitrate removal.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. Activated Aqua consists of TSB, growth media, and Aqua. Carbon was added from TSB, growth media, and Aqua. Growth media added 1000 mg/L dextrose. TSB added 250 mg/L dextrose because only 10% of the TSB was added to the activated Aqua solution. Aqua added 7362.5 mg/L dextrose because about 95% of Aqua is dextrose. The activated Aqua solution contained a total of 8612.5 mg/L dextrose. About 0.32% of the activated Aqua was added to the wastewater samples. Therefore, a total of

27.56 mg/L dextrose was added. This equates to 11 mg/L dextrose as carbon. The dry Aqua added 23.75 mg/L dextrose, which equates to 9.5 mg/L dextrose as carbon. The C:N ratios were different for the samples (Table 4.101). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency and 5:1 produced the ideal nitrate removal rate (Winkler, 2005). The ammonia C:N ratios for wastewater without Aqua were not included because the natural bacteria likely experience autotrophic metabolic rates. Therefore, BOD (organic carbon) cannot be used to find C:N ratios for the autotrophic bacteria. The C:N ratio for all samples could have decreased the ammonia removal rates because C:N ratios were lower than ideal. The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.101: Carbon to nitrogen ratios for experiment 13

WW	Carbon in WW ¹	Total NH ₄ -N ²	Total NO ₃ -N ²	Total C	C:N for NH ₄ -N	C:N for NO ₃ -N
BR Secondary with Activated Aqua	70.48	58.9	25.7	81.58	1.4:1	3.2:1
BR Secondary with Aqua		56.4	25.7	79.98	1.4:1	3.1:1
BR Secondary without Aqua		59.7	26.9	70.48	-	2.6:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C for activated Aqua includes initial, Aqua, and TSB. Total C for Aqua includes initial and Aqua

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.50). The total nitrogen was steady around 80 ppm for all samples. Therefore, no total nitrogen removal occurred. The lack of total nitrogen removal was likely due to partial aeration slowing or preventing nitrate removal. A longer run time may have shown more nitrification or the start of denitrification. The addition of Aqua did not improve total nitrogen removal.

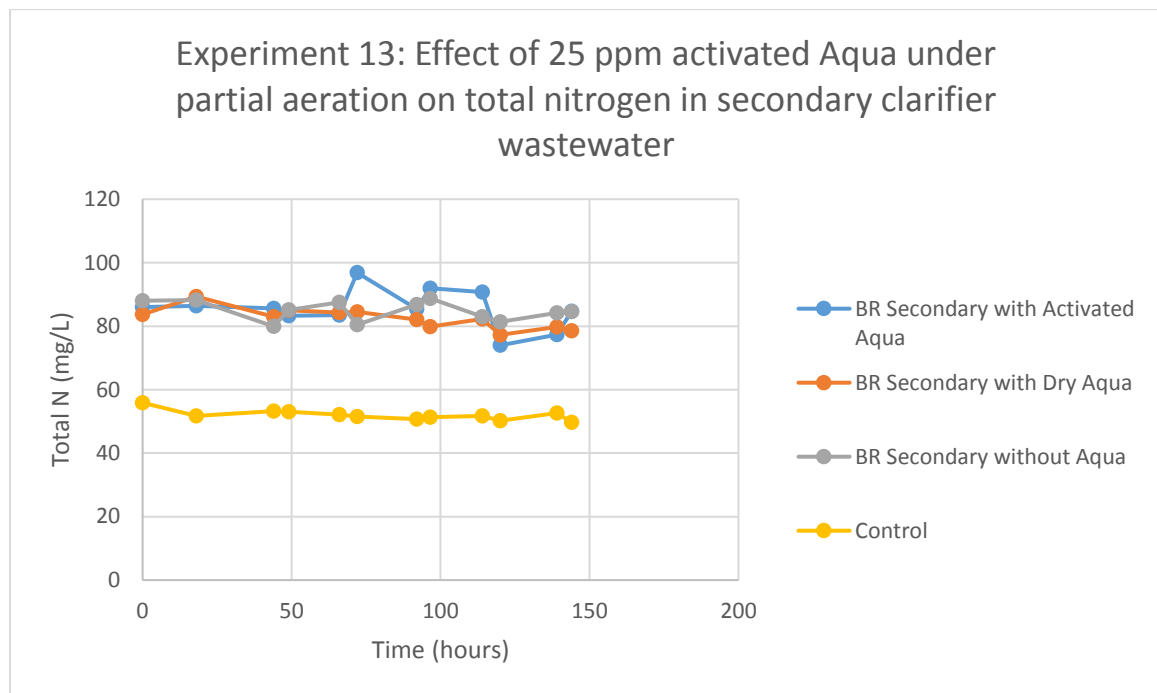


Figure 4.50: Effect of 25 ppm activated Aqua under partial aeration on total nitrogen in secondary clarifier wastewater

Indication of nitrification occurred for the natural and Aqua inoculated wastewater under partially aerobic conditions. However, nitrate removal did not occur. Normally, Aqua can undergo simultaneous nitrification and denitrification. However, aeration may have been

too low or too high for simultaneous nitrification and denitrification to occur. Therefore, a laboratory study was conducted to analyze how high aeration affects denitrification.

4.2.10 Experiment 14 – Effect of 25 ppm Biogenesis on Secondary Clarifier

Wastewater

Nitrate removal was analyzed for Aqua in secondary clarifier wastewater under anoxic conditions in lab and in field (Table 4.105, Table 4.106, Figure 4.51, and Figure 4.52). This experiment determined if a competitor's product will achieve better denitrification rates than Aqua. Temperature and pH were measured to determine their potential effects on nitrate removal (Table 4.103 and Table 4.104). Different times between the pH, temperature, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is medium because less than half the data points did not pass QA/QC.

Table 4.102: Labeling for experiment 14

Description	Label
In-field bioreactor secondary wastewater with 25 ppm Biogenesis and 25 ppm NO ₃ -N	BR Secondary with Biogenesis
In-field bioreactor secondary wastewater with 25 ppm Dry Aqua and 25 ppm NO ₃ -N	BR Secondary with Aqua
In-field bioreactor secondary wastewater with 25 ppm NO ₃ -N	BR Secondary without Aqua
In-lab secondary wastewater with 25 ppm Biogenesis and 25 ppm NO ₃ -N	Lab Secondary with Biogenesis
In-lab secondary wastewater with 25 ppm Dry Aqua and 25 ppm NO ₃ -N	Lab Secondary with Aqua
In-lab secondary wastewater with 25 ppm NO ₃ -N	Lab Secondary without Aqua
In-lab DI water with no Aqua and 25 ppm NO ₃ -N	Control

Table 4.103: pH measurements for experiment 14

Time (hours)	0	20	26	45	50	67.5	74	91	98
BR Secondary with Biogenesis	7.2	7.38	7.4	7.37	7.48	7.51	7.44	7.65	7.64
BR Secondary with Aqua	7.04	7.31	7.17	7.12	7.28	7.32	7.28	7.47	7.57
BR Secondary without Aqua	7.16	7.58	7.57	7.65	7.66	7.74	7.7	7.83	7.86
Lab Secondary with Biogenesis	6.94	7.19	7.11	7.03	7.01	6.84	6.68	6.97	7.05
Lab Secondary with Aqua	6.91	7.06	6.94	6.89	6.89	6.79	6.6	6.88	7.01
Lab Secondary without Aqua	6.92	7.16	7.02	6.92	6.88	6.76	6.89	6.96	6.98
Control	6.31	5.67	6.52	7.01	6.86	6.3	5.69	5.7	5.95

The pH for 53% of all samples was within the recommended 7 to 8 for denitrification (Metcalf & Eddy, 2003). All of the bioreactor samples are within the 7 to 8 range, but only

25% of the laboratory samples are within the range. About 4 of the DI control samples are below a pH of 6, which is where denitrification is inhibited. Therefore, pH could have an impact on the denitrification rate of the DI control samples, but not on the denitrification rate of the wastewater samples.

Table 4.104: Temperature measurements for experiment 14

Time (hours)	0	20	26	45	50	67.5	74	91	98
BR Secondary with Biogenesis	23.4	19	32.2	25.6	28.4	18.1	25.5	17.3	24.3
BR Secondary with Aqua	22.8	19.9	29.9	27.2	26.7	18.7	23	17.3	22.7
BR Secondary without Aqua	22.1	19.8	28.3	23.9	24.5	18	21.6	16.9	21
Lab Secondary with Biogenesis	23.8	26.1	26.3	26.3	26.7	26.5	25.7	25.9	26.6
Lab Secondary with Aqua	23.6	25.5	26.4	26.6	26.8	26	26	26.2	25.8
Lab Secondary without Aqua	23.7	26	26.3	26.4	26.5	25.8	26.2	26.1	26.2
Control	22.3	25.2	26.3	26.5	26.9	24.6	26.2	24.2	25.1

Laboratory temperatures were around 26°C, which was the typical temperature during the day. Bioreactor temperatures fluctuated between 17°C and 30°C, which has a significant impact on the rate of denitrification. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973). Removal rates were predicted to be faster during the day and slower at night.

Table 4.105: Nitrate concentrations in mg/L NO₃-N for experiment 14

Time (hours)	0	26	45	50	67.5	74	98	Amount Degraded
BR Secondary with Biogenesis	22.34	14.51	10.49	11.09	9.03	8.45	8.79	13.55
BR Secondary with Aqua	21.37	14.20	9.23	9.16	7.51	6.98	5.98	15.39
BR Secondary without Aqua	19.46	12.58	8.17	8.69	7.89	7.19	6.61	12.85
Lab Secondary with Biogenesis	21.58	11.29	7.96	7.97	7.17	7.09	6.19	15.39
Lab Secondary with Aqua	10.16	9.00	7.16	6.79	4.53	4.11	3.70	6.46
Lab Secondary without Aqua	1.45	ND	1.97	2.88	4.00	5.09	6.73	-5.28
Control	18.18	22.31	23.85	24.68	21.84	24.54	23.78	-5.60

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

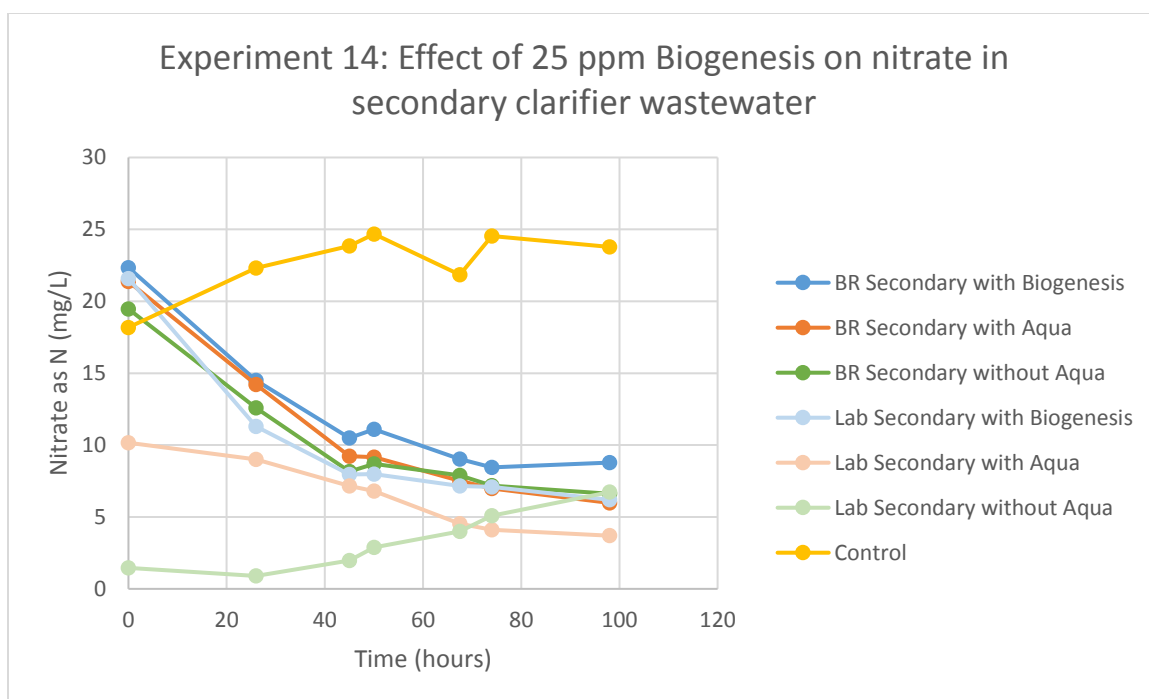


Figure 4.51: Effect of 25 ppm Biogenesis on nitrate in secondary clarifier wastewater

Table 4.106: Nitrite concentrations in mg/L NO₂-N for experiment 14

Time (hours)	0	26	45	50	67.5	74	98
BR Secondary with Biogenesis	ND	ND	ND	ND	ND	ND	ND
BR Secondary with Aqua	1.07	ND	ND	ND	ND	ND	ND
BR Secondary without Aqua	ND	1.43	ND	ND	ND	ND	ND
Lab Secondary with Biogenesis	ND	ND	ND	ND	ND	ND	ND
Lab Secondary with Aqua	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua	ND	ND	ND	ND	ND	ND	1.10
Control	ND	ND	ND	ND	ND	ND	ND

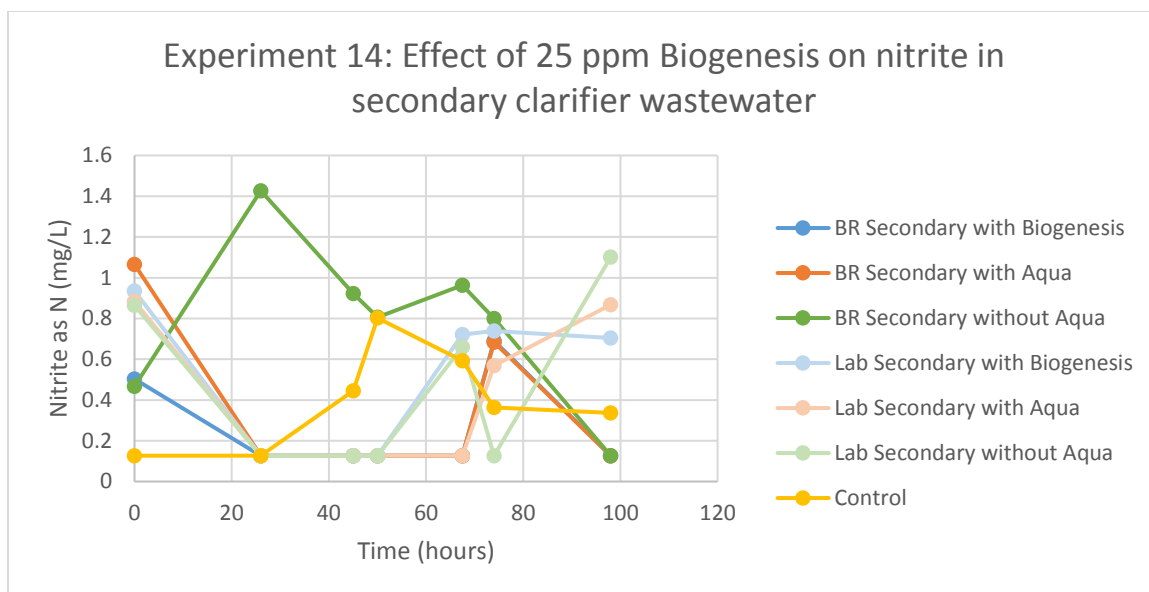


Figure 4.52: Effect of 25 ppm Biogenesis on nitrite in secondary clarifier wastewater

All samples containing nitrate and wastewater exhibited nitrate removal over a period of about 4 days. Two of the laboratory samples started at different concentrations than the other samples. This is likely due to procedural error of failing to add all the nitrate in the solution bottles. Therefore, a laboratory experiment performed under the same conditions was performed after this experiment. As nitrate decreased, nitrite only increased by about 1 ppm. Therefore, nitrate removal likely occurred via assimilation or denitrification without production of intermediate nitrite. Nitrogen gas and organic nitrogen content would need to be measured to know the exact process. Many of the nitrite samples were below the 1 ppm MDL, which means that the sample points could be inaccurate. Therefore, the nitrite increase could be due to instrumental error. The natural and Aqua/Biogenesis inoculated wastewater followed the same nitrate removal pattern. Therefore, using 25 ppm of Aqua or Biogenesis would not be beneficial for decreasing nitrate in secondary clarifier wastewater.

Zero and first order degradation rates were calculated for nitrate removal. Since the laboratory samples had different starting points, those will be disregarded. Samples followed first order kinetics. However, zero order kinetic values were compared to other literature values (Table 4.107). The amount of nitrate removed was calculated by subtracting the initial value from the final value (Table 4.108). In three days, all samples were below the discharge permit level of 10 mg/L nitrate as nitrogen for the SLO WRRF. All samples achieved a volumetric denitrification rate lower than all literature values.

Table 4.107: Denitrification rates for experiment 14

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Secondary Clarifier Wastewater with Aqua	3 – 3.6	~25°C
	Secondary Clarifier Wastewater without Aqua	2.9	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.108: Total nitrate removed and removal rates for experiment 14

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
BR Secondary with Biogenesis	13.55	98	0.1285	0.0095
BR Secondary with Aqua	15.39	98	0.1497	0.0132
BR Secondary without Aqua	12.85	98	0.1198	0.0107

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 23.75 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 9.5 mg/L as carbon. The C:N ratios were different for the samples (Table 4.109). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.109: Carbon to nitrogen ratios for experiment 14

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
BR Secondary with Biogenesis	70.48	22.3	70.48	3.2:1
BR Secondary with Aqua		21.4	79.98	3.7:1
BR Secondary without Aqua		19.5	70.48	3.6:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C for Aqua includes initial and Aqua. Assuming Biogenesis does not add any carbon

Total nitrogen was calculated by adding the nitrate and nitrite (Figure 4.53). Total nitrogen decreased by about 15 ppm for all bioreactor samples. Laboratory samples started at different concentrations due to sample preparation error. Therefore, total nitrogen cannot be analyzed for laboratory samples. Experiment 15 will run the same test in lab only. The bioreactor samples showed that addition of Aqua or Biogenesis did not improve total nitrogen removal.

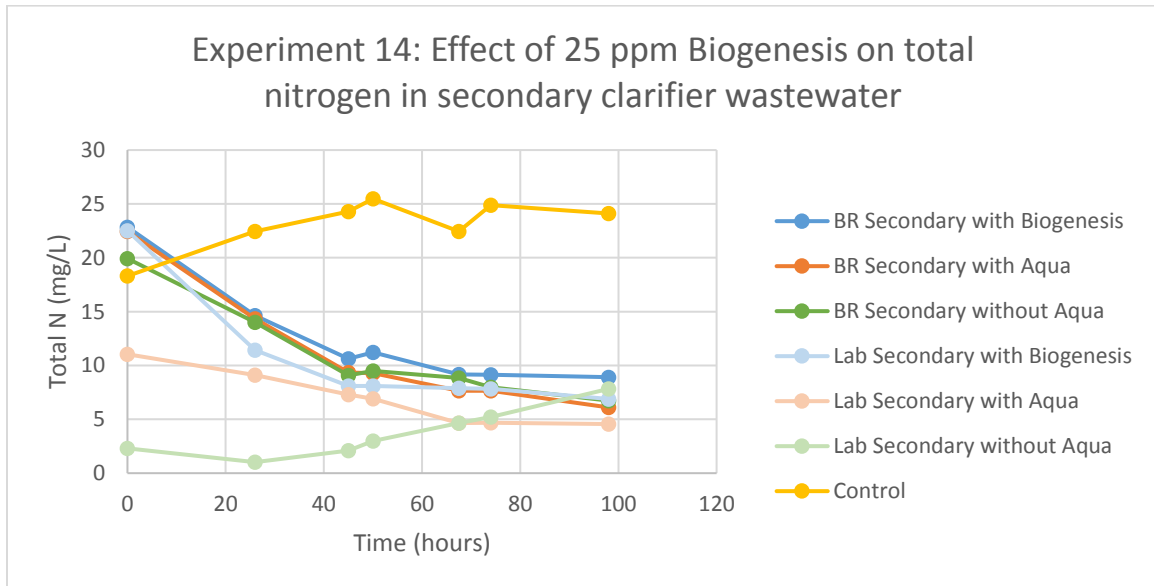


Figure 4.53: Effect of 25 ppm Biogenesis on total nitrogen in secondary clarifier wastewater

Nitrate removal rates for Aqua and Biogenesis were the same as the natural bacteria when 25 ppm of Aqua/Biogenesis was used. Therefore, any nitrate removal was due to the natural bacteria. This experiment was performed again in a lab setting to determine if the same conclusions can be made and if outside factors impacted the bioreactor samples.

4.3 Laboratory Results for Field Wastewater During Cold Weather

Laboratory experiments were conducted during cold weather conditions (below 70°F) because slower degradation rates were expected in cold temperatures. Laboratory experiments analyzed the impact of Aqua on nitrified final clarifier wastewater with and without an external carbon source. Comparison of Aqua with a competitor bacterial mixture was also analyzed. The effect of high aeration on Aqua denitrification was also analyzed.

4.3.1 Experiment 15 – Effect of 25 ppm Biogenesis on Secondary Clarifier

Wastewater Conducted in Lab

Nitrate removal was analyzed for Aqua in secondary clarifier wastewater under anoxic conditions in lab and in field (Table 4.113, Table 4.114, Figure 4.54, and Figure 4.55). This experiment determined if a competitor's product would achieve better denitrification rates than Aqua. Temperature and pH were measured to determine their potential effects on nitrate removal (Table 4.111 and Table 4.112). Different times between the pH, temperature, nitrate, and nitrite concentrations occurred because data that did not pass

quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.110: Labeling for experiment 15

Description	Label
In-lab secondary wastewater with 25 ppm Biogenesis and 25 ppm NO ₃ -N	Secondary with Biogenesis
In-lab secondary wastewater with 25 ppm Dry Aqua and 25 ppm NO ₃ -N	Secondary with Aqua
In-lab secondary wastewater with 25 ppm NO ₃ -N	Secondary without Aqua or Biogenesis
In-lab DI water with no Aqua and 25 ppm NO ₃ -N	Control

Table 4.111: pH measurements for experiment 15

Time (hours)	0	20.5	27	43.5	52.5	67.5	74.5	91.5	98.5	116	122.5
Secondary with Biogenesis	7.22	7.03	7.49	7.39	7.21	7.46	7.4	6.9	7.31	6.8	6.86
Secondary with Aqua	7.18	7.39	7.39	7.32	7.13	7.43	7.04	7.39	7.24	6.78	6.77
Secondary without Aqua or Biogenesis	7.19	7.19	7.19	7.36	7.25	6.93	7.39	6.89	7.23	6.81	6.77
Control	6.23	5.73	5.54	5.63	5.9	5.14	5.33	5.16	5.81	5.41	5.38

The pH for 54.5% of all samples was within the recommended 7 to 8 for denitrification (Metcalf & Eddy, 2003). None of the wastewater samples and 91% of the DI control samples were below a pH of 6, which is where denitrification is inhibited. Therefore, pH did not have an impact on the denitrification rate, besides for the DI control.

Table 4.112: Temperature measurements for experiment 15

Time (hours)	0	20.5	27	43.5	52.5	67.5	74.5	91.5	98.5	116	122.5
Secondary with Biogenesis	18.7	22.6	24.5	22.6	24.5	22.4	25.3	23.5	25	24.5	25.1
Secondary with Aqua	18.8	22.4	24.4	23.2	24.4	22.5	25.3	23.9	25.6	24.7	25.9
Secondary without Aqua or Biogenesis	18.8	23	23.2	23.1	24.6	24.3	25.1	24.7	25.1	25.4	25.9
Control	20.7	22.2	23.6	22.6	24.4	24.4	25.2	21.7	25.2	24.5	24.4

Laboratory temperatures were around 25°C to ensure good bacterial growth. The samples fluctuated between 19°C to 26°C, which means the bacteria were kept at a good temperature for growth. The fluctuations in temperature could impact denitrification rates. Decreasing by 5°C can increase the lag time and decrease the removal rates by at least 20% (Lekang, 2013; Jenkins, 1973).

Table 4.113: Nitrate concentrations in mg/L NO₃-N for experiment 15

Time (hours)	0	20.5	27	43.5	52.5	67.5	74.5	98.5	116	122.5	Amount Degraded
Secondary with Biogenesis	20.23	16.08	17.82	17.97	16.35	17.29	15.78	16.01	15.42	17.71	4.81
Secondary with Aqua	19.33	14.29	14.38	14.05	12.06	12.17	12.63	10.78	12.01	10.95	7.32
Secondary without Aqua or Biogenesis	20.03	20.85	17.52	16.74	16.49	17.64	16.63	16.92	17.14	15.39	2.89
Control	21.31	23.34	23.01	21.53	23.74	24.06	24.47	23.37	23.65	23.61	-2.34

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

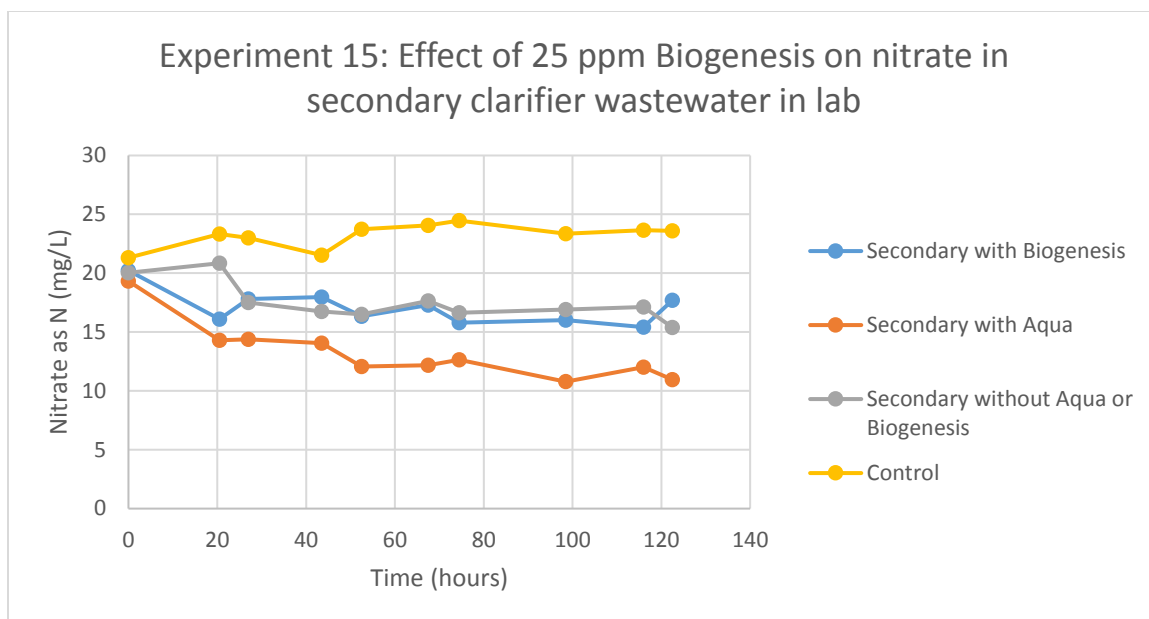


Figure 4.54: Effect of 25 ppm Biogenesis on nitrate in secondary clarifier wastewater in lab

Table 4.114: Nitrite concentrations in mg/L NO₂-N for experiment 15

Time (hours)	0	20.5	27	43.5	52.5	67.5	74.5	98.5	116	122.5
Secondary with Biogenesis	ND	ND	3.59	ND	ND	ND	1.07	ND	2.16	ND
Secondary with Aqua	1.25	ND	3.53	ND	ND	ND	ND	ND	1.83	ND
Secondary without Aqua or Biogenesis	1.30	7.95	6.22	ND	ND	ND	1.11	ND	2.54	ND
Control	ND	ND	1.63	ND	ND	1.13	ND	ND	ND	ND

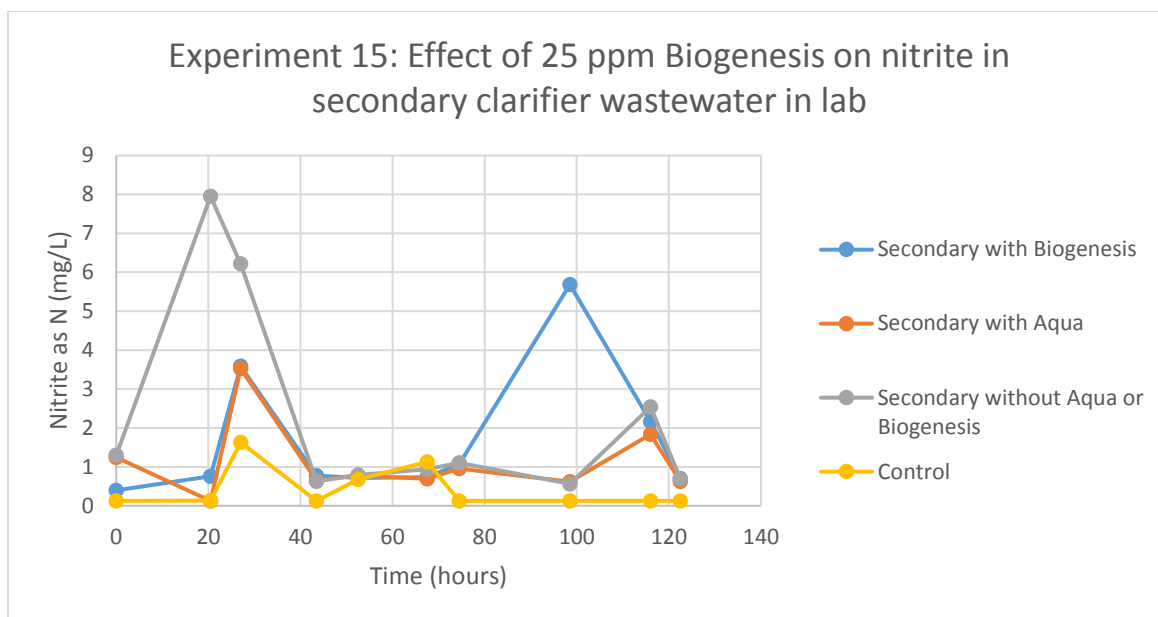


Figure 4.55: Effect of 25 ppm Biogenesis on nitrite in secondary clarifier wastewater in lab

All samples containing nitrate and wastewater exhibited nitrate removal over a period of about 5 days. As nitrate decreased, nitrite only increased by 1 ppm, except for a couple of spikes. Therefore, nitrate removal likely occurred via assimilation or denitrification without production of intermediate nitrite. Nitrogen gas and organic nitrogen content would need to be measured to know the exact process. The spikes could be due to procedural error, the intermediate step of nitrification, or another metabolic process. The sample with Biogenesis followed the same trend as the sample without anything added. The Aqua degraded about 5 ppm nitrate more than the other two samples. Therefore, using 25 ppm of Aqua could be helpful for decreasing nitrate in secondary clarifier wastewater. A cost to benefit analysis should be conducted to determine whether using Aqua would be beneficial.

Zero and first order degradation rates were calculated for nitrate removal. Typically denitrification follows zero order kinetics (Lee, 2012). All wastewater samples followed a zero order degradation rate for nitrate removal. Therefore, zero order kinetic values were compared to other literature values (Table 4.115). The amount of nitrate removed was calculated by subtracting the initial value from the final value (Table 4.116). In the five days total for this experiment, all samples failed to reach below the discharge permit level of 10 mg/L nitrate as nitrogen for the SLO WRRF. All samples achieved a volumetric denitrification rate lower than all other literature values.

Table 4.115: Denitrification rates for experiment 15

Source	System	Volumetric Denitrification Rate (mg NO ₃ -N/L/d)	Temperature
This Study	Secondary Clarifier Wastewater with Biogenesis	0.5	~25°C
This Study	Secondary Clarifier Wastewater with Aqua	1.2	~25°C
This Study	Secondary Clarifier Wastewater without Aqua or Biogenesis	0.7	~25°C
(Metcalf & Eddy, 2003)	Preanoxic Tanks	95 – 995 ¹	N/A
	Postanoxic Tanks	24 – 95 ¹	N/A
(Maurer, Fux, Graff, & Siegrist, 2011)	Moving-Bed Biological Treatment	240	10°C
		730	20°C
(Lee, 2012)	Column Tests with Mineral Media	4.56	30°C
(Dincer & Kargi, 2000)	Two Series Reactors (postanoxic)	10 – 30	N/A

¹Assumes typical MLVSS value of 2370 mg/L (Metcalf & Eddy, 2003)

Table 4.116: Total nitrate removed and removal rates for experiment 15

Sample	Amount Nitrate Removed (mg N/L)	Removal Time Period (hours)	Zero Order Removal Rate (mg N/L/hr)	First Order Removal Rate (1/hr)
Secondary with Biogenesis	2.52	122.5	0.0189	0.0011
Secondary with Aqua	8.37	122.5	0.0494	0.0035
Secondary without Aqua or Biogenesis	4.64	122.5	0.0285	0.0016

*Amount degraded assumes the non-detect values are 1 mg/L, which is the detection limit. This gives a conservative estimate of the amount of nitrate removed.

According to research done by Eva Lee for BiOWiSH™, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. The Aqua added 23.75 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 9.5 mg/L as carbon. The C:N ratios were different for the samples (Table 4.117). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratio for all samples did not impact the nitrate removal rates because the ratios were in the ideal range.

Table 4.117: Carbon to nitrogen ratios for experiment 15

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Secondary with Biogenesis	70.48	20.2	70.48	3.5:1
Secondary with Aqua		19.3	79.98	4.1:1
Secondary without Aqua or Biogenesis		20.0	70.48	3.5:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C for Aqua includes initial and Aqua. Assuming Biogenesis does not add any carbon

Total nitrogen was calculated by adding the nitrate and nitrite (Figure 4.56). About 3 ppm total nitrogen was removed for samples with Biogenesis and natural bacteria (no Aqua or Biogenesis added). The sample with Aqua removed about 9 ppm total nitrogen. Therefore, Aqua achieved better nitrogen removal than natural bacteria and Biogenesis. However, the experiment was conducted in an ideal setting. The bioreactor data showed that neither Aqua nor Biogenesis improved total nitrogen removal. Therefore, field conditions, such as temperature, affected total nitrogen removal.

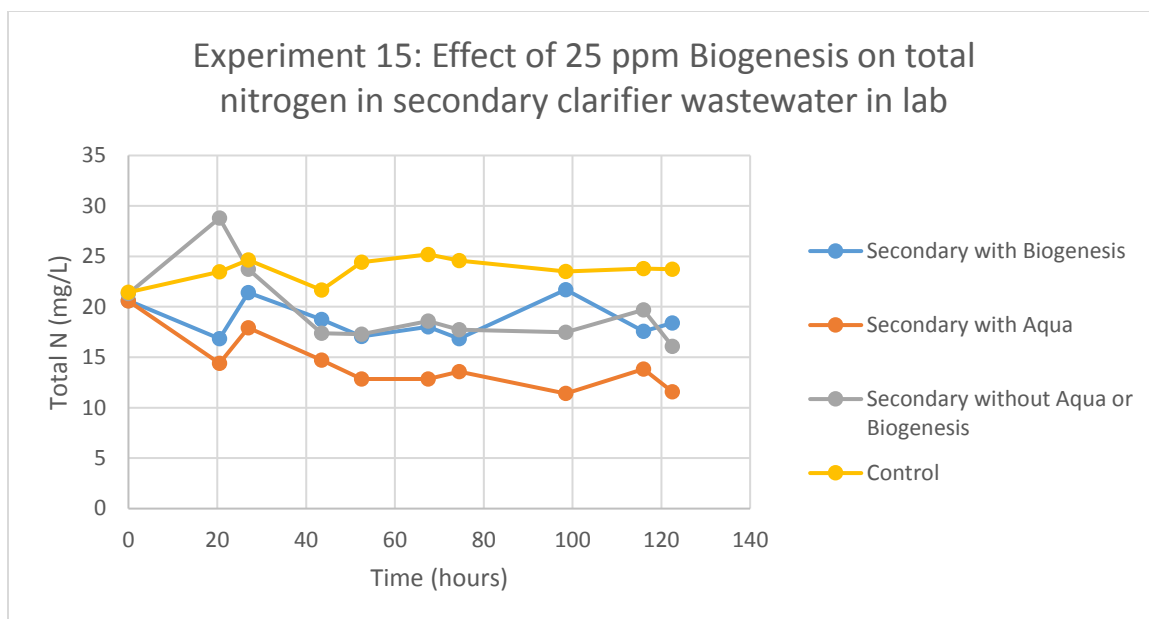


Figure 4.56: Effect of 25 ppm Biogenesis on total nitrogen in secondary clarifier wastewater in lab

The pattern for nitrate degradation was the same for Biogenesis and natural bacteria when 25 ppm of Biogenesis was used. However, Aqua removed 5 ppm more nitrate than the natural and Biogenesis bacteria. A cost and benefit analysis should be performed to determine if the higher degradation is beneficial. The laboratory data did not match with the bioreactor data in Experiment 14. Therefore, outside factors did influence the removal rates.

For the SLO WWTP, it would be beneficial to remove nitrate in the final clarifier because it has the highest nitrate levels of all the clarifiers. Therefore, laboratory experiments were performed using final clarifier wastewater.

4.3.2 Experiment 16 – Effect of 25 ppm Activated Aqua on Final Clarifier

Wastewater

Nitrate removal was analyzed for Aqua in final clarifier wastewater under anoxic conditions in lab and in field (Table 4.121, Table 4.122, Table 4.123, Figure 4.57, Figure 4.58, and Figure 4.59). This experiment determined if Aqua can denitrify with a limited carbon source. Temperature and pH were measured to determine their potential effects on nitrate removal (Table 4.119 and Table 4.120). Different times between the pH, temperature, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.118: Labeling for experiment 16

Description	Label
In-lab final clarifier wastewater with no additions	Final without Aqua or nitrate
In-lab final clarifier wastewater with 25 ppm Activated Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	Final with Activated Aqua
In-lab final clarifier wastewater with 25 ppm dry Aqua, 25 ppm NO ₃ -N, and 25 ppm NH ₄ -N	Final with dry Aqua
In-lab final clarifier wastewater with 25 ppm NO ₃ -N and 25 ppm NH ₄ -N	Final without Aqua
In-lab DI water with 25 ppm NO ₃ -N and 25 ppm NH ₄ -N	Control

Table 4.119: pH measurements for experiment 16

Time (hours)	0	17.5	23.5	42.5	47.5	65.5	70.5	89.5	95	112.5	118.5	137.5	142.5
Final with Activated Aqua	6.5	6.47	6.52	6.25	6.33	6.55	6.35	6.48	6.15	6.34	6.25	6.31	6.36
Final with dry Aqua	6.55	6.37	6.37	6.13	6.3	6.46	6.3	6.3	6.1	6.3	6.41	6.38	6.33
Final without Aqua	6.37	6.59	6.55	6.43	6.38	6.56	6.58	6.37	6.03	6.33	6.47	6.28	6.34
Final without Aqua or nitrate	6.45	6.54	6.54	6.4	6.35	6.49	6.33	6.28	6.09	6.39	6.59	6.34	6.47
Control	6.58	7.01	6.91	6.21	6.3	6.62	6.67	6.77	6.12	6.79	6.36	6.6	6.64

None of the samples were within the recommended pH range of 7.5 to 8 for nitrification (Metcalf & Eddy, 2003). About 97% of all samples were below a pH of 6.8, which is where nitrification rates decline significantly (Metcalf & Eddy, 2003). Therefore, pH had an impact on the nitrification rate. The pH for 1.5% of all samples was within the recommended 7 to 8 for denitrification (Metcalf & Eddy, 2003). About 98.5% of all samples were below a pH of 7 and none of the samples were above a pH of 8. None of the samples were below a pH of 6, which is where denitrification is inhibited. Therefore, pH did not have an impact on the denitrification rate.

Table 4.120: Temperature measurements for experiment 16

Time (hours)	0	17.5	23.5	42.5	47.5	65.5	70.5	89.5	95	112.5	118.5	137.5	142.5
Final with Activated Aqua	23.8	25.9	25.7	26.2	26.7	26.7	27.1	26.3	27.2	26.6	26.5	25.8	26.2
Final with dry Aqua	23.7	25.7	26	26.6	26.5	26.5	27.1	26.7	27.1	26.8	26.8	26.3	26.3
Final without Aqua	23.9	25.6	26.3	25.9	26.5	26.4	27.2	26.8	27	26.9	27	25.2	26.4
Final without Aqua or nitrate	23.9	25.4	26.1	26.5	26.4	25.8	27.1	26.7	27.2	26.6	26.7	26	26.5
Control	27.1	26	26.3	26.2	26.6	26.5	27.2	26.5	27.2	26	26.7	26	26

Laboratory temperatures were around 26°C to ensure good bacterial growth. The samples fluctuated between 24°C to 27°C, which means the bacteria were kept at a good temperature for growth. Since the temperature stayed relatively steady, it likely did not impact removal rates.

Table 4.121: Ammonia concentrations in mg/L NH₄-N for experiment 16

Time (hours)	0	17.5	23.5	42.5	47.5	65.5	70.5	89.5	95	112.5	118.5	137.5	142.5
Control	27.72	28.83	26.39	27.93	26.60	28.21	26.41	27.78	25.87	25.53	25.90	24.45	25.30
Final without Aqua	24.54	26.84	24.58	26.95	23.08	27.08	24.70	25.95	22.91	19.66	21.43	20.41	19.96
Final with dry Aqua	24.94	24.15	23.07	23.65	24.82	25.36	24.57	25.08	24.44	21.73	23.00	24.24	22.04
Final with Activated Aqua	27.19	25.03	22.38	23.92	24.13	24.25	26.36	24.43	24.62	23.54	25.67	25.95	22.29
Final without Aqua or nitrate	-0.45	-0.39	-0.37	-0.26	-0.35	-0.37	-0.28	-0.44	-0.40	0.26	0.09	0.08	0.10

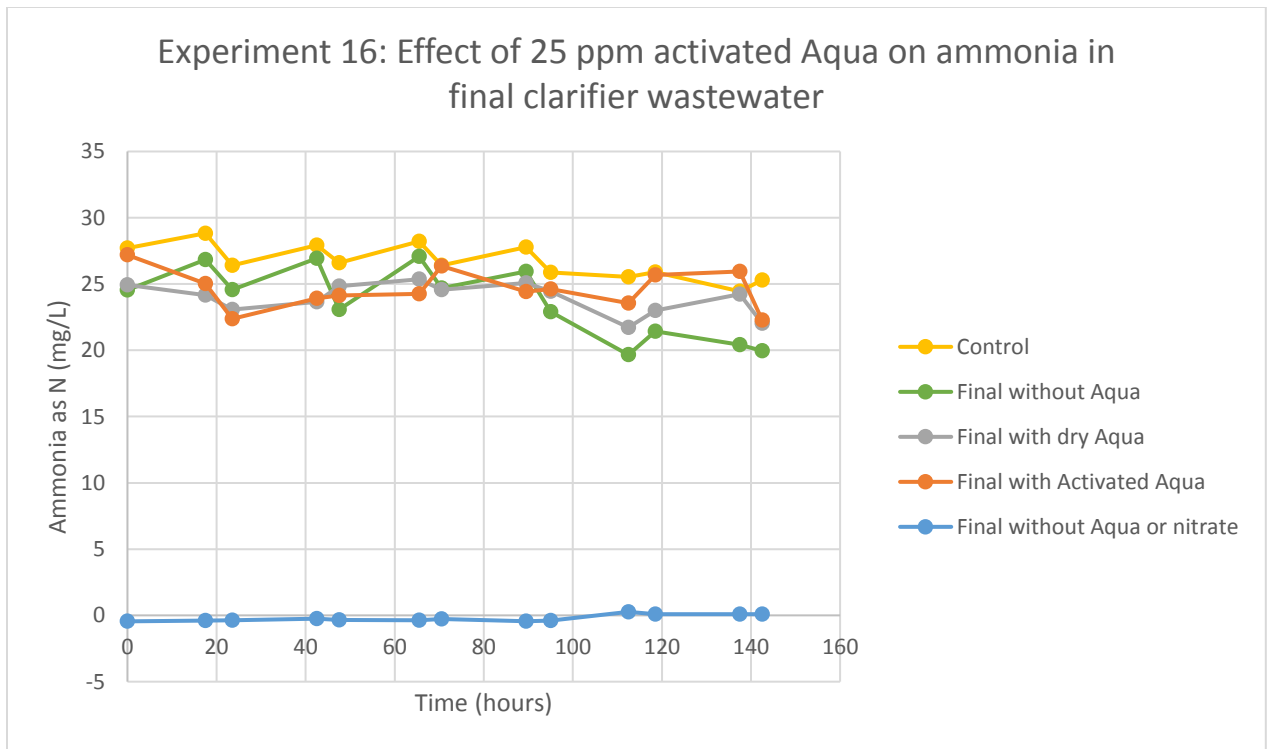


Figure 4.57: Effect of 25 ppm activated Aqua on ammonia in final clarifier wastewater

The ammonium concentration fluctuated between 20 to 27 ppm. There was a slight decrease over time. However, a slight decrease in the DI control sample occurred as well. Therefore, the decrease is due to a procedural error, such as contamination or accidental dilution. Therefore, bacteria did not process ammonia. The anoxic conditions likely inhibited nitrification and assimilation of ammonia. The samples containing Aqua behaved the same as natural bacteria samples. Therefore, Aqua had no impact on changes in ammonia concentration. The activated Aqua sample behaved the same as the dry Aqua inoculated sample. Therefore, activated Aqua did not help degrade ammonium.

Table 4.122: Nitrate concentrations in mg/L NO₃-N for experiment 16

Time (hours)	0	17.5	23.5	42.5	47.5	65.5	70.5	89.5	95	112.5	118.5	137.5	142.5
Final without Aqua or nitrate	30.31	31.97	31.38	31.27	31.16	32.56	32.21	32.21	31.42	29.11	32.46	31.78	32.23
Final with Activated Aqua	62.01	59.34	52.20	55.66	56.62	57.36	61.28	56.58	57.30	55.98	62.22	63.16	55.24
Final with dry Aqua	56.54	56.00	54.57	53.14	55.32	55.20	54.80	54.21	55.42	48.47	54.09	58.64	53.80
Final without Aqua	56.13	57.58	57.29	59.04	54.58	59.02	62.31	58.37	58.25	51.90	58.95	59.50	57.53
Control	26.22	25.21	24.46	24.47	26.47	24.24	24.21	23.73	25.73	23.82	25.61	26.17	24.00

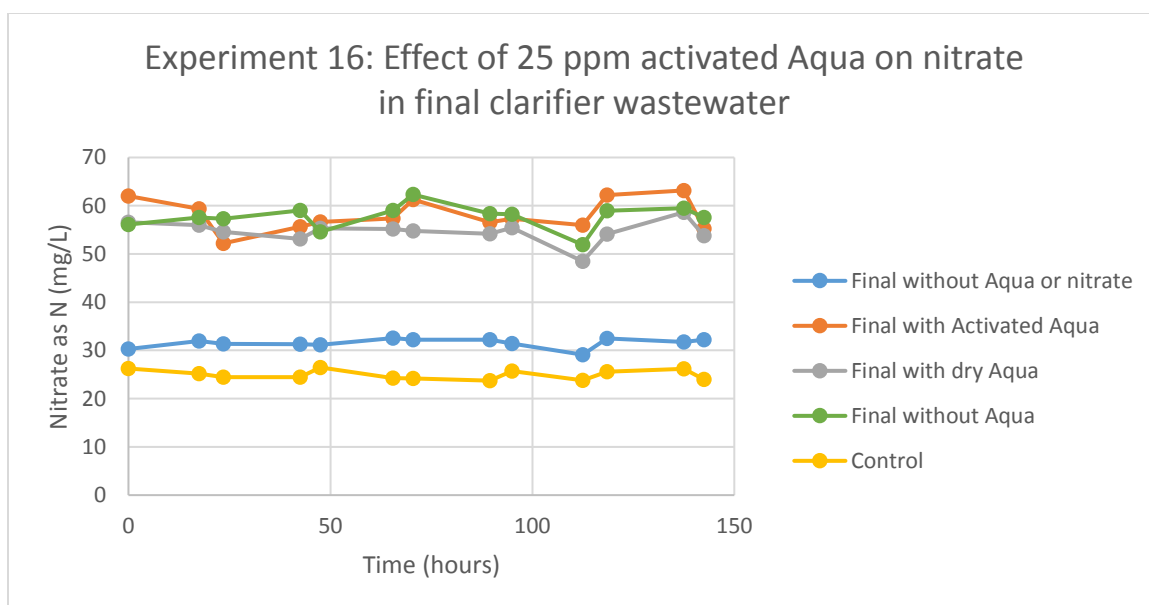


Figure 4.58: Effect of 25 ppm activated Aqua on nitrate in final clarifier wastewater

Table 4.123: Nitrite concentrations in mg/L NO₂-N for experiment 16

Time (hours)	0	17.5	23.5	42.5	47.5	65.5	70.5	89.5	95	112.5	118.5	137.5	142.5
Final without Aqua or nitrate	ND	ND	ND	ND	1.10	ND	ND	ND	ND	ND	ND	ND	ND
Final with Activated Aqua	ND	ND	1.40	0.78	1.47	1.06	1.06	1.13	1.31	1.20	1.26	1.45	1.40
Final with dry Aqua	ND	1.61	1.80	1.66	2.26	1.94	1.83	2.01	1.97	1.30	1.52	1.71	1.41
Final without Aqua	ND	ND	1.00	1.64	1.29	1.29	1.31	1.40	1.58	1.19	ND	1.54	1.43
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

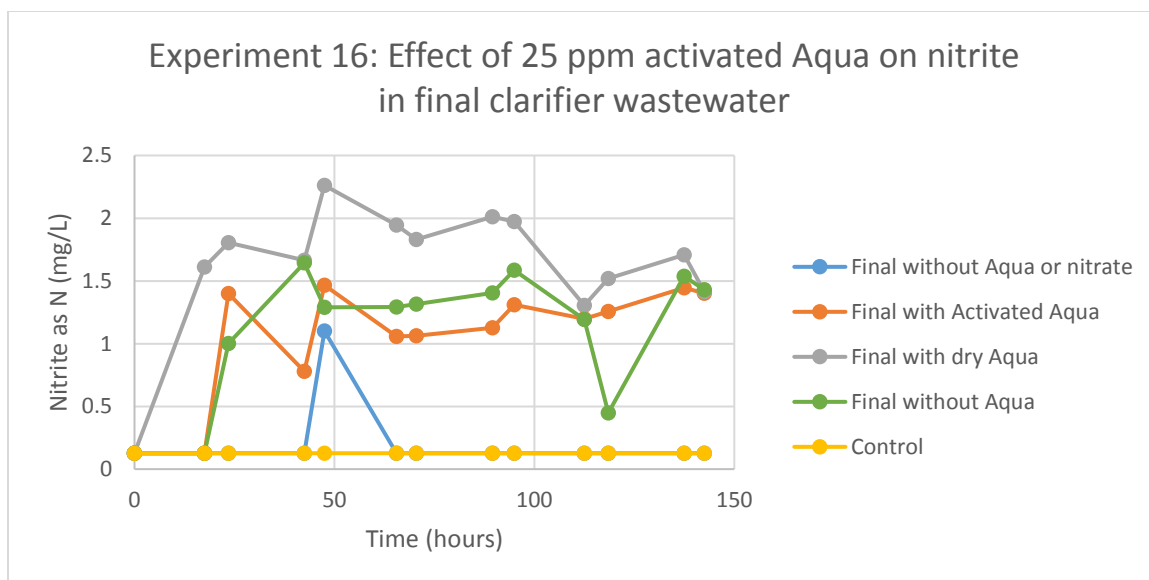


Figure 4.59: Effect of 25 ppm activated Aqua on nitrite in final clarifier wastewater

The nitrate concentration fluctuated around 60 ppm and around 30 ppm. Therefore, bacteria did not process nitrate. The nitrite increased over time and fluctuated around 1 to 2 ppm. The increase was likely due to procedural error, some small amount of nitrification or denitrification, or other metabolic processes. Using 25 ppm of Aqua would not be beneficial for decreasing nitrate because nitrate removal in this experiment did not occur. The final clarifier likely did not have enough carbon for nitrate removal to occur. The activated Aqua followed the same pattern as dry Aqua. Therefore, activated Aqua did not produce better results for nitrate removal.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. At the SLO WRRF, BOD was assumed to be the main carbon source. Data obtained from the SLO WRRF showed the average carbon amount for final clarifier wastewater in 2014 was 7.1

mg/L. In activated Aqua, TSB, growth media, and Aqua provide additional carbon to the solutions. Growth media added 1000 mg/L dextrose. TSB added 250 mg/L dextrose. Aqua added 7362.5 mg/L dextrose. Therefore, activated Aqua solution contained a total of 8612.5 mg/L dextrose. About 0.32% of the activated Aqua was added to the wastewater samples. Therefore, a total of 27.56 mg/L dextrose was added. This equates to 11 mg/L dextrose as carbon. The dry Aqua added 23.75 mg/L dextrose, which equates to 9.5 mg/L dextrose as carbon.

Due to the addition of activated Aqua and dry Aqua, the C:N ratios were different for the samples (Table 4.124). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Ratios below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The low C:N ratios for all samples likely decreased or inhibited the nitrate removal rates.

In this experiment, low C:N ratios resulted in no nitrate removal. In previous experiments, higher C:N resulted in nitrate removal. Therefore, the Aqua bacteria likely follow heterotrophic metabolic processes rather than autotrophic for denitrification.

Table 4.124: Carbon to nitrogen ratios for experiment 16

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Final with Activated Aqua	7.1	62.0	18.1	0.3:1
Final with dry Aqua		56.5	16.6	0.3:1
Final without Aqua		56.1	7.1	0.1:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C for Aqua includes initial and Aqua. Assuming Biogenesis does not add any carbon

Total nitrogen was calculated by adding the nitrate, nitrite, and ammonia (Figure 4.60).

The total nitrogen was steady around 80 ppm for samples with activated Aqua, with dry Aqua, and without Aqua. The total nitrogen was steady around 30 ppm for the sample without Aqua or nitrate.

The total nitrogen was steady around 30 ppm for the sample without Aqua or nitrate. Therefore, no total nitrogen removal occurred for all samples.

Also, the addition of Aqua did not improve total nitrogen removal.

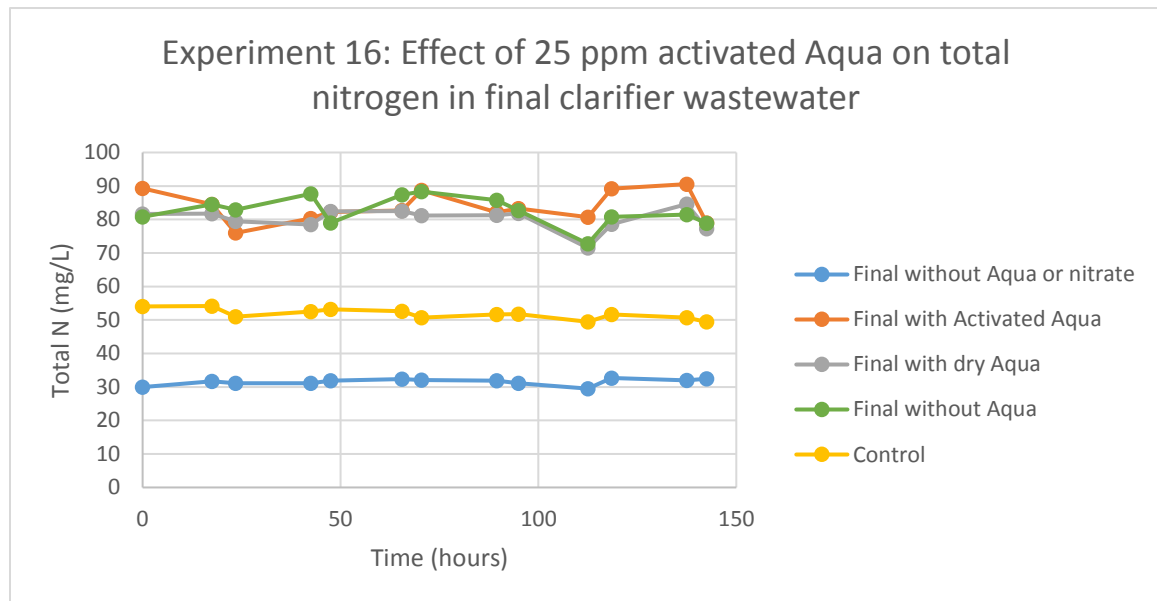


Figure 4.60: Effect of 25 ppm activated Aqua on total nitrogen in final clarifier wastewater

Nitrate removal did not occur for any samples. Nitrate removal likely did not occur because of a limited carbon source. Typically, if not enough carbon is present, another carbon source will be added to aid in denitrification. The next experiment focused on adding an external carbon source. Primary clarifier wastewater was added to the final clarifier wastewater to see if denitrification can occur with additional carbon.

4.3.3 Experiment 17 – Effect of 25 ppm Aqua on Final Clarifier Plus 5% Primary Clarifier Wastewater

Nitrate removal was analyzed for Aqua in final clarifier wastewater with 5% primary clarifier wastewater under anoxic conditions in lab and in field (Table 4.128, Table 4.129, Figure 4.61, and Figure 4.62). This experiment determined if Aqua can denitrify with limited carbon source. The solution consisted of 95% final clarifier wastewater and 5% primary clarifier wastewater. Temperature and pH were measured to determine their potential effects on nitrate removal (Table 4.126 and Table 4.127). Different times between the pH, temperature, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.125: Labeling for experiment 17

In-lab final clarifier wastewater with 5 % primary wastewater, 25 ppm dry Aqua, and 25 ppm NO ₃ -N	Final + 5% Primary with Aqua 1
In-lab final clarifier wastewater with 5 % primary wastewater, 25 ppm dry Aqua, and 25 ppm NO ₃ -N replicate	Final + 5% Primary with Aqua 2
In-lab final clarifier wastewater with 25 ppm NO ₃ -N	Final without Aqua
In-lab DI water with 25 ppm NO ₃ -N	Control

Table 4.126: pH measurements for experiment 17

Time (hours)	0	20	26	45	50	67.5	74	91	98
Final + 5% Primary with Aqua 1	6.34	6.37	6.27	6.37	6.34	6.24	6.15	6.4	6.43
Final + 5% Primary with Aqua 2	6.42	6.35	6.23	6.36	6.4	6.27	6.08	6.58	6.42
Final without Aqua	6.39	6.45	6.34	6.45	6.43	6.35	6.2	6.46	6.41
Control	6.31	5.67	6.52	7.01	6.86	6.3	5.69	5.7	5.95

The pH for 2.8% of all samples was within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). About 97.2% of all samples were below a pH of 7 and none are above a pH of 8. About 11.1% of all samples were below a pH of 6, which is where denitrification is inhibited. The pH was below 6 only for the DI control samples. Therefore, pH could have an impact on the denitrification rate for the DI control samples, but not on the denitrification rate of the wastewater samples.

Table 4.127: Temperature measurements for experiment 17

Time (hours)	0	20	26	45	50	67.5	74	91	98
Final + 5% Primary with Aqua 1	23.1	26	26.2	26.5	26.8	26.3	25.7	26.1	25.9
Final + 5% Primary with Aqua 2	23.1	25.3	26.3	26.5	26.7	25.6	25.9	25.6	25.7
Final without Aqua	23.1	25.3	26.4	26.6	26.4	26.3	25.9	25.9	26.3
Control	22.3	25.2	26.3	26.5	26.9	24.6	26.2	24.2	25.1

Laboratory temperatures were around 26°C to ensure good bacterial growth. The samples fluctuated between 23°C to 27°C, which means the bacteria were kept at a good temperature for growth. Since the temperature stayed relatively steady, it likely did not impact removal rates.

Table 4.128: Nitrate concentrations in mg/L NO₃-N for experiment 17

Time (hours)	0	26	74	91	98
Final + 5% Primary with Aqua 1	50.50	63.73	50.35	51.04	54.59
Final + 5% Primary with Aqua 2	53.85	59.44	49.73	51.83	53.60
Final without Aqua	58.46	61.94	55.82	56.77	57.85
Control	18.18	22.31	24.54	22.24	23.78

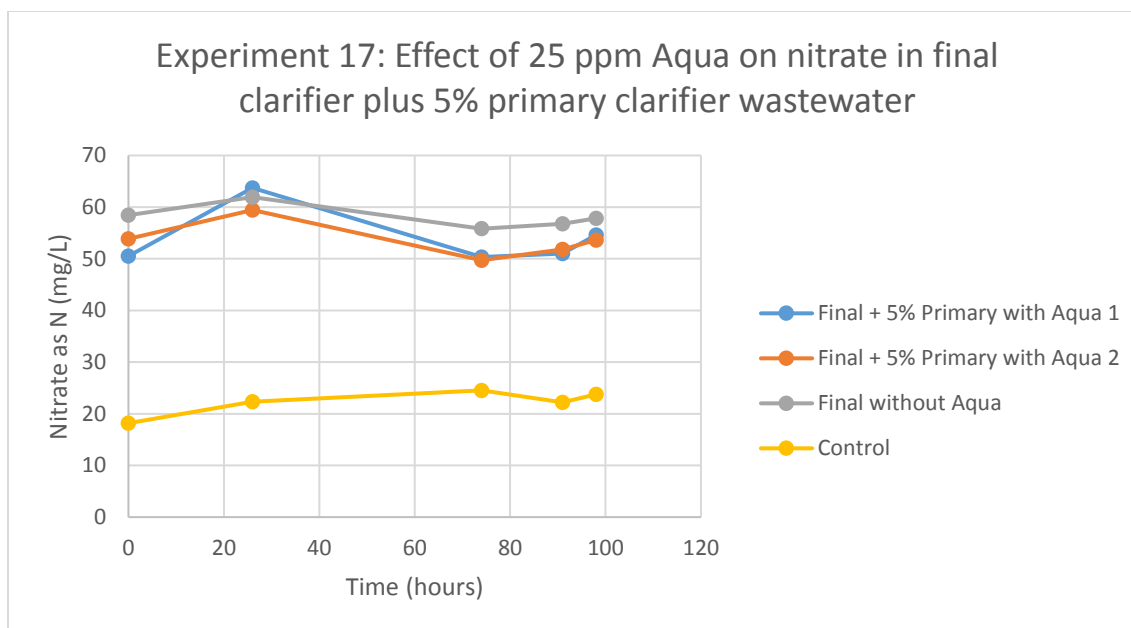


Figure 4.61: Effect of 25 ppm Aqua on nitrate in final clarifier plus 5% primary clarifier wastewater

Table 4.129: Nitrite concentrations in mg/L NO₂-N for experiment 17

Time (hours)	0	26	74	91	98
Final + 5% Primary with Aqua 1	ND	1.37	1.08	1.11	1.01
Final + 5% Primary with Aqua 2	ND	1.32	1.06	1.33	1.15
Final without Aqua	ND	1.02	ND	1.07	ND
Control	ND	ND	ND	ND	ND

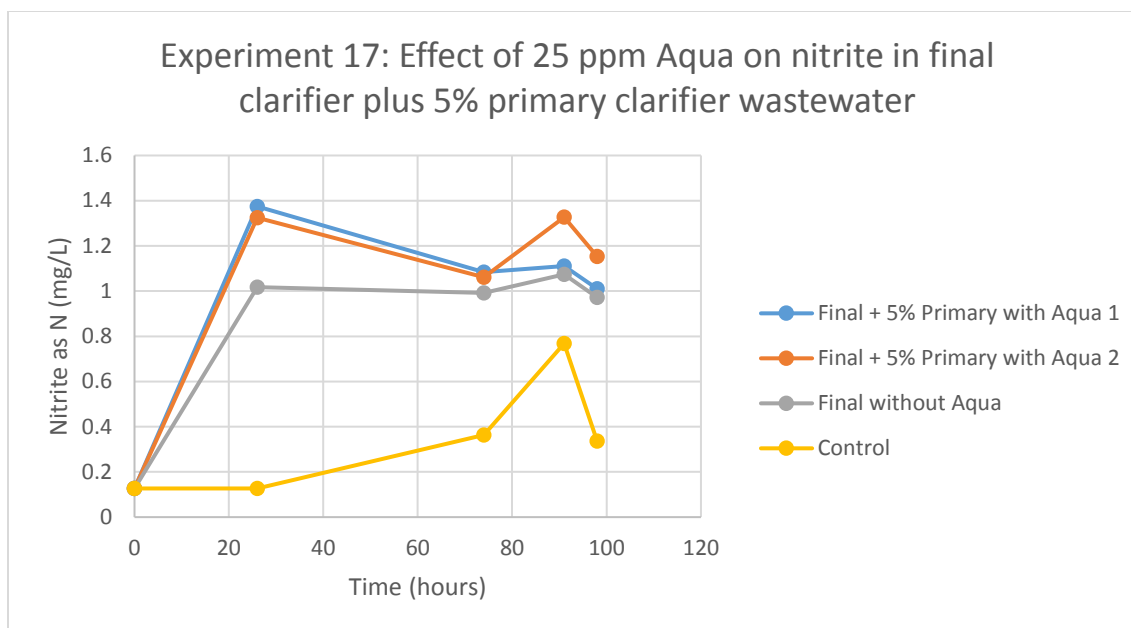


Figure 4.62: Effect of 25 ppm Aqua on nitrite in final clarifier plus 5% primary clarifier wastewater

The nitrate concentration fluctuated between 50 and 60 ppm. Therefore, bacteria did not process nitrate. The nitrite increased in the first 20 hours, then fluctuated between 1 and 1.4 ppm. The increase was likely due to procedural error or other metabolic processes. Using 25 ppm of Aqua under partially aerobic conditions would not be beneficial for decreasing nitrate because nitrate removal did not occur in this experiment. Even with primary clarifier wastewater added, the final clarifier did not have enough carbon for nitrate removal to occur.

According to research done by Eva Lee for BiOWiSHTM, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. To determine the carbon amounts at the SLO WRRF, BOD was assumed to be the main carbon

source. Data obtained from the SLO WRRF showed the average carbon amounts in 2014 for the different wastewaters. Primary clarifier wastewater has about 181.6 mg/L carbon and about 35 mg/L ammonia. The Aqua added a total of 23.75 mg/L of dextrose to the lab and bioreactor sample, since about 95% of Aqua is dextrose. This resulted in 9.5 mg/L as carbon. With 95% final and 5% primary, the total initial carbon concentration was 15.825 ppm. The C:N ratios were different for the samples (Table 4.130). For C:N ratios higher than 6:1, nitrate removal rates were still high, but not as high as C:N ratio of 2:1. For C:N ratios less than 2:1, nitrate removal rates were slower. Typical natural bacteria needed C:N ratios of 2.5:1 to 5:1 for denitrification. Below 2.5:1 reduced nitrate removal efficiency (Winkler, 2005). The C:N ratios were low, even with primary clarifier wastewater added. The low C:N ratios for samples with and without Aqua likely decreased or inhibited the nitrate removal rates.

Table 4.130: Carbon to nitrogen ratios for experiment 17

WW	Carbon in WW ¹	Total NO ₃ -N ²	Total C	C:N for NO ₃ -N
Final + 5% Primary with Aqua	15.825	50.5 – 53.9	25.325	0.5:1
Final without Aqua		58.5	15.825	0.3:1

¹Values obtained from the SLO WRRF data

²Obtained from initial points from data collected

*Total C for Aqua includes initial and Aqua. Assuming Biogenesis does not add any carbon

Total nitrogen was calculated by adding the nitrate and nitrite (Figure 4.63). The total nitrogen was steady around 55 ppm for all wastewater samples. Therefore, total nitrogen removal did not occur. The lack of total nitrogen removal was likely due to low C:N ratios. Also, the addition of Aqua did not improve total nitrogen removal.

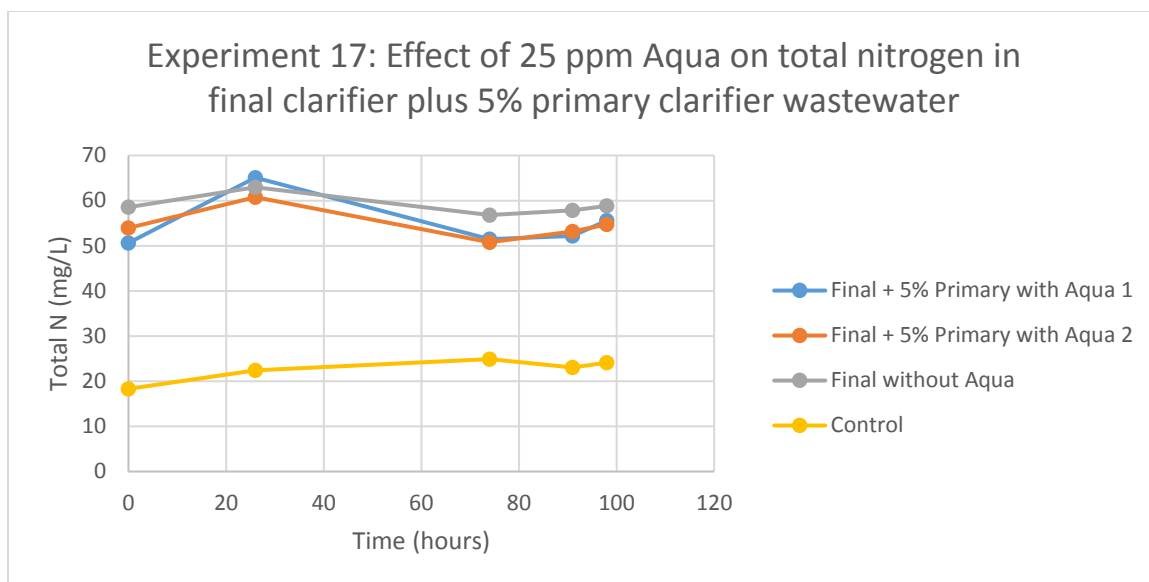


Figure 4.63: Effect of 25 ppm Aqua on total nitrogen in final clarifier plus 5% primary clarifier wastewater

Nitrate removal did not occur for all samples likely because of a limited carbon source. Even adding some primary clarifier wastewater did not help, likely because not enough was added. Ideally, primary wastewater would be added to produce a 2:1 C:N ratio. However, this will also add ammonia, which reverses the intended nitrogen removal. If 45% primary clarifier wastewater was added, a 2:1 ratio would result for the final clarifier wastewater with Aqua. However, about 15.75 ppm of ammonia would be added as well. The solution of final and primary clarifier wastewater may need to be added to the beginning of the treatment plant to remove all nitrogen. An external carbon source that does not add nitrogen would also be beneficial to use for denitrification.

4.3.4 Experiment 18 – Effect of 500 ppm Activated Aqua under High Aeration on Growth Media

Nitrate removal was analyzed for Aqua in secondary clarifier wastewater in lab and in field (Table 4.136, Table 4.137, Figure 4.65, and Figure 4.66). This experiment analyzed the impact of high aeration on Aqua. The concentration of Aqua was increased to see the changes in a shorter period of time. Duplicates of the aerated samples were performed. A DI control and another sample were also performed under anoxic conditions. Temperature, pH, and dissolved oxygen (DO) were measured to determine their potential effects on denitrification (Table 4.132, Table 4.133, Table 4.134, and Table 4.135). Different times between the pH, temperature, nitrate, and nitrite concentrations occurred because data that did not pass quality assurance and quality control tests was discarded. Confidence in data reliability is high because little to none of the data points did not pass QA/QC.

Table 4.131: Labeling for experiment 18

Description	Label
In-lab growth media with 500 ppm activated Aqua and 50 ppm NO ₃ -N 1	Growth Media with Aqua 1
In-lab growth media with 500 ppm activated Aqua and 50 ppm NO ₃ -N 2	Growth Media with Aqua 2
In-lab growth media with 500 ppm activated Aqua and 50 ppm NO ₃ -N with no aeration	Growth Media without air
In-lab DI water with no Aqua and 50 ppm NO ₃ -N	Control

Table 4.132: pH measurements for experiment 18

Time (hours)	0	3	4.5	9	12	22	24	27	29	31	46.5	49.5	51	53	55
Growth Media with Aqua 1	6.33	5.45	4.75	6.48	6.9	7.76	7.82	7.78	7.62	7.78	8.02	8.02	8.08	8.11	8.02
Growth Media with Aqua 2	6.38	5.65	4.72	6.42	6.87	7.72	7.76	7.67	7.67	7.72	7.84	7.92	7.86	7.89	7.89
Growth Media without air	6.28	5.66	4.78	3.85	3.52	3.44	3.42	3.44	3.36	3.56	3.4	3.46	3.53	3.5	3.43
Control	5.48	5.37	4.98	5.3	5.11	5.07	6.16	5.15	5.66	4.71	5.13	5.74	4.82	5.26	5.38

The pH for 25% of all samples was within the recommended pH range of 7 to 8 for denitrification (Metcalf & Eddy, 2003). About 66.7% of all samples were below a pH of 7 and 8.3% are above a pH of 8. About 53.3% of all samples were below a pH of 6, which is where denitrification is inhibited. Only the growth media without air and DI control samples were below 6. Therefore, pH could have an impact on the denitrification rate for the growth media without air and DI control samples. The pH did not impact the growth media with Aqua samples that were aerated. The pH increased for the aerated samples. Aerating Aqua could cause the bacteria to undergo a metabolic process that causes an increase in pH. The pH decreased over time in the anoxic wastewater sample.

Table 4.133: Temperature measurements for experiment 18

Time (hours)	0	3	4.5	9	12	22	24	27	29	31	46.5	49.5	51	53	55
Growth Media with Aqua 1	23.3	26.6	27.3	27	27.4	25.9	24.8	25.9	25.8	25.1	24.9	25.5	25.9	26.5	26
Growth Media with Aqua 2	23.6	26.2	26.5	25.8	27	23.8	23.8	25.1	25.1	25.4	23.8	24.3	25.3	25.7	24.4
Growth Media without air	23.3	28.1	28.3	29.3	29.2	27.6	26.6	26.7	26.7	26.7	27	27.4	27.4	27.3	28.4
Control	24.9	29.5	29.9	28.9	28.5	26.1	26.3	26.5	25.5	28.8	26.2	28.1	28.1	28.3	27.8

Laboratory temperatures were around 25°C to ensure good bacterial growth. The samples fluctuated between 24°C to 28°C, which means the bacteria were kept at a good temperature for growth. Since the temperature stayed relatively steady, it likely did not impact removal rates.

Table 4.134: DO for growth media with Aqua 1 in mg/L for Experiment 18

Dist. On Beaker (mL)	0 hr	3 hr	4.5 hr	9 hr	12 hr	22 hr	24 hr	27 hr	29 hr	31 hr	46.5 hr	49.5 hr	51 hr	53 hr	55 hr
500	5.46	0.28	0.43	2.95	3.58										
400	5.32	0.11	0.15	2.67	2.53	5.84	7.53	7.12	6.53	5.91	7.33	6.6			
200	5.08	0.14	0.15	2.48	1.87	5.6	5.74	5.32	5.65	5.55	6.11	6.13	6.52	6.64	7.28
50	4.82	0.11	0.11	2.09	1.67	5.32	5.51	4.82	4.78	4.79	5.25	5.35	5.96	5.9	6.48

Note: Blanks mean that evaporation occurred and a sample at that distance could not be obtained

Table 4.135: DO for growth media with Aqua 2 in mg/L for experiment 18

Dist. On Beaker (mL)	0 hr	3 hr	4.5 hr	9 hr	12 hr	22 hr	24 hr	27 hr	29 hr	31 hr	46.5 hr	49.5 hr	51 hr	53 hr	55 hr
500	5.62	0.15	1.99	4.03	3.16										
400	5.51	0.1	1.53	3.2	2.56	6.24	7.98	7.52	7.67	6.78	7.68				
200	5.55	0.1	0.13	2.84	2.04	6.01	6.75	6.7	6.8	6.31	6.79	7.16	6.36		
50	5.44	0.11	0.16	2.71	1.83	5.9	6.11	5.82	5.49	5.9	6.31	5.62	6.07	6.95	8.01

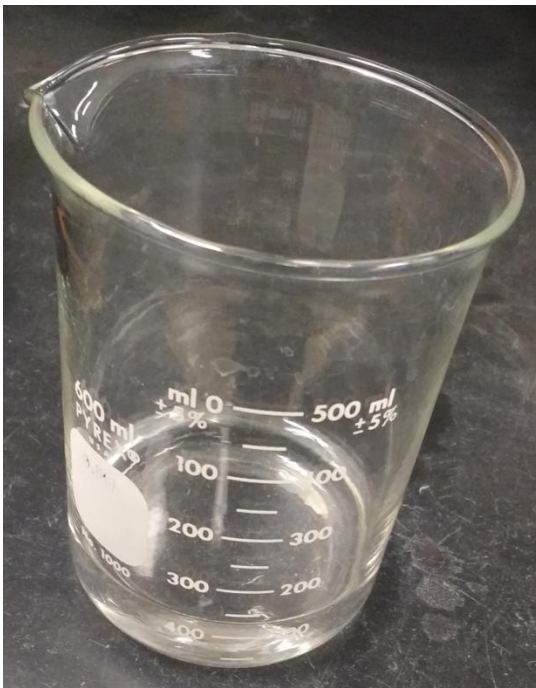


Figure 4.64: Example of beaker with distances where dissolved oxygen was measured

The samples evaporated over time, which will be factored into the concentration analysis.

The DO for A2 was higher than for A1. The bubbler used in A2 seemed to have a higher aeration rate, which explains why the DO is higher.

Table 4.136: Nitrate concentrations in mg/L NO₃-N for experiment 18

Time (hours)	0	3	4.5	9	12	22	24	27	29	31	46.5	49.5	51	53	55
Control	53.09	51.54	51.85	52.07	52.34	51.87	52.65	52.21	51.82	57.09	52.07	51.86	54.52	47.95	52.20
GM with Aqua 1	39.51	44.45	41.10	45.35	47.31	50.36	53.28	53.64	52.97	48.84	67.44	56.88	58.76	60.27	65.18
GM with Aqua 2	40.59	45.22	44.86	46.64	49.26	53.37	58.29	49.05	60.86	70.95	79.28	79.78	89.62	89.29	98.29
GM without air	42.27	43.82	43.15	43.49	45.39	43.45	44.64	44.67	43.68	42.08	39.81	40.60	39.11	39.76	41.00

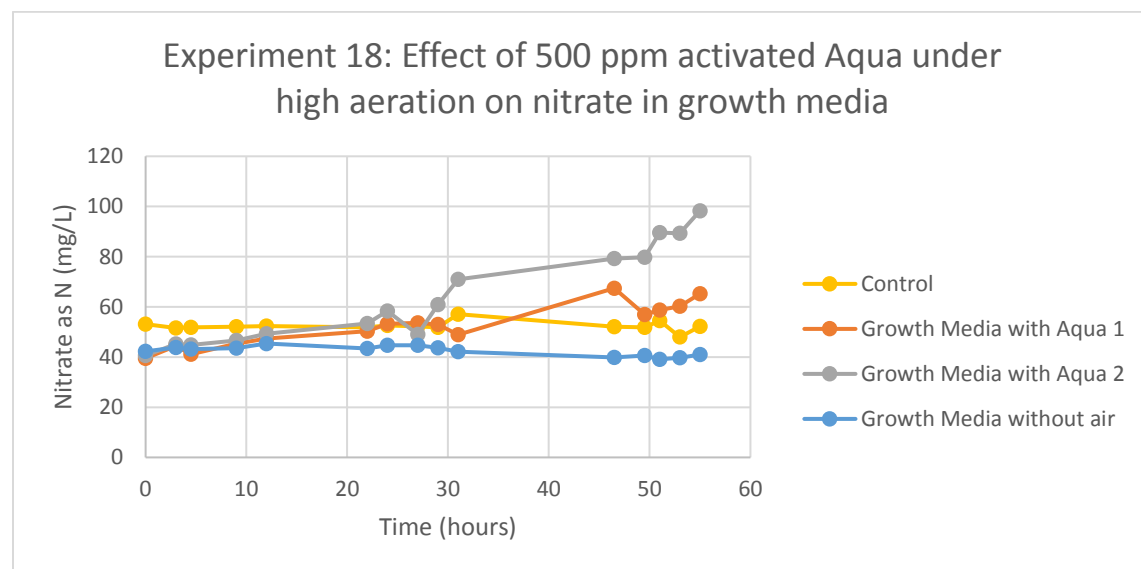


Figure 4.65: Effect of 500 ppm activated Aqua under high aeration on nitrate in growth media

Table 4.137: Nitrite concentrations in mg/L NO₂-N for experiment 18

Time (hours)	0	3	4.5	9	12	22	24	27	29	31	46.5	49.5	51	53	55
Control	3.53	ND	ND	ND	ND	ND	1.00	ND	ND	ND	ND	ND	4.94	ND	ND
Growth Media with Aqua 1	7.02	ND	6.35	9.48	7.47	14.83	10.79	14.13	ND	9.00	9.60	ND	ND	ND	ND
Growth Media with Aqua 2	ND	7.06	6.77	ND	ND	14.95	10.32	ND	0.58	2.49	ND	15.12	12.07	ND	ND
Growth Media without air	10.51	6.29	13.81	5.87	ND	ND	8.40	ND	ND	9.72	ND	ND	ND	ND	ND

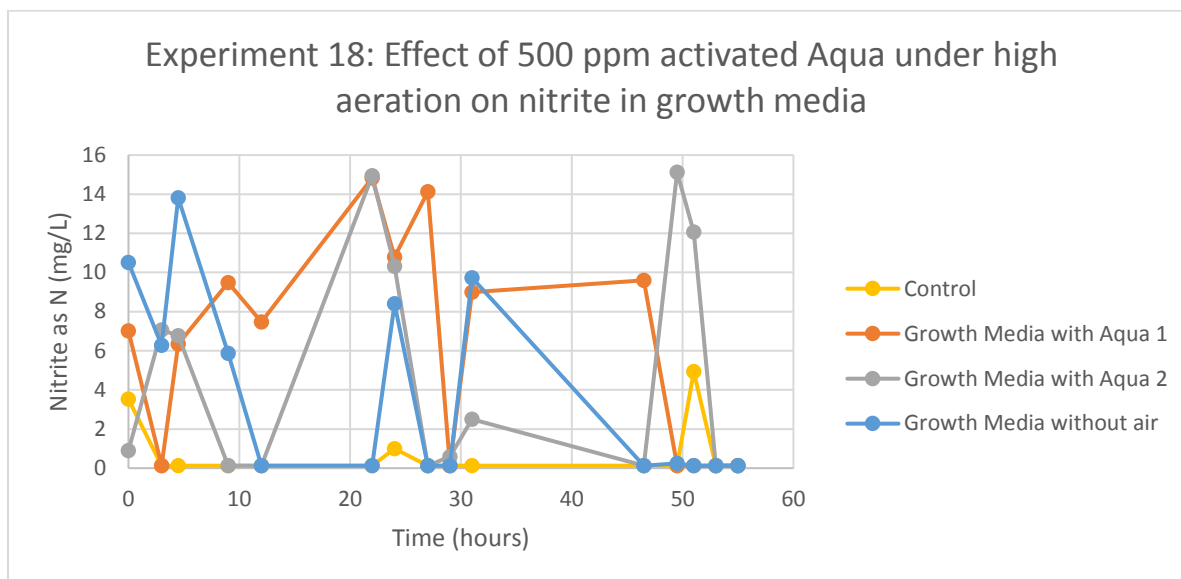


Figure 4.66: Effect of 500 ppm activated Aqua under high aeration on nitrite in growth media

The nitrate concentration fluctuated between 50 and 60 ppm for the first 30 hours. After 30 hours, the two aerated samples increased in concentration. The sample at a higher aeration rate increased by 60 ppm nitrate. The sample at a lower aeration rate increased by about 30 ppm nitrate. Since TSB added ammonia, the nitrate increase was likely because of nitrification and some evaporation. The anoxic sample did not denitrify at all, which is contrary to what was seen in Experiment 1. Although all the components in this growth media were the same as in Experiment 1, the activation method was different. In Experiment 1, activation only occurred with Aqua and TSB, not with growth media. Also, the activation was anoxic in Experiment 1. Activating the bacteria under aerobic conditions may have inhibited denitrification. The nitrite for each sample fluctuated and had no pattern. The fluctuations in nitrite were likely caused by procedural error or nitrification.

The two duplicated samples did not follow the same trend. DI control samples above 1 ppm were likely caused by procedural error. Activating the Aqua like the field experiments resulted in denitrifying bacteria inhibited by aeration. However, nitrification can occur under high aerobic conditions with a high Aqua concentration.

According to research done by Eva Lee for BiOWiSH™, a C:N ratio of 2:1 was ideal for ammonia removal, nitrate removal, and simultaneous ammonia and nitrate removal. In this experiment, carbon was added from TSB, growth media, and Aqua. Growth media added 1000 mg/L dextrose. TSB added 250 mg/L dextrose because only 10% of the TSB was added to the solution. The concentration of Aqua in the solution was 500 mg/L. Aqua added 475 mg/L dextrose because about 95% of Aqua is dextrose. The solution contained a total of 1725 mg/L dextrose. This equates to 690 mg/L dextrose as carbon. The initial nitrate concentration in the samples was about 40 ppm. This 40 ppm concentration created a carbon to nitrogen ratio of 17.3:1, which could slow the nitrate removal.

Total nitrogen was calculated by adding the nitrate and nitrite (Figure 4.67). The total nitrogen increased over time for the aerated samples. The total nitrogen stayed constant for the anoxic samples. Therefore, total nitrogen removal did not occur for all samples. An increase in total nitrogen likely occurred because ammonia was removed and nitrate was produced via nitrification. However, ammonia was not measured for this experiment, so nitrification cannot be concluded.

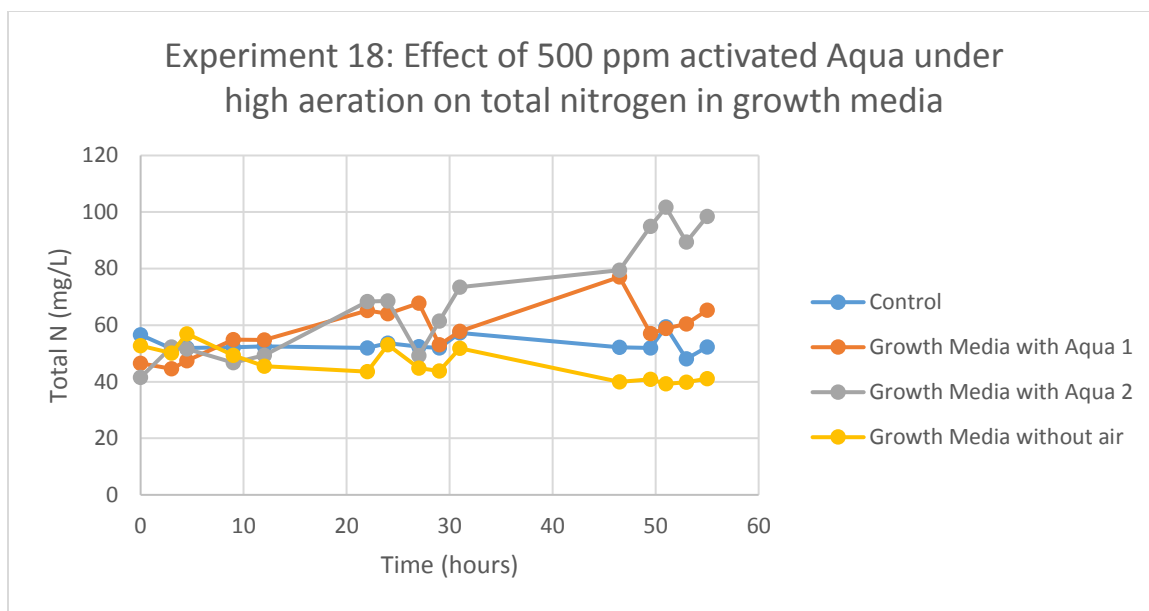


Figure 4.67: Effect of 500 ppm activated Aqua under high aeration on total nitrogen in growth media

Nitrate increased over time for the aerated samples, indicating nitrification. Nitrite fluctuated drastically over time from intermediate step in nitrification. Nitrate removal did not occur because high aeration and different activation methods can inhibit nitrate removal. Simultaneous nitrification and denitrification could be possible if the activation method was changed.

CHAPTER 5: CONCLUSIONS

BiOWiSH™ was tested in simple and complex media (growth media and wastewater). Based on the eighteen experiments conducted, various conclusions were made.

Bacillus licheniformis prevailed in Aqua for denitrification. It degraded 6.6 mg N/L/hour. The *Bacillus amyloliquefaciens* assimilated or denitrified without the production of intermediate nitrite. It removed 3.2 mg N/L/hour. *Bacillus mojavensis subtilis* prevailed in Aqua for slow nitrite removal. It denitrified 2.5 mg N/L/hour.

When Aqua was tested in different wastewaters in the treatment plant, Aqua did not produce better nitrification rates than the natural bacteria. However, Aqua did remove nitrate at a better rate than the natural bacteria. The nitrate degradation was likely due to assimilation rather than denitrification in all experiments using unsterilized wastewater from the treatment plant. In partially sterilized wastewater, denitrification was observed. The secondary clarifier wastewater produced the best nitrate removal results. In secondary clarifier wastewater, Aqua degraded 2.67 mg N/L/hour. In unsterilized secondary clarifier wastewater, Aqua assimilated 15 ppm more nitrate when compared to the natural bacteria.

Secondary clarifier wastewater was used in the lab to determine: 1) if the addition of trace mineral media helped increase nitrate removal, 2) if a powder containing a higher concentration of microbial cultures increases nitrate removal, and 3) if dosing in a concentrated liquid form increases nitrate removal. The 500 ppm Aqua concentration degraded nitrate quicker than the 50 ppm Aqua concentration. However, the 50 ppm Aqua

concentration degraded more nitrate than the natural bacteria. The natural bacteria did not remove any nitrate. Nitrate removal was observed for all samples containing Aqua in the 10 hours of the experiment. Adding trace mineral media to the samples did not improve the nitrate degradation rate. The concentrated microbial cultures removed nitrate at a higher rate than regular Aqua. About 6 ppm more nitrate was removed in the microbial cultures. Adding Aqua in a concentrated liquid form rather than as a dry powder did not make a difference in the nitrate removal rates. When the dry powder was used, the highest weight measured was 50 mg and the lowest weight measured was 5 mg. When 50 mg was used, the difference between the liquid and dry inoculation was negligible. When 5 mg was used, it showed that Aqua did help remove more nitrate. This means that weighing out 5 mg of Aqua can still contain enough microbial cultures to represent Aqua capabilities and remove more nitrate than the natural bacteria. It would be beneficial to analyze lower weights to determine what weight does not contain enough microbial cultures to represent Aqua. Duplicating this experiment would also be beneficial to determine if the outcome is the same.

Bioreactors were set up at the secondary clarifier in an anoxic environment. Doses of 50, 5, and 2.5 ppm Aqua were used in the bioreactors. The 50 ppm Aqua dose was the only concentration that produced better nitrate removal rates when compared to the natural bacteria. It degraded about 10 ppm more nitrate.

Bioreactors were also set up at the sludgewash in an anoxic environment. Doses of 25, 10, 5, and 2.5 ppm Aqua were used in the bioreactors. None of the doses were able to perform

nitrification or denitrification better than the natural bacteria. The high concentrations of ammonia and nitrate already existing in the wastewater were likely toxic to the bacteria responsible for nitrogen removal. Salinity, other toxins, or deficiency in essential minerals in the wastewater could also prevent Aqua growth.

Bioreactors were set up again at the secondary clarifier in an anoxic environment. The effect of activated Aqua on nitrogen removal was tested. Activating the Aqua should produce a large cell count before inoculation. This would ideally skip the lag time needed for bacterial growth, producing a faster nitrate removal rate. Nitrate removal occurred for 25 ppm Aqua. It degraded about 5 to 10 ppm more nitrate than the natural bacteria. However, the activated Aqua performed the same nitrate removal as the dry Aqua. Therefore, activation made no difference on the performance of Aqua.

Bioreactors were set up at the secondary clarifier in an aerobic environment. Activated Aqua was tested in an aerobic environment to determine if the increased cell count would help in nitrification. Nitrification occurred near the end of the run. However, the nitrification rate for all samples was the same, which means the natural bacteria was responsible for the nitrification and 25 ppm of Aqua did not help in nitrification. This reflects results found in preliminary experiments where partial aeration was used in the lab.

Bioreactors were set up at the secondary clarifier again to test Aqua against a competitor product called Biogenesis. The bioreactor data showed that the natural bacteria were responsible for nitrate removal, and that 25 ppm of Aqua and Biogenesis did not help in

nitrate removal. This concentration of Aqua has shown better nitrate removal results in past experiments, so constituents in the wastewater could have changed to prevent nitrate removal or field conditions affected nitrate removal. Nitrate in lab wastewater started at different concentrations due to procedural error. Therefore, this experiment was redone in lab to determine if bioreactor data was different from laboratory data. The laboratory experiment showed that Biogenesis did not help in nitrate removal because it followed the same trend as the natural bacteria. The Aqua in lab removed 5 ppm more nitrate than the natural bacteria and Biogenesis. Experiments 12 and 14 showed that using 25 ppm Aqua in secondary clarifier wastewater resulted in about 5 to 10 ppm nitrate removal. Therefore, a cost-benefit analysis should be performed before implementing Aqua.

Laboratory studies using final clarifier wastewater were performed because the clarifier is located after all BOD and ammonia removal technologies. It should ideally have little to no nitrogen. However, the clarifier still has about 25 ppm of nitrate. If Aqua can remove the nitrate at this stage, it would be very beneficial for the SLO WRRF. Using Aqua in final clarifier wastewater showed that neither ammonia nor nitrate removal occurred because of very low C:N amounts in the wastewater. About 5% of primary clarifier wastewater was added to increase the carbon content in the wastewater. However, not enough primary clarifier wastewater was added because 5% did not show any nitrate removal. To achieve the ideal 2:1 carbon to nitrogen ratio, about 45% of the total solution should be primary clarifier wastewater. However, primary clarifier wastewater will increase the ammonia concentration in the final clarifier by 15.75 ppm. This treated wastewater from the final clarifier could be mixed back into the beginning of the treatment

train to remove that excess ammonia. An external carbon source, such as glucose, could be used instead of primary clarifier wastewater. That way, only carbon is added to the wastewater, not ammonia. Since low C:N ratios resulted in no nitrate removal and higher C:N ratios resulted in nitrate removal, the Aqua bacteria likely follow heterotrophic metabolic processes rather than autotrophic. A laboratory experiment controlling inorganic carbon amounts for Aqua would help prove or disprove this statement.

The impact of high aeration on denitrification was also studied in the laboratory using a mineral medium, including Aqua, GM, and TSB, instead of wastewater. In this experiment, nitrate increased over time. Therefore, denitrification likely did not occur under high aeration. The causes of the nitrate increases were likely nitrification and evaporation. The ammonia used in nitrification came from the TSB that was used for Aqua growth. Denitrification likely did not occur because an aerated activation method and high aeration of the solutions likely inhibited nitrate removal.

CHAPTER 6: RECOMMENDATIONS

Aqua in unsterilized wastewater did not perform nitrification. A higher concentration of Aqua and a larger amount of aeration is likely needed for Aqua to achieve nitrification, as seen in Experiment 18. Duplicating Experiment 18 and running the experiment for a longer time would be beneficial because nitrification rates could be calculated. Also, performing this experiment to determine ideal aeration rates for nitrification would be beneficial. Performing another experiment with ideal aeration and concentration of Aqua in unsterilized wastewater would be beneficial. The experiment could determine if competition between Aqua and the natural bacteria occurs under ideal nitrification conditions.

Testing Aqua with different dissolved oxygen amounts in a laboratory setting would be beneficial. The ideal dissolved oxygen concentration can be determined for simultaneous nitrification and denitrification. Once ideal dissolved oxygen concentrations are determined in lab, conducting the same test with complex media, such as wastewater, would be beneficial. The unsterilized wastewater should be as close to ideal conditions as possible to determine if natural bacteria or Aqua will prevail in aerobic nitrification and denitrification.

Testing Aqua with different temperatures and pHs in a laboratory setting would be beneficial. Analyzing ammonia, nitrate, and nitrite would be ideal to observe how temperature and pH affects ammonia and nitrate removal. The different temperatures and pHs could cause different strains of bacteria in Aqua to prevail over others. Once ideal

temperatures and pHs are determined in lab, conducting the same test with complex media, such as wastewater, would be beneficial.

It would be interesting to see how dissolved oxygen, salinity, essential minerals, trace elements, metals, and toxic chemicals effect Aqua bacteria growth. These different parameters should be tested in mineral media to determine ideal and limiting concentrations of each.

Since Aqua did not nitrify well, it would be beneficial to test the different strains of bacteria in Aqua and other bacteria known for their nitrification capabilities in a laboratory setting. The C:N ratio, dissolved oxygen concentration, temperature, salinity, and pH can be analyzed to find optimal amounts. The bacteria producing the highest rate should be tested in unsterilized wastewater under ideal conditions to see if it prevails over the natural bacteria. Using a larger amount of the nitrifying bacteria in Aqua could help Aqua achieve nitrification rates better than natural bacteria.

Analyzing different activation techniques would also be beneficial. Similar to the study conducted by Yao, Ni, Chen, and Borthwick, laboratory experiments could be performed to determine the best way to activate the Aqua to achieve optimal nitrification and denitrification rates (Yao, Ni, Chen, & Borthwick, 2012). Then, the activated Aqua could be tested in unsterilized wastewater and compared to the natural bacteria to ensure that Aqua achieves better nitrogen removal rates. Researching into different activation techniques would also be helpful for studying aerobic denitrification. According to Yao,

Ni, Chen, and Borthwick, doing a multi-step DO increase rather than a single DO over the entire run will help increase the amount and efficiency of aerobic denitrification bacteria.

Testing how acidification of samples in the IC would be beneficial. The acidification could raise the conductivity of the samples. If acidification does impact the concentrations of the samples, then it is recommended to acidify the calibrations used for the IC.

REFERENCES

- Biotech Solabia Group. (2008, March 28). *PANCREATIC DIGEST OF CASEIN – A1403 / A1433*. Retrieved May 12, 2017, from <http://solabia.com/solabia/content/NT00004416.pdf>
- Biotech Solabia Group. (2008, March 25). *PAPAIC DIGEST OF SOYBEAN MEAL USP – A1601*. Retrieved May 12, 2017, from <http://solabia.com/solabia/content/NT000043E6.pdf>
- BiOWiSH. (2016, May 18). *Technical Data Sheet of BiOWiSH Aqua*. Retrieved from BiOWiSH Technologies: <http://b14279a71a966e52ae36-fad5b9385501cb7f7cac4f09f1c56222.r14.cf5.rackcdn.com/TDS/tds-aqua-en.pdf>
- BiOWiSH Technologies. (2016, May 18). *Technical Data Sheet*. Retrieved April 20, 2017, from <http://b14279a71a966e52ae36-fad5b9385501cb7f7cac4f09f1c56222.r14.cf5.rackcdn.com/TDS/tds-aqua-en.pdf>
- Blackwell, S., Bowen, C., Parker, L., & Crowe, B. (2015). *Total Ammonia Nitrogen Analysis Using the Timberline TL-2800*. Standard Operating Procedure, Cal Poly San Luis Obispo, Environmental Engineering.
- Capodaglio, A. G., Hlavinek, P., & Raboni, M. (2015, May 21). *Physio-chemical technologies for nitrogen removal from wastewaters: a review*. Retrieved April 4, 2017, from http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1980-993X2015000300481
- Chen, J., Gu, S., Hao, H., & Chen, J. (2016, September 27). *Characteristics and metabolic pathway of Alcaligenes sp. TB for simultaneous heterotrophic nitrification-aerobic denitrification*. Retrieved July 31, 2017, from Environmental Biotechnology: <https://link-springer-com.ezproxy.lib.calpoly.edu/content/pdf/10.1007%2Fs00253-016-7840-x.pdf>
- Choi, K.-J., Zhang, S., Song, J., & Hwang, S.-J. (2016, December 30). *Aerobic Denitrification by a Heterotrophic Nitrifying-aerobic Denitrifying (HN-AD) Culture Enriched Activated Sludge*. Retrieved July 31, 2017, from KSCE Journal of Civil Engineering: <https://link-springer-com.ezproxy.lib.calpoly.edu/content/pdf/10.1007%2Fs12205-016-1287-6.pdf>
- Choubert, J.-M., Racault, Y., Grasmick, A., Beck, C., & Heduit, A. (2005). *Maximum nitrification rate in activated sludge processes at low temperature: key parameters, optimal value*. Retrieved April 25, 2017,

- from E-WATER (EWA): http://www.ewa-online.eu/tl_files/_media/content/documents_pdf/Publications/E-Water/documents/56_2005_09.pdf
- Dawson, R. N., & Murphy, K. L. (1972). The temperature dependency of biological denitrification. In *Water Res.* (pp. 71-83).
- DeCoste, D. J., & Zumdahl, S. S. (2010). *Introductory Chemistry*. Belmont, CA, USA: Brooks/Cole, Cengage Learning.
- Dincer, A., & Kargi, F. (2000, January 18). *Kinetics of sequential nitrification and denitrification process*. Retrieved April 20, 2017, from Dokuz Eylul University: https://www.researchgate.net/publication/256968019_Kinetics_of_sequential_nitrification_and_denitrification_processes
- Dunn, I., Tanaka, H., Uzman, S., & Denac, M. (1984). *Biofilm fluidized bed reactors and their application to wastewater nitrification*. Retrieved April 25, 2017, from Biotech. Bioengr.: <http://www.eubios.info/TTEC/TTECPY.htm>
- Ehrlich, H. L., & Newman, D. K. (2009). *Geomicrobiology* (Fifth ed.). Boca Raton, Florida, USA: CPC Press.
- EPA. (2017, March 22). *Aquatic Life Criteria - Ammonia*. Retrieved March 24, 2017, from United States Environmental Protection Agency: <https://www.epa.gov/wqc/aquatic-life-criteria-ammonia>
- EPA. (1998, September). *How nitrogen oxides affect the way we live and breathe*. Retrieved July 31, 2017, from United States Environmental Protection Agency: <https://web.archive.org/web/20080716063437/http://www.epa.gov/air/urbanair/nox/noxfldr.pdf>
- EPA. (2010, August). *Nutrient Control Design Manual*. Retrieved July 31, 2017, from United States Environmental Protection Agency: <https://nepis.epa.gov/Exe/ZyPDF.cgi/P1008KTD.PDF?Dockkey=P1008KTD.PDF>
- EPA. (2017, March 9). *Nutrient Pollution: Harmful Algal Blooms*. Retrieved March 13, 2017, from United States Environmental Protection Agency: <https://www.epa.gov/nutrientpollution/harmful-algal-blooms>
- EPA. (2017, March 10). *Nutrient Pollution: Sources and Solutions*. Retrieved March 13, 2017, from United States Environmental Protection Agency: <https://www.epa.gov/nutrientpollution/sources-and-solutions>
- EPA. (2017, April 7). *Nutrient Pollution: The Effects: Environment*. Retrieved April 9, 2017, from United States Environmental Protection Agency: <https://www.epa.gov/nutrientpollution/effects-environment>

- EPA. (2017, March 10). *Nutrient Pollution: The Effects: Human Health*. Retrieved March 13, 2017, from United States Environmental Protection Agency: <https://www.epa.gov/nutrientpollution/effects-human-health>
- EPA. (2017, March 10). *Nutrient Pollution: The Problem*. Retrieved March 13, 2017, from United States Environmental Protection Agency: <https://www.epa.gov/nutrientpollution/problem>
- EPA. (2017, April 6). *The Nitrogen Cycle*. Retrieved April 7, 2017, from https://www3.epa.gov/caddis/ssr_amm_nitrogen_cycle_popup.html
- EPA. (2017, March 10). *The Sources and Solutions: Fossil Fuels*. Retrieved April 4, 2017, from Environmental Protection Agency: <https://www.epa.gov/nutrientpollution/sources-and-solutions-fossil-fuels>
- Evans, J., & Perlman, H. (n.d.). *Biogeochemical cycles: Figure 4*. Retrieved from USGS: <http://cnx.org/contents/s8Hh0oOc@9.10:1KV9fus6@4/Biogeochemical-Cycles>
- Francis-Floyd, R., Watson, C., Petty, D., & Pouder, D. B. (2009, February). *Ammonia in Aquatic Systems*. Retrieved April 4, 2017, from University of Florida IFAS Extension: <http://edis.ifas.ufl.edu/pdf/FA/FA03100.pdf>
- Gorsuch, J. P., Roberts, J. P., Lenhoff, E. A., & Showell, M. S. (n.d.). *BiOWiSH Aqua Research Study*. Retrieved April 4, 2017, from BiOWiSH Technologies: <https://cead874f5b added4b43a5cfad5b9385501cb7f7cac4f09f1c56222.ssl.cf5.rackcdn.com/Research%20Studies/Water%20Treatment/research-report-nitrogen-management-in-water-treatment.pdf>
- Harrison, J. A. (2003). *The Nitrogen Cycle: Of Microbes and Men*. Retrieved March 13, 2017, from Vision Learning: <http://www.visionlearning.com/en/library/Earth-Science/6/The-Nitrogen-Cycle/98>
- Hartmand, P., & Cleland, J. (2007, May 31). *Wastewater Treatment Performance and Cost Data to Support an Affordability Analysis for Water Quality Standards*. Retrieved July 31, 2017, from http://www.kysq.org/docs/Wastewater_2007.pdf
- HDR. (2014, December 18). *Water Resource Recovery Facility Draft Facilities Plan*. Retrieved March 10, 2017, from SLO City: <http://www.slocity.org/home/showdocument?id=5841>
- Herrero, M., & Stuckey, D. C. (2014, November 12). *Bioaugmentation and its application in wastewater treatment: A review*. Retrieved April 4,

- 2017, from
<http://www.sciencedirect.com/science/article/pii/S0045653514012181>
- Hu, Z., Lotti, T., Loosdrecht, M. v., & Kartal, B. (2013, April 1). *Nitrogen removal with the anaerobic ammonium oxidation process*. Retrieved July 31, 2017, from Springer Science+Business Media Dordrecht: <https://link-springer-com.ezproxy.lib.calpoly.edu/content/pdf/10.1007%2Fs10529-013-1196-4.pdf>
- Jenkins, S. (1973). *Advances in Water Pollution Research*. Elmsford, New York, USA: Pergamon Press Inc.
- Kim, J. K., Park, K. J., Cho, K. S., Nam, S.-W., Park, T.-J., & Bajpai, R. (2005, March 29). *Aerobic nitrification-denitrification by heterotrophic Bacillus strains*. Retrieved April 4, 2017, from <http://www.sciencedirect.com.ezproxy.lib.calpoly.edu/science/article/pii/S0960852405000970>
- Lee, E. (2012, May). *Investigation of a Commercial Product (BiOWiSH) for Nitrogen Management*. Retrieved April 20, 2017, from California Polytechnic University San Luis Obispo: <https://pdfs.semanticscholar.org/7ffd/bdc20a10575cae1c379ed93519e5dd51b92e.pdf>
- Lekang, O.-I. (2013). *Aquaculture Engineering*. Chichester, West Sussex, UK: Wiley-Blackwell.
- Li, B., & Wu, G. (2014, March 27). *Effects of Sludge Retention Times on Nutrient Removal and Nitrous Oxide Emission in Biological Nutrient Removal Processes*. Retrieved April 25, 2017, from International Journal of Environmental Research and Public Health: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4024994/>
- Li, J., Qiang, Z., Yu, D., Wang, D., Zhang, P., & Li, Y. (2016, July 21). *Performance and microbial community of simultaneous anammox and denitrification (SAD) process in a sequencing batch reactor*. Retrieved July 31, 2017, from Bioresource Technology: http://ac.els-cdn.com.ezproxy.lib.calpoly.edu/S0960852416310604/1-s2.0-S0960852416310604-main.pdf?_tid=b938e36e-77e2-11e7-8200-00000aacb35f&acdnat=1501720312_76932735fb018e7ebbd69f2c4a2e5f3f
- Lunquist, T. (2017, January 18). Lecture: N Cycle Diagram. San Luis Obispo, CA.
- Maurer, M., Fux, C., Graff, M., & Siegrist, H. (2011). *Moving-bed biological treatment (MBBT) of municipal wastewater: Denitrification*. Retrieved April 20, 2017, from Water Science and Technology: <https://www-engineeringvillage->

com.ezproxy.lib.calpoly.edu/search/doc/abstract.url?pageType=quickSearch&usageOrigin=searchresults&usageZone=resultslist&searchtype=Quick&SEARCHID=1ce30d0aMc34dM4360Mab27M8adaec36ea64&DOCINDEX=6&database=1&format=quickSearchAbstractFormat&dedupResultCount=&SEARCHID=1ce30d0aMc34dM4360Mab27M8adaec36ea64&referer=%2Fsearch%2Fresults%2Fquick.url

- Metcalf & Eddy. (2003). *Wastewater Engineering Treatment and Reuse* (Fourth ed.). New York, New York: McGraw-Hill.
- MWH. (2005). *Water Treatment: Principles and Design* (Second ed.). Hoboken, New Jersey: John Wiley and Sons, Inc.
- New Hampshire Department of Environmental Services. (2006). *Nitrate and Nitrite: Health Information Summary*. Retrieved April 4, 2017, from Department of Environmental Services: <https://www.des.nh.gov/organization/commissioner/pip/factsheets/ard/documents/ard-ehp-16.pdf>
- NOAA. (2008, March 24). *Nutrient Pollution - Eutrophication*. Retrieved March 15, 2017, from NOAA Ocean Service Education: http://oceanservice.noaa.gov/education/kits/estuaries/media/supp_estuar09b_eutro.html
- NPDES. (2011). *Basic Soil Science and Soil Fertility*. Retrieved July 31, 2017, from United States Environmental Protection Agency: https://www.epa.gov/sites/production/files/2015-08/documents/cafo_permitmanual_appendixa.pdf
- Perlman, H. (2016, December 2). *Wastewater Treatment Water Use*. Retrieved March 10, 2017, from USGS: <https://water.usgs.gov/edu/wuww.html>
- Phillips, J. (1995, April). *Control and Pollution Prevention Options for Ammonia Emissions*. Retrieved April 7, 2017, from EPA: <https://www3.epa.gov/ttnecat1/dir1/ammonia.pdf>
- Postgate, J. (1998). *Nitrogen Fixation* (Third ed.). Cambridge, United Kingdom: Press Syndicate of the University of Cambridge.
- Power, J. F., & Prasad, R. (1997). *Soil Fertility Management for Sustainable Agriculture*. Boca Raton, Florida, USA: CRC Press.
- Pynaert, K., Smets, B., Wyffels, S., Beheydt, D., Siciliano, S., & Verstraete, W. (2003, February 26). *Characterization of an Autotrophic Nitrogen-Removing Biofilm from a Highly Loaded Lab-Scale Rotating Biological Contactor*. Retrieved July 31, 2017, from American Society for Microbiology: <http://aem.asm.org/content/69/6/3626.full>
- Rabalais, N. N. (2002, March 31). *Nitrogen in aquatic ecosystems*. Retrieved April 4, 2017, from <https://www.ncbi.nlm.nih.gov/pubmed/12077998>

- Reardon, R., Kolby, T., & Odo, M. (1996). *The LOTT Nitrogen Removal Facilities: A First Year Evaluation*. Retrieved April 20, 2017, from Water Environment Federation 69th Annual Conference & Exposition: www.dtic.mil/get-tr-doc/pdf?AD=ADA358621
- Tarre, S., & Green, M. (2004, November). *High-Rate Nitrification at Low pH in Suspended- and Attached-Biomass Reactors*. Retrieved April 25, 2017, from Applied and Environmental Microbiology: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC525248/>
- Thermo Scientific. (2012, October). *Dionex ICS-1600 Ion Chromatography System Operator's Manual*. Retrieved April 4, 2017, from <http://www.canitec.com.mx/doc/ICS1600.pdf>
- Tijhuis, L., Van Loosdrecht, M., & Heijnen, J. (1992). *Nitrification with biofilms on small suspended particles in airlift reactors*. Retrieved April 25, 2017, from Water Science Tencology: <http://www.eubios.info/TTEC/TTECPY.htm>
- van Heeswijk, W. C., Westerhoff, H. V., & Boogerd, F. C. (2013). *Nitrogen Assimilation in Escherichia coli: Putting Molecular Data into a Systems Perspective*. Retrieved July 31, 2017, from American Society for Microbiology: <http://mmbr.asm.org/content/77/4/628.full>
- Wang, C., Liu, S., Xu, X., Zhao, C., Yang, F., & Wang, D. (2017, January 11). *Potential coupling effects of ammonia-oxidizing and anaerobic ammonium-oxidizing bacteria on completely autotrophic nitrogen removal over nitrite biofilm formation induced by the second messenger cyclic diguanylate*. Retrieved July 31, 2017, from Environmental Biotechnology: <https://link-springer-com.ezproxy.lib.calpoly.edu/content/pdf/10.1007%2Fs00253-016-7981-y.pdf>
- Winkler, M. (2005, October 12). *Practice Report Laboratory & Process Analysis Wastewater Treatment Nutrients*. Retrieved from Hach: <https://ro.hach.com/asset-get.download.jsa?id=25593611376>
- Winkler, M., Yang, J., Kleerebezem, R., Plaza, E., Trela, J., Hultman, B., et al. (2012, March 29). *Nitrate reduction by organotrophic Anammox bacteria in a nitrification/anammox granular sludge and moving biofilm reactor*. Retrieved July 31, 2017, from Bioresource Technology: <http://winklerlab.com/wp-content/uploads/2015/11/5.pdf>
- Yang, X.-P., Wang, S.-M., Zhang, D.-W., & Zhou, L.-X. (2010, September 7). *Isolation and nitrogen removal characteristics of an aerobic heterotrophic nitrifying-denitrifying bacterium, Bacillus subtilis A1*. Retrieved July 31, 2017, from Bioresource Technology: https://www.researchgate.net/publication/46577919_Isolation_and_nitrogen_removal_characteristics_of_an_aerobic_heterotrophic_nitrifying-denitrifying_bacterium_Bacillus_subtilis_A1

- Yao, S., Ni, J., Chen, Q., & Borthwick, A. (2012). *Enrichment and characterization of a bacteria consortium capable of heterotrophic nitrification and aerobic denitrification at low temperatures*. Peking University, Department of Environmental Engineering. Beijing: Elsevier Ltd.
- Zhang, Q.-L., Liu, Y., Ai, G.-M., Miao, L.-L., Zheng, H.-Y., & Liu, Z.-P. (2012, January 9). *The characteristics of a novel heterotrophic nitrification-aerobic denitrification bacterium, Bacillus methylotrophicus strain L7*. Retrieved July 31, 2017, from Bioresource Technology: http://ac.els-cdn.com.ezproxy.lib.calpoly.edu/S0960852411019353/1-s2.0-S0960852411019353-main.pdf?_tid=a80caa14-7ad8-11e7-8b61-00000aacb35d&acdnat=1502045841_d5093fc358a50b3baba6e49a879a9bb2
- Zhu, I., & Getting, T. (2012, August 2). *A review of nitrate reduction using inorganic materials*. Retrieved July 31, 2017, from Taylor & Francis Online: <http://www.tandfonline.com/doi/full/10.1080/09593330.2012.706646>

APPENDICIES

Appendix A: Other Experiments

Testing for bioreactors set up at the secondary clarifier started with 25 ppm and 10 ppm of Aqua. However, the existing nitrate levels in the SLO WRRF wastewater had changed since experiments 2 and 3. Nitrate started at low concentrations for these experiment A.1 and A.2, therefore the data was put in the appendix. Conclusions for using a dose of 25 ppm Aqua can be made because other experiments used 25 ppm Aqua.

A.1 Experiment A.1 – Secondary Wastewater with 25 ppm Aqua in Field

A.1.1 Methods and Materials

Ammonia, nitrate, nitrite, pH, and temperature were measured during this experiment. BiOWiSHTM was added to the wastewater samples (Table A.1a). All samples used secondary clarifier wastewater as the media, except the controls which used DI water as the media. Nitrate was not added because previous experiments showed nitrate in the wastewater. Ideally, an initial sample of the wastewater would have been run first. However, due to time constraints and running multiple experiments at once, this was not done. Ammonia was not added because the wastewater naturally contained around 25 ppm of ammonia. Duplicates of laboratory samples was conducted. The bioreactor was set up according to Section 3.10. Dry Aqua was added according to Section 3.4 and Section 3.10.

During the experiment, lab samples needed to be in an anoxic environment so nitrate removal could be studied. Lab samples also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab samples in an anoxic and warm

environment, samples were capped with a solid lid and kept in the incubator at 27°C, as described in Section 3.5. Sampling was conducted twice a day for six days. Samples were prepared and analyzed according to Section 3.6 and 3.7.

Table A.1a: Weights and concentrations for experiment A.1

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Total Volume (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Aqua	Field	4000	25	-	-	151
Lab Secondary with Aqua 1	Lab	25	25	-	-	1
Lab Secondary with Aqua 2	Lab	25	25	-	-	1
Lab Secondary without Aqua 1	Lab	-	-	151.8	25	1
Lab Secondary without Aqua 2	Lab	-	-	151.8	25	1
Lab Secondary without Aqua or nitrate 1	Lab	-	-	-	-	1
Lab Secondary without Aqua or nitrate 2	Lab	-	-	-	-	1
Control with nitrate	Lab	-	-	151.8	25	1
Control without nitrate	Lab	-	-	-	-	1

A.1.2 Results

Ammonia, nitrate, nitrite, temperature, and pH were measured. The time stamps for ammonia concentrations (Table A.1d and Figure A.1a) may be different from nitrate (Table A.1e and Figure A.1b) and nitrite (Table A.1f and Figure A.1c) concentrations because data that did not pass control verification standards, spikes, and splits was discarded. The pH (Table A.1b) and temperature (Table A.1c) time stamps may also be different from the ammonia, nitrate, and nitrite concentrations.

Table A.1b: pH measurements for experiment A.1

Time (hours)	0	7	24	31	48	55	72	79	96	103	120	127
BR Secondary with Aqua	7.2	7.2	7.42	7.5	7.53	7.39	7.6	-	7.54	7.55	7.61	7.73
Lab Secondary with Aqua 1	7.09	6.93	6.84	6.88	7.57	6.92	6.88	6.83	6.91	7.03	6.92	6.88
Lab Secondary with Aqua 2	7	6.97	6.88	6.97	6.9	6.93	6.86	6.84	6.84	6.98	6.89	6.86
Lab Secondary without Aqua or nitrate 1	7.31	7.21	7.14	6.98	6.99	7.02	6.98	6.89	6.97	7.1	7.09	7.01
Lab Secondary without Aqua or nitrate 2	7.15	7.2	7.07	6.97	6.9	7	6.96	6.88	6.95	7.06	7.09	6.97
Lab Secondary without Aqua 1	7.71	7.65	7.46	7.42	6.7	7.39	7.33	7.25	7.24	7.46	7.45	7.26
Lab Secondary without Aqua 2	7.74	7.74	7.72	7.44	7.36	7.41	7.4	7.47	7.35	7.56	7.58	7.34
Control with nitrate	7	6.8	6.74	5.69	7.07	5.33	5.72	6.83	6.44	6.57	6.75	6.6
Control without nitrate	6.9	7.4	7.4	3.62	6.96	3.91	5.81	6.79	6.12	6.52	6.38	6.72

Table A.1c: Temperature measurements for experiment A.1

Time (hours)	0	7	24	31	48	55	72	79	96	103	120	127
BR Secondary with Aqua	24.4	-	26.4	30.9	24.6	29	23.4	-	24.7	27.4	23.3	28.5
Lab Secondary with Aqua 1	27.2	-	26.2	27.6	27.6	27	26.8	28.2	27.6	27.4	26.3	27.8
Lab Secondary with Aqua 2	26.3	-	26.1	27.3	26.8	26.7	26.4	27.9	27.4	27.5	26.1	27.2
Lab Secondary without Aqua or nitrate 1	26.5	-	26.3	27.1	26.6	26.6	26.1	27.8	27.5	27.2	26.6	27.5
Lab Secondary without Aqua or nitrate 2	25.7	-	26.3	27.7	26.2	26.7	25.7	27.8	26.5	27.2	26.8	27.3
Lab Secondary without Aqua 1	26.8	-	25.5	27.2	25.7	26.4	25.8	27.9	26.6	27.4	26.6	27.3
Lab Secondary without Aqua 2	26.5	-	27	26.2	25.9	26.5	26	27.8	26.8	27.5	26.8	27.4
Control with nitrate	26	-	25.1	26.3	27	26.3	25.7	27.7	26.6	27	26.4	28.5
Control without nitrate	26.3	-	26.2	27.3	25.7	26.4	25.4	27.5	26.4	27.1	25.9	27.1

The pH and temperature for the bioreactor for this experiment were not taken on-site. Instead the sample was brought back to the lab to be measured, which introduced procedural error. After getting permission to bring the pH and temperature probe to the treatment plant, this data was measured on-site for the bioreactor experiments.

Table A.1d: Ammonia concentrations in mg/L NH₄-N for experiment A.1

Time (hours)	0	7	24	31	55	72	96	120	127
Control without nitrate	ND	ND	ND	ND	ND	ND	ND	ND	ND
Control with nitrate	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua or nitrate 2	23.15	25.76	27.76	24.61	26.66	23.80	29.76	28.06	24.17
Lab Secondary without Aqua or nitrate 1	24.70	25.93	23.35	23.17	24.89	22.16	25.03	23.04	25.23
Lab Secondary without Aqua 2	23.70	26.21	24.81	23.94	26.96	25.60	25.27	26.80	27.30
Lab Secondary without Aqua 1	18.08	23.87	25.65	23.76	25.89	24.45	29.48	24.54	23.88
Lab Secondary with Aqua 2	23.38	24.76	21.32	24.94	24.60	24.28	23.34	24.27	22.92
Lab Secondary with Aqua 1	26.89	25.16	25.95	23.28	23.17	22.05	25.31	23.21	24.36
BR Secondary with Aqua	23.91	21.80	25.23	22.56	23.77	21.25	23.69	22.18	22.29

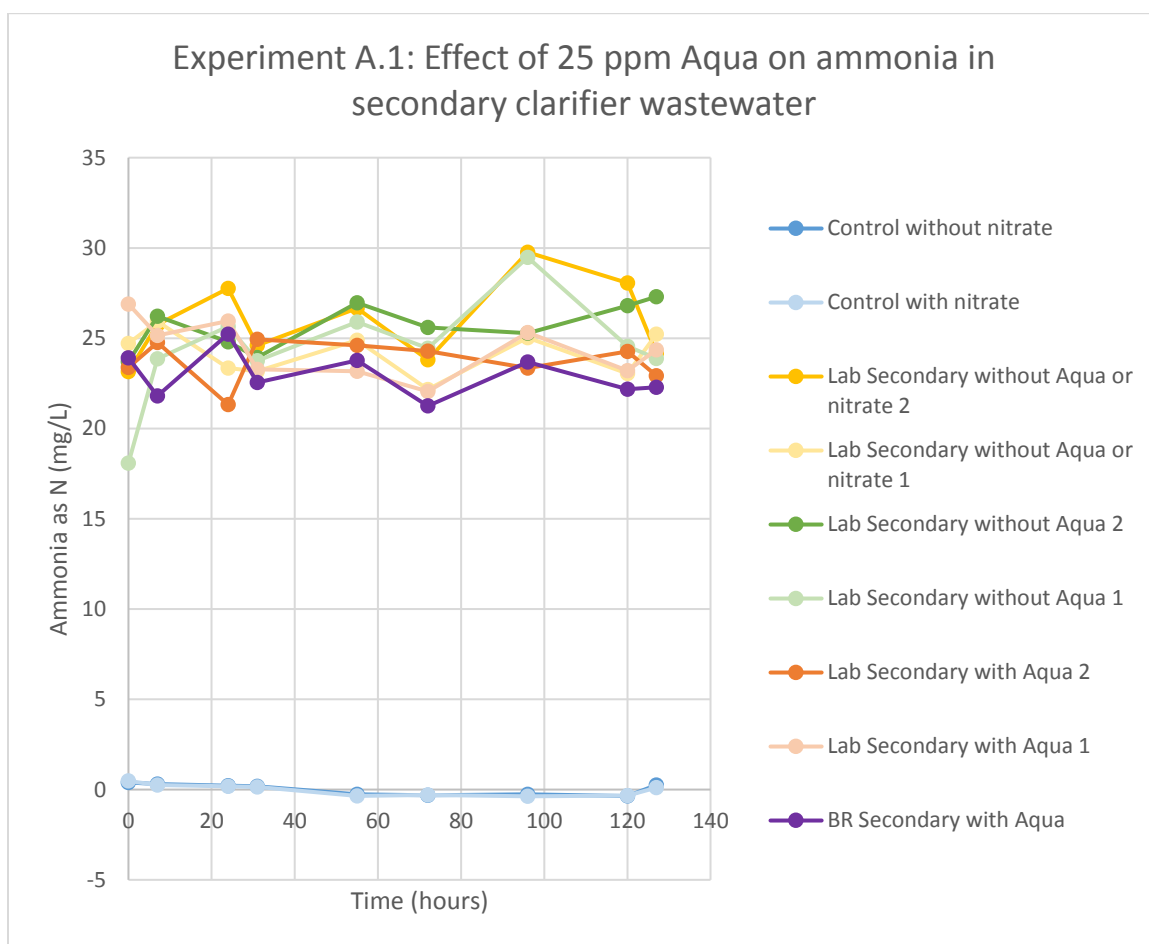


Figure A.1a: Effect of 25 ppm Aqua on ammonia in secondary clarifier wastewater

The ammonia concentration fluctuated between 20 and 30 ppm $\text{NH}_4\text{-N}$ with no noticeable trend. Therefore, bacteria did not process ammonia.

Table A.1e: Nitrate concentrations in mg/L NO₃-N for experiment A.1

Time (hours)	0	24	31	55	72	96	120	127
BR Secondary with Aqua	ND	ND	1.09	ND	1.14	ND	ND	ND
Lab Secondary with Aqua 1	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary with Aqua 2	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua or nitrate 1	ND	ND	ND	ND	1.01	ND	ND	ND
Lab Secondary without Aqua or nitrate 2	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua 1	5.67	4.78	3.86	3.14	2.71	2.34	1.81	1.61
Lab Secondary without Aqua 2	6.44	3.84	3.28	2.29	1.89	1.26	1.04	0.99
Control with nitrate	25.30	25.25	23.49	21.12	21.21	22.95	25.36	24.72
Control without nitrate	1.25	ND	ND	ND	1.23	1.18	1.11	0.82

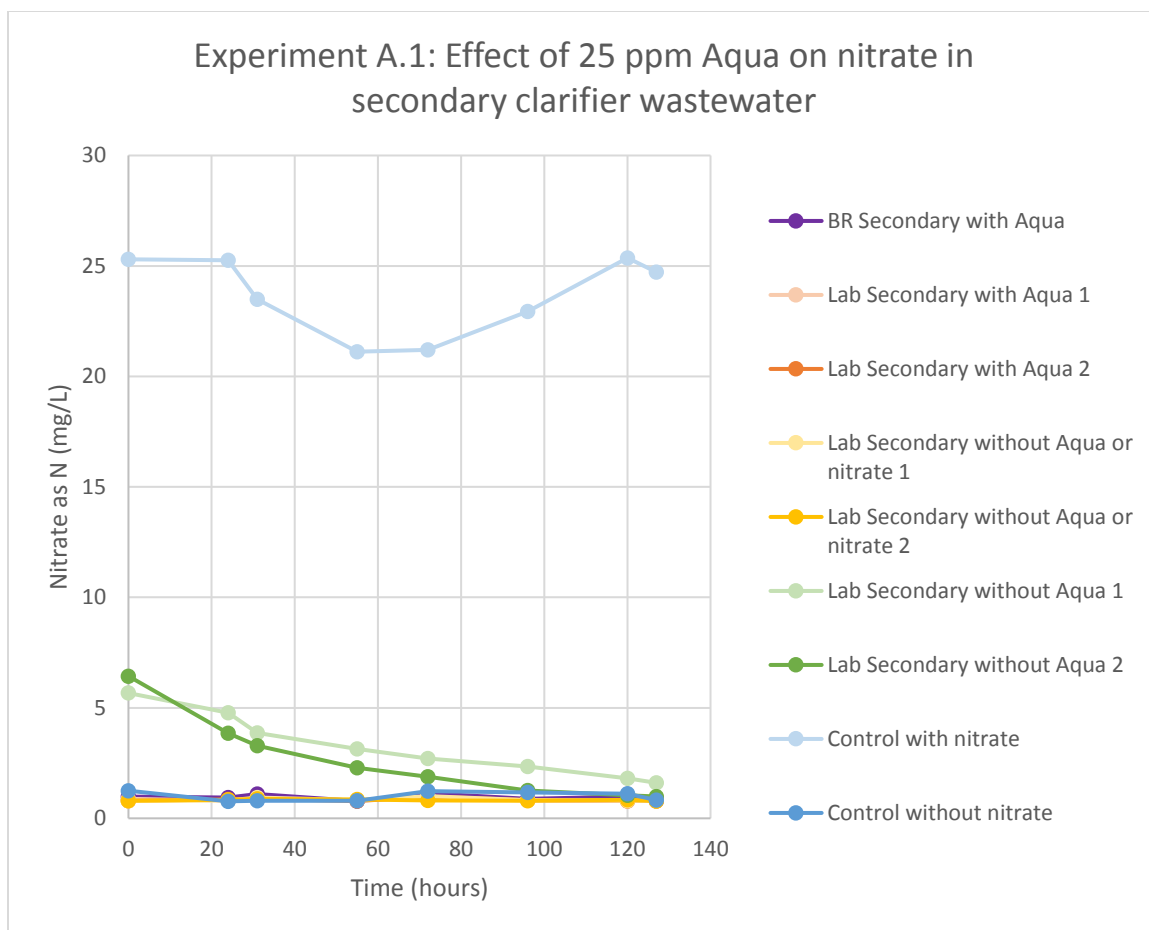


Figure A.1b: Effect of 25 ppm Aqua on nitrate in secondary clarifier wastewater

Nitrate was only added to two natural bacteria wastewater laboratory samples. Therefore, no conclusions about Aqua on denitrification can be made. Nitrate removal can be observed for the natural bacteria in the wastewater.

Table A.1f: Nitrite concentrations in mg/L NO₂-N for experiment A.1

Time (hours)	0	24	31	55	72	96	120	127
BR Secondary with Aqua	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary with Aqua 1	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary with Aqua 2	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua or nitrate 1	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua or nitrate 2	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua 1	ND	ND	ND	ND	ND	ND	ND	ND
Lab Secondary without Aqua 2	ND	ND	ND	ND	ND	ND	ND	ND
Control with nitrate	ND	ND	ND	ND	ND	ND	ND	ND
Control without nitrate	ND	ND	ND	ND	ND	ND	ND	ND

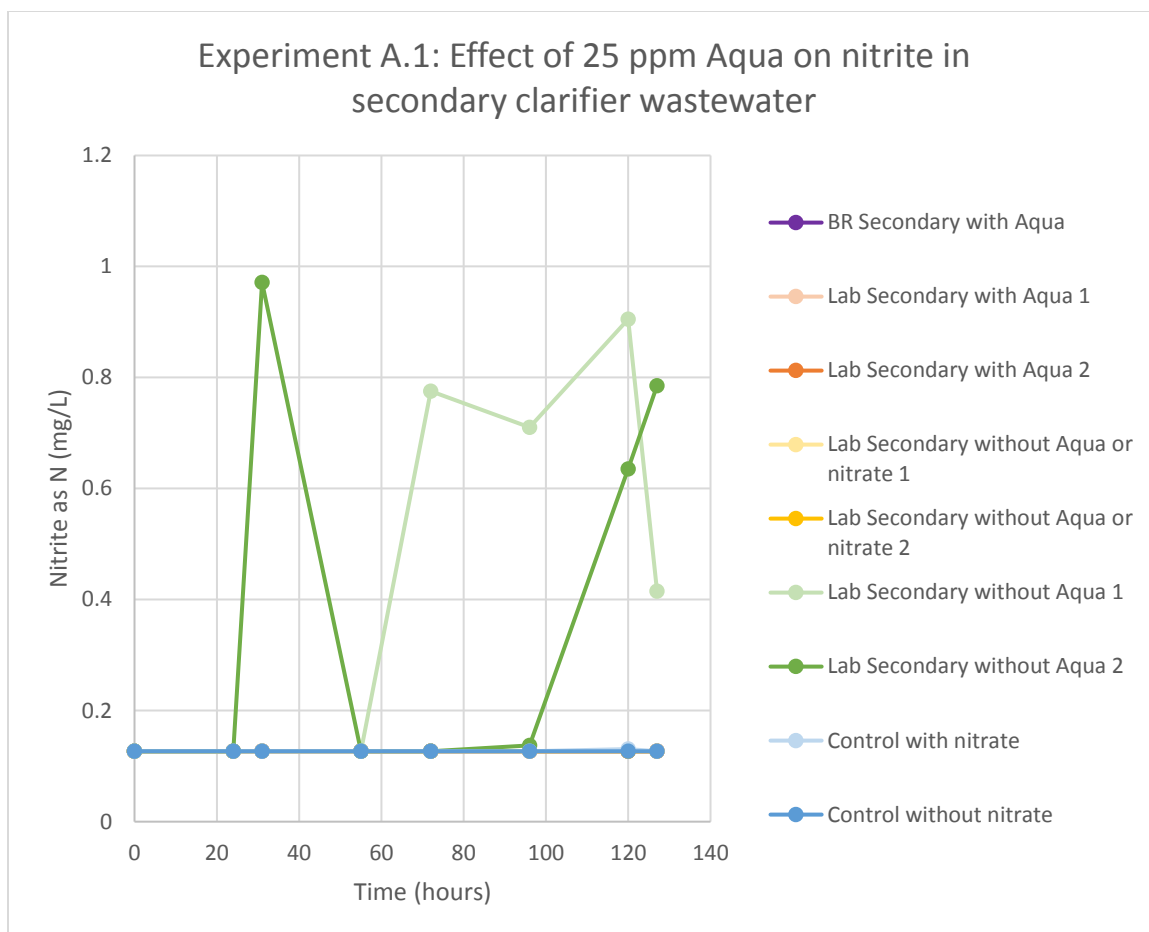


Figure A.1c: Effect of 25 ppm Aqua on nitrite in secondary clarifier wastewater

Nitrite production was observed for the samples that experienced denitrification. However, since the measured concentrations were below the 1 ppm MDL, the exact amount of nitrite cannot be accurately concluded.

A.2 Experiment A.2 – Secondary Wastewater with 10 ppm Aqua in Field

A.2.1 Methods and Materials

Ammonia, nitrate, nitrite, pH, and temperature were measured during this experiment. BiOWiSHTM and nitrate were added to the wastewater samples (Table A.2a). All samples used secondary clarifier wastewater as the media, except the controls which used DI water

as the media. Duplicates of laboratory samples was conducted. The bioreactor was set up according to Section 3.10. Ammonia was not added because the wastewater naturally contained around 25 ppm of ammonia. Dry Aqua was added according to Section 3.4. For laboratory samples, 25 ppm Aqua was accidentally added instead of 10 ppm Aqua. Nitrate was not added to Aqua samples initially. After 12 hours, nitrate was added to Aqua samples. However, the natural bacteria wastewater sample started with nitrate in it. Therefore, nitrate removal rates for Aqua and natural bacteria could not be compared.

During the experiment, lab samples needed to be in an anoxic environment so nitrate removal could be studied. Lab samples also needed to be in a warm environment so bacterial growth could occur. In order to keep the lab samples in an anoxic and warm environment, samples were capped with a solid lid and kept in the incubator at 27°C, according to Section 3.5. Sampling was conducted twice a day for seven days. Samples were prepared and analyzed according to Section 3.6 and 3.7.

Table A.2a: Weights and concentrations for experiment A.2

Label	Sample in Field or Lab	BiOWiSH™		Nitrate (NaNO ₃)		Total Volume (L)
		Weight (mg)	Conc (mg/L)	Weight (mg)	Conc as N (mg/L)	
BR Secondary with Aqua	Field	1500	10	-	-	151
Lab Secondary with Aqua 1	Lab	25	25	-	-	1
Lab Secondary with Aqua 2	Lab	25	25	-	-	1
Lab Secondary without Aqua 1	Lab	-	-	151.8	25	1
Lab Secondary without Aqua 2	Lab	-	-	151.8	25	1
Lab Secondary without Aqua or nitrate 1	Lab	-	-	-	-	1
Lab Secondary without Aqua or nitrate 1	Lab	-	-	-	-	1
Control with nitrate	Lab	-	-	151.8	25	1
Control without nitrate	Lab	-	-	-	-	1

A.2.2 Results

Ammonia, nitrate, nitrite, temperature, and pH were measured. The time stamps for ammonia concentrations (Table A.2d and Figure A.2a) may be different from nitrate (Table A.2e and Figure A.2b) and nitrite (Table A.2f and Figure A.2c) concentrations because data that did not pass control verification standards, spikes, and splits was discarded. The pH (Table A.2b) and temperature (Table A.2c) time stamps will also be different from the ammonia, nitrate, and nitrite concentrations.

Table A.2b: pH measurements for experiment A.2

Time (hours)	0	17	24	44	48	67	72	89	96	113	120	137
BR Secondary with Aqua	7.54	7.77	7.94	7.92	8.07	7.93	-	7.46	8.06	8.08	8.03	8.13
Lab Secondary with Aqua 1	7.81	7.48	7.62	7.55	7.54	7.37	7.51	8.03	7.45	7.47	7.47	7.48
Lab Secondary with Aqua 2	7.82	7.51	7.58	7.56	7.5	7.31	7.47	7.4	7.35	7.41	7.41	7.42
Lab Secondary without Aqua or nitrate 1	7.75	7.81	7.74	7.5	7.45	7.3	7.4	7.31	7.37	7.37	7.38	7.3
Lab Secondary without Aqua or nitrate 2	7.75	7.79	7.75	7.5	7.44	7.29	7.46	7.34	7.35	7.34	7.38	7.33
Lab Secondary without Aqua 1	7.74	7.84	7.79	7.65	7.6	7.45	7.71	7.53	7.5	7.47	7.47	7.42
Lab Secondary without Aqua 2	7.71	7.87	7.71	7.61	7.58	7.42	7.44	7.5	7.48	7.51	7.44	7.41
Control with nitrate	9.06	9.38	9.14	9.24	8.93	8.89	9.38	8.97	8.93	8.79	6.92	8.49
Control without nitrate	8.71	8.94	8.78	8.77	8.64	8.51	7.5	8.6	8.63	8.71	8.4	8.45

Table A.2c: Temperature measurements for experiment A.2

Time (hours)	0	17	24	44	48	67	72	89	96	113	120	137
BR Secondary with Aqua	25.9	24.9	31.5	27.3	27.2	22.3	-	25.3	27.1	22.9	27.3	23.4
Lab Secondary with Aqua 1	26.5	24.9	27.5	26.1	26.6	25.4	26.8	21.6	26.2	26.2	26.6	26.1
Lab Secondary with Aqua 2	26.5	25.1	27.4	26	26.5	25.4	26.8	25.4	26.4	26	26.4	26
Lab Secondary without Aqua or nitrate 1	26.5	25.8	27.2	26	26.4	25.5	26.6	24.6	26.1	25.9	26.3	26.1
Lab Secondary without Aqua or nitrate 2	26.3	25.9	27.5	26.1	26.5	25.1	26.5	24.7	26.4	25.7	26.4	25.9
Lab Secondary without Aqua 1	26.4	25.7	27.7	25.9	26.7	25.3	26.4	24.5	26.1	25.4	26.4	25.8
Lab Secondary without Aqua 2	26.2	26	27.3	25.7	26.6	25.1	26	24.4	25.9	26.1	26.4	25.7
Control with nitrate	24.4	26.2	27.3	25.4	25.7	24.7	26.4	24.9	25.9	26	26.3	25.8
Control without nitrate	25.1	26.8	27.3	25.2	25.8	24.4	26.1	24.8	26.2	26.2	26.1	25.9

Table A.2d: Ammonia concentrations in mg/L NH₄-N for experiment A.2

Time (hours)	17	24	67	89	96	113	120
Control without nitrate	0.20	0.18	0.14	0.14	1.54	0.28	0.04
Control with nitrate	0.11	0.11	-0.03	-0.04	0.06	0.04	-0.02
Lab Secondary without Aqua or nitrate 2	27.66	29.65	31.41	29.87	32.37	28.97	32.89
Lab Secondary without Aqua or nitrate 1	28.11	31.33	32.60	31.99	31.91	35.19	33.20
Lab Secondary without Aqua 2	27.35	31.02	32.61	33.24	30.85	30.20	32.45
Lab Secondary without Aqua 1	28.11	35.72	30.57	33.29	32.82	29.56	32.16
Lab Secondary with Aqua 2	32.19	29.60	33.30	34.62	32.90	32.68	32.76
Lab Secondary with Aqua 1	26.91	29.36	31.94	32.63	32.22	32.33	32.81
BR Secondary with Aqua	22.68	24.32	23.87	26.27	26.89	24.01	25.64

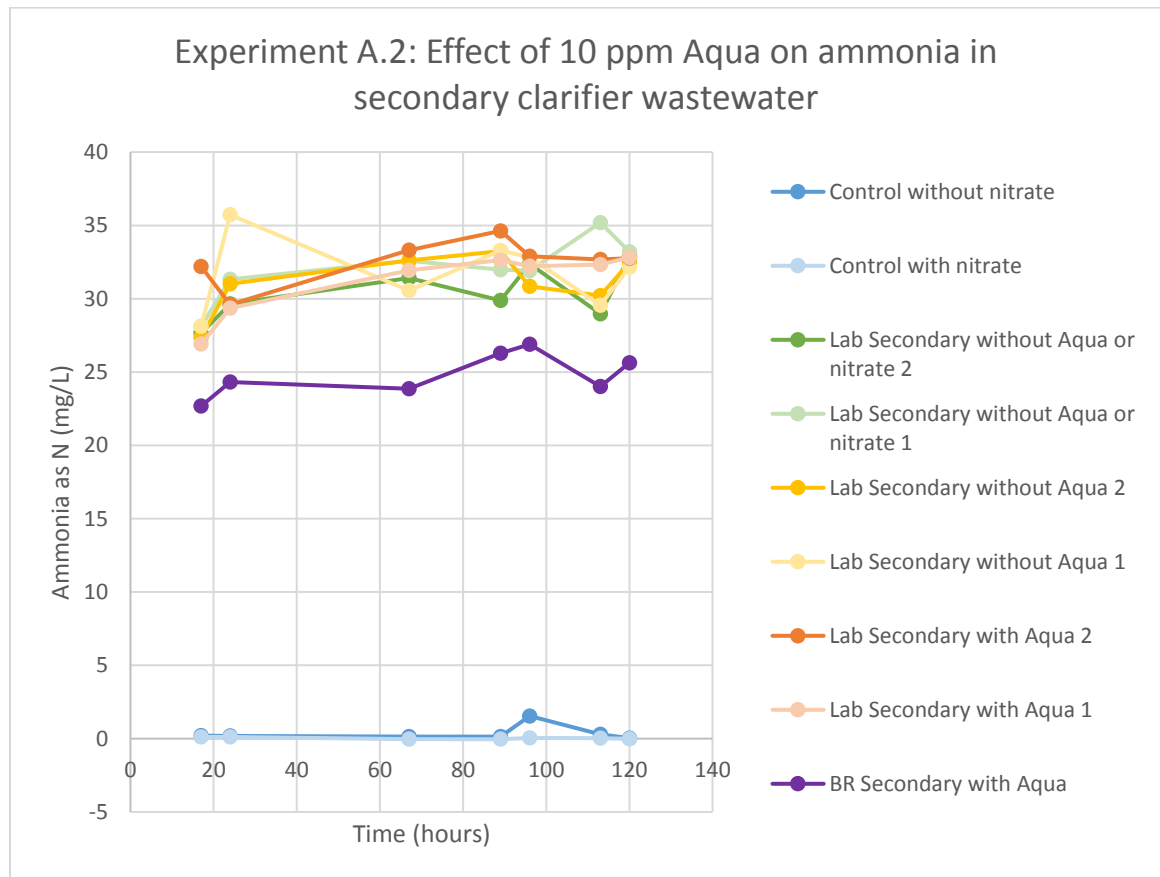


Figure A.2a: Effect of 10 ppm Aqua on ammonia in secondary clarifier wastewater

Initial data did not pass quality assurance and quality control tests. The ammonia concentration fluctuated between 25 and 35 ppm $\text{NH}_4\text{-N}$ with no noticeable trend. Therefore, bacteria did not process ammonia.

Table A.2e: Nitrate concentrations in mg/L $\text{NO}_3\text{-N}$ for experiment A.2

Time (hours)	0	17	24	48	67	89	113
BR Secondary with Aqua	0.92	0.83	13.02	13.64	13.47	13.83	11.57
Lab Secondary with Aqua 1	3.86	0.81	18.48	14.59	13.74	13.23	11.95
Lab Secondary with Aqua 2	4.07	0.81	19.29	14.63	13.41	12.79	11.79
Lab Secondary without Aqua or nitrate 1	4.04	0.80	0.82	0.83	0.86	0.87	0.86
Lab Secondary without Aqua or nitrate 2	3.97	0.78	0.79	0.81	0.86	0.98	0.88
Lab Secondary without Aqua 1	24.23	18.76	18.10	13.98	13.56	13.18	13.39
Lab Secondary without Aqua 2	11.98	19.83	18.73	16.75	14.10	12.68	11.53
Control with nitrate	24.20	24.52	24.59	23.96	21.31	23.54	21.98
Control without nitrate	1.01	0.78	1.14	0.78	0.80	0.92	0.90

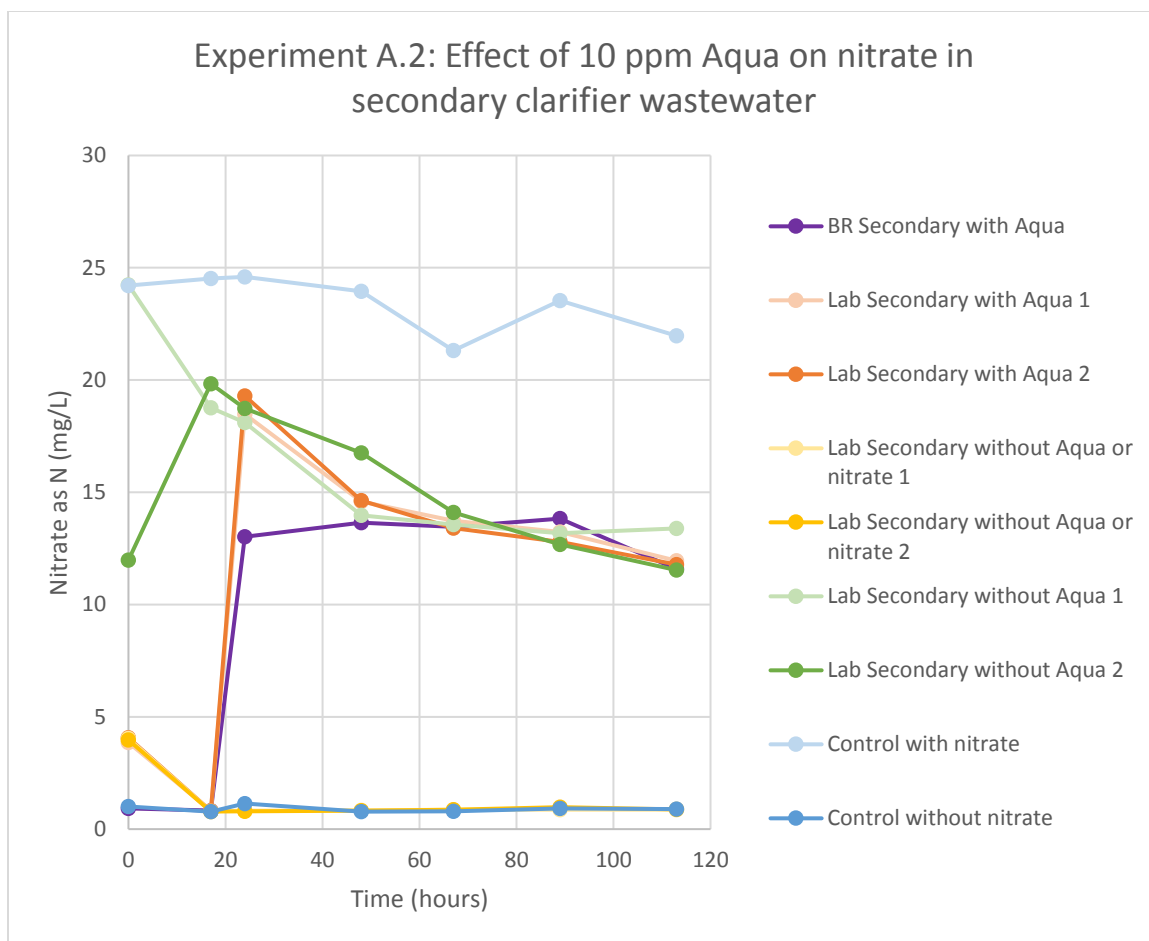


Figure A.2b: Effect of 10 ppm Aqua on nitrate in secondary clarifier wastewater

Nitrate was only initially added to two natural bacteria wastewater laboratory samples. After about 24 hours, nitrate was added to the samples with Aqua. Denitrification and assimilation of nitrate can be observed for both the natural bacteria and Aqua in the wastewater. The natural bacteria wastewater samples degraded at the same rate as the samples containing Aqua. Bacteria competition likely occurred, resulting in natural bacteria prevailing over the Aqua bacteria.

Table A.2f: Nitrite concentrations in mg/L NO₂-N for experiment A.2

Time (hours)	0	17	24	48	67	89	113
BR Secondary with Aqua	0.13	0.13	1.51	0.13	0.13	0.13	0.13
Lab Secondary with Aqua 1	1.44	0.13	0.85	0.13	0.13	0.13	0.13
Lab Secondary with Aqua 2	1.49	0.13	0.75	1.08	0.13	0.13	0.13
Lab Secondary without Aqua or nitrate 1	1.44	0.13	0.13	2.56	0.13	0.13	0.13
Lab Secondary without Aqua or nitrate 2	1.41	0.13	0.13	2.14	0.13	0.13	0.13
Lab Secondary without Aqua 1	1.33	0.13	0.13	3.13	0.13	0.13	0.90
Lab Secondary without Aqua 2	1.36	0.13	0.13	1.20	0.13	0.13	0.13
Control with nitrate	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Control without nitrate	0.94	0.13	0.13	0.13	0.13	0.13	0.13

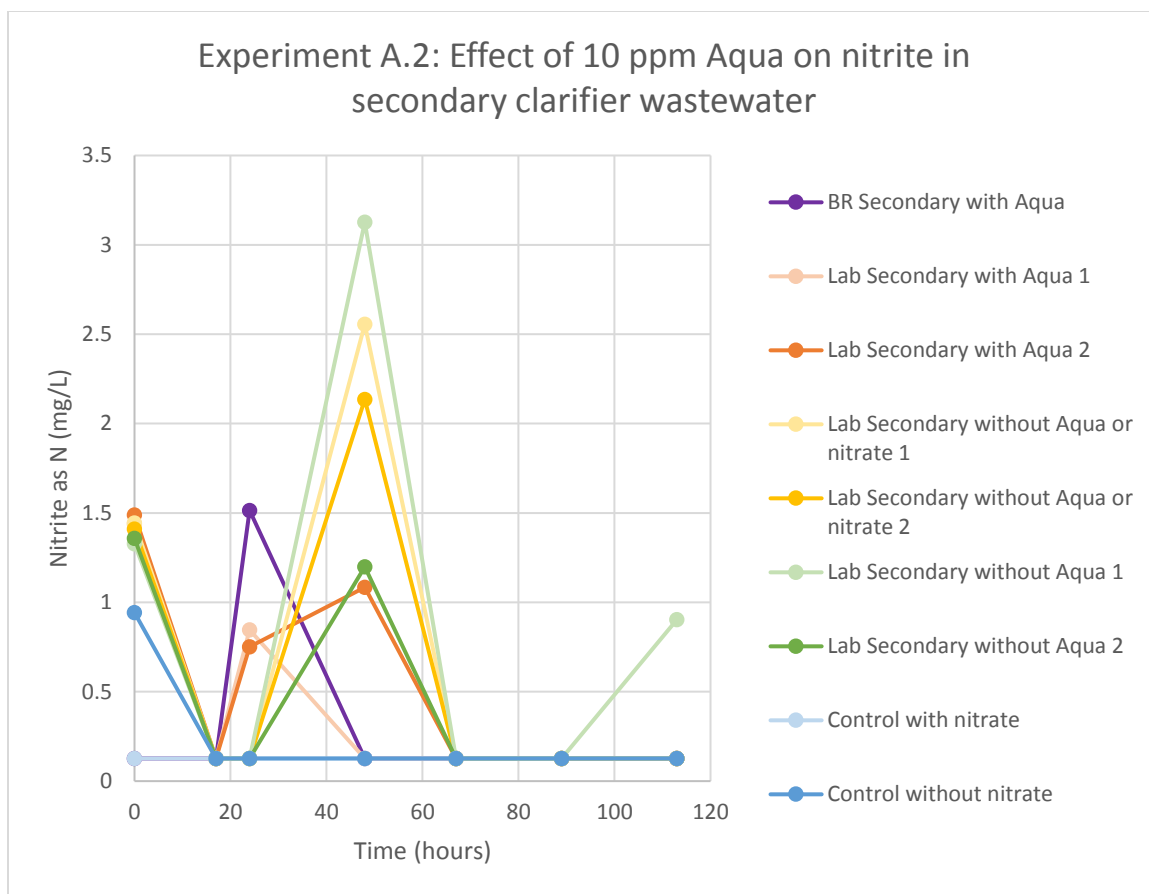


Figure A.2c: Effect of 10 ppm Aqua on nitrite in secondary clarifier wastewater

Nitrite production can be observed for most wastewater samples. A slight lag occurred from nitrate removal to nitrite production for some samples. The nitrite production could also be due to the nitrification intermediate step.

Appendix B: Rate Constant Calculations

Many of the experiments in this study contain a lag phase. They can also drop and then continue to stay constant over time. No changes in concentration over time are also known as flat-line. For both first order (Figure B.1 and Figure B.2) and zero order kinetics (Figure B.3 and Figure B.4), the lag phase data points and the data points that flat-line are omitted when determining the degradation rate constants. First order graphs were calculated by natural logging the original data.

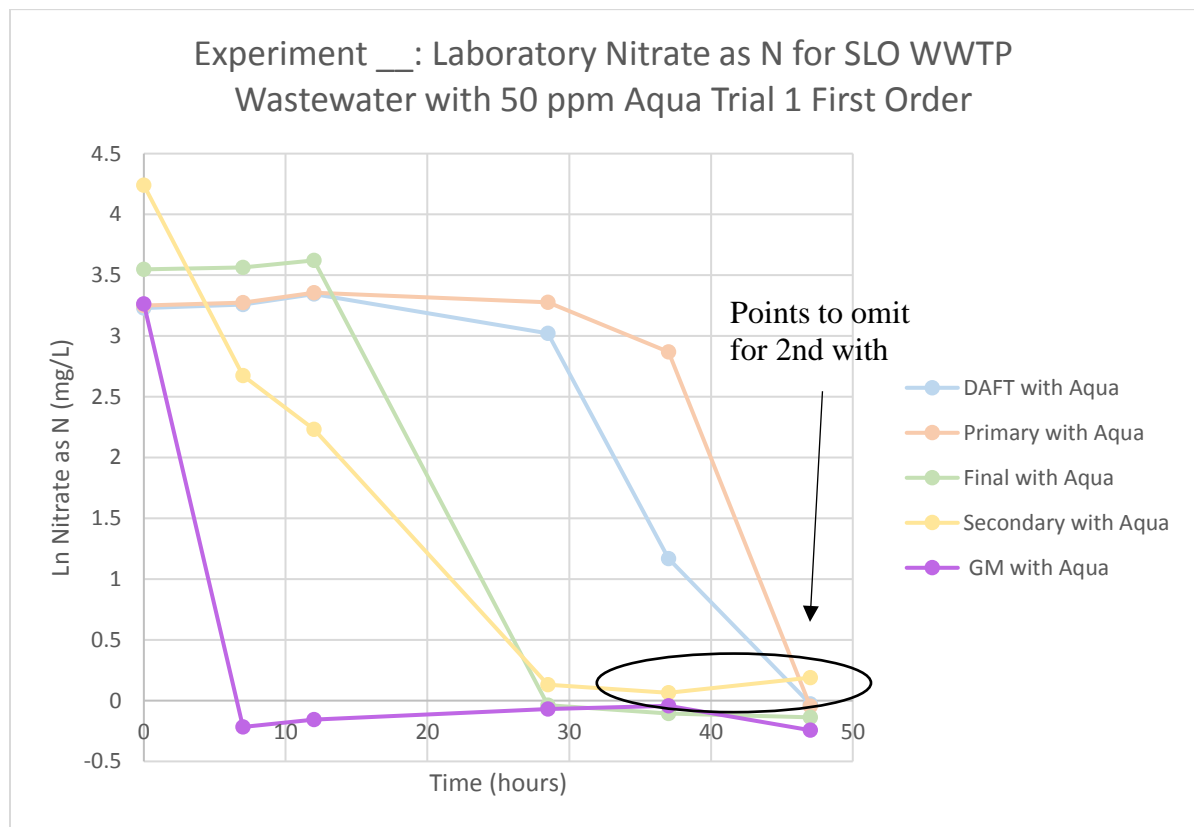


Figure B.1: Example of points omitted for first order kinetics determination

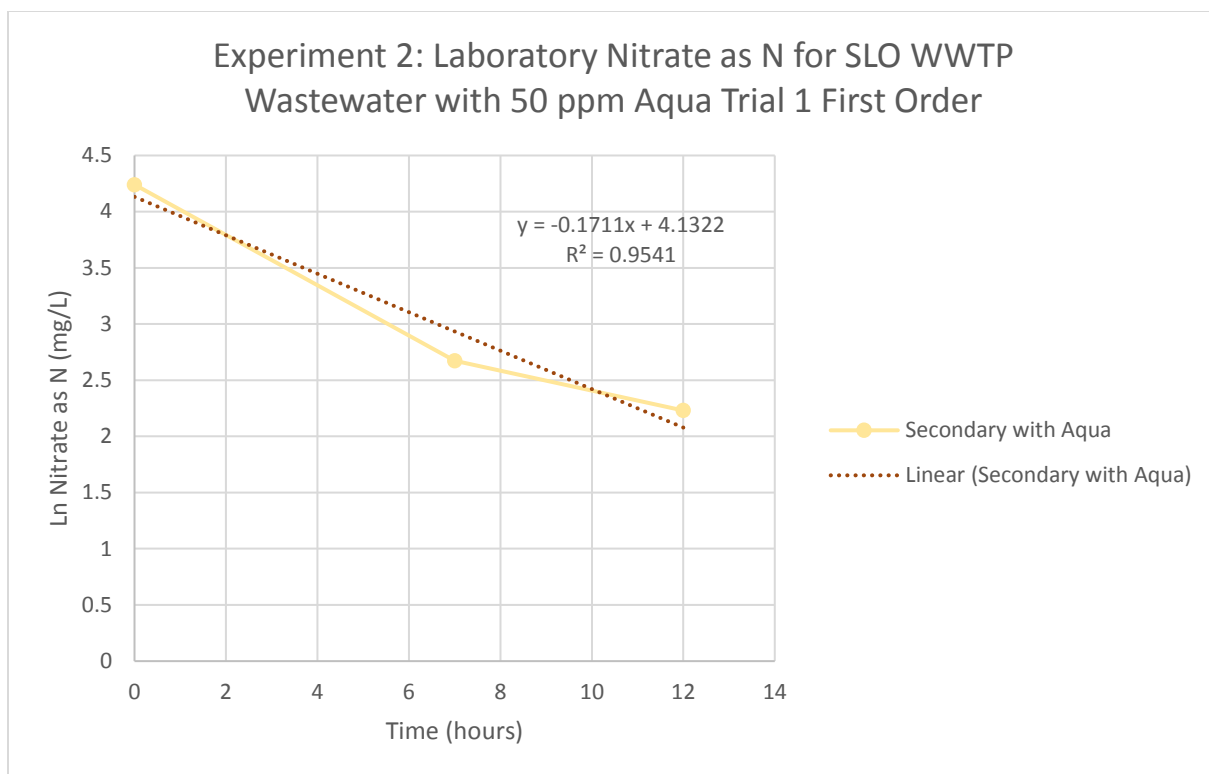


Figure B.2: Example of first order kinetics determined once points were omitted

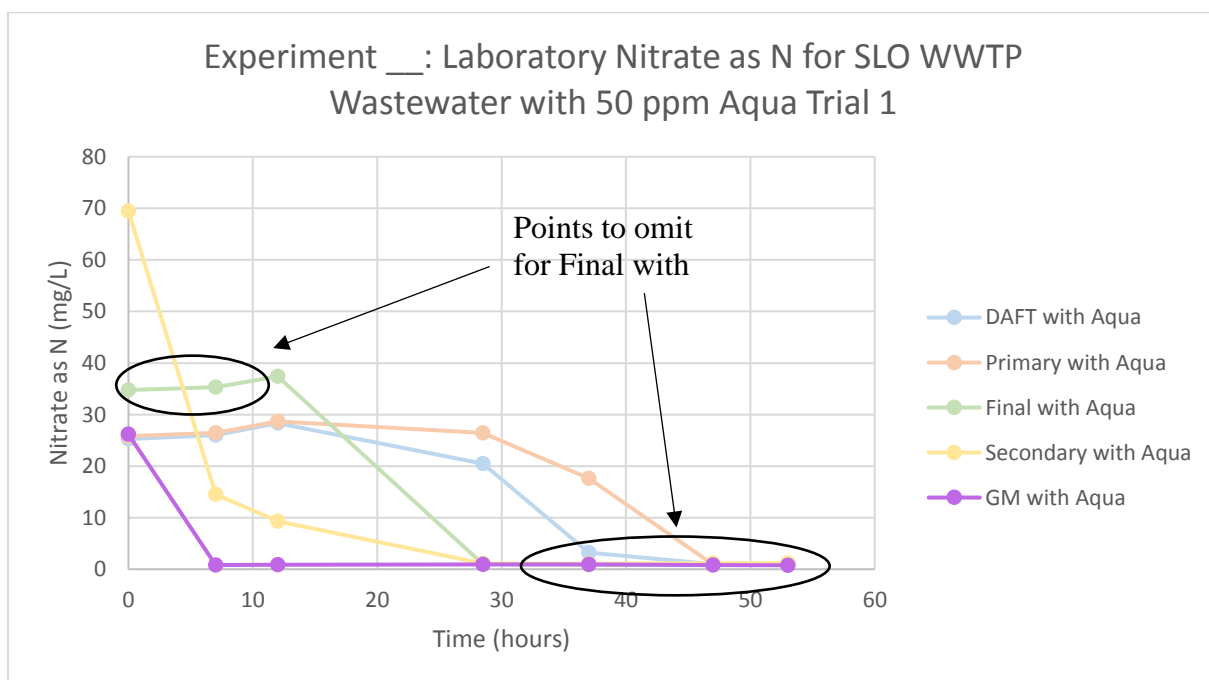


Figure B.3: Example of points omitted for zero order kinetics determination

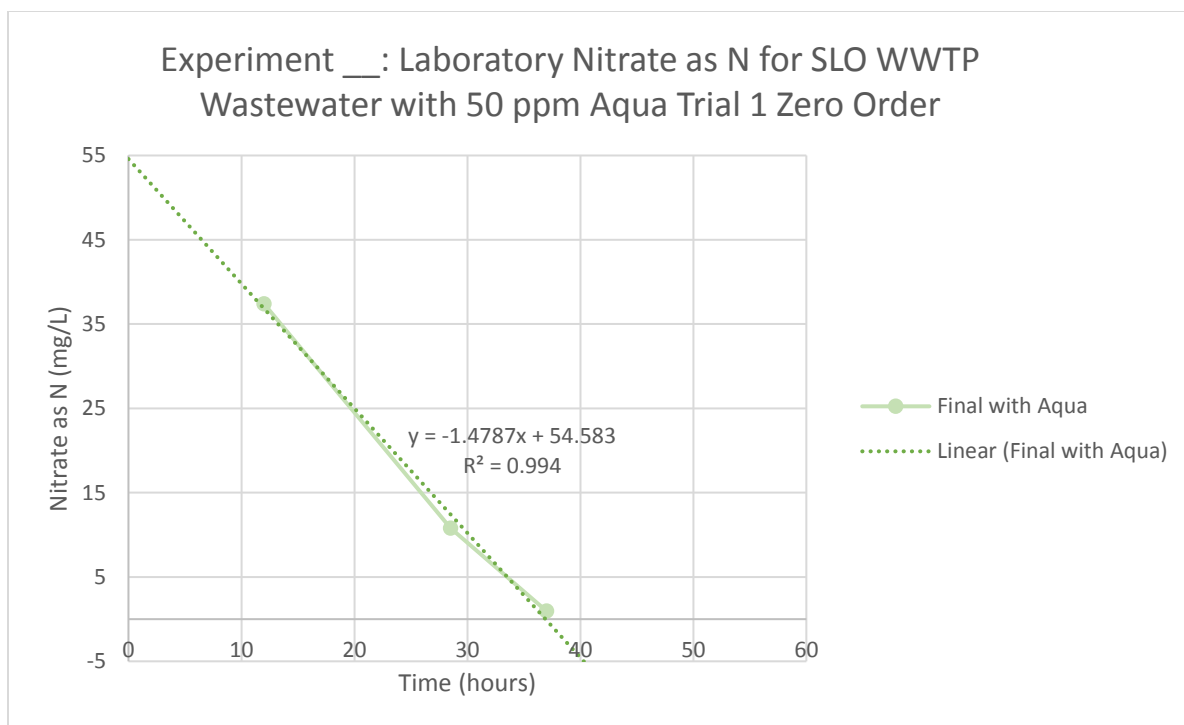


Figure B.4: Example of zero order kinetics determined once points were omitted

Appendix C: Sample Calculations for Oxygen and Alkalinity Required for Nitrogen Removal

To obtain 4.57 g O₂/g N oxidized:



$$1 \text{ g NH}_4 \left(\frac{1 \text{ mol NH}_4}{18 \text{ g NH}_4} \right) \left(\frac{2 \text{ mols O}_2}{1 \text{ mol NH}_4} \right) \left(\frac{32 \text{ g O}_2}{1 \text{ mol O}_2} \right) = 3.55 \text{ g O}_2$$

$$3.55 \frac{\text{g O}_2}{\text{g NH}_4} \left(\frac{18 \text{ g NH}_4}{1 \text{ mol NH}_4} \right) \left(\frac{1 \text{ mol NH}_4}{1 \text{ mol NH}_4 - N} \right) \left(\frac{1 \text{ mol NH}_4 - N}{14 \text{ g NH}_4 - N} \right) = 4.57 \text{ g O}_2/\text{g NH}_4 - N$$

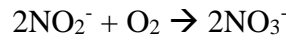
To obtain 3.43 g O₂/g N oxidized:



$$1 \text{ g NH}_4 \left(\frac{1 \text{ mol NH}_4}{18 \text{ g NH}_4} \right) \left(\frac{3 \text{ mols O}_2}{2 \text{ mols NH}_4} \right) \left(\frac{32 \text{ g O}_2}{1 \text{ mol O}_2} \right) = 2.67 \text{ g O}_2$$

$$2.67 \frac{\text{g O}_2}{\text{g NH}_4} \left(\frac{18 \text{ g NH}_4}{1 \text{ mol NH}_4} \right) \left(\frac{1 \text{ mol NH}_4}{1 \text{ mol NH}_4 - N} \right) \left(\frac{1 \text{ mol NH}_4 - N}{14 \text{ g NH}_4 - N} \right) = 3.43 \text{ g O}_2/\text{g NH}_4 - N$$

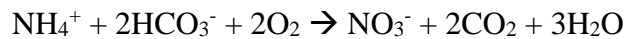
To obtain 1.14 g O₂/g N oxidized:



$$1 \text{ g NO}_2 \left(\frac{1 \text{ mol NO}_2}{46 \text{ g NO}_2} \right) \left(\frac{1 \text{ mol O}_2}{2 \text{ mols NO}_2} \right) \left(\frac{32 \text{ g O}_2}{1 \text{ mol O}_2} \right) = 0.35 \text{ g O}_2$$

$$0.35 \frac{\text{g O}_2}{\text{g NO}_2} \left(\frac{46 \text{ g NO}_2}{1 \text{ mol NO}_2} \right) \left(\frac{1 \text{ mol NO}_2}{1 \text{ mol NO}_2 - N} \right) \left(\frac{1 \text{ mol NO}_2 - N}{14 \text{ g NO}_2 - N} \right) = 1.14 \text{ g O}_2/\text{g NO}_2 - N$$

To obtain 7.14 g CaCO₃/g N oxidized:



$$1 \text{ g } NH_4 \left(\frac{1 \text{ mol } NH_4}{18 \text{ g } NH_4} \right) \left(\frac{2 \text{ mols } HCO_3}{1 \text{ mol } NH_4} \right) \left(\frac{61 \text{ g } HCO_3}{1 \text{ mol } HCO_3} \right) = 6.78 \text{ g } HCO_3$$

$$6.78 \frac{\text{g } HCO_3}{\text{g } NH_4} \left(\frac{18 \text{ g } NH_4}{1 \text{ mol } NH_4} \right) \left(\frac{1 \text{ mol } NH_4}{1 \text{ mol } NH_4 - N} \right) \left(\frac{1 \text{ mol } NH_4 - N}{14 \text{ g } NH_4 - N} \right) = 8.7 \text{ g } HCO_3 / \text{g } NH_4 - N$$

$$8.7 \frac{\text{g } HCO_3}{\text{g } NH_4 - N} \left(\frac{1 \text{ mol } HCO_3}{61 \text{ g } HCO_3} \right) \left(\frac{1 \text{ eq}}{1 \text{ mol } HCO_3} \right) \left(\frac{1 \text{ mol } CaCO_3}{2 \text{ eq}} \right) \left(\frac{100 \text{ g } CaCO_3}{1 \text{ mol } CaCO_3} \right) = 7.14 \text{ g } CaCO_3 / \text{g } NH_4 - N$$

Appendix D: Sample Calculations for Nitrogen Content and Eluent

To determine stock solutions for calibrations, ammonium and nitrate concentrations to be added to solutions, and eluent concentrations, the following calculations are needed:

If 25 ppm of nitrate as nitrogen is required in solution, 151.8 mg/L of NaNO₃ is added:

$$25 \frac{mg}{L} NO_3 - N \left(\frac{1 mmol NO_3 - N}{14 mg NO_3 - N} \right) \left(\frac{1 mmol NaNO_3}{1 mmol NO_3 - N} \right) \left(\frac{85 mg NaNO_3}{1 mmol NaNO_3} \right) = 151.8 \frac{mg}{L} NaNO_3$$

If 25 ppm of ammonium as nitrogen is required in solution, 95.5 mg/L of NH₄Cl is added:

$$25 \frac{mg}{L} NH_4 - N \left(\frac{1 mmol NH_4 - N}{14 mg NH_4 - N} \right) \left(\frac{1 mmol NH_4Cl}{1 mmol NH_4 - N} \right) \left(\frac{53.5 mg NH_4Cl}{1 mmol NH_4Cl} \right) = 95.5 \frac{mg}{L} NH_4Cl$$

To obtain a concentration of 9 mM carbonate, 953.9 mg/L of Na₂CO₃ is added:

$$9 \frac{mol}{L} CO_3^{2-} \left(\frac{1 mol Na_2CO_3}{1 mol CO_3^{2-}} \right) \left(\frac{106 g Na_2CO_3}{1 mol Na_2CO_3} \right) = 954 \frac{g}{L} Na_2CO_3$$