ABSTRACT

SALILING, WILLIE JONES BAYOTLANG. **DENITRIFICATION OF AQUACULTURE WASTEWATER USING AGRICULTURAL BY-PRODUCTS AS BIOFILTER MEDIA** (UNDER THE DIRECTION OF DR. PHILIP W. WESTERMAN AND DR. THOMAS M. LOSORDO)

This research evaluated wood chips and wheat straw as inexpensive and readily available biofilter media for denitrification as alternatives to commonly used plastic media. Nine 3.6-L laboratory scale reactors (40 cm packed height x 10 cm dia) were built to evaluate performance of wood chips, wheat straw, and Kaldnes plastic media to reduce nitrate from synthetic and real aquaculture wastewater. The upflow bioreactors were loaded at a constant flow rate and three influent concentrations of 50, 120, and 200 mg NO_3-N/L each for at least four weeks, in sequence.

Experiments showed that both wood chips and wheat straw are comparable to Kaldnes plastic media in terms of reducing nitrate. As high as 99% of nitrate (as high as 200 mg NO_3-N/L) were reduced. Pseudo-steady state denitrification rates at 200 mg NO_3-N/L influent averaged 1365 ± 39 g NO_3-N/m^3/day for wood chips, 1361 ± 80 g NO_3-N/m^3/day for wheat straw, and 1326 ± 74 g NO_3-N /m^3/day for Kaldnes. These values were not even the maximum potential since nitrate profile along reactors showed substantial reduction in the lower half of the reactors with rates more than double than what was estimated for the whole reactor. Treatment of real wastewater also showed comparable performance with that at the 50 mg NO_3-N /L synthetic experiments.

COD consumption per NO_3-N removed was highest in Kaldnes media (3.41 to 3.95) compared to wood chips (3.34 to 3.64) and wheat straw (3.26 to 3.46). COD/NO_3-N ratios for the real wastewater were low (<2.0). Effluent ammonia concentrations were close to zero while nitrites were around 2.0 mg NO_2-N/L for all reactor types. There was

alkalinity production and pH increased corresponding to NO_3-N removed. On the other hand, the oxidation-reduction potential also decreased with nitrate removal.

Wood chips and wheat straw lost 16.2% and 37.7% of their masses, respectively over 140 days. There were physical signs of degradation like discoloration and structural transformation. Carbon/Nitrogen (C/N) ratio and lignin mass of the media also decreased. Considerable amount of microbial biomass also developed inside the reactors decreasing porosity.

By Polymerase Chain Reaction –Denaturing Gradient Gel Electrophoresis (PCR-DGGE) analysis, it was shown that the bacterial populations differed among reactor types and with column height.

DENITRIFICATION OF AQUACULTURE WASTEWATER USING AGRICULTURAL BY-PRODUCTS AS BIOFILTER MEDIA

by
WILLIE JONES BAYOTLANG SALILING

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

BIOLOGICAL AND AGRICULTURAL ENGINEERING

Raleigh

2005

APPROVED BY:

Co-Chair of the Advisory Committee   Co-Chair of the Advisory Committee

Para kay Yvonne, aking mahal
At kina Walbert at Caroline
Na sa kalinga ng ama'y naghihintay

Sa magsasaka at mamalakayang Pilipino, isang pagpupugay!
Sa Maykapal aking alay!

To Yvonne, my love
And to Walbert and Caroline
Who longed for a father's care

To the Filipino farmers and fisherfolks, my salute.
To God Almighty, I dedicate this work!

- Willie Jones B. Saliling

BIOGRAPHY

The researcher spent half of his life in the coastal city of Panabo, Davao Province in Southern Mindanao, Philippines. During his tender years, he, together with other kids, used to pass by along the fish ponds on their way to gather fuel wood trapped on the mangroves in the coastline. There were times they devised short bamboo poles to trap crabs digging holes on the fishpond dikes. That was his first brush with aquaculture.

He attended Panabo Central Elementary School where he graduated class valedictorian in 1988. He pursued his secondary schooling at Panabo High School where he again topped the class. Using a full scholarship privilege as the high school’s class valedictorian, he enrolled in the agricultural engineering program at the University of Southern Mindanao (USM), Kabacan, Cotabato. It was during his stint in USM, where he learned more and was enlightened about the other side of agriculture – small-scale family farming which is entirely opposite to the large corporate farming (e.g. banana plantations) he was exposed to while growing up. The seemingly poor plight of farmers had made agricultural engineering not just a mere career to him but rather a passion. His participation in various student activities and organizations has expanded his view of the social responsibility of engineers towards their clients. With his good scholastic performance, he graduated as *magna cum laude* in 1997. Thereafter, he took the licensure examination and fortunately ranked second place nationwide.

After taking the board exam, he worked in the University of Southeastern Philippines, Tagum City as an Instructor in the Department of Agricultural Engineering. When the university was tasked to conduct research on irrigation, he took part as a research associate. This job provided him the opportunity to travel around many points in Mindanao. In his travels, he witnessed the need for environmental protection of land, water and air resources. He thought of pursuing an advanced education in environmental engineering. He went back to USM in June 2000 to teach in the College of Engineering while taking advanced courses in soil and water engineering.

While in USM, he tried applying for various scholarship programs. With God's grace, an opportunity for graduate studies was finally accorded to him through the Fulbright Philippine Agriculture Scholarship Program (FPASP). He started his Master of Science program at the Department of Biological and Agricultural Engineering, North Carolina State University (NCSU) on Fall 2003. To complement his knowledge on land and water resources, he concentrated on waste management and took civil engineering as minor to strengthen his coursework in environmental engineering. He was delighted with his worthwhile experiences in NCSU for he was able to study the different facets of environmental engineering (soil, water, and air) as it is applied to agriculture. He was also fortunate to be involved in the world-renowned aquaculture engineering venture – the NCSU Fish Barn.

Upon completion, he intends to strengthen relevant programs of the University of Southern Mindanao by teaching agricultural waste management, aquaculture engineering, and environmental engineering. He plans also to help the government and civil societies in some of their environmental undertakings in Mindanao.

Someday, he would trek again Davao's shorelines in its more fascinating view and bountiful natural resources. That time, definitely, he would do it together with his family, her wife-Yvonne and their two smart kids – Dods and Carol.

ACKNOWLEDGMENT

As with any major undertaking, it takes a great team to make all elements come together. This work is a result of the help extended by several individuals and institutions. I want to extend my personal and sincere thanks to:

Dr. Tom Losordo – whose logistic support has kept my project going despite the “expense”. His portraits of a family waiting back in the Philippines has kept my spirit alive as well as to work harder.

Dr. Phil Westerman – whose guidance throughout my academic pursuit allowed me to keep pace with time and let me wander the world of research and eagerly listened to my ideas. And when faced with challenges, says: “That’s research”.

Dr. Francis de los Reyes – whose expertise strengthen my fundamental understanding of biological wastewater treatment.

My professors and instructors who made my coursework challenging and rewarding as well: Dr. Mort Barlaz, Dr. John Classen, Dr. Sanjay Shah, Dr. Joel Ducoste, Dr. Jason Osborne, and Dr. Rog Woodard.

Mr. Dennis De Long and Mike Adcock – for assisting me in the procurement of materials and getting samples from the NCSU Fish Barn.

Rachel, Heather, Hiroshi, Tracy of the BAE Environmental Analysis Laboratory – who gave priority in the analyses of my samples.

Cesar Mota – for running DGGE analysis of my biomass samples at the Wastewater Biotechnology and Process Engineering Research Lab.

Dr. Ratna Sharma and James Shi – for allowing me to use some of their equipment for lignin analysis.

Dr. Nancy Love and Mr. Brian Brazil of Virginia Tech for sharing information about their work on recirculatiing aquaculture system.

The staff at the Research Shop and the USDA Lab.

The Philippine-American Educational Foundation (PAEF), Fulbright Scholarship Board and the Institute of International Education (IIE) – whose belief in me brought me to the land of Uncle Sam.

US Department of Agriculture – CREES – whose grant to NCSU Fish Barn financed my study.

Serge, Pam, and Andre – for the company.

Kuya Dave, Ate Feds, Ading David – for showing me different sights and insights of US and North Carolina living.

Kuya Audi and Ate Tess – for the parties and “discounts” at Walmart.

The NC State Pinoys: Ric, Lizza, Remil, Jun, Jovy, Carl.

My parents, Walton and Concepcion, brother, Princeton and sister, Bai Janice.

My Dods and Carol – whose giggles and whispers keep me homeward bound.

My everdearest Yvonne – whose love sustained me.

And above all, to HIM who gives me wisdom, courage, and strength amidst trials, HE be praised forever!

TABLE OF CONTENTS

LIST OF TABLES x
LIST OF FIGURES xiii
1 INTRODUCTION **1**
1.1 BACKGROUND AND SIGNIFICANCE........ 1
1.1.1 AQUACULTURE INDUSTRY........ 1
1.1.2 ADVENT OF RECIRCULATING AQUACULTURE SYSTEM 1
1.1.3 REARING WATER QUALITY 2
1.1.3.1 NITROGEN CONSTRAINTS........ 3
1.1.4 RAS EFFLUENT QUALITY........ 4
1.1.5 THE NEED FOR LOW COST NITRATE REMOVAL 6
1.2 BIOLOGICAL DENITRIFICATION........ 6
1.2.1 ATTACHED GROWTH BIOREACTORS 8
1.2.2 BACTERIAL SUPPORT /BIOFILM CARRIERS 9
1.2.3 DENITRIFICATION IN AQUACULTURE 10
1.2.4 ALTERNATIVE CARBON SOURCES 10
1.2.5 USE OF NATURALLY-OCCURRING ORGANICS........ 12
1.2.6 DEGRADATION OF ORGANIC MATERIALS........ 13
1.2.7 DENITRIFYING MICROBIAL COMMUNITY........ 14
1.3 RESEARCH QUESTIONS........ 15
2 MATERIALS AND METHODS **17**
2.1 LABORATORY SETUP........ 17
2.1.1 BIOREACTOR CONFIGURATION........ 17
2.1.2 PERISTALTIC PUMPS 20
2.1.3 WASTE STORAGE TANK 21
2.2 SYNTHETIC WASTEWATER........ 21
2.3 PERFORMANCE STUDY........ 23
2.3.1 INOCULATION/STARTUP........ 24
2.3.2 VARIABLE NITRATE LOADING 24
2.3.3 REAL AQUACULTURE WASTEWATER 26
2.3.4 COD/NITRATE-N RATIO 27
2.4 EFFLUENT QUALITY 27
2.5 DEGRADATION OF FILTER MEDIA 29
2.5.1 MASS LOSS 29
2.5.2 POROSITY........ 30
2.5.3 FILTER MEDIA C/N RATIO........ 31
2.5.4 LIGNIN AND OTHER CONSTITUENTS........ 32
2.5.4.1 ASH CONTENT........ 32
2.5.4.2 ACID-INSOLUBLE LIGNIN 32
2.5.4.3 ACID-SOLUBLE LIGNIN........ 34
2.6 MICROBIAL MASS AND PRELIMINARY CHARACTERIZATION........ 34
2.6.1 MICROBIAL COMMUNITY........ 34
2.6.2 BIOMASS DEVELOPMENT........ 35

3 BIOFILTER DENITRIFICATION PERFORMANCE ……………………………….. 36
3.1 DENITRIFICATION UNDER SYNTHETIC WASTEWATER………………………. 36
3.1.1 NITRATE REDUCTION IN KALDNES MEDIA REACTORS……………….. 36
3.1.2 NITRATE REDUCTION IN WOOD CHIPS REACTORS……………………. 39
3.1.3 NITRATE RED UCTION IN WHEAT STRAW REACTORS………………… 41
3.1.4 COMPARISON OF DENITRIFICATION RATES AMONG REACTOR TYPES…. 44
3.2 NITRATE PROFILE ALONG COLUMN HEIGHT……………………………….. 47
3.2.1 NITRATE PROFILE IN KALDNES MEDIA………………………………. 48
3.2.2 NITRATE PROFILE IN WOOD CHIPS REACTORS……………………….. 50
3.2.3 NITRATE PROFILE IN WHEAT STRAW REACTORS…………………….. 52
3.2.4 COMPARISON OF NITRATE PROFILES…………………………………. 53
3.3 NITRATE REDUCTION WITH REAL AQUACULTURE WASTEWATER……….. 57
3.4 COD REDUCTION WITH SYNTHETIC WASTEWATER……………………… 58
3.4.1 COD REDUCTION IN KALDNES MEDIA………………………………. 58
3.4.2 COD REDUCTION IN WOOD CHIP MEDIA…………………………….. 60
3.4.3 COD REDUCTION IN WHEAT STRAW MEDIA…………………………. 61
3.4.4 COMPARISON OF COD REDUCTION RATES………………………….. 63
3.5 COD AND NITRATE…………………………………………………….. 64
3.6 COD REDUCTION UNDER REAL WASTEWATER…………………………….. 68
3.7 ESTIMATE OF EXTERNAL CARBON NEEDS………………………………….. 70
4 EFFLUENT QUALITY ……………………………………………………… 72
4.1 AMMONIA………………………………………………………………. 72
4.1.1 AMMONIA ASSIMILATION OR GENERATION USING SYNTHETIC WASTEWATER……………………………………………………. 72
4.1.2 AMMONIA ASSIMILATION OR GENERATION USING REAL WASTEWATER 76
4.2 NITRITE………………………………………………………………… 77
4.2.1 NITRITE GENERATION USING SYNTHETIC WASTEWATER…………….. 77
4.2.2 NITRITE REDUCTION WITH REAL WASTEWATER……………………… 80
4.3 ALKALINITY……………………………………………………………. 81
4.3.1 ALKALINITY CHANGES USING SYNTHETIC WASTEWATER…………… 81
4.3.2 ALKALINITY CHANGES USING REAL WASTEWATER………………….. 83
4.4 PH AND OXIDATION-REDUCTION POTENTIAL………………………………. 84
4.4.1 PH AND ORP USING SYNTHETIC WASTEWATER……………………… 84
4.4.2 PH AND ORP USING REAL WASTEWATER……………………………. 85
4.4.3 PH, ORP, AND NITRATE REDUCTION………………………………… 86
5 DEGRADATION OF BIOFILTER MEDIA AND BIOMASS CHARACTERIZATION …… 90
5.1 CHANGES IN PHYSICAL CHARACTERISTICS……………………………... 90
5.1.1 MASS AND BED HEIGHT LOSS……………………………………….. 90
5.1.2 CHANGES IN POROSITY……………………………………………. 91
5.1.3 VISUAL DESCRIPTION OF DEGRADATION…………………………….. 94
5.2 CHANGES IN CHEMICAL CHARACTERISTICS……………………………….. 95
5.2.1 CHANGES IN CARBON AND NITROGEN CONTENT…………………… 95
5.2.2 CHANGES IN LIGNIN AND OTHER CONSTITUENTS…………………… 96
5.3 BIOMASS DEVELOPMENT IN REACTORS…………………………………... 99
5.3.1 BIOMASS GROWTH ALONG THE REACTOR COLUMN HEIGHT ………… 101

5.3.2 Biomass buildup with time 104
5.4 Differences in Microbial Community 108
5.4.1 Microbial community along column height 110
6 Summary and conclusion 116
References 120
Appendices 124
Appendix A. Laboratory procedure and wastewater characterization 125
A.1 Analytical Procedure followed by the BAE-Environmental analysis Laboratory (updated April 2004) 125
A.2 A description of PCR-DGGE 126
A.3 Wastewater Characterization 127
Appendix B. Nitrate Data 128
Appendix C. COD and Alkalinity Data 143
Appendix D. Ammonia and Nitrite raw data 146
Appendix E. pH, DO, and ORP raw data 147
Appendix F. Physical and chemical characteristics 158
Appendix G. SAS source codes and partial outputs 164
G.1 Denitrification rates at LR1 (overall) 164
G.2 Denitrification rates at LR2 (overall) 165
G.3 Denitrification rates at LR3 (overall) 166
G.4 Denitrification rates at LR1 (steady) 167
G.5 Denitrification rates at LR2 (steady) 168
G.6 Denitrification rates at LR3 (steady) 169
G.7 COD removed at LR1 (overall) 170
G.8 COD removed at LR2 (overall) 171
G.9 COD removed at LR3 (overall) 172
G.10 COD removed at LR1 (steady) 173
G.11 COD removed at LR2 (steady) 174
G.12 COD removed at LR3 (steady) 175
G.13 COD/NO_3-N ratio at LR1 (overall) 176
G.14 COD/NO_3-N ratio at LR2 (overall) 177
G.15 COD/NO_3-N ratio at LR3 (overall) 178
G.16 COD/NO_3-N ratio at LR1 (steady) 179
G.17 COD/NO_3-N ratio at LR2 (steady) 180
G.18 COD/NO_3-N ratio at LR3 (steady) 181

LIST OF TABLES

Table 1.1	Wastewater characteristics at various points in the waste treatment system at NCSU Fish Barn (Chen et al, 2002)	4
Table 1.2	Relevant denitrification studies using attached growth bioreactors. . .	11
Table 2.1	Composition of synthetic wastewater used in this study…………….	22
Table 2.2	Variable nitrate loading summary……………………………………	25
Table 2.3	Summary of water analyses done in this study………………………	28
Table 3.1	Mean NO_3-N effluent concentrations and percent reduction (Kaldnes media)……………………………………………………………....	37
Table 3.2	Mean NO_3-N effluent concentrations and percent reduction (wood chips reactors)……...	40
Table 3.3	Mean NO_3-N effluent concentrations and percent reduction (wheat straw reactors)……………………………………………………..	42
Table 3.4	Average percent NO_3-N reduction along column height under LR1...	54
Table 3.5	Average percent NO_3-N reduction along column height under LR2...	55
Table 3.6	Average percent NO_3-N reduction along column height under LR3...	56
Table 3.7	Influent, effluent nitrate concentrations and denitrification rates of reactors fed with actual wastewater (November 18, 2004)…………..	58
Table 3.8	Average COD removed in Kaldnes reactors in each loading rate with corresponding percentage…………………………………………….	60
Table 3.9	Average COD removed in wood chips reactors in each loading rate with corresponding percentage……………………………………….	61
Table 3.10	Average COD removed in wood chips reactors in each loading rate with corresponding percentage………………………………………	62
Table 3.11	Comparison of average COD removal in each loading rate with corresponding percentage……………………………………………	64
Table 3.12	Mean COD/NO_3-N ratios…………………………………………….	66

Table 3.13	Total and Soluble COD reduction in reactors under actual Fish Barn wastewater (November 18, 2004)……………………………………….	69
Table 3.14	Methanol Requirement to completely reduce NO_3-N from actual wastewater………………………………………………………………	71
Table 4.1	Influent and effluent ammonia of reactors subject to real wastewater (November 18, 2004)………………………………………………………	76
Table 4.2	Influent and effluent NO_2-N concentration of the reactors under real wastewater……………………………………………………………….	81
Table 4.3	Average alkalinity produced and its ratio with NO_3-N removed of the three media types……………………………………………….	82
Table 4.4	Influent, effluent alkalinity of the reactors and its corresponding relationship with reduced nitrate during real wastewater…………..	83
Table 4.5	Influent and effluent pH and ORP of reactors under synthetic wastewater………………………………………………………….	84
Table 4.6	Influent and effluent pH and ORP of reactors under real wastewater..	86
Table 5.1	Mass loss of wood chips and wheat straw after 140 day experiment..	90
Table 5.2	Porosity of the reactor and its sections before and after the denitrification tests………………………………………………………	92
Table 5.3	Carbon and nitrogen content of new and used wood chips and wheat straw…………………………………………………………………….	95
Table 5.4	Lignin, ash and other constituents of new and used wood chips (WC) and wheat straw (WS) media…………………………………	97
Table 5.5	Solids Analyses of 9 reactors to estimate microbial biomass………..	100
Table 6.1	Estimated volume and corresponding economics of constructing denitrification biofilter at a recirculating aquaculture facility……….	118
Table A.1	Fish barn wastewater analyses (samples taken on July 12, 2004) compared with wastewater characteristics of a RAS facility at Virginia Tech (Brazil, 2000). TOC, COD, BOD, and soluble COD samples were taken on December 2, 2004……………………………	127

Table B.1	Data on influent and effluent nitrates and its respective denitrification rates from July to November 2004 with synthetic wastewater as feed…………………………………………………	128
Table B.2	Data on nitrate profile and denitrification rates of reactors at 50 mg/L measured on September 7, 2004 ……………………………………..	136
Table B.3	Data on nitrate profile and denitrification rates of reactors at 120 mg/L on October 4, 2004..……………………………………………	138
Table B.4	Data on nitrate profile and denitrification rates of reactors at 200 mg/L measured on November 8, 2004 ………………………………	140
Table B.5	Nitrate effluent and denitrification rates of reactors fed with NCSU Fish Barn waste ……………………………………………………	142
Table C.1	Synthetic COD raw data…………………………………………...	143
Table C.2	COD removed and percentage removal………………………………	144
Table C.3	Alkalinity measurements at the influent and effluent under synthetic wastewater experiments……………………………………………	145
Table D.1	Ammonia (mg NH_3-N/L) concentrations at the influent and effluent under wastewater experiments……………………………………..	146
Table D.2	Nitrite (mg NO_2-N/L) concentrations at the influent and effluent under wastewater experiments……………………………………..	146
Table E.1	pH, DO, and ORP, wter temperature under synthetic wastewater……	147
Table E.2	pH along column height (November 8, 2004)………………………..	157
Table E.3	ORP (mV) along column height (November 8, 2004)………………..	157
Table F.1	Acid-insoluble lignin content of wood chips and wheat straw……….	158
Table F.2	Acid-soluble lignin content of wood chips and wheat straw…………	159
Table F.3	Ash content of wood chips and wheat straw …………………………	160
Table F.4	Moisture content of wood chips and wheat straw…………………….	161
Table F.5	Volume of water drained (to estimate porosity) from reactors (empty, with media (before and after denitrification))……………….	162

LIST OF FIGURES

Figure 1.1	Unit processes typical to recirculating aquaculture production system (after Losordo et al, 1998) modified to include wastewater management	3
Figure 1.2	Schematic diagram of sampling points reported in Chen et al (2002): (1) inflow 1, (2) inflow 2, (3) outflow, and (4) effluent pond………………………………………………………………..	4
Figure 1.3	Several possible pathways of nitrate transformation: Denitrification (solid lines), dissimilatory nitrate reduction to ammonia (DNRA), assimilatory nitrate reduction or assimilation to form amino groups (R-NH_2) in microbial cells, and anaerobic ammonia oxidation (ANAMMOX) . . . . ………………..	7
Figure 2.1	(a) Laboratory setup showing the randomly mounted reactors, multi-port peristaltic pump, and nitrogen tank. (b) Schematic diagram showing the dimensions of a bioreactor and the sampling locations ……………………...	18
Figure 2.2	The effluent side of the laboratory setup during a typical sampling activity. Sampling of an intermediate port is also shown (lower left inset)……………………………………………………………...	20
Figure 3.1	Influent and effluent NO_3-N concentrations of Kaldnes reactors for three loading rates (LR) separated by vertical broken lines. “Eff” NO3-N represents the samples taken at the overall effluent of all nine reactors…………………………………………………	38
Figure 3.2	Denitrification (DN) rates of the three Kaldnes media reactors for three loading rates…………………………………………………	39
Figure 3.3	Influent and effluent NO_3-N concentrations of wood chips reactors……………………………………………………………….	40
Figure 3.4	Denitrification rates of the three wood chips reactors for three loading rates……………………….………………………………	41
Figure 3.5	Influent and effluent NO_3-N concentrations of wheat straw reactors…………………………….………………………………	42
Figure 3.6.	Denitrification rates of the three wheat straw reactors……………	43
Figure 3.7	Mean influent and effluent NO_3-N concentrations……………….	44

Figure 3.8	Estimated denitrification rates of the three reactor types for three loading rates (LR)……………………………………………….	46
Figure 3.9	Nitrate profile of Kaldnes media reactors at three loading rates…	48
Figure 3.10	Estimated denitrification rates in each section of the Kaldnes reactors at each nitrate loading…………………………………...	49
Figure 3.11	Nitrate profile of wood chips reactors at each nitrate loading……	50
Figure 3.12	Estimated denitrification rates in each section of the wood chips reactors at each nitrate loading…………………………………….	51
Figure 3.13	Nitrate profile of wheat straw reactors at each nitrate loading…….	52
Figure 3.14	Estimated denitrification rates in each section of the wheat straw reactors at each nitrate loading…………………………………….	53
Figure 3.15	Nitrate profiles of different reactors and the corresponding denitrification rates at 50 mg NO_3-N/L(September 7, 2004)……...	54
Figure 3.16.	Nitrate profiles of different reactor types and their corresponding denitrification rates at 120 mg NO_3-N/L (October 4, 2004)……….	55
Figure 3.17	Nitrate profiles of different reactors and their corresponding denitrification rates at 200 mg/L (November 8, 2004)…………….	56
Figure 3.18	Influent and Effluent COD values of Kaldnes plastic media……...	59
Figure 3.19	Influent and Effluent COD values of wood chips reactors………...	61
Figure 3.20	Influent and effluent COD values of wheat straw reactors……….	62
Figure 3.21	Influent COD and mean NO3-N effluent of different reactors…...	65
Figure 3.22	Average COD/NO_3-N ratios of the different reactor types……….	68
Figure 4.1	Influent and effluent NH_3-N of Kaldnes media reactors………...	72
Figure 4.2	Influent and effluent NH_3-N of wood chips reactors…………….	73
Figure 4.3	Influent and effluent NH_3-N of wood chips reactors…………….	74
Figure 4.4	Mean NH_3-N concentration of three reactor types……………….	75

Figure 4.5	Influent and effluent NO_2-N of Kaldnes media……………………	77
Figure 4.6	Influent and effluent NO_2-N of wood chips reactors………………	78
Figure 4.7	Influent and effluent NO_2-N of wheat straw reactors……………..	79
Figure 4.8	Mean influent and effluent NO_2-N of three media types…………..	80
Figure 4.9	Average influent and effluent alkalinity of each media type………	82
Figure 4.10	pH and ORP profile of the three media types (November 8, 2004).	85
Figure 4.11	Relationship of pH, ORP, and NO_3-N in Kaldnes reactors ……….	87
Figure 4.12	Relationship of pH, ORP, and NO_3-N in wood chips reactors…….	88
Figure 4.13	Relationship of pH, ORP, and NO_3-N in wheat straw reactors……	89
Figure 5.1	A comparison of raw (new) and spent (used) wheat straw media…	94
Figure 5.2	Mean changes of masses of lignin, ash, and other constituents of wood chips and wheat straw before and after the experiments……	98
Figure 5.3	Kaldnes beads taken in each level – 0, 10, 20, 30, 40 cms (shown above from left to right). The lower portion showed the beads freshly taken out……………………………………...................	102
Figure 5.4	Wood chips with biomass attached in varying thickness. Lowest position biofilter (A) to highest position (D)…………………….....	103
Figure 5.5	Biomass on wheat straw from the lowermost portion of the reactor (A) to the uppermost section (D)………………………………......	103
Figure 5.6	A photo log of the biomass buildup on Reactor 3 – Kaldnes media………………………………………………………….	105
Figure 5.7	A photo log of biomass buildup on reactor 4 – wood chips…….....	106
Figure 5.8	A photo log showing biomass buildup on reactor 8 – wheat straw..	107
Figure 5.9	The DGGE photograph showing nine gels in order (left to right): 5A, 5C, 5B - (wood chips); 2A, 2C, 2B – (Kaldnes); 7C, 7B, and 7A – (wheat straw)…………………………………………………	109

Figure 5.10	Gels from reactor 5 (wood chips) arranged from port A to C (1,3,2)……………………………………………………………	111
Figure 5.11	Gels from reactor 2 (Kaldnes) arranged from port A to C (4, 6, 5)………………………………………………………………...	113
Figure 5.12	Gels from reactor 7 (wheat straw) arranged from port a to c (9,8,7)……………………………………………………………	114
Figure 5.13	Brownish layer of biomass covered the wood chips (leftmost picture). A white grayish layer remained even after washing – sprayed with running water (two middle pictures). The rightmost was the chip after brushing off grayish layer………………………	115
Figure 5.14	A straw of wheat showing whitish layer covered by black biomass………………………………………………………….	115

1. INTRODUCTION

1.1 BACKGROUND AND SIGNIFICANCE

1.1.1 AQUACULTURE INDUSTRY

Aquaculture is growing more rapidly than all other animal food producing sectors. FAO (2002) indicated that aquaculture's contribution to global supplies of fish, crustaceans and mollusks continues to grow, increasing from 3.9 percent of total production by weight in 1970 to 27.3 percent in 2000. Worldwide, this sector has increased at an average compounded rate of 9.2 percent per year since 1970 (FAO, 2002). This rate of growth is higher than the 1.4 percent reported for capture fisheries and the 2.8 percent for terrestrial farmed meat production systems.

In order to meet future demand using traditional technologies, the industry will have to utilize more land and water. Traditional pond-based production requires approximately 9.3 million liters per hectare to rear approximately 2,300 kg of fish a year (Losordo et al, 1998). In many areas in the United States and Europe, however, aquaculture investments have been moving away from pond aquaculture. Water supplies are limited and areas suitable for pond construction are less available in these countries. Thus, aquaculturists have been finding new ways to produce more fish with less water and less land.

1.1.2 ADVENT OF RECIRCULATING AQUACULTURE SYSTEM (RAS)

Recirculating systems are highly intensive culture systems. Water quality in the rearing tanks is maintained for optimum fish growth by relatively complex water treatment systems and other support equipment. Aquaculture researchers have been investigating these technologies for more than three decades.

Aside from reducing land and water requirements, Masser et al. (1999) also pointed out other advantages of this technology, including: a higher degree of environmental control allowing year-round optimal production and the feasibility of locating production in close proximity to prime markets thereby cutting transportation costs. In this type of production system, better feed conversion can be achieved, biosecurity enhanced and better inventory control can be implemented (Losordo, 2004).

1.1.3 Rearing Water Quality

The key to success in aquaculture is the maintenance of a good water quality. A good recirculating production system design must incorporate mechanisms that remove solid waste, remove carbon dioxide, add oxygen, control ammonia and nitrite-nitrogen concentrations, pH and pathogenic bacteria at a rate equal to or greater than the rate of generation (Losordo, 2004). Figure 1.1 shows the different unit processes typical of recirculating aquaculture systems (RAS).

The incorporation of these unit processes in the design can be costly. High initial investment, technology not properly designed or sized, and lack of a good track record has made this technology unattractive to financial lenders and/or investors. Recent advancements have been geared towards reducing the costs of these unit processes to achieve profitable production. It is estimated that, in many cases when using recirculating technologies, fishes need to be reared at least 0.06 kg per liter (60 kg / m^3) to be cost-effective (Losordo, 1998).

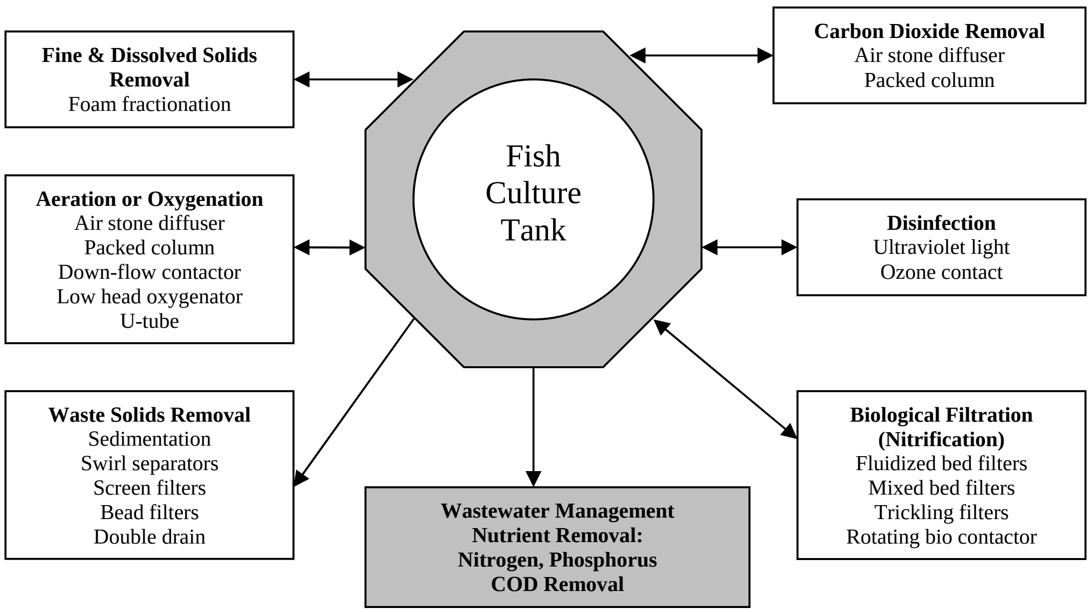

Figure 1.1 Unit processes typical to recirculating aquaculture production system (after Losordo et al, 1998) modified to include wastewater management.

1.1.3.1 NITROGEN CONSTRAINTS

The metabolism of fishes and the decomposition of feces and unutilized feeds produce ammonia-nitrogen, which in un-ionized form is very toxic to the reared fish. To mitigate this hazard, the current industry uses nitrification as means of oxidizing the ammonia-nitrogen to nitrate-nitrogen. Since nitrates are not toxic to many freshwater fishes and concentrations of up to 300 ppm can be tolerated (Masser et al, 1999), water can be recycled for extended periods of time before being discharged from the system.

Under certain conditions, nitrite-nitrogen can also accumulate inside the tanks particularly in spaces where oxygen is deficient. Nitrification is an aerobic process wherein nitrogen is transformed by autotrophic bacteria in two steps: ammonia to nitrite, then nitrite to nitrate. If the biofilters are not working properly, nitrification is not complete, thus, accumulation of nitrite occurs inside the tanks. Nitrite at a certain level (e.g. 5 mg/L for Tilapia) is lethal to fishes (Masser et al, 1999).

1.1.4 RAS EFFLUENT QUALITY

Recirculating systems are designed to minimize water requirements which results in a low-volume, highly concentrated waste stream. A recirculating facility produces effluent that is high in solids, nutrients, and COD (Twarowska et al, 1997). In a study conducted at the North Carolina State University (NCSU) Fish Barn, Chen et al (2002) characterized the wastewater (Table 1.1) at different points of the waste treatment system (Figure 1.2).

Table 1.1 Wastewater characteristics at various points in the waste treatment system at NCSU Fish Barn (Chen et al, 2002).

Parameter	TKN (mg/L)	NH_3-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	TP (mg/L)	PO_4-P (mg/L)	COD (mg/L)	TS (%)	TSS (mg/L)
Settling tank 1 (inflow)	50.3	2.96	5.35	109.0	28.6	5.98	1043	0.22	752
Settling tank 2 (inflow)	47.5	2.42	31.17	78.5	22.7	11.50	690	0.18	364
Settling tank 2 (outflow)	37.7	3.42	44.00	36.4	17.6	12.20	409	0.16	205
Receiving pond effluent	8.94	0.12	1.93	8.2	4.95	3.68	153	0.11	44

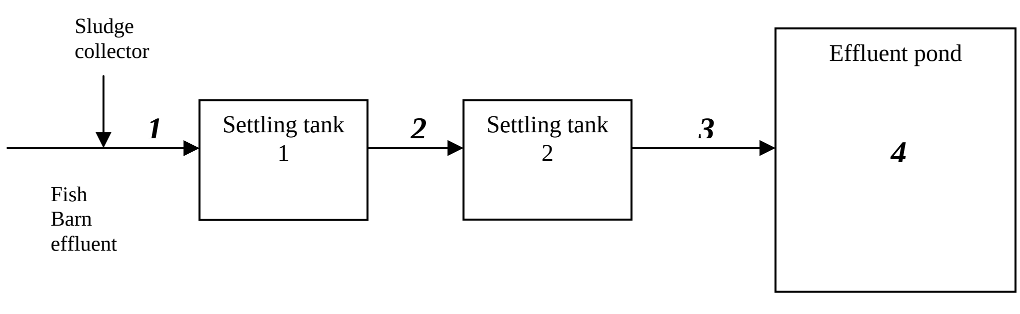

Figure 1.2 Schematic diagram of sampling points reported in Chen et al (2002): (1) inflow 1, (2) inflow 2, (3) outflow, and (4) effluent pond.

The waste treatment system of NCSU Fish Barn described above was mainly implemented to treat solids. Forty percent of the solid waste produced are collected in a sludge collector and composted. The remaining 60% of the solids are treated in two serial primary settlers (septic tanks) and then polished in an effluent pond. As can be seen in Table 1.1, nitrogen in the effluent is primarily in the form of nitrate-nitrogen. As noted previously, this is because recirculating systems are actively employing a process of biological nitrification. As water is used over and over again, one can expect accumulation of nitrates in the recirculating system (van Rijn, 1996). Thus, aquaculture wastewater leaving the system has relatively high concentrations of nitrates. Though substantial nitrate reduction (Table 1.1) occurred in the serial tanks (with waste solids as carbon source), nitrate concentrations at the outflow were still high (36 mg NO_3-N/L) and had to be treated (with longer residence time and large space) in effluent retention ponds where algae were assumed to consume NO_3-N (down to 8 mg NO_3-N/L).

Recognizing the potential environmental impact, the Environmental Protection Agency (2004) has come up with a technical document for the final effluent limitation guidelines for concentrated aquatic animal production like RAS. This means that more stringent guidelines are underway to regulate effluents from the industry. Introduction of nitrate-nitrogen in surface waters may induce eutrophication that could result in toxic algal blooms. Nitrate-nitrogen can also contaminate groundwater drinking water supplies which can cause methemoglobinemia or “blue baby” syndrome.

1.1.5 THE NEED FOR LOW COST NITRATE REMOVAL

The considerations discussed in Section 1.1.4 have made nitrate removal imperative for aquaculture wastewater. Several technologies have been employed for nitrate removal. Some farmers have resorted to retention ponds, sprayfields, and hydroponics for waste treatment. These approaches require more land which is contrary to the objective of intensive aquaculture (rearing fish in less space). Moreover, this approach adds another layer of technology to an already complex farming operation. In places where land application is limited due to nitrate groundwater contamination, spraying wastewater over fields is becoming less an option.

This has led to the interest in removing nitrate-nitrogen from aquaculture effluents in a more compact way. Biofiltration is thought to be a good candidate for this approach. Aside from being compact, biofilters maintain a great diversity of microorganisms (Ouyang et al, 2000). However, the high initial cost of construction, media, and required external carbon source makes it less attractive to aquaculturists. This study examines the suitability of agricultural by-products and residues as biofilter media.

1.2 BIOLOGICAL DENITRIFICATION

Biological denitrification is the reduction of nitrate to nitrogen gas using bacteria in an anoxic environment. The necessary ingredients to achieve denitrification include an electron donor (carbon source), an electron acceptor, denitrifying bacteria, and water. Conversion of nitrate to nitrogen gas is not instantaneous. There are several sequential steps in the process as shown in Figure 1.3.

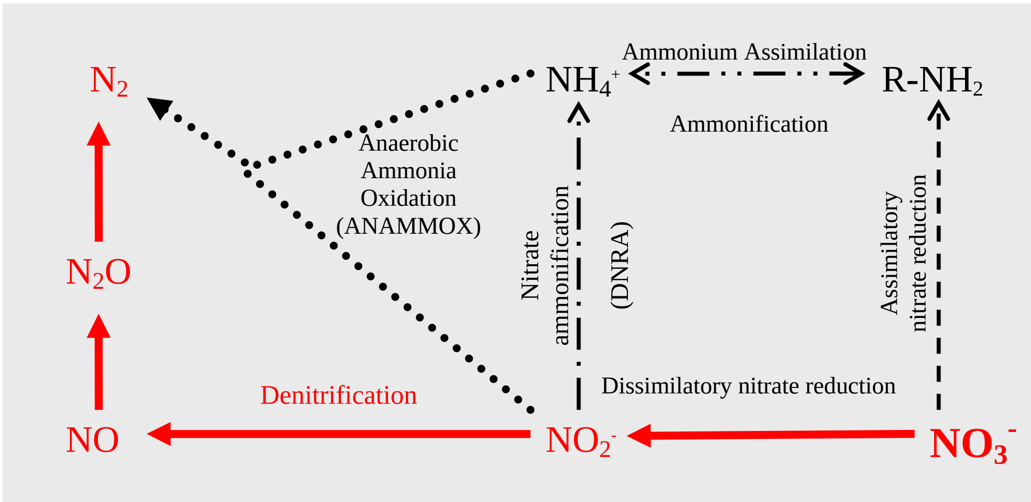

Figure 1.3 Several possible pathways of nitrate transformation: Denitrification (solid lines), dissimilatory nitrate reduction to ammonia (DNRA), assimilatory nitrate reduction or assimilation to form amino groups (R-NH_2) in microbial cells, and anaerobic ammonia oxidation (ANAMMOX).

Denitrifying bacteria are facultative which means that they can shift from using oxygen to nitrate as the electron acceptor once the dissolved oxygen (DO) falls below a certain threshold. Thus, dissolved oxygen should be kept low because these microorganisms prefer oxygen over nitrate. Activities of the denitrification enzymes are inhibited with oxygen concentrations as little as 0.5 mg/L or less. Nitrous oxide reductase, responsible for conversion to dinitrogen gas, are inhibited at 0.2 mg/L DO (Maier et al, 1999). In activated sludge systems, the reported values are 0.3 to 1.5 mg/L possibly due to diffusion limitations within microbial floc (EPA, 1993). Diffusion limitation may also occur in attached biofilm reactors, thus, even at higher bulk liquid DO, denitrification can still occur in the inner layers of the biofilm.

Denitrifying bacteria need an electron donor for energy and a carbon source for cell synthesis. The most common organic compounds used as carbon sources in controlled denitrification reactions are acetic acid and methanol. An unbalanced biochemical reaction is outlined in Equation 1.1 as:

Equation 1.1

$NO_3^- + \text{carbon source} + H^+ \rightarrow C_5H_7O_2N\ (\text{cells}) + N_2 + CO_2 + H_2O$

The amount of carbon needed would depend on the chemical characteristics of the organic compound. In complex sources like carbonaceous wastewater, the proper carbon-to-nitrogen ratio is determined through experiments and "rules of thumb". A carbon-rich environment may induce dissimilatory nitrate reduction to ammonia (DNRA) instead of denitrification (Maier et. al, 2000). Some nitrates are also reduced via assimilation by bacteria. In an anaerobic environment, ammonia and nitrite may react with each other to form dinitrogen gas (Madigan et al, 2003).

Temperature can also affect microbial processes. Low temperature ranges (6 to 10^{o}C) significantly slowed denitrification rates (Carrera, 2003).

Denitrification can be achieved in either suspended or attached growth bioreactors. However, the nature of the intensive aquaculture would favor use of attached bioreactor to save space.

1.2.1 ATTACHED GROWTH BIOREACTORS

As the name implies, attached growth bioreactors are reactors where microorganisms are attached to a solid support. EPA (1993) described two attached growth systems used in denitrification: downflow packed-bed reactors and upflow fluidized-bed systems. Packed bed systems provides the necessary hydraulic detention time for biological reactions to take place, and the open water surface in the filter is limited to minimize oxygen transfer. In fluidized bed system systems, media are not in contact with other particles resulting in high specific surface area.

The use of attached growth bioreactors (e.g. fluidized bed and submerged biofilters) is popular in the aquaculture industry with applications mainly for nitrification. The familiarity of aquaculturists with biofiltration allows its application to denitrification to be easily accepted.

1.2.2 BACTERIAL SUPPORT /BIOFILM CARRIERS

Biofilm carriers should have some desirable physical and chemical properties in order to successfully support bacterial growth. Dussert and Tramposch (1996) suggest that media for biofiltration should have good porosity and surface roughness, a high adsorptive capacity, and favorable surface chemistry (not reacting with the bulk liquid causing its rapid degradation). Desirable attributes for a biological filter include high removal efficiencies at steady state, short acclimation time and suitable response to influent water quality variation (e.g. temperature, shock loads, starvation, and presence of biologically toxic compounds). Son et al (2004) also suggests that microorganisms will immobilize on surfaces where there is little resistance to fluid flow. In addition, the bulk media should have sufficient interstitial volume to entrap suspended microorganisms.

The presence of micronutrients in a bacterial support media may also influence denitrification if these trace elements are missing from the reactor and needed for the growth of denitrifying bacteria. Saka (2001) has identified several trace chemical constituents in wood like Ca, K, Mg, Na, B, Mn, Fe, Mo, Cu, Zn, Co, Ni, and other trace elements that are considered essential micronutrients for denitrifying bacteria. However, micronutrient requirements vary widely from one species to another. Thus, nutrients for denitrification should not be entirely dependent on these naturally occurring elements on the media substrates.

1.2.3 DENITRIFICATION IN AQUACULTURE

There are several studies that have used biofiltration for the denitrification of aquaculture wastewater. Most of these studies used plastic media and other inorganic granules and supplied an external carbon source (e.g. methanol, acetate).

Sauthier et al (1998) demonstrated a denitrification treatment capacity of 0.1 kg (NO_3-N) $m^{-3}h^{-1}$ using crushed brick as media and ethanol as carbon source in a closed marine aquaculture system. Biodegradable polymers were also used as the carbon source and biofilm carrier in a study conducted by Boley et al (2000). The biodegradable media exhibited denitrification comparable to more traditional media with methanol used as the carbon source. However, these polymers are costly and may not prove to be cost-effective. Table 1.2 summarizes some studies on denitrification using attached growth bioreactors for treating drinking water, aquaculture wastewater, and runoff.

1.2.4 ALTERNATIVE CARBON SOURCES

The search for low-cost denitrification has included investigations testing alternative carbon sources. Aboutboul et al (1995) concluded that volatile fatty acids resulting from anaerobic treatment of effluent solids (feces and unutilized feeds) is adequate to support denitrification in RAS. These results are similar to a related study by Aesoy et al (1998) where they used hydrolyzed sludge from a municipal wastewater as the carbon source for the denitrification of domestic wastewater.

Table 1.2 Relevant denitrification studies using attached growth bioreactors.

Influent Nitrate concentration (mg NO_3-N/L)	**Flow rate** (mL/min)	**Water velocity – Total hydraulic loading (cm/hr)**	**Reactor dimensions** (reactor height, cm x diameter, cm)	**Type of wastewater**	**Filter Media**	**Carbon source**	**Denitrification rates** (g NO_3-N removed/m^3/day or otherwise noted)	**Researcher**
25	294 - 1472	100-500	200 x 15	Municipal wastewater	Biolite	Hydrolyzed organic matter	2500	Aesoy et al, 1998
22.6	4.42, 22.12	2, 10	14.5 x 13	Nitrate amended Drinking water	Wheat straw	Wheat straw	18 g wheat/ g N removed	Aslan and Turkman, 2003
3 - 6	6.94- 41.67		unspecified (200 liters)	Tile drainage	Tree bark, wood chips, leaf compost	Tree bark, wood chips, leaf compost	0.3 to 0.9	Blowes et al, 1994
56.1	5 to 8.33		unspecified (0.375 liters)	Aquaculture wastewater	PHB, PCL, Bionelle	PHB, PCL, Bionelle	36 - 3984	Boley at al, 2003
2.07 to 7.93	2.14 to 10.72	4 to 20	40 x 6.4	Runoff	Wood chips	Wood chips	10.26 (13.2 mg N/day)	Kim et al, 2003
21 to 44	7.5		1 x 7.5 , 2 x 7.5, 3 x 7.5	Treated sewage effluent or synthetic sol.	Wheat straw	Wheat straw	11.8 g N/kg wheat straw	Lowengart et al, 1993
38.5, 85.3, 64.8	453, 907.29	300, 150	400 x 15.2	Aquaculture wastewater	BioStyr	Fermented fish feeds	0.81 to 2.21	Phillips, 1998
14.2 to 37.7	694 to 50694	0.08 to 0.79	9 to 360 m^3	Septic effluent	Nitrex (wood chips)	Wood chips	7 to > 10	Robertson et al, 2005
22.6	1.92 to 7.7	2.3 to 5.4	50 x 8	Nitrate amended Drinking water	Wheat straw	Wheat straw	32 to 53 (11 g wheat straw/ g N removed)	Soares and Abeliovich, 1998
10, 30, 60	2094	400	150 x 10	Marine aquaculture	Crushed brick	Ethanol	2400	Sauthier et al, 1998

Phillips (1998) also reported the viability of using VFAs from fermented feed in the denitrification of aquaculture wastewater in an upflow biofilter at loading rates of 1.13 and 2.52 kg NO_3-N/m^3/day. The author described a COD to NO_x -N consumption ratio of 4.62 ± 0.28 mg COD/L per mg NO_x-N/L for complete nitrogen removal. This confirmed the findings of Aboutboul, et al (1995) that reported COD to NO_x-N consumption ratios in the range of 3.2 to 8.5.

1.2.5 USE OF NATURALLY-OCCURRING SOLUBLE ORGANIC CARBON

The use of naturally occurring soluble organic solid substrates were also investigated but mainly in drinking water and agricultural runoff. Soares and Abeliovich (1998) reported a drinking water denitrification of 0.053 g N removed $L^{-1}d^{-1}$ using wheat straw as solid substrate packed in a laboratory column. Aslan and Turkman (2003) reported 63% reduction of nitrates but observed 23.5% weight loss of the wheat straw substrate.

Wheat straw was also used in a biofilter for nitrate removal of turbid and nitrogen rich irrigation water (Lowengart et al, 1993). Additionally, Blowes et al (1994) reported substantial nitrate removal of an influent concentration 3-6 mg /L NO_3-N at a flow rate of 10-60 L/day (0.3 to 0.9 g NO_3-N/m^3/day) over a 1 year period study in reactors packed with wood chips. Recently, Kim et al (2003) investigated the use of newspaper, wood chips, and wheat straw as organic sources in denitrification in a storm water bioretention study. The authors reported nitrate mass removal rates of 15.1 mg N/day (11.74 g NO_3-N/m^3/day) for newspaper and 13.2 mg N/day (10.26 g NO_3-N/m^3/day) for wood chips.

These experiments were conducted in 40 cm x 6.4 cm i.d. laboratory columns at 4 cm/h water velocity.

Recently, Robertson et al (2005) reported a 96% nitrate removal of pretreated (nitrified on a sand filter) septic effluent. They used wood-based Nitrex filter (9 to 360 m^3) to treat an average of 14.2 to 37.7 mg NO_3-N/L of inflow (7 to more than 10 g NO_3-N/m^3 /day). Earlier, Robertson et al (2000) also reported 0.7 to 32 g NO_3-N/m^3/day nitrate removal rates in pilot studies in treating septic systems using saw dust, leaf compost, unprocessed grain seed, and wood mulch as reactive barriers.

Using agricultural by-products as substrate for denitrification has mostly been studied using low nitrate concentration water. Though Robertson et al (2005) demonstrated treatment of high NO_3-N influent, their experiment was performed using relatively large media volume. This study investigates the performance of agricultural by-products as denitrification biofilter media under higher nitrate loading typical of aquaculture wastewater with external carbon addition to reduce volumetric requirements.

1.2.6 Degradation of Organic Materials

Agricultural by-products (e.g. wood chips and wheat straw) are biodegradable. It is expected, therefore, that the filter will decay and degrade somewhat during the process of denitrification. Measurement of this parameter is an important factor in the evaluation of the desirability of these materials as biofilter media.

Eiland et al (2001) in a composting study, pointed out that organic materials with a low initial carbon-to-nitrogen (C/N) ratio has faster degradation as compared to materials with high C/N ratio. A reduction in C/N ratio would mean that degradation has occurred since microorganisms utilize and synthesize the carbon and nitrogen present in the biomass. With high initial C/N ratios, lower degradation is expected because microorganisms are nitrogen limited.

Shah et al (2002) has demonstrated a slight decrease of C/N ratio in a wheat straw biofilter treating dairy wastewater. The lignin concentration also increased as a result of hemicellulose degradation. Similarly, Lowengart et al. (1993) observed decreases in hemicellulose and cellulose components. The authors also reported a reduction of C/N ratio of the wheat straw biofilter due to a decrease in organic carbon and nitrogen enrichment.

Lignin resists biological degradation (Richard, 2004). The physical state of the material can also affect the rate of degradation. Nikitin (1966) stressed that the higher the degree of comminution (breaking into smaller pieces) of wood, the more easy the cellulose is degraded. Therefore, degradation of wood chips is more rapid than when it is in solid hardwood state (not chopped or broken into pieces).

Degradation can also be ascertained by measuring the loss of mass as the decayed materials are being degraded and/or being assimilated by microorganisms. Along with weight loss, it is also possible that the bulk volume and bed height would also be reduced.

1.2.7 Denitrifying Microbial Community

Microorganisms that denitrify are found widely in the environment (Maier et al, 2000). Microorganisms most prominent in wastewater treatment include *Achromobacter, Aerobacter, Alcaligenes, Bacillus, Flavobacterium, Micrococcus, Proteus*, and *Pseudomonas* (Grady et al, 1999).

Attached growth bioreactors also maintain diverse microbial communities which include fungi and other saprophytic organisms (Grady et al, 1999). With media such as wood chips and wheat straw, there is the possibility that microbial communities may differ between each media. Microbial communities may also vary along the length of a reactor because of the changes in the chemical composition as fluid flows through the reactor.

There are limited studies found in the literature that identify the microorganisms involved in nitrogen transformation processes, particularly in denitrification in a recirculating aquaculture system. Tal et al (2003) has detected *Pseudomonas* sp. in moving bed bioreactors of a closed mariculture system. *Planctomycetes* sp. which are responsible for anaerobic ammonia oxidation or converting ammonia to N_2 by using nitrite as acceptor were also detected.

1.3 RESEARCH QUESTIONS

No previous studies have investigated the use of agricultural by-products as biofilter media in denitrifying aquaculture wastewater. While there are several studies that deal with naturally-occurring organics as substrates and carbon sources, they focused on drinking water or nitrate reduction of groundwater, which have relatively low nitrate concentration.

Thus, this study attempts to utilize the knowledge developed in other studies and apply it to aquaculture wastewater treatment. This study attempts to answer the following questions:

1. Is it possible to use wheat straw and wood chips as alternatives to plastic media in denitrifying biofilter? If possible, what is the degree of its potential? How much of it is needed?
2. How much external carbon supply is needed to sustain a high reaction rate? What reaction rates can be achieved?
3. What is the final effluent quality? Will it conform to standards set forth by regulations for effluent discharge?
4. Given the biodegradability of these alternative media, how long will they last?
5. What are differences of microbial community among the different filter media? Within a reactor type, will the community differ along height?

To address the questions above, the following objectives were set:

1. To determine performance of agricultural by-products as biofilter media for aquaculture wastewater denitrification under different nitrate loading.
2. To determine effluent quality by measuring pH, ORP, nitrite, ammonia, alkalinity and to evaluate relationships among these parameters with denitrification.
3. Estimated possible long-term use by assessing degradation of filter media by analyzing the changes in physical and chemical properties.
4. Observed differences in microbial (bacterial) community in each media and along its respective heights.

2. MATERIALS AND METHODS

2.1 LABORATORY SETUP

2.1.1 BIOREACTOR CONFIGURATION

Nine 3.8-L bench scale biofilters were fabricated for this study (Figure 2.1). Three biofilters were each packed with one of the three types of media – wood chips, wheat straw, and Kaldnes plastic media (Kaldnes Miljøteknologi AS, Norway). Kaldnes is made of high-density polyethylene with an average diameter of 10 mm and height of 7 mm and effective specific surface area of 500 m^2/m^3 (Ødegaard et al, 2000). The dimensions of the wood chips particles were approximately: 8 to 50 mm on a side and 2 to 15 mm thickness while the wheat straws were chopped at lengths of around 20 to 35 mm.

The dimensions of the 3.2-L packed column made of clear PVC pipes were 10.16 cm (4” i.d.) by 40 cm. The ends of the PVC column were covered with plastic screens to hold the media and were fitted with 10.16 cm by 2.54 cm (4” by 1”, insert) reducer tees. The 2.54 cm outlets of the reducer tees were fitted with a 2.54 cm by 0.635 cm (1” x ¼”, insert by barbed) reducer coupling. These reducers served as the influent and the effluent ports for the reactors.

Three holes were bored along the column at different heights – 10 cm, 20 cm, and 30 cm and 0.635 cm (¼” barbed by NPT) adapters were fitted to these holes. These openings were used to extract samples for the analysis of nitrate concentration and

(a) (b)

Figure 2. 1 (a) Laboratory setup showing the nine experimental reactors, multi-port peristaltic pump, and nitrogen tank. (b) Schematic diagram showing the dimensions of a bioreactor and the sampling locations.

microbial biomass profiles. These intermediate ports were fitted with PVC tubing and sealed with clamps.

To minimize void space within the bottom and upper reducer tees, thin polyethylene disks were inserted before sealing both ends of the reactors with PVC plugs. The reactor components were joined and sealed with silicone sealant. The nine bioreactors were placed on a wooden stand and the media type was randomly assigned to each reactor.

The effluent line was fitted with a T-connector so that effluent could be diverted to a sampling container. Samples from the intermediate ports were taken in sequence from the top to bottom. When sampling a particular port, the sampling tube was looped over the top of the reactor (with effluent port clamped tight) before draining it to a sampling container. This was done to ensure that samples were representative of the fluid on a particular height and not diluted by the possible rush of water due to water pressure above it. The typical sampling procedure is shown in Figure 2.2.

The waste influent was fed through 0.635 cm (¼”) clear vinyl tubing. Likewise, a similar type of vinyl tubing was used to carry effluent to a laboratory sink for disposal. The vinyl tubing was cleaned at least once every two weeks to minimize biofilm and solids buildup inside the influent line. This maintenance procedure was conducted to minimize nitrate reduction in the influent lines.

Figure 2. 2 The effluent side of the laboratory setup during a typical sampling activity. Sampling of an intermediate port is also shown (lower left inset).

2.1.2 PERISTALTIC PUMPS

A multiport peristaltic pump set (Masterflex L/S drive, 8-roller pump head, small cartridge model no. 07519-85, Cole-Parmer, Illinois) provided energy for fluid flow to the nine bioreactors. The pump head speed (RPM) was adjusted every other day to ensure that flow rate was close to 15 mL/min. If a particular port was below 10 mL/min, the peristaltic tubing (Santoprene) was replaced. If a majority of the ports were below 15 mL/min, pump speed was adjusted.

In the early part of the study, the investigator employed 9 individual peristaltic pumps. However, it was difficult to obtain uniform flow-rate. Equally problematic, the peristaltic pump tubing wore out easily whereby wastewater flowed out from the system. A metal pan was fabricated to catch the spills as a result of the unpredictable wearing out of the pump tubing. It was decided to replace the nine reactors with a single multi-port peristaltic pump as described beforehand.

A peristaltic pump (Fisher Scientific Variable Flow Mini-Pump, Control Company, Texas) was also installed to extract influent samples through a T-connector tapped on the clear vinyl tubing between the multi-port pump and the waste storage tank.

2.1.3 WASTE STORAGE TANK

A 473 liter (125-gallon) tank served as the synthetic and real wastewater storage tank. The tank held a two-day supply of synthetic wastewater and all inlets and outlets were sealed with silicone sealant to minimize air entry.

Nitrogen gas was released through two diffuser stones on the bottom of the tank to keep a low level of DO in the synthetic wastewater.

2.2 SYNTHETIC WASTEWATER

Synthetic wastewater was prepared for 50, 120, and 200-mg/L NO_3-N concentrations dissolved from fertilizer grade sodium nitrate (Hi-Yield Nitrate of Soda, Voluntary Purchasing Group, Texas). The composition of the synthetic aquaculture wastewater is outlined in Table 2.1. The mixture was formulated by reviewing and comparing synthetic wastewater from previous studies (Menoud, 1999; Phillips, 1998; Chen, 1996).

Table 2.1 Composition of synthetic wastewater used in this study.

Constituents	Concentration (mg/L)	Commercial Source	Ionic/Elemental Constituents	Expected Concentration (mg/L)
Nitrate of Soda	379.51, 767.01, 1278.35	Voluntary Purchasing Group, Texas	**NO_3-N**	50, 120, 200
			Carbon	56.25, 112.5 187.5
Methanol	150, 300, 500	Acros Organics	**Na^+**	130.28, 214.17, 376.74
$CaCl_2$*$2H_2O$	82.90	Fisher Scientific	**Ca**	22.6
$CoCl_2$*$6H_2O$	0.40	Fisher Scientific	**Mg**	9.80
$CuSO_4$*$5H_2O$	0.12	Fisher Scientific	**K**	15.13
$FeCl_3$*$6H_2O$	1.00	Fisher Scientific	**Fe**	0.20
KH_2PO_4	52.65	Fisher Scientific	**NH_4**	0.05
$MgCl_2$*$2H_20$	81.96	Fisher Scientific	**Co**	0.10
$MnCl_2$*$4H_2O$	0.32	Fisher Scientific	**Mn**	0.09
$(NH4)_6Mo_7O_{24}$*$4H_2O$	0.6	Fisher Scientific	**Cu**	0.03
NaCl	56.52	Fisher Scientific	**Zn**	0.05
$ZnCl_2$	0.11	Fisher Scientific	**Mo**	0.05
			PO_4-P	11.98
Na_2SO_3	70.92	Fisher Scientific	**Cl^-**	103.4
			SO_4^{2-}	54.08

To confirm that this synthetic wastewater was similar to real wastewater from fish culture systems, a characterization of the NCSU Fish Barn wastewater was undertaken analyzing TP, Cl, Na, K, Ca, Mg, Cu, Zn, Fe, Ni, and Zn (See Appendix A.1 for the Reference Methods and Appendix A.2 for the results of wastewater analyses). Since SO_4^{2-} was not analyzed, it was estimated from Brazil (2000). Cl^- is higher in recirculating systems than SO_4^{2-} as large amounts of either sodium chloride or calcium chloride are used to maintain salinity levels to provide for protection against nitrite toxicity to the fish.

Methanol was first added at a methanol to nitrate-nitrogen (MeOH/N) ratio (mass basis) equal to 3 as recommended by EPA (1993) to ensure that carbon does not limit the reaction. However, the COD values were still high at the effluent ports prompting the researcher to lower the ratio. The MeOH/NO_3-N ratio was consequently reduced to 2.5

which conformed to stoichiometric values given by McCarty et. al. (1969) shown in Equation 2.1. Since the nitrite and DO concentrations were low compared to the nitrate concentration, a ratio of 2.5 was thought to be high enough to account for nitrite and DO. Assuming all influent COD is from methanol, the equivalent COD/NO_3-N would be 3.75.

Equation 2.1

$$\text{Methanol} = 2.47\ (NO_3\text{-}N) + 1.53\ (NO_2\text{-}N) + 0.87\ (DO)$$

On the other hand, departure of methanol requirements from Equation 2.1 is likely due to variations in biomass net yield. Equation 2.2 is an expression presented by EPA (1993) to estimate COD/NO_3-N under varying growth yield.

Equation 2.2

$$\frac{COD}{NO_3 - N} = \frac{2.86}{1 - 1.134 Y_{S_{NET}}}$$

where $Y_{S_{NET}}$ = biomass net yield based on COD, g VSS per g COD removed

2.3 PERFORMANCE STUDY

Knowledge of denitrification rates is essential for proper sizing of an attached-growth reactor (EPA, 1993). Although denitrification rates can be expressed in units of nitrate removed per unit of media surface area (as in nitrification rates reported for plastic media), in this study they will be expressed as mass of nitrate removed per bulk volume of media per day (g NO_3-N/m^3/day). The inherent difficulty in measuring the effective surface area of the wood chips and wheat straw prompted the researcher to settle for volumetric loading rates. On the other hand, Robertson et al (2000) reported that surface area did not affect the denitrification rates arguing that reaction rims penetrate into the carbonaceous solids and are not surface restricted to the grain surfaces.

2.3.1 INOCULATION / STARTUP

The bioreactors were filled up with nitrate-rich aquaculture wastewater from the NCSU Fish Barn and allowed to stand for 48 hours. The wastewater used for this procedure was taken from the NCSU Fish Barn effluent just before the retention pond. This effluent was thought to have substantial denitrifying bacterial cells after running through a sludge-settling tank. After 48 hours, the waste storage tank was filled with another batch of Fish Barn wastewater and circulated through the reactors in a closed loop, returning to the waste storage tank. That is, recirculation of the wastewater was continued until there was an indication of a substantial reduction in nitrate inside the waste storage tank. It was thought that recirculation would allow for bacterial acclimation. During this period, wastewater in the feed tank was amended through the addition of nitrate and methanol to prevent starvation of the bacteria. After recirculating for 9 days, feeding synthetic wastewater at concentration of 50 mg NO_3-N/L was begun.

2.3.2 VARIABLE NITRATE LOADING

Each reactor was fed with synthetic wastewater in concentrations of 50, 120, and 200 mg/L NO_3-N. These concentrations were fed at approximately 15 mL/min to the bioreactors, with approximately a month per concentration level. The sequential incremental increase of nitrate loading was thought to mimic the behavior of an aquaculture facility where nitrates are expected to rise as the fishes grow and mature and as the rearing water is recirculated for a longer period of time. Table 2.2 summarizes the nitrogen loading of the reactors under the various concentrations.

Table 2.2 Variable nitrate loading summary.

Concentration (mg NO_3-N/L)	**Flow rate** (mL/min)	**Total Hydraulic Loading-Water velocity** (cm/hr)	**Mass Loading** (g NO_3-N/day)	**Volumetric Loading** (g NO_3-N/m^3/day)	**Empty bed HRT** (hrs)
50 (LR1)	15	11.10	1.08	333	3.60
120 (LR2)			2.59	800	
200 (LR3)			4.32	1350	

Samples were collected at influent and effluent ports twice per week and submitted for nitrate-nitrogen analysis to the NC State University Department of Biological and Agricultural Engineering Environmental Analysis Lab (BAE-EAL). The rate of denitrification was computed using Equation 2.3.

Equation 2.3

$$DNR = 1440 \frac{F\left[(NO_3 - N)_i - (NO_3 - N)_e\right]}{V_M}$$

where: DNR = is the denitrification rate (g/m^3/day)
F = flow rate (mL/min)
V_M = bulk volume of the media (cm^3)
$(NO_3\text{-}N)_i$ = nitrate concentration at the influent (mg/L)
$(NO_3\text{-}N)_e$= nitrate concentration at the effluent (mg/L)

The pseudo-steady state denitrification rate for each loading rate was assumed when the denitrification rates were nearly constant (within 10%) over two weeks (at least 5 successive samplings). A near horizontal line on a graph of the denitrification rate vs. time indicated these pseudo-steady state conditions.

Samples were also extracted from each intermediate port when the reactors achieved a pseudo-steady state condition and analyzed for nitrate-nitrogen at each of the nitrate concentrations (50, 120, and 200 mg/L NO_3-N). Denitrification rates in each section were estimated by using Equation 2.3 and the bulk volume of the section considered.

2.3.3 Real aquaculture wastewater

At the end of the synthetic wastewater tests, a single test was done to determine the performance of the biofilters using real wastewater as feed. The wastewater was taken from NCSU Fish Barn, using the effluent after the drum screen filter and immediately before the sludge settling tank. It should be noted that the wastewater used in Section 2.3.1 is different from this phase of the study.

Two influent samples were taken – one at the start and one at the end of sampling. The time between these two samples was determined from the estimated average hydraulic retention time (HRT), in this case, around 2 hours. These two influent samplings were taken to try to account for any nitrate reduction activity in the tank during the sampling time.

Effluent samples were taken after one estimated HRT of each reactor. Thus, samples were taken at different times from the first influent sampling. The theoretical HRT's were different due to the different porosities of the bulk volume. The Kaldnes HRT was estimated to be approximately 2.75 hours while both wheat straw and wood chips were approximately 1.75 hours. These HRT's estimated the time needed for the fluid to pass through the porous reactor.

2.3.4 COD / Nitrate-N ratio

In order to assess the need for additional organic carbon, the COD/Nitrate-N ratio (COD/N) was calculated by comparing the COD removed per unit nitrate-nitrogen removed. For the real wastewater, Biochemical Oxygen Demand (BOD) and Total Organic Carbon (TOC) analyses were also performed to compare and assess the carbon content for a particular COD reading of the real wastewater. BOD samples were analyzed by TriTest Lab (Raleigh, NC) using EPA Method 405.1. Both COD and TOC were performed at BAE-EAL (Appendix A.1). TOC and BOD were not performed for the synthetic wastewater since relationships between COD and carbon of methanol is well known.

2.4 Effluent quality

Recognizing that two of the filter media are organic in nature, there is a potential for the reaction of the media with the wastewater. It was therefore important to examine the possibility of the introduction of constituents that are not desirable. Influent and effluent samples were collected for water analysis.

In addition to nitrate+nitrite-nitrogen (NO_3+NO_2-N), the influent and effluent were analyzed for total ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), COD, pH, DO and alkalinity. The chemical analyses were performed in the BAE-EAL. Table 2.3 shows a summary of the water analyses and frequency of sampling. A short description of methods used by the BAE-EAL is listed in Appendix A.1.

Table 2.3 Summary of water analyses done in this study.

Parameter	Sampling locations					Standard Methods #/ Instruments
	INF	Port A[1] (10 cm)	Port B[1] (20 cm)	Port C[1] (30 cm)	Port D Effluent (40 cm)	
NO_3-N	Twice a week	Once per level	Once per level	Once per level	Twice a week	4500-NO3-E (1998)
NO_2-N	Once a week	---	---	---	Once a week	4500-NO2-A (1998)
NH_3-N	Once a week	---	---	---	Once a week	4500-NH3-G (1998)
COD	Once a week	---	---	---	Once a week	5220 D (1998)
Alkalinity	Once a week	---	---	---	Once a week	2320 B (1998)
pH	At least once a week	Once at 200 mg/L level	Once at 200 mg/L level	Once at 200 mg/L level	At least once a week	pH meter WTW pH330i SET
ORP	At least once a week	Once at 200 mg/L level	Once at 200 mg/L level	Once at 200 mg/L level	At least once a week	pH meter WTW pH330i SET
DO	At least twice a week	---	---	---	Only the Final effluent	YSI 52 DO meter
Water Temperature	At least twice a week	---	---	---	At least twice a week	YSI52 DO meter

[1]*Intermediate ports were only sampled once after pseudo-steady state conditions were reached for each loading regime.*

NH_3-N was examined to determine if there was generation of ammonia in the reactor and effluent as a result of the biodegradation of the test media. The organic nature of the media may add carbon (in COD equivalent) to the bulk liquid due to biodegradation. This will result in lower net COD removal than plastic media. To assess this possibility, analysis of both the influent and effluent was performed.

Nitrite analysis was conducted to ascertain if complete denitrification was occurring inside the reactors. Alkalinity analysis served as check for denitrification activity (not simply filter adsorption) since stoichiometry indicates that an increase in alkalinity should take place with denitrification reactions.

The water temperature, pH and ORP of the influent and effluent were also monitored. Influent pH was maintained around 7 (6 to 7.9) while the laboratory room temperature was set around 20^{o}C and actual temperature recorded ranged from17 to 24.9^{o}C.

2.5 DEGRADATION OF FILTER MEDIA

One of the important characteristics of the wood chips and wheat straw as a biofiltration media is its apparent suitability as habitat for microorganisms. The wood chips and wheat straw's relatively rough surface facilitates entrapment and subsequent adsorption to form biofilm which can be estimated by porosity. However, wood chips and wheat straw are prone to biodegradation. Degradation of the media was evaluated by various methods described below.

2.5.1 MASS LOSS

Degradation of the media should not be too rapid. To estimate degradation, reduction in media mass was measured. Both wheat straw and wood chips were soaked in water for at least 24 hours. This enabled the media to swell to assume the moisture content and particle size that the media would have inside the wastewater-filled reactor. Reactors were filled with the swollen media. After securing the needed volume for each reactor, the media were taken from the reactors.

Before denitrification tests, the media were oven-dried at 70 - 104 ^{o}C for at least 24 hrs. The resulting oven-dry weight was recorded. The media were soaked again to test if it would be restored to its original volume. However, the bed heights were not the same as before (around 3.5 cm reduction), prompting the researcher to add more wood chips. The reduction of bed height may be attributed to the differences on the degree of compaction and the effect of shrinkage during drying. Extra wood chips were first dried before adding to the columns. Each mass addition was recorded. Prior measurements of oven-dry weights were amended to account for these additions.

In the case of wheat straw, some straws were taken from the reactor until the media had approximately the same dry weight. Straws were taken from heavier groups until it was within 1 gram of the lightest group. Removal of straw was done before placing it back in the reactors.

Upon the completion of the denitrification tests, the mass of the media was measured. The media were thoroughly washed until minimal biofilm was attached on the media. Then, the media were oven dried following the same procedure as before, and weighed. The mass loss provides a quantification of degradation of the media materials.

The appearance of the media was also observed during the course of the study. This included the change in color, and packed height of the columns.

2.5.2 POROSITY

A disadvantage of the biofilm processes is the tendency for excessive biomass growth (biofouling) and gas entrapment that could lead to clogging and headloss (Henze, 2000). Clogging and head loss can be assessed by measuring porosity. Porosity is a physical property which can be indirectly attributed to degradation, microbial solids and gaseous accumulation. An increase in porosity may mean that filter media have disintegrated, creating hollow spaces. On the other hand, denitrification evolves nitrogen gas that may accumulate in between media. A decrease of porosity may also indicate that solids may have been trapped and accumulated in the filter media bed.

The porosity of the reactor media before and after denitrification tests was determined. The packed bed was divided into four sections depending on the location of the sampling port. An empty reactor was filled with water keeping track of the volume occupied by the fluid in each part. It was first filled up to the center of the influent port. The water was then added at the rim of the bottom of the lower screen. This process continued up to each three sampling ports, to the upper screen and to effluent port. To countercheck, the upper third port was opened and the water collected was compared to the value obtained before. The middle port was then opened, followed by the lower and then influent ports.

The swollen (soaked) media was then poured into the reactors. The reactors were then filled and drained as before without the media. The collected water should correspond to the void volume. Porosity can be calculated by dividing void volume by the original (empty bed) volume.

2.5.3 FILTER MEDIA C/N RATIO

To support the assessment of degradation by weight loss, the C/N ratio of the filter media were determined. Dried wheat straw and wood chip samples were ground with a laboratory Wiley Mill (2 mm) and submitted to BAE-EAL for analysis (Appendix A.1). After the denitrification tests, samples of the media were taken from each section of the column height. Half of the dried samples were shredded and submitted to BAE-EAL for analysis. Media samples were selected making sure that each section of column was represented. Some ground samples were also set aside for lignin analysis.

2.5.4 LIGNIN AND OTHER CONSTITUENTS

Only one set (triplicate) of ground samples each for wood chips and wheat straw were analyzed to represent the initial concentrations of lignin and ash for each raw (new) media type. After the denitrification tests, however, triplicate samples from each reactor were taken for lignin and ash analyses.

2.5.4.1 ASH CONTENT

Clean and empty crucibles were placed in a muffle furnace at 500 ^{o}C for at least 30 minutes and placed in a desiccator and weighed and recorded as empty crucible. Three empty crucibles were filled each with approximately 1 gram of moisture free sample. Samples were ignited in a muffle furnace for at least 2 hours. Samples were then cooled in a desiccator and weighed. Ash content is determined using Equation 2.4.

Equation 2.4

$$\%\ \text{Ash Content} = \left(\frac{W_3 - W_1}{W_2}\right) \times 100\%$$

where W_1 *= empty crucible (g)*
W_2 *= moisture-free sample(g)*
W_3 *= ignited samples and crucible (g)*

2.5.4.2 ACID-INSOLUBLE LIGNIN

Ground samples (approximately 0.3 g) of wood chips and wheat straw were placed in 10 ml beakers and 3 ml of 72% H_2SO_4 were added to the beakers and mixed until samples were thoroughly wet with acid. Samples were allowed to hydrolyze for 2 hours, stirring every 15 minutes.

The hydrolyzate was transferred to a serum bottle and diluted to a 4% acid concentration by adding 84 ml deionized water. To ensure that all contents were transferred, 50 ml of water was first transferred to the serum bottle and the remaining 34 ml was used to rinse the beakers. Bottles were plugged with rubber stoppers and aluminum seals were crimped into place. Serum bottles were placed in an autoclave set to the liquid vent cycle for 1 hr at 121 ^{o}C. After allowing 20 minutes for cooling, seals were removed.

The hydrolysis solution was vacuum filtered through a dry crucible previously ignited in the furnace. The filtrate was saved for acid soluble lignin determination by storing in refrigerator at 4 ^{o}C. Remaining particles from the serum bottles were rinsed with hot deionized water. This was also used to wash the filtered residue free of acid. Crucibles with residue (containing acid-insoluble lignin and ash) were dried in an oven at 105 ^{o}C overnight. After cooling in a desiccator, its weight was recorded. Thereafter, it was placed in muffle furnace at 500 ^{o}C for at least two hours. What remained was the acid-insoluble ash. Acid-insoluble lignin was calculated using Equation 2.5.

Equation 2.5

$$\%\ \textit{acid-insoluble lignin} = \frac{W_2 - W_3}{W_1 \times (100 - \%\, moisture\, content)} * 100$$

where: W_1 = *initial sample weight(g)*
W_2 = *weight of crucible, acid-insoluble lignin and acid-insoluble ash(g)*
W_3 = *weight of crucible and acid-insoluble ash (g)*

2.5.4.3 Acid-Soluble Lignin

Filtrate obtained from the acid insoluble lignin test was used for this analysis. A reference blank diluted to 4% by adding 84 ml deionized water to 3 ml 72% H_2SO_4 was prepared as reference blank for spectrophotometer.

After setting up and calibrating the spectrophotometer, absorbance of the hydrolyzate was measured at 205 nm, using a 1-cm light path cuvette. The hydrolyzate was diluted in order for absorbance not to exceed 1.0.

Acid-Soluble Lignin (ASL) was determined with Equation 2.6.

Equation 2.6

$$\%ASL = \frac{\frac{A}{b*a} * df * V * \frac{Liter}{1000mL}}{\frac{W * T_{final}}{100}} * 100$$

where: A = absorbance at 205 nm
df = dilution factor
b = cell path length, 1 cm
a = absorptivity, equal to 110 L/g-cm
V = filtrate volume, 87 mL
W = initial biomass sample weight
T_{final} = % total solids content of biomass sample

2.6 Microbial Mass and Preliminary Characterization

2.6.1 Microbial Community

Differences in bacterial community were determined by comparing the different ribotypes as imprinted on gels generated using polymerase chain reaction – denaturing gel electrophoresis (PCR-DGGE). Only three reactors were considered for this analysis, one for each media type. Biomass samples were taken from the intermediate ports A, B, C. Samples were placed in centrifuge tubes, sealed and placed in an ice bath. These samples were submitted to Wastewater Biotechnology and Process Engineering Research Group Lab at the Department of Civil, Construction, and Environmental Engineering of NCSU.

2.6.2 BIOMASS DEVELOPMENT

At the end of the experiments, the drained water from void determination was poured into a bucket. After removing media from the reactors, the media were washed with deionized water. To ensure that none of filter media is mixed with the biomass, the solution was sieved (2 mm). With the wood chips, biomass was also brushed and scraped off the media.

The washwater (suspended biomass diluted with deionized water used to wash the filter media) was analyzed for Total Solids (TS), Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), and Volatile Solids (VS) at BAE-EAL. The results were adjusted taking into account the initial volume of water before and after washing. The initial water represented the water that was drained from the reactors during porosity determination.

3. BIOFILTER DENITRIFICATION PERFORMANCE

3.1 DENITRIFICATION USING SYNTHETIC WASTEWATER

Nitrate+nitrite-nitrogen (NO_3+NO_2-N) values will be discussed separately for each reactor type before comparing the results with other reactor types. Comparisons are presented based on the average effluent concentration (mg NO_3+NO_2-N/L), reduction percentages, and denitrification rates (g NO_3+NO_2-N/m^3/day). Two sets of data are presented in this case: (a) overall data considering all time points and (b) pseudo-steady state considering the last five time-points in each load (except for November 10 at 200 mg NO_3+NO_2-N/L). The pseudo-steady state points were selected as defined in Section 2.3.2. Hereinafter, the term nitrate (NO_3-N) shall refer to nitrate + nitrite-nitrogen (NO_3+NO_2-N).

3.1.1 NITRATE REDUCTION IN KALDNES MEDIA REACTORS

Table 3.1 gives the average nitrate effluent concentration as well as its corresponding percent reduction at the different loading rates (LR). In most instances, the average effluent concentrations are consistent for the three reactors. Examining Figure 3.1, there were at least two instances wherein reactor 3 (R3) had higher effluent values than the other two Kaldnes reactors. This can be attributed to a higher flow rate (increased load) during those particular sampling periods (17 mL/min (October 4) and 16 mL/min (November 3)). “Spikes” were also attributed to some instances of COD influence, notably on October 20 and November 10. Details of the COD influence will be discussed in Section 3.5. However, examining percentage of reductions in Table 3.1

reveals that the Kaldnes reactors' performance in reducing NO_3-N was 90% or more for all three loading rates for pseudo-steady state period.

The mean effluent NO_3-N concentrations under pseudo-steady state were generally lower than the overall mean. As seen in Figure 3.1, high effluent NO_3-N were observed in the early stages of a loading rate while lower values were obtained under pseudo-steady state points. The reduction percentages were higher under pseudo-steady state points than considering all points (Table 3.1).

Table 3.1 Mean NO_3-N effluent concentrations and percent reduction (Kaldnes media).

Influent feed (mg NO_3-N/L±s.d.)	Effluent Concentration (mg NO_3-N/L± s.d.)				Percentage Reduction (%±s.d.)			
	R1	R2	R3	Mean (RKM)	R1	R2	R3	Mean (RKM)
Overall								
48.0±7.1 (LR1)	11.17 ±13.39	10.91 ±13.12	11.02 ±11.83	11.03 ±12.28	75.89 ±28.70%	76.33 ±28.35%	75.69 ±25.70%	75.97 ±26.53%
120.2±7.9 (LR2)	16.84 ±13.24	19.74 ±16.90	22.55 ±16.74	19.71 ±14.99	86.23 ±10.75%	83.65 ±14.28%	81.51 ±13.93%	83.79 ±12.51%
203.5±10.2 (LR3)	12.81 ±23.83	16.97 ±27.58	24.79 ±24.91	18.19 ±23.83	93.44 ±12.18%	91.55 ±13.82%	87.47 ±12.74%	90.82 ±12.17%
Pseudo-steady state								
51.8±5.8 (LR1)	5.25 ±7.70	5.08 ±2.49	5.04 ±3.67	5.13 ±2.80	89.51 ±15.03%	89.81 ±5.23%	89.86 ±7.71%	89.73 ±5.76%
120.4±9.6 (LR2)	10.07 ±9.33	9.02 ±7.67	16.13 ±13.34	11.74 ±9.66	91.90 ±7.33%	92.66 ±6.27%	87.13 ±10.04%	90.56 ±7.50%
203.6±10.4 (LR3)	0.06 ±0.05	0.17 ± 0.06	13.12 ±17.43	4.45 ±5.83	99.97 ±0.03%	99.92 ±0.03%	93.36 ±8.77%	97.75 ±2.93%

With increased loading, denitrification rates (Figure 3.2) also increased. After startup on July 26, the rate of denitrification was low on August 2 but showed a steady increase until August 16 when rates started to become more or less stable (Figure 3.2). This trend also occurred on September 13 when the influent was again increased, this time from 50 to 120 mg NO_3-N/L.

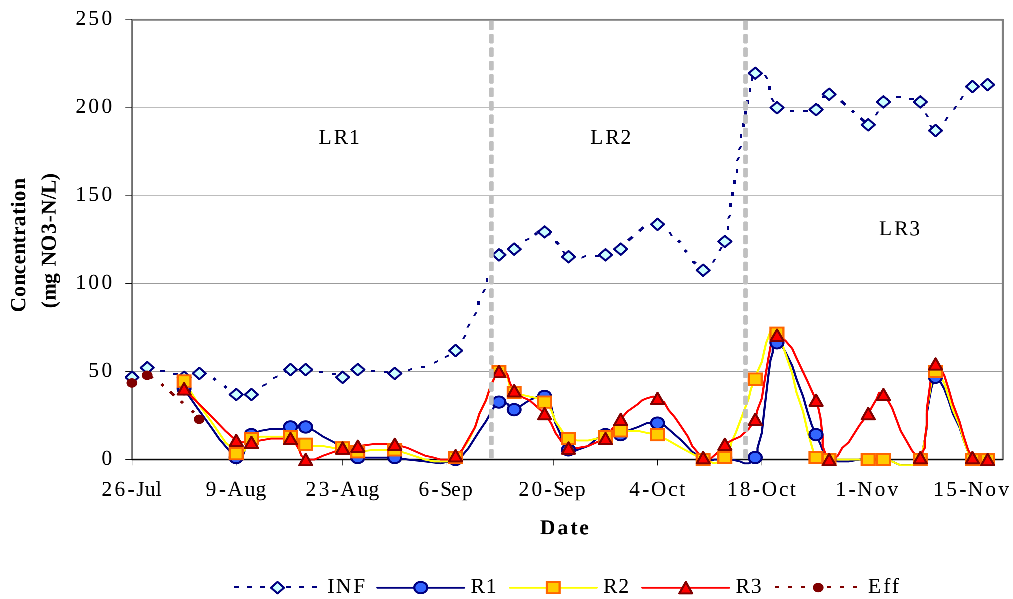

Figure 3.1 Influent and effluent NO_3-N concentrations of Kaldnes reactors for three loading rates (LR) separated by vertical broken lines. "Eff" NO_3-N represents the samples taken at the overall effluent of all nine reactors.

At LR3, the reactors responded well, possibly due to mature biomass in each reactor. However, the reactors had some problems due to low COD input on October 20 and November 10 (Section 3.5). This is shown in Figure 3.2 as two steep drops in the denitrification rates at the input concentration LR3. Pseudo- steady state averages were 313 ± 45, 743 ± 36, 1326 ± 74 g NO_3-N/m^3/day for LR1, LR2 and LR3, respectively.

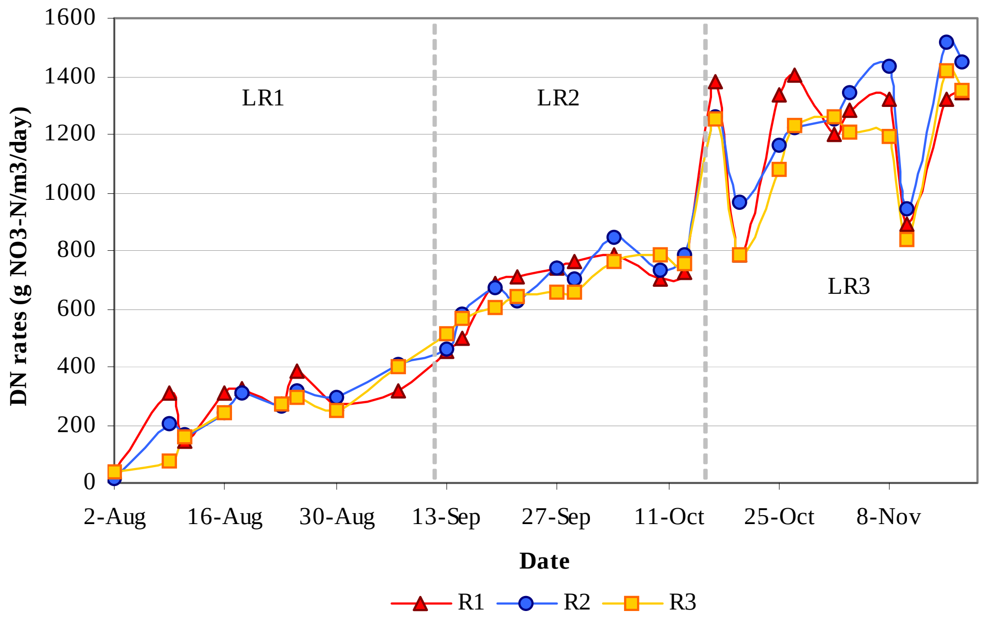

Figure 3. 2 Denitrification (DN) rates of the three Kaldnes media reactors for three loading rates.

3.1.2 NITRATE REDUCTION IN WOOD CHIPS REACTORS

The overall average reduction of NO_3-N for all three loading rates averaged approximately 94% (Table 3.2). The average overall effluent concentrations, however, increased slightly from 3 mg NO_3-N / L to 10 mg NO_3-N /L as the influent concentration increased. Considering pseudo-steady state, the mean effluent NO_3-N concentrations were relatively lower in all loading rates. The mean effluent NO_3-N concentrations at LR3 were even less than 1 mg NO_3-N/L when considering only steady-state points.

Results displayed in Figure 3.3 indicate that the wood chip reactors responded well to higher loading as evidenced by minimal spikes at the start of each increased loading period. Figure 3.4 shows the trend of the denitrification rates of the wood chip reactors. Except for the LR3, rates rose immediately with increased concentration and stabilized thereafter. On average, the pseudo-steady state denitrification rates at LR1, LR2, and LR3 were 333 ± 32, 760 ± 46, 1365 ± 39 g NO_3-N/m^3/day, respectively.

Table 3.2 Mean NO_3-N effluent concentrations and percent reduction (wood chips reactors).

Influent feed (mg NO_3-N/L±s.d.)	Effluent Concentration (mg NO_3-N/L± s.d.)				Percentage Reduction (%±s.d.)			
	R1	R2	R3	Mean (RKM)	R1	R2	R3	Mean (RKM)
Overall								
48.0±7.1 (LR1)	4.04 ±6.19	2.51 ±2.86	2.89 ±3.50	3.15 ±3.79	91.73 ±13.03%	94.69 ±5.91%	94.09 ±6.99%	93.50 ±7.80%
120.2±7.9 (LR2)	7.42 ±7.57	7.70 ±5.49	4.66 ±6.07	6.59 ±5.54	93.91 ±5.89%	93.64 ±4.62%	96.16 ±5.10%	94.57 ±4.49%
203.5±10.2 (LR3)	10.60 ±20.90	10.51 ±20.25	9.10 ±18.47	10.07 ±19.85	94.56 ±10.75%	94.62 ±10.37%	95.31 ±9.52%	94.83 ±10.20%
Pseudo-steady state								
51.8±5.8 (LR1)	3.06 ±4.73)	1.14 ±1.59	2.17 ±3.67	2.12 ±3.33	94.04 ±9.22%	97.78 ±3.09%	95.78 ±7.16%	95.87 ±6.48%
120.4±9.6 (LR2)	4.20 ±4.44	5.24 ±3.38)	0.81 ±1.23)	3.42 ±2.39)	96.46 ±3.81%	95.69 ±2.87%)	99.37 ±0.91%	97.17 ±2.05%
203.6±10.4 (LR3)	0.31 ±0.18	1.13 ±0.55	0.30 ±0.54	0.58 ±0.30	99.85 ±0.10%	99.44 ±0.28%	99.84 ±0.29%	99.71 ±0.16%

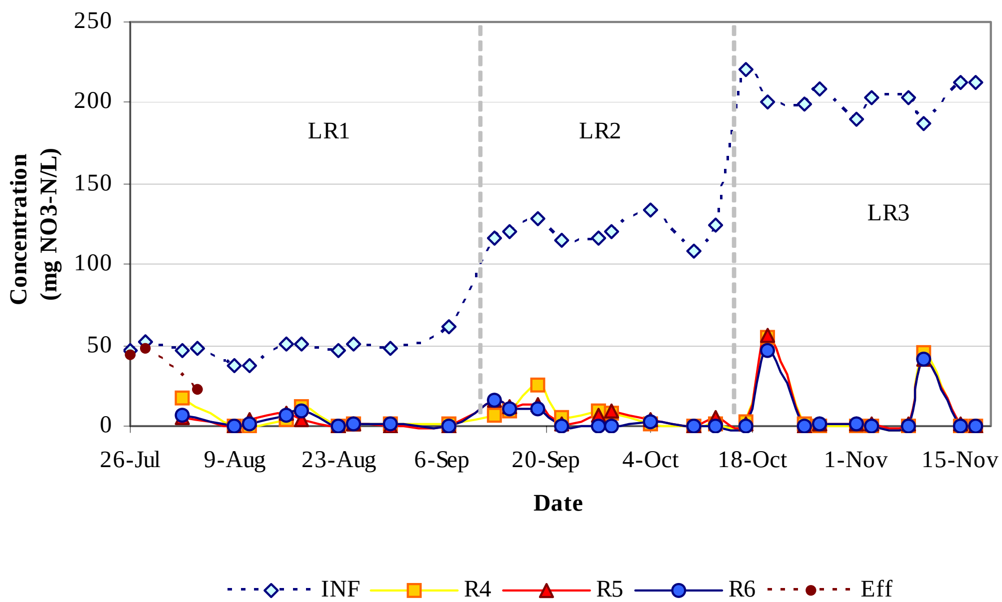

Figure 3. 3 Influent and effluent NO_3-N concentrations of wood chips reactors.

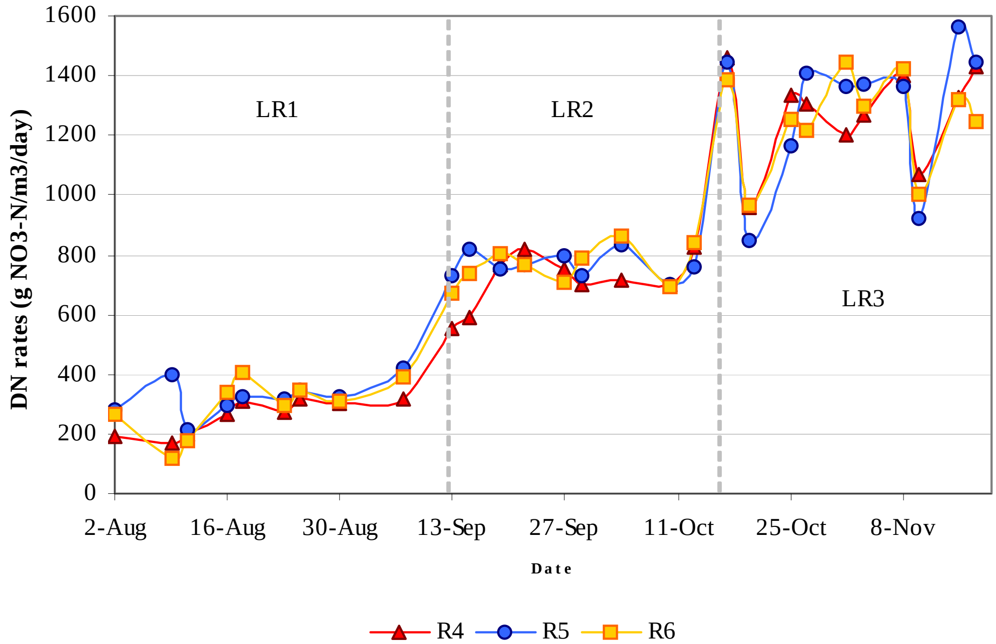

Figure 3. 4 Denitrification (DN) rates of the three wood chips reactors for three loading rates.

3.1.3 NITRATE REDUCTION IN WHEAT STRAW REACTORS

With increased loading, the average effluent concentration of NO_3-N in wheat straw reactors also increased slightly from 6 to 11 mg / L (Table 3.3). However, the LR3 had increased values on October 20 and November 10 as previously mentioned for the other reactors (Figure 3.5). The three reactors' effluent concentrations were relatively close, except for three instances where a reactor had a higher or lower effluent value than the others (Figure 3.5).

Under pseudo-steady state conditions, the mean effluent NO_3-N concentrations were very low compared to overall data. This shows that much of the higher values happened at the early or transition period.

Table 3.3 Mean NO_3-N effluent concentrations and percent reduction (wheat straw reactors).

Influent feed (mg NO_3-N/L±s.d.)	Effluent Concentration (mg NO_3-N/L± s.d.)				Percentage Reduction (%±s.d.)			
	R1	R2	R3	Mean (RKM)	R1	R2	R3	Mean (RKM)
Overall								
48.0±7.1 (LR1)	5.97 ±10.31	6.38 ±8.03	4.77 ±6.60	5.71 ±7.62	87.64 ±22.03%	85.61 ±19.27%	89.63% ±14.32%	87.63% ±16.54%
120.2±7.9 (LR2)	4.99 ±5.41	4.98 ±5.61	6.98 ±11.86	5.65 ±5.65	95.88 ±4.57%	95.95 ±4.54%	94.09 ±10.20%	95.31 ±5.95%
203.5±10.2 (LR3)	13.60 ±20.95	9.91 ±20.63	10.44 ±19.94	11.32 ±20.23	93.16 ±10.65%	94.89 ±10.64%	94.65 ±10.31%	94.23 ±10.42%
Pseudo-steady state								
51.8±5.8 (LR1)	1.90 ±2.04	1.65 ±3.38	1.63 ±3.47	1.73 ±2.86	96.27 ±3.98%	96.78 ±6.59%	96.82 ±6.76%	96.63 ±5.56%
120.4±9.6 (LR2)	1.85 ±1.25	1.87 ±2.57	1.56 ±0.59	1.76 ±1.29	98.51 ±0.90%	98.52 ±1.98%	98.71 ±0.47%	98.58 ±0.96%
203.6±10.4 (LR3)	0.34 ±0.22	0.02 ±0.01	0.07 ±0.09	0.14 ±0.09	99.83 ±0.12%	99.99 ±0.00%	99.97 ±0.04%	99.93 ±0.05%

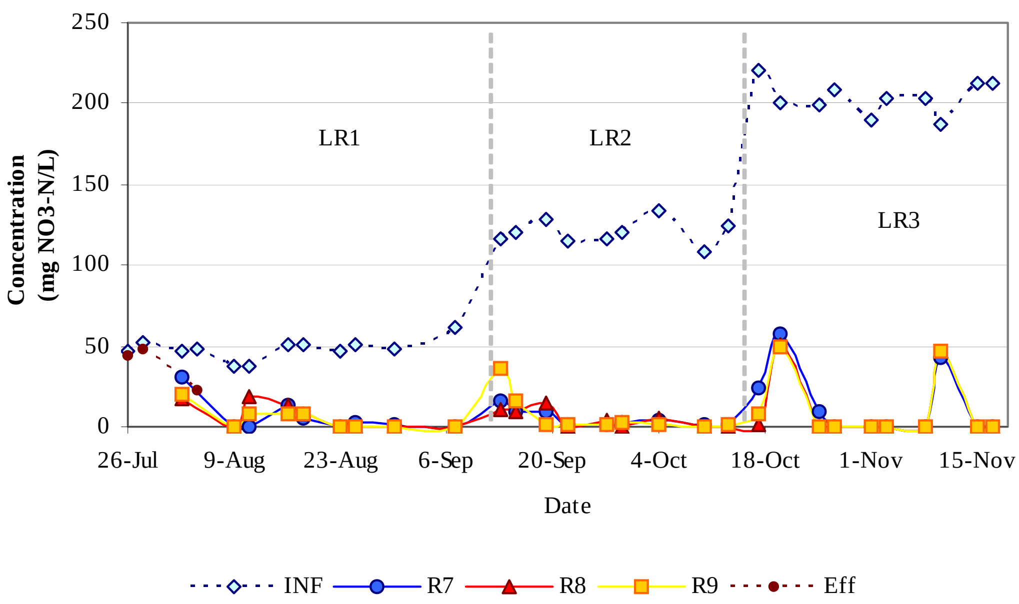

Figure 3. 5 Influent and effluent NO_3-N concentrations of wheat straw reactors.

On August 11, reactor 7 had no nitrate-nitrogen in the effluent which can be attributed to a 10 mL/min flow rate and influent concentration of 37.3 NO_3-N mg/L instead of the target flow rate of 15 mL/min and influent concentration of 50 mg NO_3-

N/L. On September 13, the effluent nitrate of reactor 9 was much higher than other wheat straw reactors. This was due to a higher flow rate of 16.6 mL/min. Reactor 7 when compared with the other wheat straw reactors did not respond well on October 17 (an effluent concentration of 24.72 mg NO_3-N /L) when the nitrate load was increased from 120 to 200 mg/L.

Figure 3.6 shows the denitrification rates over time and with varying nitrate loadings. The mean pseudo steady-state denitrification rates of these reactors were calculated to be 328 ± 42, 806 ± 42, 1361 ± 80 g NO_3-N/m^3/day at LR1, LR2, and LR3 respectively.

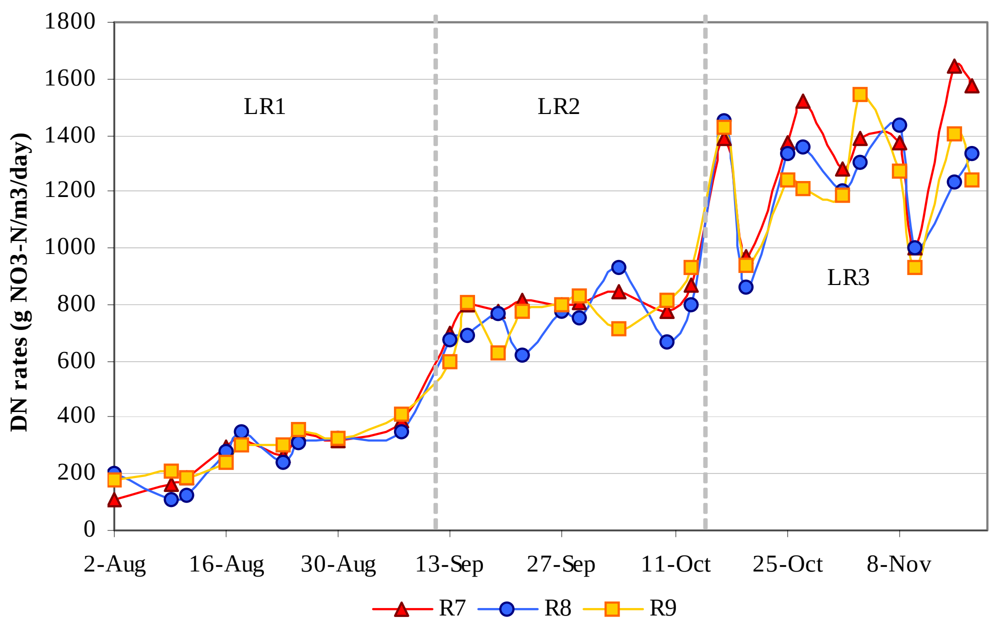

Figure 3. 6 Denitrification (DN) rates of the three wheat straw reactors.

3.1.4 COMPARISON OF DENITRIFICATION RATES AMONG REACTOR TYPES

Except for the last few weeks of these experiments, the results of this study show that the Kaldnes media usually allowed slightly higher effluent NO_3-N concentrations values (Figure 3.7) than the other media types. During the final weeks, the effluent concentrations were very similar. Biomass appeared to develop more slowly in the Kaldnes media reactors (to be discussed in Chapter 5). At the start of the experiments, nitrate effluent values for the Kaldnes media did not decrease as quickly as with other two reactor types. The Kaldnes media responded more slowly to increased loads. Effluent NO_3-N concentrations surged when higher concentrations were introduced.

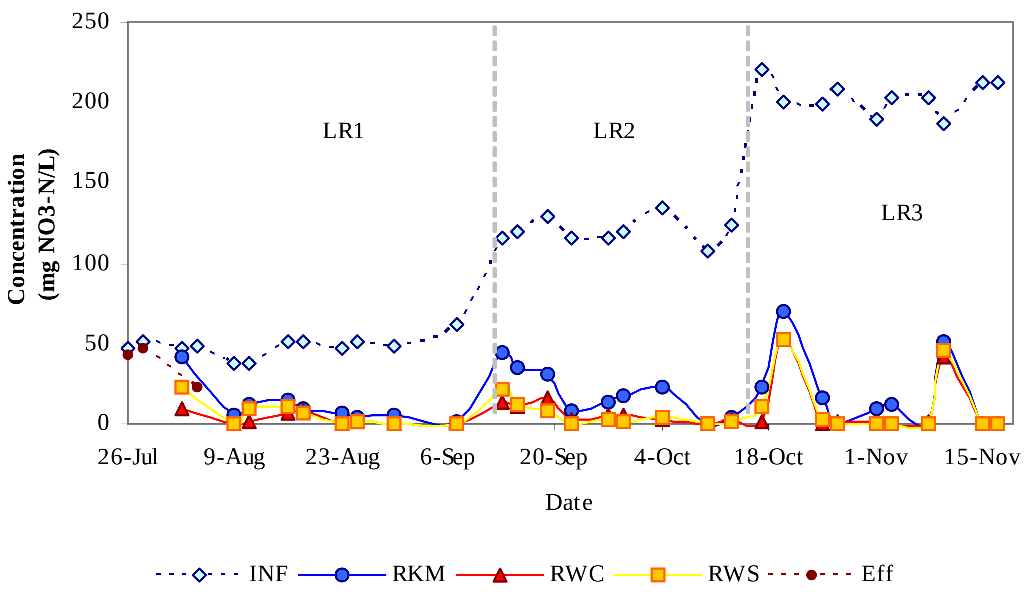

Figure 3. 7 Mean influent and effluent NO_3-N concentrations of three reactor types.

Comparing the effluent from the two organic medias – wood chips and wheat straw, the nitrate concentrations similar. To further analyze these findings, the nitrate values were translated to denitrification rates to account for influent nitrate, media volume, and flow rates.

Split-plot analyses were carried out for each loading rate using overall and pseudo-steady-state conditions. Split-plot analysis was employed to account for variation due to repeated measurements. Since multiple samples were taken over time on each reactors, it was thought that analysis would fall on split-plot – a repeated measures analysis (Rao, 1998). The analyses considered media type as whole-plot factor and days as within-plot factor. The reactors which were nested on each corresponding media type were the whole-plot units. Analyses were carried out using PROC MIXED on SAS 8.2 software (SAS Institute, Cary, NC).

At LR1, only Kaldnes media and wood chips showed significant difference ($p = 0.05$) in denitrification rates when the overall data were considered. It can be inferred that wood chips performed better than Kaldnes, but not better than wheat straw at this particular LR. Under steady state conditions, however, analysis of variance (ANOVA) showed no significant difference among reactor types ($p = 0.38$). Since pseudo-steady state considered the last five data points, it can be said that differences (overall) were due to effect of startup.

On the other hand, ANOVA at LR2 indicated differences among reactor types for both overall ($p = 0.01$) and pseudo-steady state ($p = 0.02$) conditions. Least square means of denitrification (overall) rates for wheat straw and wood chips exceeded Kaldnes at $p = 0.0029$ and $p = 0.0089$, respectively. Overall denitrification rates of wheat straw and wood chips were not significantly different ($p=0.34$). But considering pseudo-steady state condition, only wheat straw showed significant difference over the other two media (wood chips, $p = 0.03$; Kaldnes, $p = 0.01$). The relatively lower performance of Kaldnes was more pronounced during the transition period (data points near the vertical line separating LR1 to LR2 in Figure 3.8). However during the pseudo-steady state conditions, Kaldnes had developed comparable performance with wood chips ($p = 0.32$).

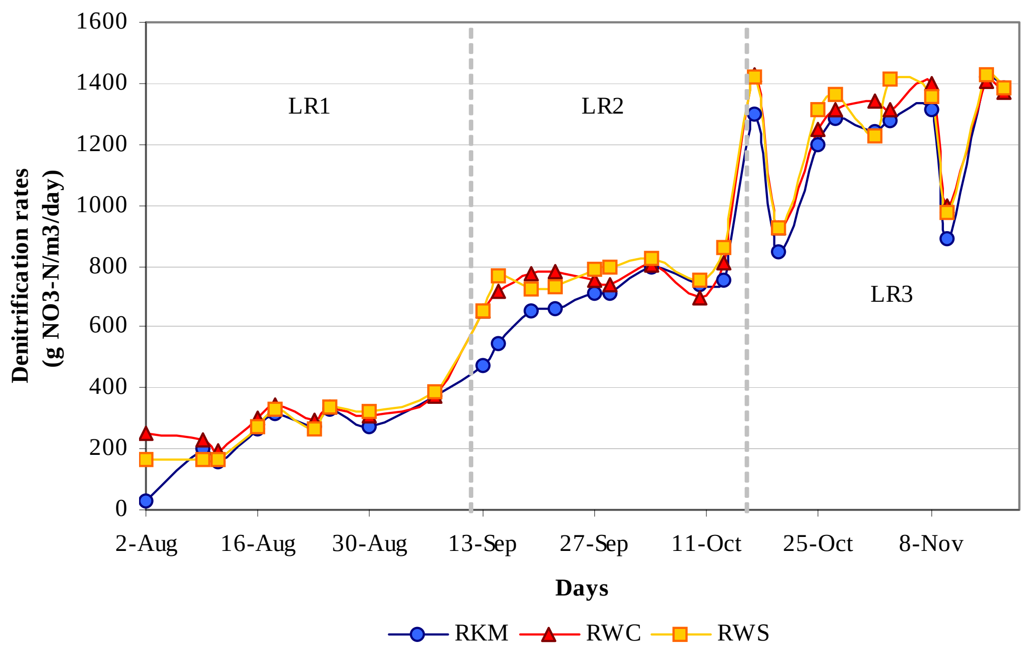

Figure 3. 8 Estimated denitrification (DN) rates of the three reactor types for three loading rates (LR).

During much of LR3, the denitrification rates of Kaldnes media reactors were similar to the other media types. ANOVA for both overall and pseudo-steady state conditions showed no significant difference among reactor types (overall, $p = 0.23$; pseudo-steady state, $p = 0.74$). This could be attributed to the possibility that the denitrifying biomass have already developed with age and thus, Kaldnes performance caught up.

Statistical analysis as well as Figure 3.8 confirmed that generally Kaldnes media were slow in adapting to new loads (transition) and startup but was comparable in performance under pseudo-steady state conditions. The slow adaptation of bacteria on the Kaldnes media favors wood chips and wheat straw. The organic nature of wood chips and wheat straw may have enhanced bacterial growth on their surfaces. Perhaps, the inherent nutrient availability on these surfaces makes it attractive to bacterial adhesion.

Moreover, age of the reactor seems to influence the performance of the reactors. In all instances (all LRs and both overall and pseudo-steady state), “days” factor explained much of the variation. This can be related to differences during startup, transition period and seemingly better performance of all reactor types during the later stages of the experiments.

3.2 NITRATE PROFILE ALONG COLUMN HEIGHT

To further quantify denitrification potential of the each reactor type, nitrate concentration profiles were taken by analyzing samples of water at each intermediate port – A (10 cm), B (20 cm) and C (30 cm) on one date for each loading rate after the reactor reached steady state performance. These results were added to the influent and effluent values reported in Section 3.1 to complete the picture. Denitrification rates were also computed for each section considering the media volume and nitrate removed at each section.

3.2.1 NITRATE PROFILE IN KALDNES MEDIA

On average, 83% of the nitrates were eliminated by the time the water reached 20 cm high port during sampling for LR1 (Fig 3.9). With influent concentrations of 120 mg/L nitrate-nitrogen, approximately 54 % of the influent nitrate was removed at 10 cm with 67% removed by the 20 cm port. However, only a small percentage was removed thereafter. As discussed in Chapter 5, minimal biomass was formed on the upper portion of the columns. This may explain the reduced removal in the upper sections. The reactors with Kaldnes media performed well with influent concentrations of 200 mg NO_3-N /L. Approximately 71 % of the NO_3-N was removed in the first 10 cm, and about 91% removed before the 20 cm sampling port.

The improved performance at LR3 is attributed mostly to improved nitrate reduction in the lower half of the reactors.

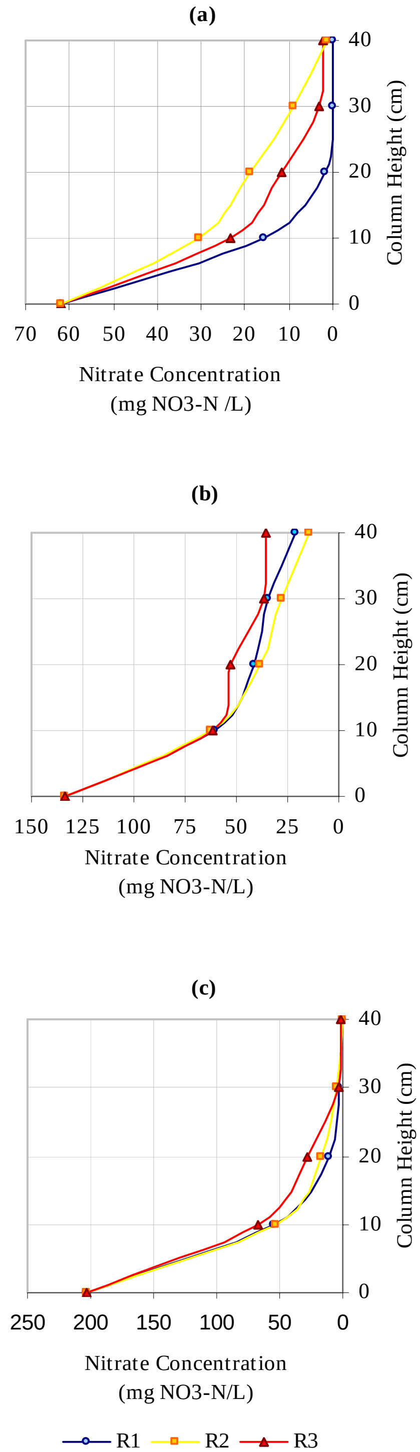

Figure 3. 9 Nitrate profile of Kaldnes media reactors at three loading rates: (a) LR1, (b) LR2, and (c) LR3.

Nitrate reduction in the upper half did not appreciably improve compared to the previous influent treatment (120 mg NO_3-N/L).

Calculated denitrification rates (Figure 3.10) indicate that much of the denitrification activity occurred in the lowest quarter of the reactor. The rates decreased moving up the reactor. Looking at sectional denitrification rates, one can conclude that rates as high as 3800 g/m^3/day can be achieved with Kaldnes media. This is higher compared to 1326 ± 74 g NO_3-N/m^3/day when considering nitrate removal rates of the whole reactor.

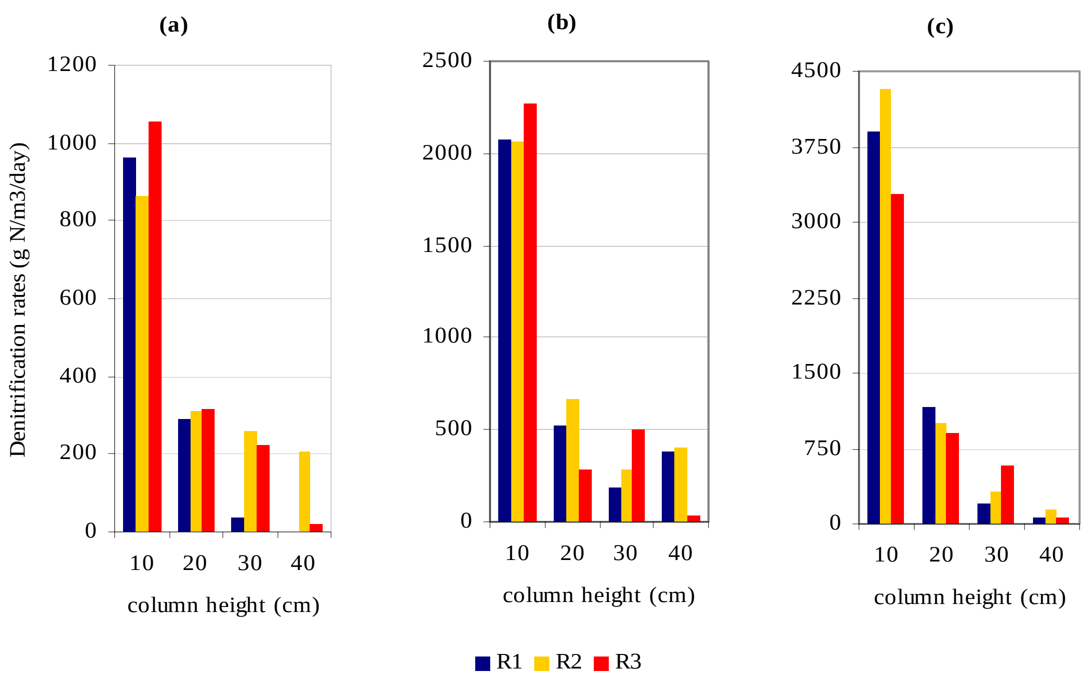

Figure 3. 10 Estimated denitrification rates in each section of the Kaldnes reactors at each nitrate loading: (a) LR1, (b) LR2, and (c) LR3.

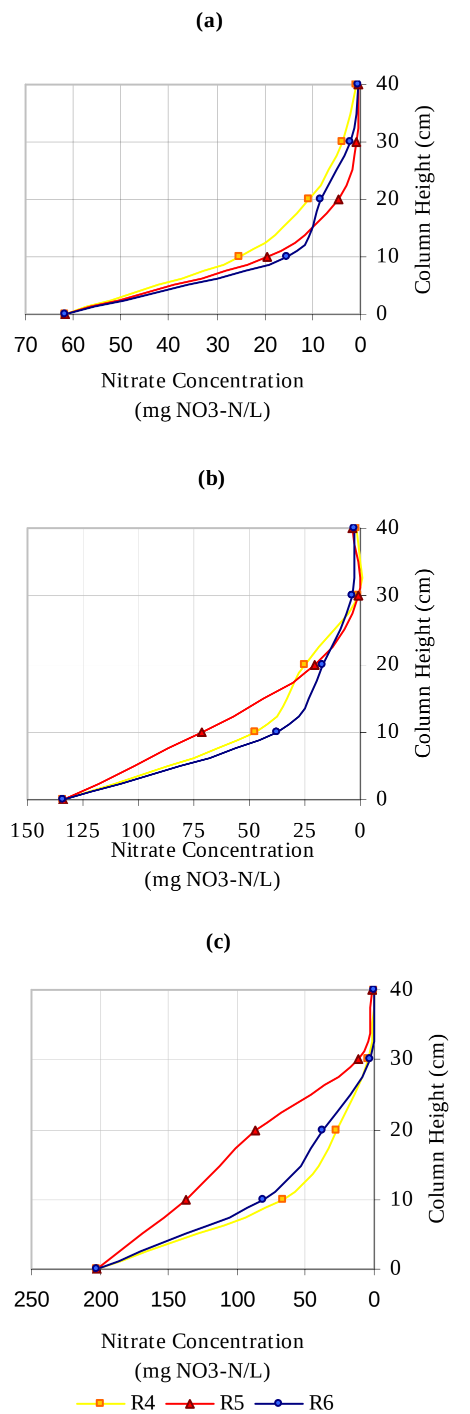

Figure 3. 11 Nitrate profile of wood chips reactors at each nitrate loading: (a) LR1, (b) LR2, and (c) LR3.

3.2.2 NITRATE PROFILE IN WOOD CHIPS REACTORS

Nitrate reduction averaged approximately 67% in the first 10 cm and about 87% in the first 20 cm of the reactors with wood chip media at LR1. Removal of nearly 100% of influent nitrate at 50 mg NO_3-N/L influent concentration proved that this media can treat higher nitrate loading rates.

When the reactors were fed with 120 mg NO_3-N/L, nitrate reduction averaged about 61% by the 10 cm sampling port and about 84% by 20 cm port (Figure 3.11). As with the LR1, the nitrate removal was greater than 95% by 30 cm with LR2. With the 200 mg NO_3-N/ L influent concentration, nitrate reduction averaged 53% by 10 cm and 75% by 20 cm. As with other loading rates, nitrate removal was greater than 95% by 30 cm.

The nitrate reduction for R5 was

lower compared to other results particularly on ports A and B. There is the possibility that channeling or short-circuiting of fluid directly to sampling ports occurred. This is more probable with greater biomass buildup. However, even if this event would occur in the field application the remaining bulk media above can still remove any highly concentrated waste effluent bypassing a particular section. In Figure 3.12, the lowest section of reactor 5 had not improved much in terms of denitrification rates from 1163 to 1625 and to 1816 g NO_3-N/m^3/day while other reactors surged by more than 3000 g NO_3-N /m^3/day.

While the lowest section had not improved much, other sections provided the denitrification needed to come up with a good nitrate effluent for the reactor as a whole. Generally, these reactors followed a pattern of tapering denitrification rates from very high rates to lower rates moving up the effluent ports.

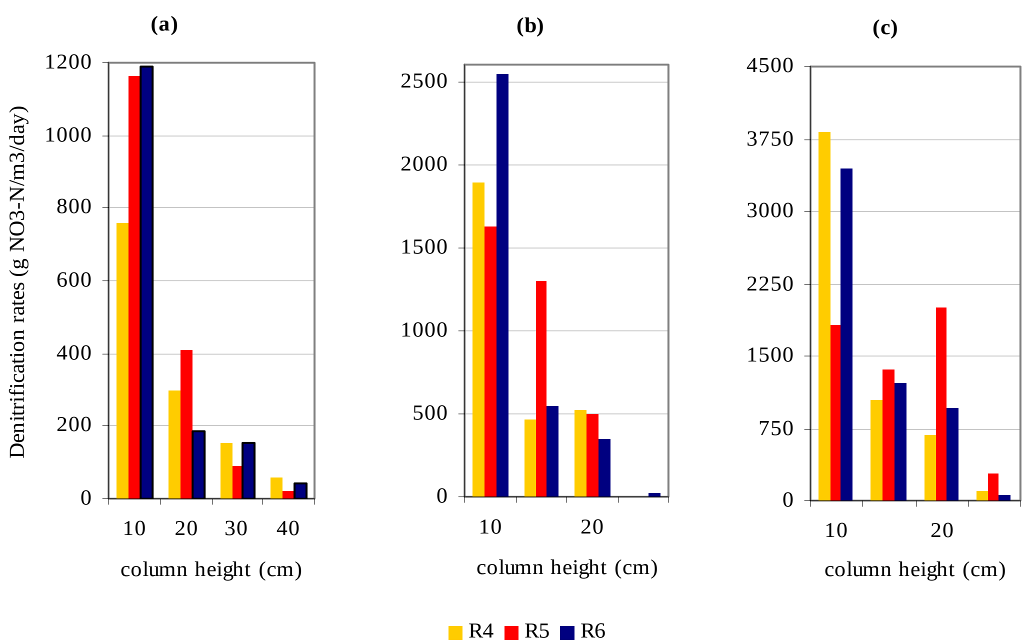

Figure 3. 12 Estimated denitrification rates in each section of the wood chips reactors at each nitrate loading: (a) LR1, (b) LR2, and (c) LR3.

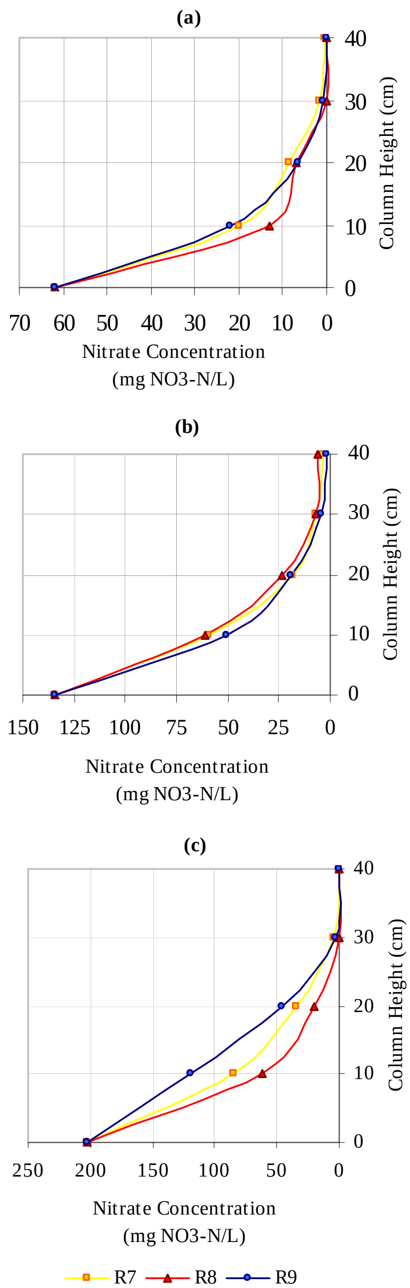

Figure 3. 13 Nitrate profile of wheat straw reactors at each nitrate loading: (a) LR1, (b) LR2, and (c) LR3.

3.2.3 NITRATE PROFILE IN WHEAT STRAW REACTORS

On average, about 70% of the influent NO_3-N was removed within the 10 cm of these reactors at LR1. Approximately 80% was removed within 20 cm and 98% within 30 cm. Nitrate concentration profiles with 120 mg NO_3-N /L influent concentrations were consistent between reactors throughout the total column height as seen in Figure 3.13. On average, 57% of NO_3-N was removed in the first 10 cm. Within the first 20 cm, 85% were removed. More than 95% were removed within 30 cm. This suggests that the reactors had the capacity to treat nitrate loading higher than 120 mg/L.

An average of 50% of NO_3-N influent were removed within the first 10 cm under LR3. Similar to LR2, more than 95% were removed within the first 30 cm. It can be seen that reactor 9 had 119 mg NO_3-N/L at the 10 cm sampling port. Biomass buildup at this section may have caused short-circuiting that resulted in apparent lower nitrate removal.

Looking at the calculated denitrification in each section (Figure 3.14), the first 10 cm of reactor 9 was low. Even though reactor 9 has the lowest denitrification at this section, it made up its shortcoming by having better rates at the end of 20 and 30 cm. This can be seen as higher bars compared to R7 and R8 on LR3 on Figure 3.14. Denitrification rates of reactor 7 and 8 showed a sharp decline of rates up the column.

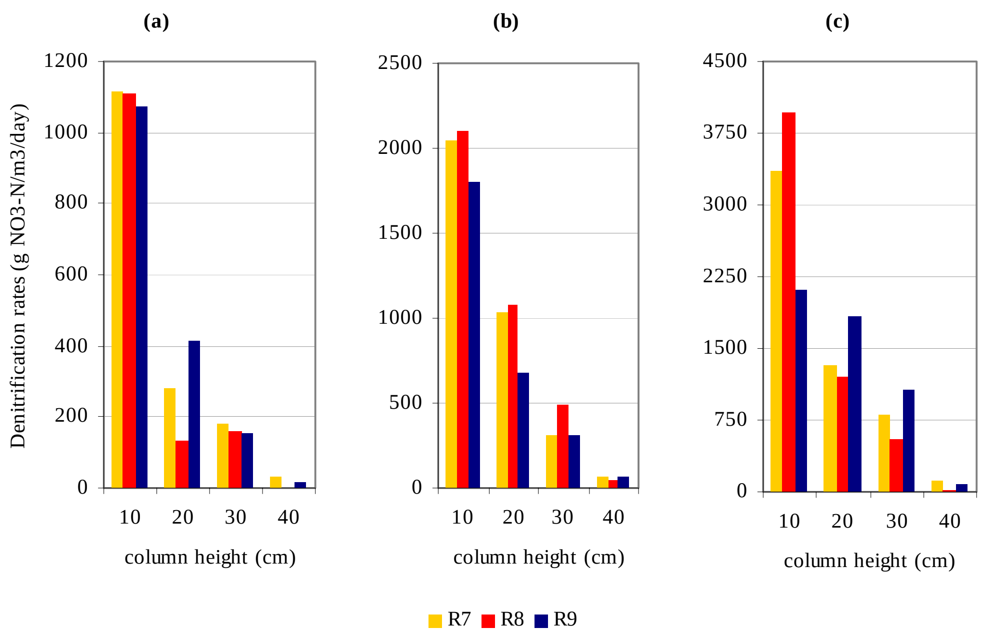

Figure 3. 14 Estimated denitrification rates in each section of the wheat straw reactors at each nitrate loading: (a) LR1, (b) LR2, and (c) LR3.

3.2.4 Comparison of Nitrate Profiles

The trends of the nitrate reduction upward through the reactors followed the same trend for all media types at LR1. As shown in Figure 3.15, the Kaldnes media profile line is situated slightly to the left suggesting that Kaldnes did not remove nitrates as well compared to other reactor types at this nitrate concentration. Within the first 10 cm, the denitrification rate for Kaldnes (959 ± 96 g NO_3-N /m^3/day) was lower than the other two reactor types (wood chips, 1037 ± 242 g NO_3-N /m^3/day; wheat straw, 1102 ± 23 g NO_3-N /m^3/day). At this particular nitrate loading rate, the performance of the first section determined the overall performance. This high performance can also be attributed to higher NO_3-N entering a particular section. With lower NO_3-N concentration entering the upper section, the rates of denitrification also declined. Table 3.4 shows that less reduction happened on the 20-cm and upwards compared to the first 10 cm.

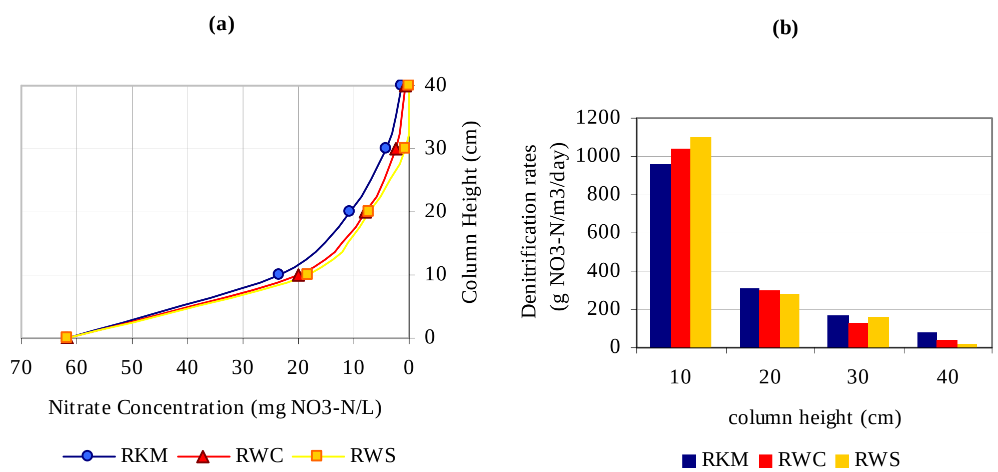

Figure 3. 15 (a) Nitrate profiles of different reactors and the (b) corresponding denitrification rates at LR1 (September 7, 2004).

Table 3.4 Average percent NO_3-N reduction along column height under LR1.

Port (cm)	**Percentage reduction of influent NO_3-N**		
	RKM	RWC	RWS
10 (A)	62.28 ± 11.80%	67.33 ± 8.18%	70.43 ± 7.67%
20 (B)	82.62 ± 13.87%	87.24 ± 5.33%	88.03 ± 1.95%
30 (C)	93.38 ± 7.41%	96.32 ± 2.03%	98.68 ± 1.22%
40 (D)	97.90 ± 1.80%	99.15 ± 0.38%	99.75 ± 0.25%

At LR2, the Kaldnes media had the highest mean denitrification rate in the first 10 cm section (Kaldnes, 2137 ± 120g NO_3-N/m^3/day; wood chips, 2019 ± 469 g NO_3-N/m^3/day;

wheat straw, 1982 ± 159 g NO_3-N /m^3/day). The higher flowrate (16 mL/min) on this date contributed to high denitrification rate although there was lower reduction percentage (Table 3.5). The rate of denitrification of the second section of Kaldnes (491 ± 197 g NO_3-N g/m3/day) was lower compared to 767 ± 461 g NO_3-N /m^3/day (wood chips) and 931 ± 221 g NO_3-N /m^3/day (wheat straw). The lower nitrate removal rates at the lower half of the Kaldnes reactor resulted in an average NO_3-N effluent concentration of 23 mg NO_3-N/L. This is seen as the nitrate profile line deviating left from the other two reactor types in Figure 3.16.

Table 3.5 Average percent NO_3-N reduction along column height under LR2.

Port (cm)	Percentage reduction of influent NO_3-N		
	RKM	RWC	RWS
10 (A)	54.00 ± 0.76%	61.28 ± 13.16%	57.42 ± 4.27%
20 (B)	66.96 ± 5.65%	84.36 ± 3.10%	84.73 ± 2.24%
30 (C)	75.23 ± 3.27%	98.50 ± 1.07%	95.40 ± 1.06%
40 (D)	82.50 ± 8.06%	97.92 ± 0.96%	97.20 ± 1.49%

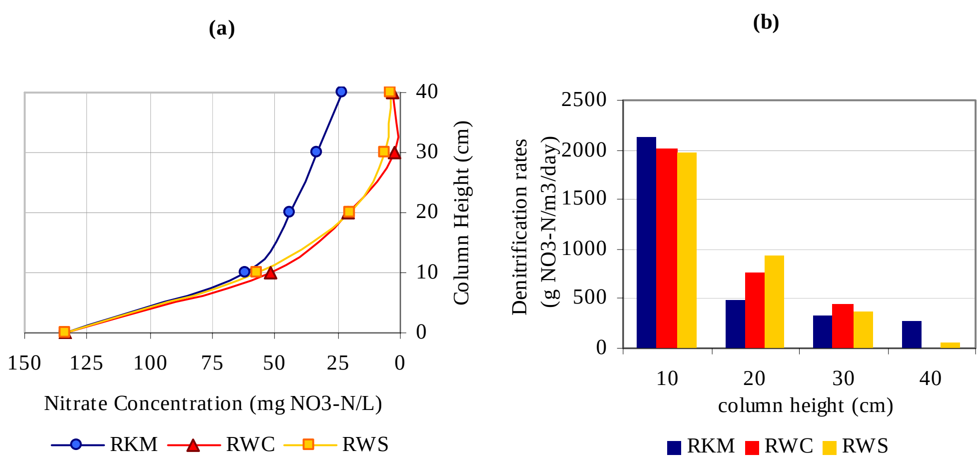

Figure 3. 16 (a) Mean nitrate profiles of different reactor types and the (b) corresponding denitrification rates at LR2 (October 4, 2004).

At LR3, the Kaldnes media's performance improved. As shown in Figure 3.17, Kaldnes NO_3-N concentration profile line has deviated to the right suggesting that it removed more nitrate than wood chips and wheat straw in these sections of the reactor.

Within 10 cm, the nitrate concentration declined to 58.20 mg NO_3-N/L from 203 mg NO_3-N/L compared to 94.44 mg NO_3-N/L and 88.68 mg NO_3-N/L for wood chips and wheat straw respectively. This high removal rate is tabulated as a percentage of influent NO_3-N removed in Table 3.6. In the first 10-cm section alone, denitrification rates were 3841 ± 522 g NO_3-N/m^3/day, 3027 ± 1066 g NO_3-N/m^3/day, and 3151 ± 946 g NO_3-N/m^3/day for Kaldnes media, wood chips, and wheat straw, respectively. The good performance of Kaldnes suggests that at this point, the biomass in the reactor must have fully developed.

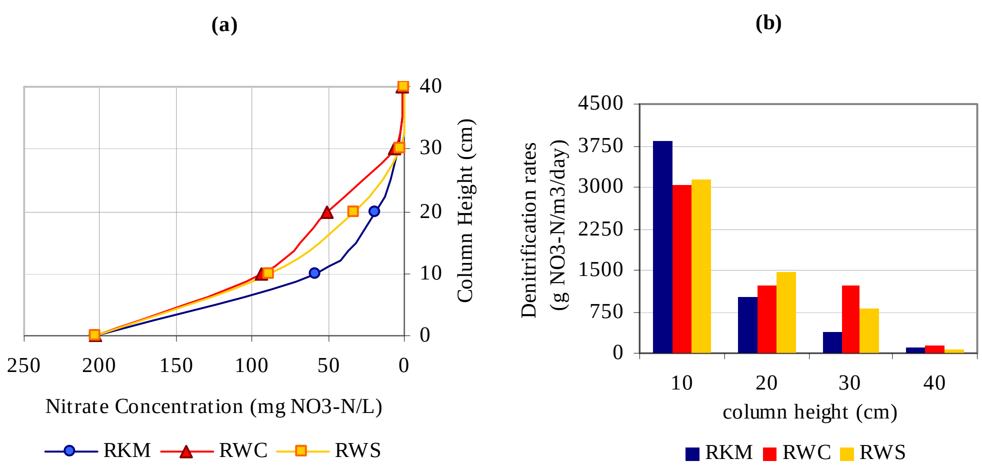

Figure 3. 17 Nitrate profiles of different reactors and their corresponding denitrification rates at 200 mg/L (November 8, 2004).

Table 3.6 Average percent NO_3-N reduction along column height under LR3.

Port (cm)	**Percentage reduction of influent NO_3-N**		
	RKM	RWC	RWS
10 (A)	71.33 ± 3.72%)	53.48 ± 18.50%	56.31 ± 14.04%
20 (B)	90.81 ± 4.57%)	75.13 ± 15.52%	83.53 ± 6.32%
30 (C)	98.17 ± 0.61%)	96.99 ± 2.61%	98.63 ± 1.08%
40 (D)	99.80 ± 0.23%)	99.62 ± 0.50%	99.96 ± 0.05%

Generally, the high rates exhibited by all of the media types in the initial or lower sections of the reactors suggests that all of the media can perform better than the rates listed in Section 3.1 for the entire reactor. In almost all reactors and at different loading rates, it was observed that at the end of the third section (30 cm), nitrate concentrations were reduced more than 95%. This suggests that the reactors sizes may be reduced (h<30 cm) if 5% of 200 mg NO_3-N/L influent is the target final effluent concentration. On the other hand, the NO_3-N loading can still be increased if reactor sizes are not reduced. The flow rate may also be increased. The nitrate profiles suggest hydraulic regimes (flow rates) are limiting optimum loading of the reactors. Robertson et al (2005) hinted that sulfate reduction may happen inside reactors with longer retention and at favorable conditions (presence of residual electron donors and exhaustion of NO_3-N).

3.3 NITRATE REDUCTION WITH REAL AQUACULTURE WASTEWATER

When the reactors were fed with wastewater from the NC State Fish Barn, they removed, on average, 72.08%, Kaldnes; 68.21%, wood chips; and 83.59%, wheat straw of influent NO_3-N in a single pass. Details of the performances of each reactor are outlined in Table 3.7. The lowest nitrate effluent in R7 was attributed to a very low flow rate at the time of sampling (8.40 mL/min) while fluid flow in R5 was 16.80 mL/min.

Comparing denitrification rates, wood chips and wheat straw were near each other with average rates of denitrification of 242.72 ± 3.79 g NO_3-N/m^3/day and 242.24 ± 52.32 g NO_3-N /m^3/day respectively while Kaldnes performed at 232.67 ± 9.19 g NO_3-N /m^3/day. The denitrification rates of real wastewater is lower than the rates when the reactors were subject to 50 mg NO_3-N/L with synthetic wastewater (RKM, 313 ± 45 g NO_3-N/m^3/day; RWC, 333 ± 32 g NO_3-N/m^3/day; RWS, 328 ± 42 g NO_3-N/m^3/day). There were several possible reasons behind the above-mentioned lower rates. One was the possibility that microorganisms were still acclimatizing to new waste makeup, particularly the carbon source. Aside from acclimatizing to new carbon sources, it was possible that the wastewater carbon, being recirculated several times, were not readily available to microbial attack. In Section 3.5, it was subsequently found out that the wastewater contained less readily biodegradable organics than the methanol-fed synthetic wastewater.

Table 3.7 Influent, effluent nitrate concentrations and denitrification rates of reactors fed with actual wastewater (November 18, 2004).

reactor / media type	INF 1 (mg NO_3-N/L)	INF2 (mg NO_3-N /L)	Ave INF (mg NO_3-N /L)	Eff (mg NO_3-N /L)	NO_3 –N Removed (mg/L)	%reduction (based on Ave-Inf	DN rate (g/m^3/day) based on Ave INF	Average DN rate (g/m^3/day) based on Ave INF
R1 (KM)	55.58	46.94	51.26	11.98	39.28	76.63%	230.51	232.67 ± 9.19
R2 (KM)				15.04	36.22	70.66%	242.74	
R3 (KM)				15.92	35.34	68.94%	224.75	
R4 (WC)				15.29	35.97	70.17%	238.44	242.72 ± 3.79
R5 (WC)				18.92	32.34	63.09%	245.64	
R6 (WC)				14.67	36.59	71.38%	244.07	
R7 (WS)				1.14	50.12	97.78%	192.46	242.24 ± 52.32
R8 (WS)				8.72	42.54	82.99%	296.78	
R9 (WS)				15.38	35.88	70.00%	237.48	

3.4 COD REDUCTION WITH SYNTHETIC WASTEWATER

3.4.1 COD REDUCTION IN KALDNES MEDIA

Figure 3.18 shows the relationship of the reactor influent and effluent COD concentrations. These values were grouped into three nitrate loading rates. On August 2, both influent and effluent COD are adjacent suggesting that not much COD have been removed. Evidently, there were errors in the addition of methanol a few times during LR3. High influent COD on October 20 and 25 resulted in high effluent COD on October 25. The effluent COD was not measured on October 20 and November 10, thus this investigator was not able to ascertain its response to a high and low COD influent at these time points. Average COD removed at each loading rate is presented on Table 3.8. COD removal was relatively constant for a particular loading rate. Even with high influent COD on October 25, Kaldnes reactors removed 692 mg COD/L which was close to the overall average of COD removal of 686 ± 29 mg COD/L at LR3 and much closer to 694 ± 32 mg COD/L at pseudo-steady state.

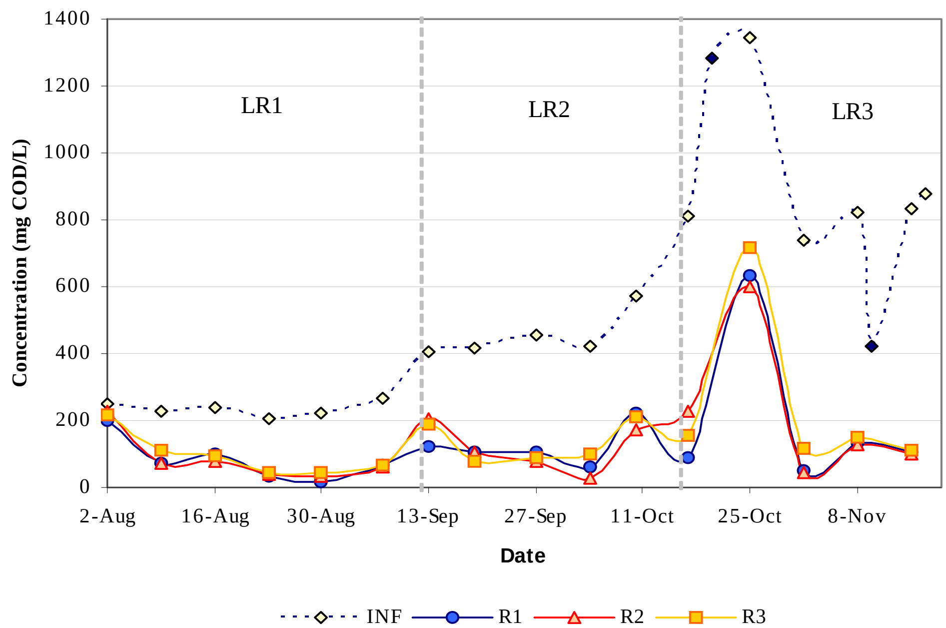

Figure 3. 18 Influent and Effluent COD values of Kaldnes plastic media.

Table 3.8 Average COD removed in Kaldnes reactors in each loading rate with corresponding percentage.

Average influent COD (mg/L	COD removed (mg COD /L)				Percentage Reduction			
	R1	R2	R3	Mean (RKM)	R1	R2	R3	Mean (RKM)
Overall								
238 ± 22 (LR1)	153.2 ± 58.6	149.8 ± 66.4	139.2 ± 60.8	147.4 ±61.3	65.94 ± 26.03%	64.42 ±28.28%	59.72 ±25.64%	63.36 ±26.35%
455 ± 68 (LR2)	332.0 ± 33.8	336.8 ± 83.1	321.4 ± 60.5	330.1 ±57.3	73.76 ±16.08%	74.03 ±16.08%	70.96 ±12.07%	72.92% ±11.33%
840 ± 297 (LR3)	707.4 ± 18.8	689.4 ± 63.2	659.8 ± 41.1	685.5 ±29.1	81.22 ±16.27%)	78.66 ±15.24%	76.01 ±16.63%	78.63 ±15.61%
Pseudo-steady state								
232 ± 34 (LR1)	193.0 ±21.2	187.7 ±22.6	180.0 ±24.1	186.9 ±21.4	83.62 ±9.09%	80.98 ±3.38%	77.55 ±1.46%	80.72 ±4.64%
483 ± 78 (LR2)	354.7 ±8.1	390.0 ±13.2	350.0 ±21.7	364.9 ±5.3	74.68 ±12.31%	81.88 ±11.53%	73.30 ±8.86%	76.62 ±10.49%
799 ± 52 (LR3)	703.3 ± 24.8	706.7 ±23.7	673.0 ±50.0	694.3 ±31.9	88.20 ±4.65%	88.62 ±4.70%	84.22 ±2.41%	87.02 ±3.53%

3.4.2 COD REDUCTION WITH WOOD CHIP MEDIA

Table 3.9 shows the average COD removed by the reactors with wood chip media at each loading rate. The extent of COD removal can be viewed in Figure 3.19 as the distance between the reactors influent and effluent concentrations. The amount of COD removed also increased as the COD load increased. The reactors responded well to transitions in the COD concentration. The effluent COD was fairly constant except for the peaks in October. The higher COD loading on October 25 resulted in a higher COD in the effluent. At LR3, the effluent COD also went up and down with increasing and decreasing load. Even at this varying influent COD, the amount of COD removed was relatively constant.

Table 3.9 Average COD removed in wood chips reactors in each loading rate with corresponding percentage.

Average influent COD (mg/L)	COD removed (mg COD/L)				Percentage Reduction			
	R4	R5	R6	Mean (RWC)	R4	R5	R6	Mean (RWC)
Overall								
238±22) (LR1)	168.0 ±37.0	165.2 ±41.7	167.0 ±34.6	166.7 ±35.9	71.84 ± 16.27%	70.37 ±17.52%	71.29 ±15.11%	71.17 ±15.51%
455±68) (LR2)	367.0 ±30.4	366.8 ±31.3	379.8 ±41.8	371.2 ±30.3	81.94 ± 12.31%	82.07 ±13.46%	84.61 ±12.88%	82.87 ±12.24%
840 (297) (LR3)	714.4 ±29.0	709.4 ±30.7	720.8 ±32.8	714.9 ±30.4	81.67% ± 14.83%	81.07% ±14.64%	82.32% ±14.71%	81.69 ±14.71%
Pseudo-steady state								
232±34) (LR1)	182.3 ± 16.5	189.3 ± 21.8	189.3 ± 18.7	187.0 ± 19.0	78.92 ± 5.37%	81.78 ± 5.10%	81.93 ±6.09%	80.88 ±5.45%
483±78) (LR2)	383.0 ± 26.9	377.0 ± 31.8	377.0 ± 31.8	387.0 ± 25.3	80.95 ± 16.72%	79.89 ±18.04%	84.72 ±16.41%	81.85 ±17.00%
799±52) (LR3)	699.0 ± 25.1	692.3 ± 25.8	692.3 ± 25.8	696.8 ± 21.8	87.66 ±4.68%	86.81 ±4.37%	87.70 ±5.24%	87.39 ±4.73%

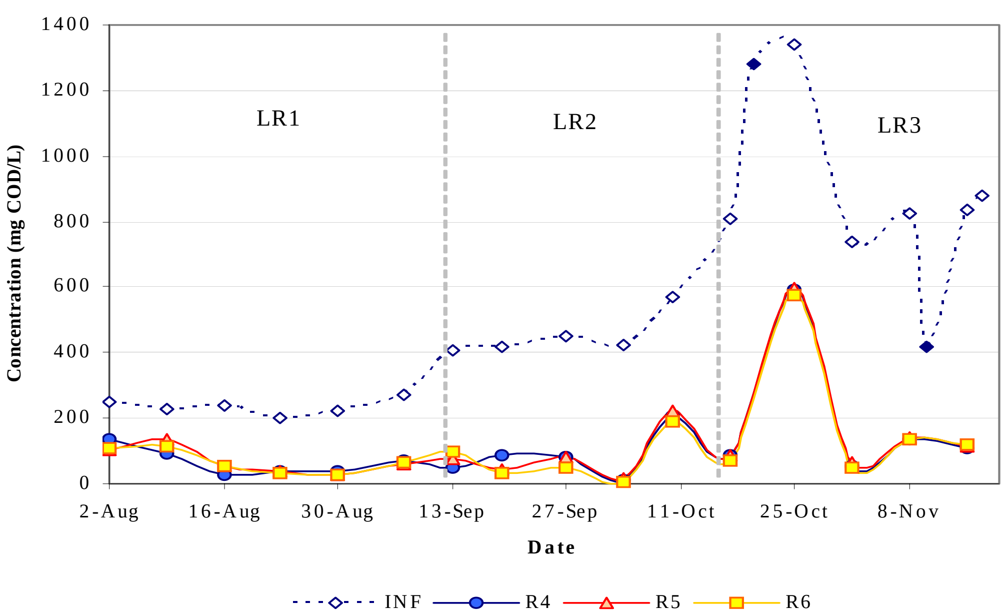

Figure 3. 19 Influent and Effluent COD values of wood chips reactors.

3.4.3 COD REDUCTION IN WHEAT STRAW MEDIA

Table 3.10 outlines the average COD removed at each loading rate with the corresponding percent reduction. It should be noted that higher average COD removal were observed at the pseudo-steady state conditions than considering the overall data.

Effluent COD in wheat straw reactors also followed the same trend with the influent COD as seen in Figure 3.20.

Table 3.10 Average COD removed in wheat straw reactors in each loading rate with corresponding percentage.

Average influent COD	COD removed (mg COD /L)				Percentage Reduction			
	R7	R8	R9	Mean (RWS)	R7	R8	R9	Mean (RWS)
Overall								
238 ± 22 (LR1)	152.7 ±43.7	141.7 ±44.2	159.2 ±40.7	151.2 ±41.2	65.51 ±19.53%	60.49 ±18.88%	68.07 ±17.37%	64.69 ±17.88%
455 ± 68 (LR2)	370.0 ±39.2	372.0 ±33.4	359.6 ± 63.0	367.2 ±44.1	82.78 ±14.38%	83.44 ±14.83	80.31 ±17.68%	82.18 ±15.18%
840 ±297 (LR3)	685.0 ±34.1	698.8 ±39.9	706.2 ±29.1	696.7 ±23.9	78.46 ±15.34%	79.73 ±14.13%	80.69 ±14.42%	79.63 ±14.33%
Pseudo-steady state								
232 ± 34 (LR1)	180.7 ±14.6	173.0 ±20.7	183.0 ± 23.6	178.9 ± 19.6	78.24 ±5.30%	74.69% ± 4.52%	78.93% ±4.49%	77.29 ±4.45%
483 ± 78 (LR2)	382.0 ±33.0	379.3 ±40.4	382.3 ± 41.2	381.2 ± 38.1	81.02 ± 18.44%	80.62% ±19.95%	81.27% ±20.17%	80..97 ±19.50%
799 ± 52 (LR3)	693.3 ±29.7	671.0 ±11.5	691.7 ± 24.8	685.3 ± 14.5	86.96% ± 5.38%	84.24% ± 6.12%	86.73% ± 4.25%	85.98 ±4.84%

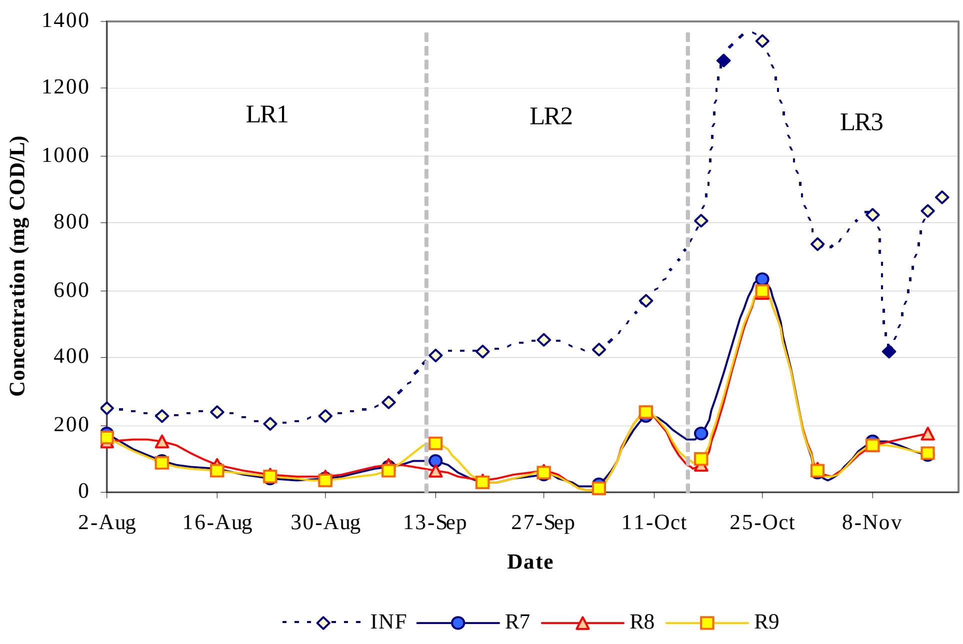

Figure 3. 20 Influent and effluent COD values of wheat straw reactors.

3.4.4 COMPARISON OF COD REDUCTION RATES

Wood chips reactors removed the most COD at all loading rates (Table 3.11). Considering overall data at LR1, split-plot analysis of variance indicated that there were significant differences among reactor types. Least square means pair-wise comparison also showed in significant differences between wood chips and other media (Kaldnes ($p = 0.12$) and wheat straw ($p = 0.03$)). There was no statistically significant difference between the Kaldnes and wheat straw ($p = 0.51$) on LR1. However, when only the last three observations (pseudo-steady state) were considered, the analysis of variance showed no significant difference among reactor types ($p = 0.19$). This means that variation among reactor types was explained by the early stages (startup) of LR1.

At LR2, ANOVA indicates significant difference ($p = 0.0009$) among reactor types. Kaldnes differed from both wheat straw ($p = 0.0008$) and wood chips ($p = 0.0005$). There is no significant difference between wheat straw and wood chips ($p = 0.53$). But if only the steady-state points were considered, no significant difference were observed ($p = 0.24$). Similar to LR1, reactor types only differ at the early period of LR2.

Similar rates of COD removal were observed for all reactor types at LR3. ANOVA shows that no significant difference (overall, $p = 0.14$; pseudo-steady state, $p = 0.56$) among reactor types. The foregoing analyses have shown that variation was due to lower performance of some reactors at the early period of a particular LR but have similar performance at the later stages. This also showed that wood chips responded well to transitions/startup.

Table 3.11 Comparison of average COD removal in each loading rate with corresponding percentage.

Average influent COD	COD removed (mg COD /L)			Percentage Reduction		
	RKM	RWC	RWS	RKM	RWC	RWS
Overall						
238 ± 22 (LR1)	147.4 ±61.3	166.7 ±35.9	151.2 ±41.2	63.36 ±26.35%	71.17 ±15.51%	64.69 ±17.88%
455 ± 68 (LR2)	330.1 ±57.3	371.2 ±30.3	367.2 ±44.1	72.92 ±11.33%	82.87 ±12.24%	82.18 ±15.18%
840 ± 297 (LR3)	685.5 ±29.1	714.9 ±30.4	696.7 ±23.9	78.63 ±15.61%	81.69 ±14.71%	79.63 ±14.33%
Pseudo-steady state						
232 ± 34 (LR1)	186.9 ±21.4	187.0 ± 19.0	178.9 ± 19.6	80.72 ± 4.64%	80.88 ± 5.45%	77.29 ± 4.45%
483 ± 78 (LR2)	364.9 ±5.3	387.0 ± 25.3	381.2 ± 38.1	76.62 ±10.49%	81.85 ±17.00%	80.97 ±19.50%
799 ± 52 (LR3)	694.3 ± 31.9	696.8 ± 21.8	685.3 ± 14.5	87.02 ± 3.53%	87.39 ± 4.73%	85.98 ± 4.84%

3.5 COD AND NITRATE

COD removal can be related to nitrate reduction to determine the appropriate amount of carbon (COD) needed to fuel denitrification. Excessive carbon addition may result in dissimilatory nitrate reduction to ammonia (DNRA). There is also the chance that hydrogen sulfide will be formed from sulfates after exhausting nitrates as the electron acceptor. A deficiency of carbon, on the other hand, can result in low nitrate removal.

Referring to Figure 3.21, there are two instances (October 20 and 25) where excessive carbon addition happened as a result of errors in adding methanol to the synthetic wastewater. DNRA did not happen in these cases since there was no increase in ammonia in the effluent on October 25 (October 20 was not sampled for ammonia). It was also interesting to note that on October 20, nitrate effluent was high compared to October 25. Evidently, the reactors did not handle well the high COD influent at first and only stabilized after 5 days (October 25). On the other hand, low COD resulted in reduced nitrate removal (low on November 10).

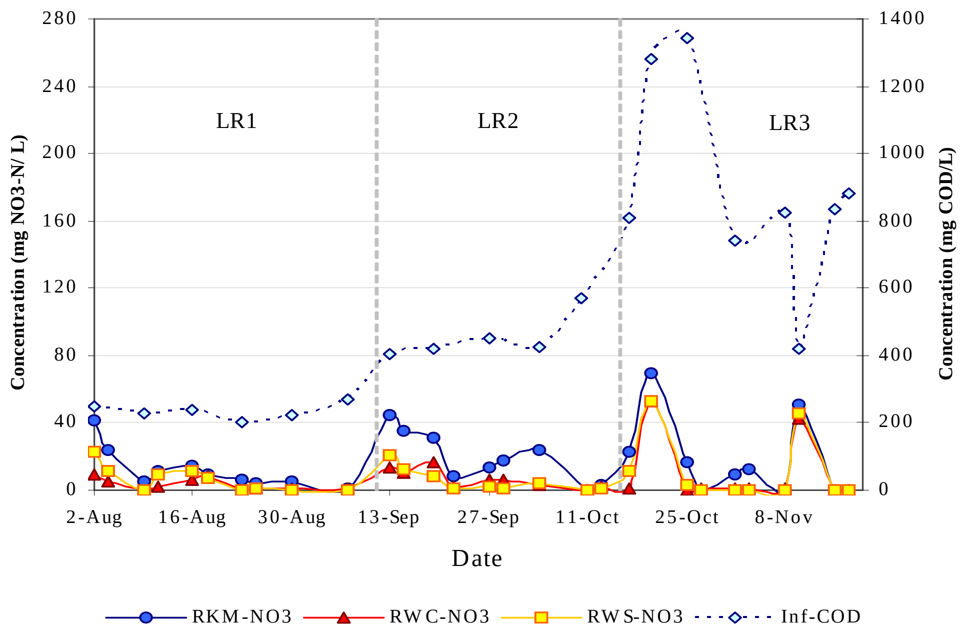

Figure 3. 21 Influent COD and mean NO_3-N effluent of different reactors.

The efficiency of each reactor type to utilize carbon to achieve the same level of denitrification rate can be assessed by measuring the COD removed per nitrate reduced. Table 3.12 shows the COD/NO_3-N ratio for the different media types during the experimental period. At LR1, it can be observed that media types differed from each other for both overall ($p = 0.01$) and steady-state points ($p = 0.0005$). The ratio of Kaldnes media was higher than wood chips (overall, $p = 0.008$; steady-state, $p = 0.002$) and wheat straw (overall, $p = 0.002$; steady-state, $p = 0.0002$). The ratio was higher at the early stages of LR1. On August 2, the ratio was excessively high due to low nitrate removal but still had some COD removed.

Table 3.12 Mean COD/NO_3-N ratios.

Loading	RKM	RWC	RWS
Overall			
LR1	4.71 ± 1.57	3.65 ± 0.44	3.59 ± 0.39
LR2	3.36 ± 0.12	3.29 ± 0.13	3.24 ± 0.13
LR3	3.52 ± 0.21	3.51 ± 0.19	3.45 ± 0.20
Pseudo-steady state			
LR1	3.95 ± 0.50	3.64 ± 0.36	3.46 ± 0.32
LR2	3.41± 0.13	3.34 ± 0.16	3.26 ± 0.18
LR3	3.50 ± 0.17	3.46 ± 0.13	3.40 ± 0.14
Overall mean	3.91± 1.12	3.49 ± 0.32	3.44 ± 0.30

At LR2, significant difference ($p = 0.007$) were detected among reactor types when considering all data points but at pseudo-steady state conditions, the reactor types have comparable ratios ($p = 0.13$). Kaldnes ratio was higher than wood chips ($p = 0.03$) and wheat straw ($p = 0.002$). Wood chips and wheat straw were not statistically different ($p = 0.06$).

At LR3, differences among reactor types were observed considering both overall ($p = 0.02$) and steady points ($p = 0.04$). In both cases, wheat straw yielded the lowest ratio among the three reactor types (overall data: KM vs WS, $p = 0.008$; WC vs WS $p = 0.02$). At the pseudo-steady state condition, only wheat straw and Kaldnes showed difference ($p = 0.01$).

The higher COD consumption per nitrate removed by the Kaldnes media may be attributed to the fact that it did not have other carbon sources other than from the external supply and lysis of microbial biomass in the reactor. The organic nature of wood chips and wheat straw may have added some COD to the reaction, thus lessening the net COD requirement. Robertson et al (2005) reported that at the early stages of their wood chips filter, it leached substantial amount of COD (from tannic acid, etc.). The wheat straw, being organic in nature may have also leached some carbonaceous COD. The leached

COD from the wood chips and wheat straw may have contributed to lower ratio of the organic media over Kaldnes.

The COD/Nitrate ratio for wheat straw was slightly lower than that for wood chips. This difference may be attributed to the observation (Chapter 5) that wheat straw degraded faster than wood chips. In this case, RWS was assumed to add more COD to the bulk liquid. While more COD was removed by RWC (Section 3.4), the wood chip media removed less nitrate compared to RWS (Section 3.1).

On the other hand, Figure 3.22 shows a decreasing trend COD/NO_3-N ratio over time. This can be attributed to solid retention time (SRT). Longer solid retention time minimizes use of electron donor (carbon) for bacterial synthesis (Grady et al, 1999). Thus, COD was consumed mainly for nitrate reduction. Using the estimated VSS yield in Section 5.3, the specific yields for all reactor types were close to 0.05 g VSS/g COD removed. Using Equation 2.2, the estimated ratio would be around 3 mg COD/mg NO_3-N (from 3.75 mg MeOH COD/mg NO_3-N). Though the calculated estimate is lower than the experimental ratios, it has shown that the COD/NO_3-N decreased over time (as SRT increased). Longer SRT is expected in biofilter due to attachment to and entrapment/filtration of solids by the filter media.

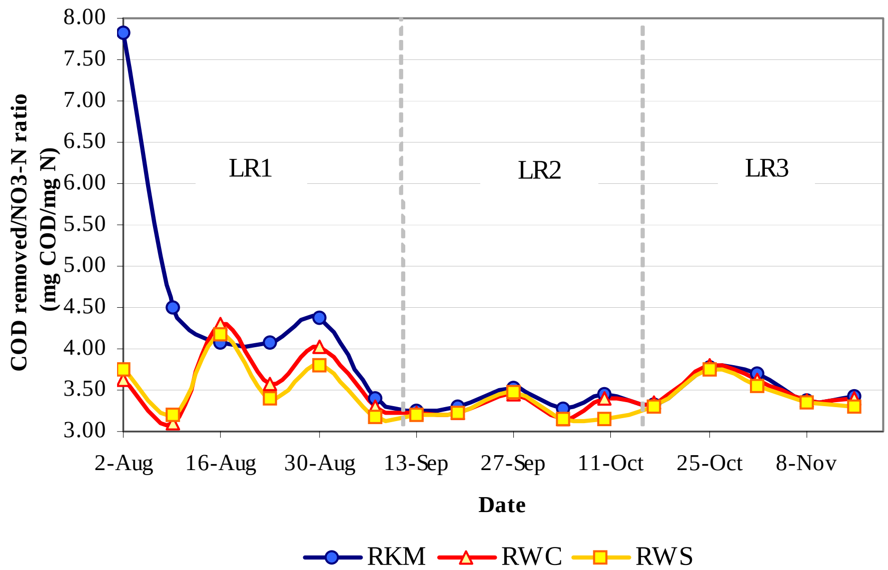

Figure 3. 22 Average COD/NO_3-N ratios of the different reactor types.

3.6 COD REDUCTION IN REAL WASTEWATER

Since the Fish Barn wastewater has more suspended solids than the synthetic wastewater used in this study, both total and soluble COD were included in this analysis. Total COD estimates both the particulate and dissolved fractions of the organics in wastewater. Table 3.13 shows the total and soluble COD reduction by the reactors under actual wastewater loading. More total COD were removed from the influent, possibly due to filtration of particulate organics.

Generally, Kaldnes media removed more COD than other media types. Comparing nitrate reduction, more COD were needed to reduce nitrate for Kaldnes media. Both wheat straw and wood chips had lower COD/NO_3-N than that of Kaldnes media. The relative ratios of the three media types conformed with the ratios observed in the synthetic wastewater experiments. That is, wheat straw and wood chips were assumed to give some COD to the bulk liquid resulting in lower COD net requirement. However, these ratios were lower compared to the results from the synthetic wastewater experiments. One explanation to this is the possibility that there were dissolved organics remaining inside the reactors after the synthetic wastewater experiments and bacteria were forced to scavenge the remaining COD because the actual wastewater contained low amount of biodegradable organics.

Table 3.13 Total and Soluble COD reduction in reactors under actual Fish Barn wastewater (November 18, 2004).

Reactor/ Media type	INF1 (mg/L)	INF2 (mg/L)	Ave (mg/L)	Eff (mg/L)	% reduction	Average reduction	COD removed/ NO3-N reduced	Calculated Carbon removed/ NO3-N reduced
Total COD								
1DKM	237	286	262	186	28.87%	26.83 ±1.92%	1.90 ±0.04	0.09 ±0.00
2DKM				192	26.58%			
3DKM				196	25.05%			
4DWC				203	22.37%	21.35 ±1.45%	1.60 ±0.03	0.07 ±0.00
5DWC				210	19.69%			
6DWC				204	21.99%			
7DWS				198	24.28%	23.39 ±1.55%	1.44 ±0.16	0.06 ±0.01
8DWS				198	24.28%			
9DWS				205	21.61%			
Soluble COD								
1DKM	98	116	107	86	19.63%	21.18 ±1.95%	0.62 ±0.08	0.18 ± 0.02
2DKM				82	23.36%			
3DKM				85	20.56%			
4DWC				111	-3.74%	12.15 ±14.14%	0.38 ±0.4	0.11 ±0.13
5DWC				82	23.36%			
6DWC				89	16.82%			
7DWS				86	19.63%	16.20 ±4.42%	0.41 ±0.12	0.12 ±0.04
8DWS				95	11.21%			
9DWS				88	17.76%			

To examine whether Fish Barn wastewater contained low organics, another sample was taken from the Fish Barn and analyzed for BOD and TOC. The results of these analyses indicate that only 4.48% of the total COD was in the form of total organic carbon (TOC/Total COD) while the BOD/Total COD was 0.24. This suggests that although the total COD was high only a small portion of the COD is biodegradable and available to facilitate denitrification. As discussed in Section 3.3, about 70% of the 51.56 mg/L average influent NO_3-N was reduced even though the total COD was 261 mg/L. This is all expected as the water in the fish barn tanks is used about 14 days and recycles through filters with bacteria every hour. Thus, any degradable carbon is degraded already.

High COD from aquaculture wastewater may not mean a good carbon source for denitrification since (aside from low organic content) these organics have recirculated in the tanks making them refractory organics. In this case, additional carbon source would be needed to remove the remaining nitrates from the effluent.

3.7 ESTIMATE OF EXTERNAL CARBON NEEDS

As reported in Section 3.5, the experiments using synthetic wastewater estimated the COD / NO_3-N requirement for efficient denitrification as 3.91 (RKM), 3.49 (RWC), and 3.44 (RWS). Translating this to a methanol (MeOH) requirement and assuming that the COD consumed were from methanol, 2.61 mg of MeOH is needed per mg of NO_3-N in Kaldnes. Wood chips needed 2.32 mg while wheat straw needed 2.29 mg of MeOH per mg of NO_3-N.

Table 3.14 outlines estimates of methanol or carbon to completely remove NO_3-N from the Fish Barn wastewater described in Section 3.6. The values in "NO_3-N still to be removed" column were taken from the effluent NO_3-N from the actual wastewater experiment. Estimates of methanol requirements were determined by multiplying the

MeOH/NO_3-N ratio and the remaining NO_3-N. These estimates may be reduced with age of biofilter as stipulated in the previous section. Reduced flow rates may also reduce needs for external carbon as exhibited in R7. Careful consideration, however, should be undertaken in using these relationships since experiments with actual wastewater were done only once. The amount of electron donor required may also differ if other carbon sources are used (e.g. VFAs from supernatant of RAS sludge).

Table 3.14 Methanol Requirement to completely reduce NO_3-N from actual wastewater.

Reactor/ Media Type	NO_3-N still need to be removed (mg/L)	MeOH/ NO_3-N from Synthetic wastewater	Needed methanol (mg/L)	Needed C (mg/L)
R1 (KM)	11.98	2.61	**31.27**	**11.73**
R2 (KM)	15.04		**39.25**	**14.72**
R3 (KM)	15.92		**41.55**	**15.58**
R4 (WC)	15.29	2.32	**35.47**	**13.30**
R5 (WC)	18.92		**43.89**	**16.46**
R6 (WC)	14.67		**34.03**	**12.76**
R7 (WS)	1.14	2.29	**2.61**	**0.98**
R8 (WS)	8.72		**19.97**	**7.48**
R9 (WS)	15.38		**35.22**	**13.21**

4. EFFLUENT QUALITY

4.1 AMMONIA

4.1.1 AMMONIA ASSIMILATION OR GENERATION USING SYNTHETIC WASTEWATER

Effluent NH_3-N from the Kaldnes media reactors were not generally affected by variations in the influent NH_3-N concentration. Figure 4.1 shows the NH_3-N values concentrations plotted over time and across the three loading rates. Influent ammonia concentrations were less than 1 mg NH_3-N/L. On average, the three reactors nearly completely removed the NH_3-N from the influent. R1 had an overall average of 0, 0.03 ± 0.07, and 0 mg NH_3-N /L for LR1, LR2, and LR3 respectively. R1's overall average did not differ much under pseudo-steady state conditions (LR1, 0; LR2, 0.05 ± 0.09; LR3, 0 mg NH_3-N /L). R2 had 0.10 ± 0.24, 0.05 ± 0.10, and 0 mg NH_3-N /L for LR1, LR2, LR3 overall data, respectively while its pseudo-steady state concentrations were: LR1, 0;

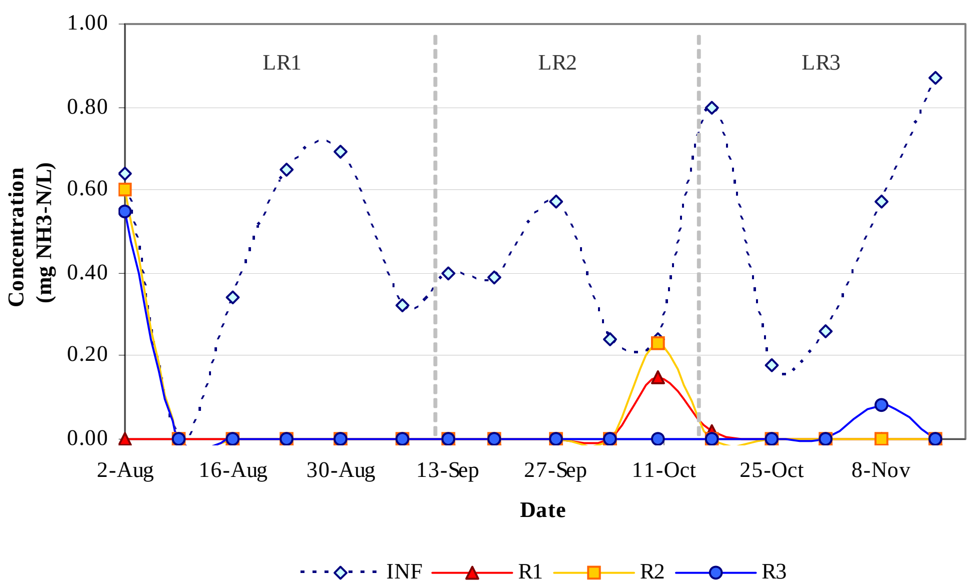

Figure 4. 1 Influent and effluent NH_3-N of Kaldnes media reactors.

LR2, 0.08 ± 0.13; and LR3, 0 mg NH_3-N/L. Ammonia effluent concentrations were: overall – LR1, 0.09 ± 0.22; LR2, 0; LR3, 0.02 ± 0.04; pseudo-steady state – LR1, 0; LR2, 0; 0.03 ± 0.05 mg NH_3-N/L.

Similarly, individual reactors using wood chip media also depleted NH_3-N except on August 9 when R5 and R6 had higher effluent NH_3-N than the influent. At this time, these reactors generated NH_3-N instead of removing it. The organic nature of the media may have caused this. Figure 4.2 shows a graph of the concentration of NH_3-N versus time for the reactors with wood chip media. The average concentrations of NH_3-N in the effluent of the wood chips reactors were: R4 (overall) – LR1, 0; LR2, 0; LR3, 0; (pseudo-steady-state) LR1, 0; LR2, 0; LR3, 0; R5 (overall) – LR1, 0.02 ± 0.05; LR2, 0; LR3, 0; (pseudo-steady-state) LR1, 0; LR2, 0; LR3, 0; R6 (overall) – LR1, 0.07 ± 0.17; LR2, 0.03 ± 0.07; LR3, 0; (pseudo-steady-state) LR1, 0; LR2, 0.02 ± 0.03; LR3, 0 mg NH_3-N/L.

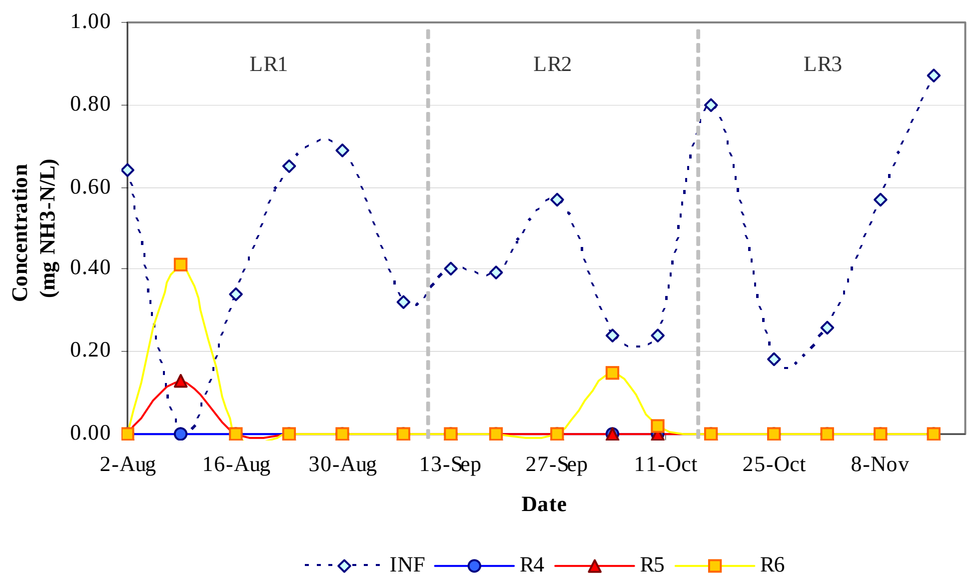

Figure 4. 2 Influent and effluent NH_3-N of wood chips reactors.

Similar to the results with the wood chip media, NH_3-N in the effluent on August 9 was greater than the influent in all three wheat straw reactors. This is shown as peaks on this date in Figure 4.3. But on average, these reactors had low concentrations of NH_3-N in the effluent. The average concentrations of NH_3-N in the effluent of wheat straw reactors were: R7 (overall) – LR1, 0.01 ± 0.03; LR2, 0.04 ± 0.06 ; LR3, 0; (pseudo-steady-state) LR1, 0; LR2, 0.07 ± 0.07; LR3, 0; R8 (overall) – LR1, 0.14 ± 0.23; LR2, 0.03 ± 0.05; LR3, 0; (pseudo-steady-state) LR1, 0.07 ± 0.02; LR2, 0.04 ± 0.06; LR3, 0; R9 (overall) – LR1, 0.03 ± 0.07; LR2, 0.04 ± 0.06; LR3, 0; (pseudo-steady-state) LR1, 0; LR2, 0.03 ± 0.05; LR3, 0 mg NH_3-N/L.

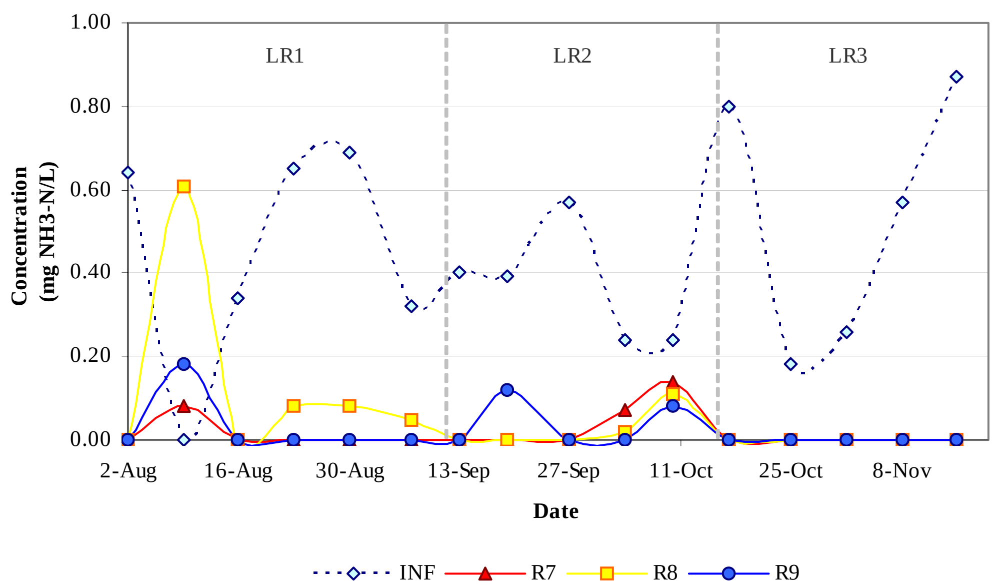

Figure 4. 3 Influent and effluent NH_3-N of wheat straw reactors.

Overall, the three types of media removed ammonia-nitrogen effectively from the system (Figure 4.4). Ammonia removal can be attributed to sorption to media and nitrogen assimilation by bacterial biomass. Generally, bacteria prefer NH_3 over NO_3 as nitrogen source for growth and cellular building blocks. On August 9, however, wood chips and wheat straw reactors had higher NH_3-N effluent than influent. Somehow, the NH_3-N from the organic surfaces may have been released (diffusion) due to very low bulk liquid NH_3-N. The mean concentrations of NH_3-N in the effluent of each reactor types were: RKM (overall) – LR1, 0.06 ± 0.00; LR2, 0.03 ± 0.07 ; LR3, 0.01 ± 0.02 ; (pseudo-steady-state) LR1, 0; LR2, 0.04 ± 0.07; LR3, 0.01 ± 0.02; RWC (overall) – LR1, 0.03 ± 0.00; LR2, 0.01 ± 0.03; LR3, 0.00 ± 0.00; (pseudo-steady-state) – LR1, 0; LR2, 0.02 ± 0.03; LR3, 0; RWS (overall) – LR1, 0.06 ± 0.01; LR2, 0.04 ± 0.06; LR3, 0; (pseudo-steady-state) – LR1, 0.02 ± 0.01; LR2, 0.05 ± 0.06; LR3, 0 mg NH_3-N/L.

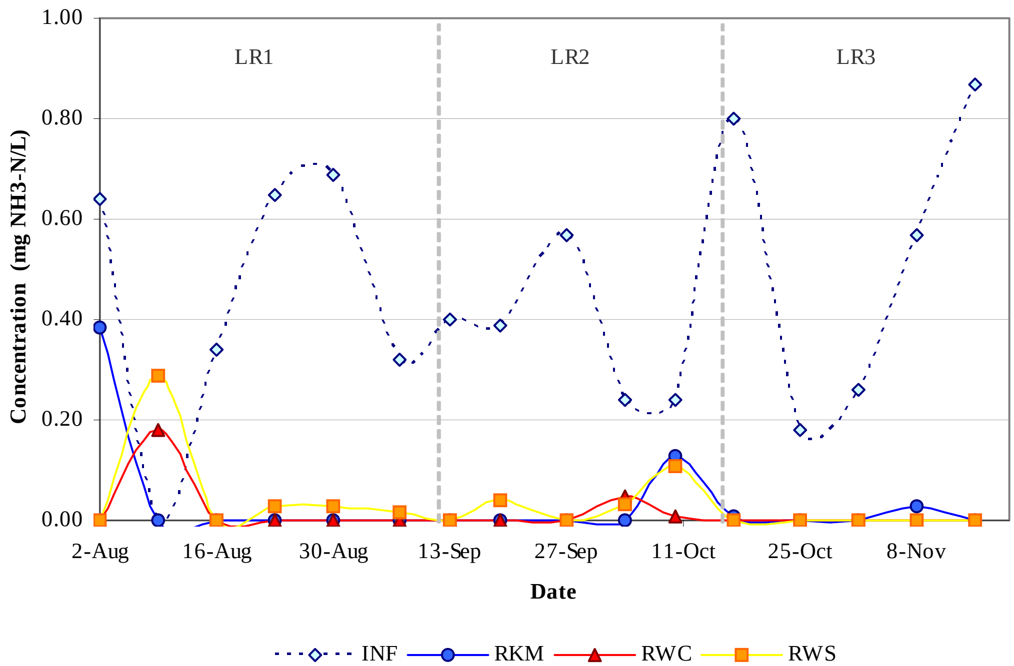

Figure 4. 4 Mean NH_3-N concentration of three reactor types.

4.1.2 AMMONIA ASSIMILATION OR GENERATION USING REAL WASTEWATER

NH_3 effluent from the reactors mostly exceeded the influent suggesting that the reactors generated NH_3 rather than removing it. Table 4.1 lists the influent and effluent NH_3 from the reactors when fed with real aquaculture wastewater. No other sources of ammonia existed inside the reactors except the organic N from wastewater, microbial biomass and the media itself. For the Kaldnes media reactors, microbial biomass and organic N from wastewater were the only possible sources. Decay of microbial biomass also released carbon aside from NH_3-N. In Section 3.6, the reduction of COD/NO_3-N ratios in real wastewater was thought to be the result of carbon supply from decaying microbial biomass and organic media. The findings herein somewhat support the idea though not conclusive due to limited samples over extended period of time.

Table 4.1 Influent and effluent ammonia of reactors subject to real wastewater (November 18, 2004).

<table>
<tr><th>Reactor/ media type</th><th>Influent 1 (mg NH_3-N/L)</th><th>Influent 2 (mg NH_3-N/L)</th><th>Average influent (mg NH_3-N/L)</th><th>Effluent (mg NH_3-N/L)</th><th>Average effluent in each media type (mg NH_3-N/L)</th></tr>
<tr><td>R1 (KM)</td><td rowspan="9">2.42</td><td rowspan="9">1.80</td><td rowspan="9">2.11</td><td>3.35</td><td rowspan="3">3.55 ± 0.22</td></tr>
<tr><td>R2 (KM)</td><td>3.50</td></tr>
<tr><td>R3 (KM)</td><td>3.79</td></tr>
<tr><td>R4 (WC)</td><td>2.76</td><td rowspan="3">2.25 ± 0.44</td></tr>
<tr><td>R5 (WC)</td><td>1.99</td></tr>
<tr><td>R6 (WC)</td><td>1.99</td></tr>
<tr><td>R7 (WS)</td><td>4.01</td><td rowspan="3">3.48 ± 0.53</td></tr>
<tr><td>R8 (WS)</td><td>3.48</td></tr>
<tr><td>R9 (WS)</td><td>2.95</td></tr>
</table>

4.2 NITRITE

4.2.1 NITRITE GENERATION USING SYNTHETIC WASTEWATER

Figure 4.5 shows the plot of influent and effluent NO_2-N in the three Kaldnes media reactors. The average concentrations of NO_2-N in the effluent of the Kaldnes reactors were: R1 (overall) – LR1, 1.26 ± 1.34; LR2, 3.11 ± 1.90 ; LR3, 1.36 ± 2.55 ; (pseudo-steady-state) – LR1, 0.68 ± 0.74; LR2, 3.11 ± 2.68; LR3, 0.06 ± 0.05; R2 (overall) – LR1, 1.60 ± 0.55; LR2, 2.82 ± 1.74; LR3, 0.84 ± 1.33; (pseudo-steady-state) – LR1, 1.53 ± 0.69; LR2, 2.10 ± 1.75; LR3, 0.09 ± 0.03; R3 (overall) – LR1, 1.97 ± 0.87; LR2, 3.30 ± 1.64; LR3, 2.79 ± 2.43; (pseudo-steady-state) – LR1, 1.79 ± 0.66; LR2, 3.26 ± 2.31; LR3, 1.40 ± 1.85 mg NO_2-N/L.

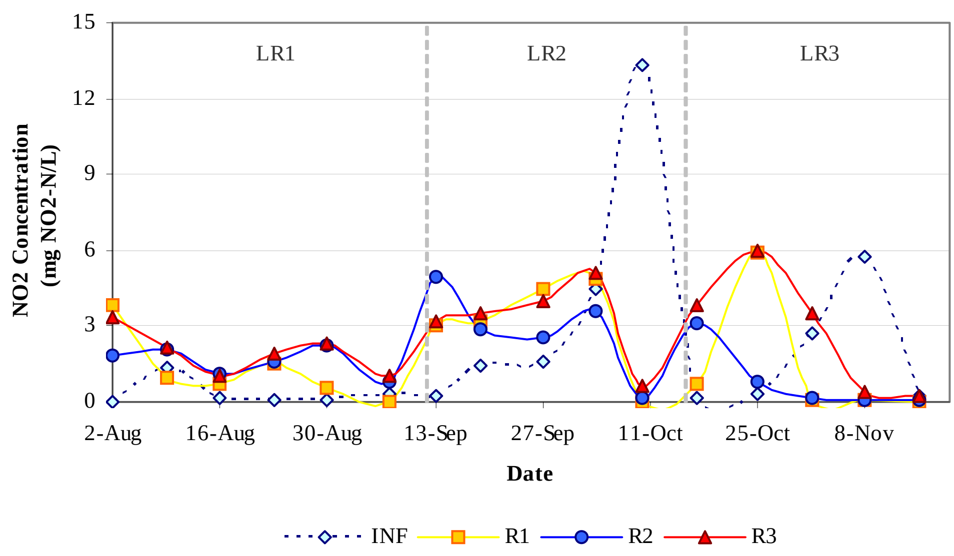

Figure 4. 5 Influent and effluent NO_2-N of Kaldnes media.

Figure 4.6 graphs the effluent NO_2-N in the reactors with wood chip media. The average concentrations of NO_2-N in the effluent of the wood chips reactors were: R4 (overall) – LR1, 2.85 ± 5.63; LR2, 1.63 ± 1.32 ; LR3, 0.40 ± 0.33 ; (pseudo-steady-state)

– LR1, 0.52 ± 0.11; LR2, 0.72 ± 0.42; LR3, 0.20 ± 0.07; R5 (overall) – LR1,1.15 ± 1.71; LR2, 2.57 ± 1.78; LR3, 0.45 ± 0.21; (pseudo-steady-state) – LR1, 0.25 ± 0.14; LR2, 1.43 ± 1.15; LR3, 0.43 ± 0.13; R6 (overall) – LR1, 1.28 ± 2.26; LR2, 1.62 ± 1.94; LR3, 0.15 ± 0.20; (pseudo-steady-state) – LR1, 0.29 ± 0.24; LR2, 0.36 ± 0.42; LR3, 0.20 ± 0.26 mg NO_2-N/L.

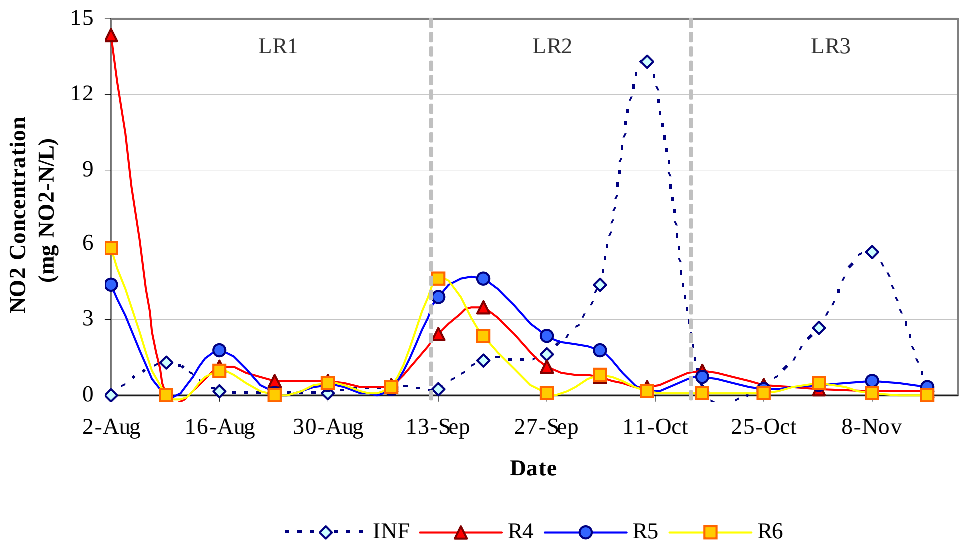

Figure 4. 6 Influent and Effluent NO_2-N of wood chips reactors.

Figure 4.7 shows the graph of effluent concentration of NO_2-N for the reactors with wheat straw media. The average concentrations of NO_2-N in the effluent of the wheat straw reactors were: R7 (overall) – LR1, 2.83 ± 6.09; LR2, 1.67 ± 1.01 ; LR3, 1.99 ± 2.41 ; (pseudo-steady-state) LR1, 0.34 ± 0.34; LR2, 1.03 ± 0.69; LR3, 0.23 ± 0.12; R8 (overall) – LR1, 2.77 ± 6.19; LR2, 2.32 ± 1.85; LR3, 0.16 ± 0.22; (pseudo-steady-state) – LR1, 0.11 ± 0.13; LR2, 1.04 ± 0.86; LR3, 0.02 ± 0.01; R9 (overall) – LR1, 2.81 ± 6.39; LR2, 1.56 ± 1.18; LR3, 0.91 ± 1.75; (pseudo-steady-state) – LR1, 0.04 ± 0.02; LR2, 0.96 ± 0.46; LR3, 0.03 ± 0.03 mg NO_2-N/L.

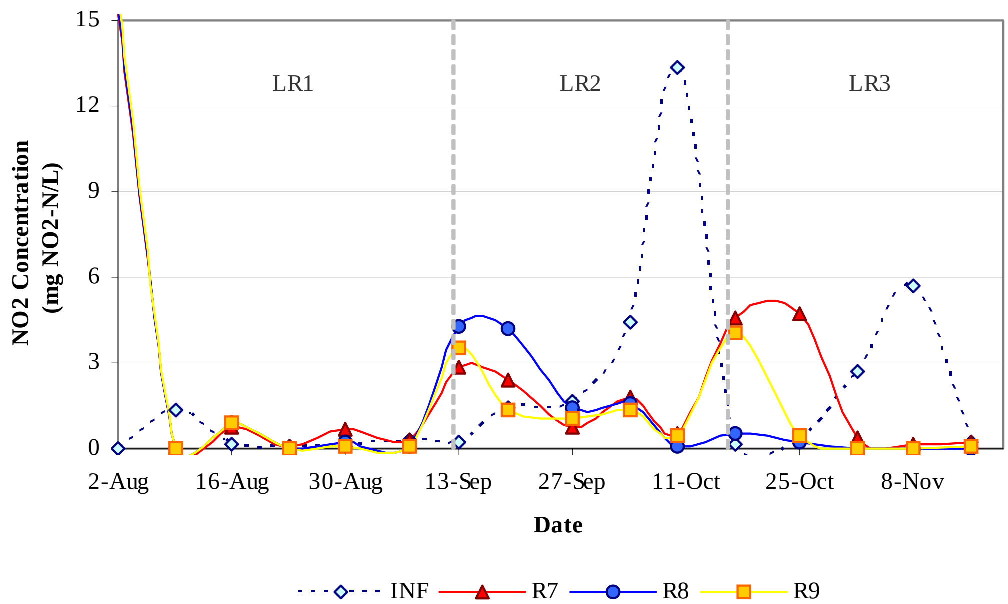

Figure 4. 7 Influent and effluent NO_2-N of wheat straw reactors.

Figure 4.8 compares the mean influent and effluent nitrite concentrations of the three reactor types. The means concentrations of NO_2-N in the effluent of each reactor types were: RKM (overall) – LR1, 1.61 ± 0.81; LR2, 3.07 ± 1.63 ; LR3, 1.66 ± 1.74 ; (pseudo-steady-state) LR1, 1.33 ± 0.60; LR2, 2.82 ± 2.24; LR3, 0.51 ± 0.62; RWC (overall) – LR1, 1.76 ± 3.17; LR2, 1.94 ± 1.56; LR3, 0.33 ± 0.16; (pseudo-steady-state) – LR1, 0.36 ± 0.13; LR2, 0.84 ± 0.56; LR3, 0.28 ± 0.11; RWS (overall) – LR1, 2.80 ± 6.22; LR2, 1.85 ± 1.27; LR3, 1.02 ± 1.35; (pseudo-steady-state) – LR1, 0.16 ± 0.16; LR2, 1.01 ± 0.63; LR3, 0.09 ± 0.05 mg NO_2-N/L.

The high influent NO_2-N values for October 10 and November 8 were attributed to partial denitrification occurring inside the waste storage tank. As observed during cleaning, slime on the walls and some solids have accumulated inside the tank. This indicated active biological activity inside the tank leading to the production of nitrite at the influent port.

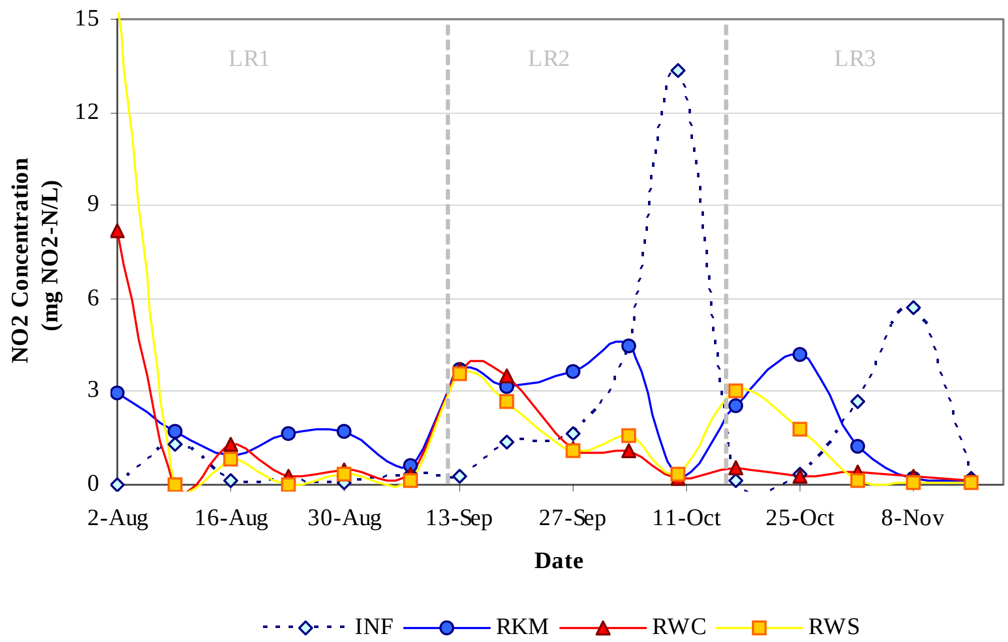

Figure 4. 8 Mean influent and effluent NO_2-N of three media types.

4.2.2 NITRITE REDUCTION WITH REAL WASTEWATER

When loaded with real wastewater, all of the reactors had nitrite concentrations in the effluent below 1 mg/L. This was relatively low when compared to average effluent values in the effluent during the synthetic wastewater experiments. It was noted that the influent NO_2-N concentration were high compared to synthetic wastewater experiments suggesting that partial denitrification occurred at the waste storage tank.

Table 4.2 Influent and effluent NO_2-N concentration of the reactors under real wastewater.

Reactor/ media type	Influent 1 (mg NO_2-N/L)	Influent 2 (mg NO_2-N/L)	Average Influent (mg NO_2-N/L)	Effluent (mg NO_2-N/L)	Average effluent in each media type (mg NO_2-N/L)
R1 (KM)	5.78	12.94	9.36	0.75	0.75 ± 0.06
R2 (KM)				0.69	
R3 (KM)				0.81	
R4 (WC)				0.84	0.90 ± 0.08
R5 (WC)				0.99	
R6 (WC)				0.87	
R7 (WS)				0.74	0.75 ± 0.10
R8 (WS)				0.66	
R9 (WS)				0.86	

4.3 ALKALINITY

4.3.1 ALKALINITY CHANGES USING SYNTHETIC WASTEWATER

Alkalinity in the effluent increased with increasing nitrate loading rates as shown in Figure 4.9. At each loading rate, the average alkalinity produced was fairly consistent as illustrated in Table 4.3. In all cases, the amount of alkalinity produced was related to amount of NO_3-N removed. The ratio of alkalinity production to corresponding NO_3-N removed averaged more than 3.00 in all loading rates and reactor types. These values were in the vicinity of 3.55 corresponding to the stoichiometric relationships (EPA, 1993) if methanol was used as carbon source.

Table 4.3 Average alkalinity produced and its ratio with NO_3-N removed of three media types.

Average influent alkalinity (mg/L as $CaCO_3$)	RKM		RWC		RWS	
	Average alkalinity produced (mg/L as $CaCO_3$)	Alkalinity produced per NO_3-N removed (mg $CaCO_3$/mg NO_3-N)	Average alkalinity produced (mg/L as $CaCO_3$)	Alkalinity produced per NO_3-N removed (mg $CaCO_3$/mg NO_3-N)	Average alkalinity produced (mg/L as $CaCO_3$)	Alkalinity produced per NO_3-N removed (mg $CaCO_3$/mg NO_3-N)
Overall						
26.4 ± 14.8 (LR1)	156.53 ± 26.12	3.77 ± 0.53	182.93 ± 16.16	3.91± 0.39	175.60 ± 21.02	3.83 ± 0.38
81.2 ± 53.0 (LR2)	302.93 ± 40.98	3.10 ± 0.18	356.80 ± 33.39	3.16 ± 0.19	356.80 ± 38.07	3.15 ± 0.22
44.8 ± 4.8 (LR3)	654.80 ± 37.34	3.36 ± 0.17	691.20 ± 17.75	3.40 ± 0.17	684.27 ± 29.48	3.39 ± 0.19
Pseudo-steady state						
34.7 ± 12.1 (LR1)	174.22 ± 12.12	3.70 ± 0.57	192.22 ± 13.89	3.74± 0.31	190.44 ± 6.41	3.70 ± 0.43
107.3 ± 55.2 (LR2)	328.22 ± 15.24	3.07 ± 0.20	362.22 ± 45.74	3.11 ± 0.15	365.56 ± 49.05	3.11 ± 0.16
47.3 ± 4.6 (LR3)	667.56 ± 46.57	3.37 ± 0.19	690.44 ± 23.86	3.44 ± 0.12	688.67 ± 31.18	3.42 ± 0.11

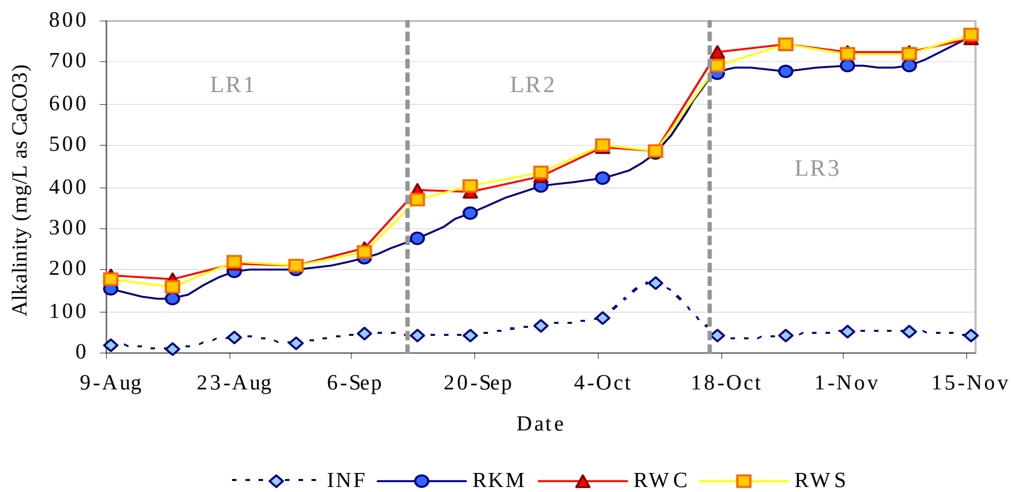

Figure 4. 9 Average influent and effluent alkalinity of each media type.

4.3.2 ALKALINITY CHANGES USING REAL WASTEWATER

Effluent alkalinity did not differ appreciably between media types (Table 4.4). The ratios of alkalinity produced to nitrate removed were higher than those found in the synthetic wastewater experiments and the estimated stoichiometric ratio. The different alkalinity/nitrate ratio compared to the synthetic experiments is due to the different carbon source. The sources of carbon in the real wastewater certainly have characteristics different from methanol. Elefsiniotis et al (2004) illustrated that alkalinity production differed with carbon sources (e.g. acetic acid, 7.36; propionic acid, 5.74; butyric acid, 5.09).

Table 4.4 Influent, effluent alkalinity of the reactors and its corresponding relationship with reduced nitrate during real wastewater.

Reactor/ Media type	Influent Alkalinity (mg $CaCO_3$/L)	Effluent Alkalinity (mg $CaCO_3$/Ll)	alkalinity produced/nitrate removed	average alkalinity produced/nitrate removed (mg/ mg NO_3-N)
R1 (KM)	214	450	6.01	6.03 ± 0.08
R2 (KM)		430	5.96	
R3 (KM)		430	6.11	
R4 (WC)		430	6.01	5.98 ± 0.21
R5 (WC)		400	5.75	
R6 (WC)		440	6.18	
R7 (WS)		500	5.71	5.82 ± 0.17
R8 (WS)		470	6.02	
R9 (WS)		420	5.74	

Nevertheless, the system under both synthetic and actual wastewater can provide enough alkalinity as buffering capacity to receiving waters as well as for recirculation to fish tanks.

4.4 pH AND OXIDATION-REDUCTION POTENTIAL

4.4.1 pH AND ORP USING SYNTHETIC WASTEWATER

The average pH values increased at the higher LR's. Table 4.5 shows the pH values in the effluent of the nine reactors as well as the oxidation-reduction potential on the last loading rate (LR3). ORP was not measured for LR1 and LR2. The results indicate that pH was high when the reactors were operating with 120 and 200 mg NO_3-N/L influent concentrations.

Table 4.5 Influent and effluent pH and ORP of reactors under synthetic wastewater.

Influent (LR)	R1	R2	R3	R4	R5	R6	R7	R8	R9
pH									
6.66 (LR1)	8.59	8.36	8.45	8.27	8.36	8.32	8.45	7.99	8.50
7.30 (LR2)	9.33	9.23	9.26	9.24	9.27	9.27	9.30	9.27	9.37
7.34 (LR3)	9.45	9.47	9.46	9.44	9.44	9.44	9.45	9.46	9.46
ORP(mV)									
-20.94 (LR3)	-144.52	-145.63	-145.09	-144.23	-144.34	-143.79	-144.52	-145.18	-144.76

The ORP values were always greater than -200 mV, the range wherein nitrite might accumulate (Lee et al, 2000). NO_2-N in effluent were, on average, more than 1 mg/L and NO_2-N species in NO_2+NO_3-N were predominant in the effluent particularly at the later period in each loading level. Both pH and ORP were also measured in the intermediate ports on LR3. Their profile along column height is graphed in Figure 4.10. Basically, large changes in ORP and pH occurred on the first 10 cm of each reactor and did not change much beyond that point. This also conformed to the findings that much of the nitrate reduction happened in this section of each reactor.

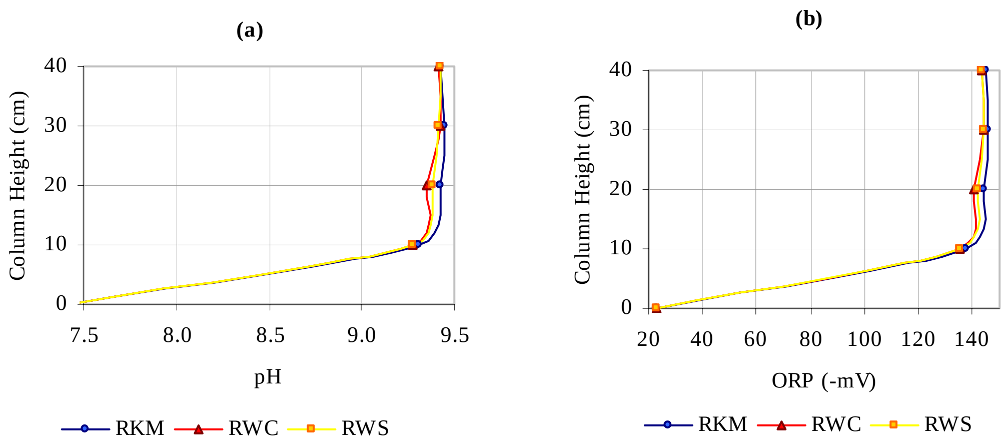

Figure 4.10 pH and ORP profile of the three media types (November 8).

4.4.2 PH AND ORP USING REAL WASTEWATER

Table 4.6 shows the pH and ORP values of the influent and effluent when the reactors were fed real wastewater from the Fish Barn. The pH ranges correspond to the values that were presented in Section 4.4.1; particularly during LR1. The reactors were fed comparable nitrate load rates to that of synthetic wastewater at LR1. Unfortunately, the ORP for LR1 and LR2 were not taken, thus, comparison with that of synthetic wastewater cannot be done.

Table 4.6 Influent and effluent pH and ORP of reactors under real wastewater.

Reactor/ media type	Inf 1	Inf 2	Average	Eff1	Eff 2	Average
pH						
R1 (KM)	7.2	7.41	7.305	8.42	8.58	8.5
R2 (KM)				8.63	8.64	8.64
R3 (KM)				8.63	8.62	8.63
R4 (WC)				8.72	8.68	8.70
R5 (WC)				8.64	8.57	8.61
R6 (WC)				8.7	8.67	8.69
R7 (WS)				8.79	8.75	8.77
R8 (WS)				8.73	8.73	8.73
R9 (WS)				8.65	8.66	8.66
ORP (mV)						
R1 (KM)	-13.2	-25.2	-19.2	-86.2	-94.2	-90.2
R2 (KM)				-96.5	-97.3	-96.9
R3 (KM)				-96.3	-96.1	-96.2
R4 (WC)				-102	-100.3	-101.15
R5 (WC)				-96.9	-94.2	-95.55
R6 (WC)				-100.8	-98.9	-99.85
R7 (WS)				-106.4	-104.1	-105.25
R8 (WS)				-102.6	-102.4	-102.5
R9 (WS)				-98.3	-98.7	-98.5

4.4.5 pH, ORP and Nitrate reduction

ORP and pH appear to be closely related. ORP went up and down with pH as seen in Figures 4.11, 4.12, and 4.13. pH also increased with higher nitrate reduction. The relationship of the ORP, pH and nitrate reduction is useful if automation of denitrification is to be implemented in RAS facilities.

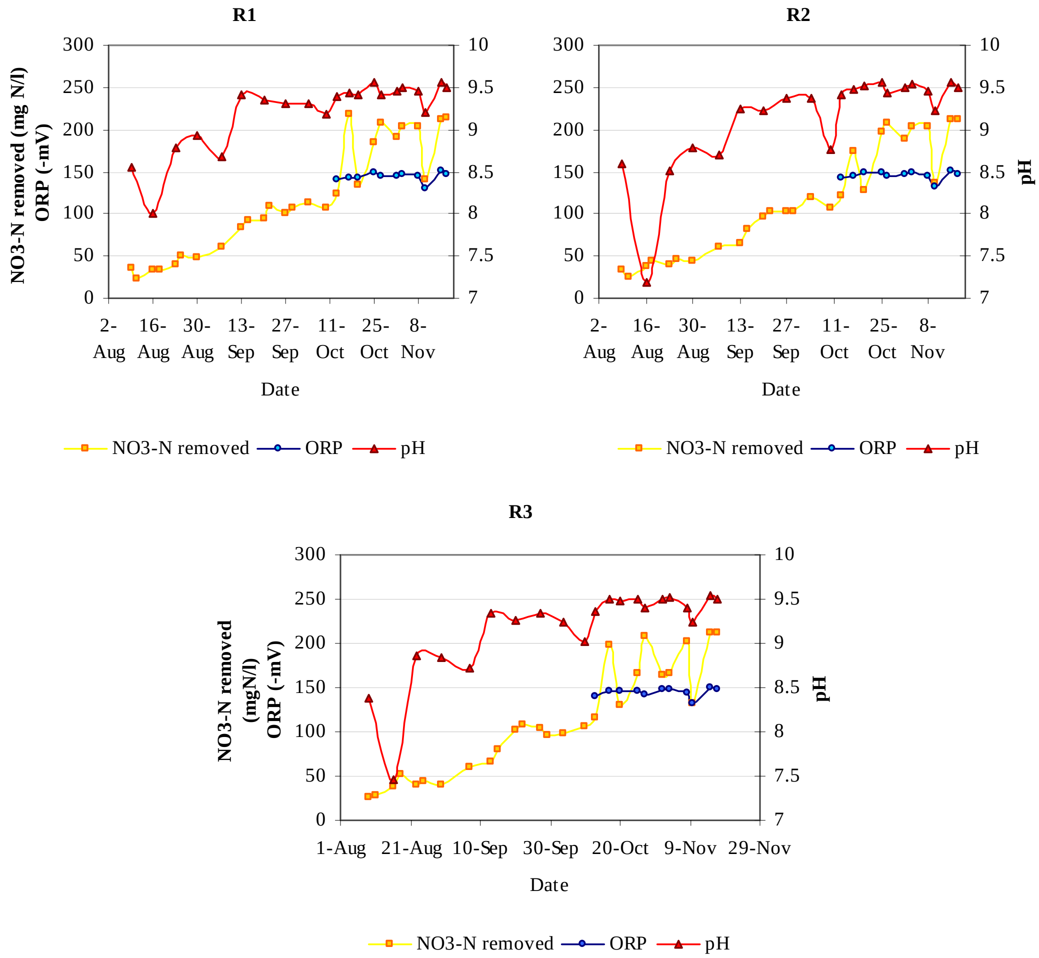

Figure 4.11 Relationship of pH, ORP, and NO_3-N in Kaldnes reactors.

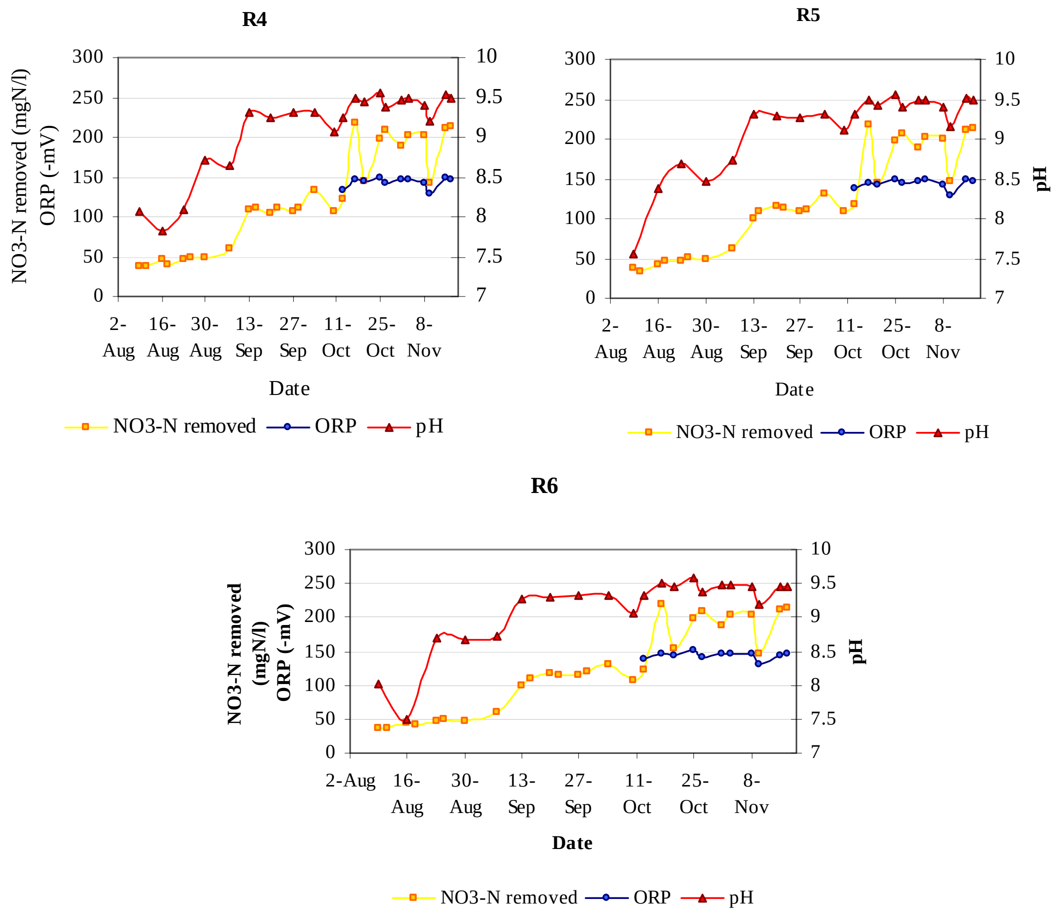

Figure 4.12 Relationship of pH, ORP, and NO_3-N in wood chips reactors.

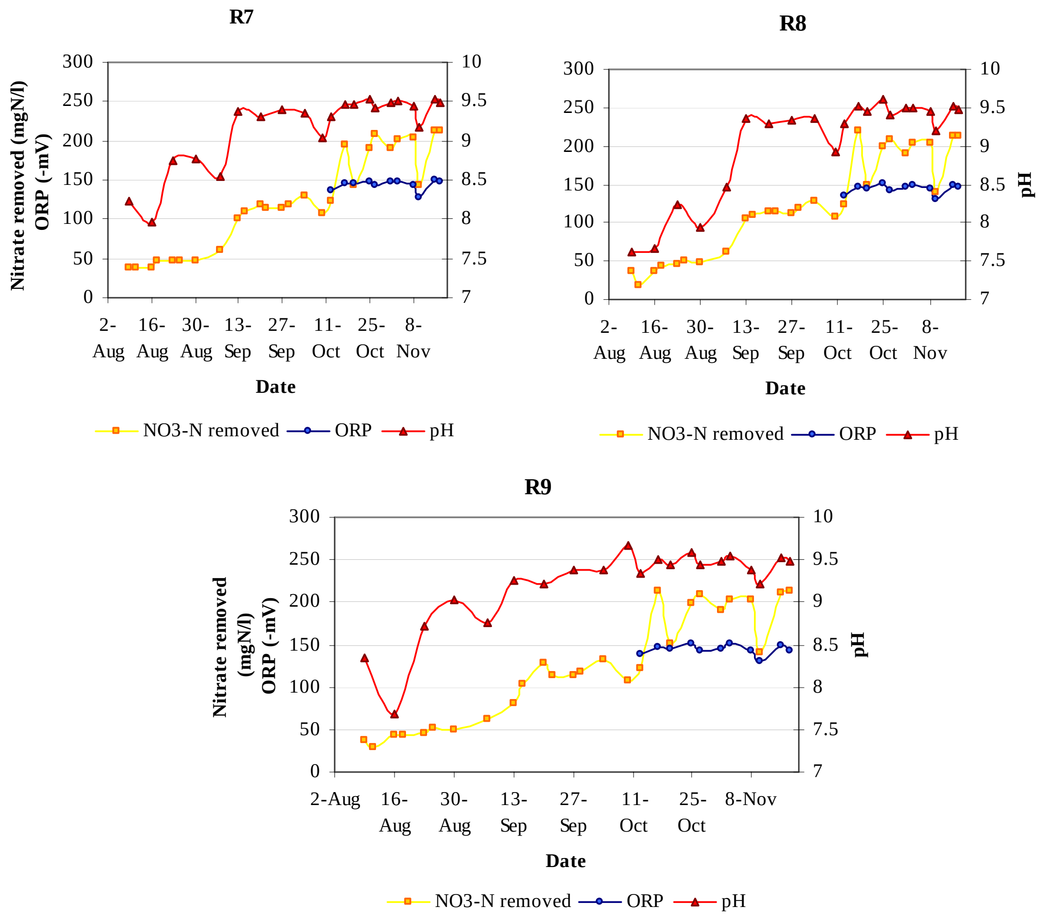

Figure 4.13 Relationship of pH, ORP, and NO_3-N in wheat straw reactors.

5. DEGRADATION OF BIOFILTER MEDIA AND BIOMASS CHARACTERIZATION

5.1 CHANGES IN PHYSICAL CHARACTERISTICS

The degradation of the wood chip and wheat straw media were assessed physically by measuring weight loss as well as changes in the media bed height. Porosities were also measured to further assess physical changes in the reactors and media types.

5.1.1 MASS AND BED HEIGHT LOSS

Wheat straw lost 37.7± 2.7% of its initial dry mass compared to only 16.2 ± 5.2% for wood chips. (Table 5.1). As such, R7 and R8 (both with wheat straw) experienced reductions in bed height. R7 decreased by 2.33 cm while R8 decreased 1.72 cm. This correlated with the results in mass reduction since R7 and R8 had higher mass reductions than R9. There was no observed media bed height loss in the wood chip reactors.

Table 5.1 Mass loss of wood chips and wheat straw after 140 day experiment.

Reactor	Media	Initial Dry mass (g)	Final Dry mass (g)	Reduction in mass (g)	Average reduction in mass (g)	Percentage Reduction (%) (initial dry mass basis)	Average Percentage Reduction (%)
R4		751.0	584.3	166.7		22.20%	
R5	wood chips	719.3	620.6	98.7	117.5 ± 43	13.72%	16.2 ± 5.2 %
R6		686.3	599.3	87.0		12.68%	
R7		161.3	97.8	63.5		39.37%	
R8	wheat straw	159.1	96.9	62.2	60.4 ± 4.3	39.09%	37.7 ± 2.7%
R9		160.3	104.8	55.5		34.62%	

Wheat straw at the bottom of the reactors appeared to have higher degradation than the wheat straw at the top. The reported bed height loss did not accurately reflect the mass loss, as the volume at the bottom of these reactors were filled primarily by biomass and liquid, as described later in this chapter. Without biomass accumulation, the bed height may have receded more. Conversely, the wood chips in the lower sections of the reactors, although degraded, remained sturdy enough to support upper wood chip media.

Based on these results, wood chips would last longer than wheat straw. Using the mass reduction rates listed in Table 5.1, wood chips would degrade by approximately 42% in a year with a rate of around 0.117% per day or around 1.17 g lost per kg of initial mass per day. On the other hand, wheat straw would degrade more than 98% in a year at a rate of 0.269% per day or 2.69 g lost per kg of initial mass per day. These estimates assumed that reduction would be linear. If the organic media's useful life is 50% of its initial biomass, then wood chips would last 1 year and wheat straw ½ year.

5.1.2 CHANGES IN POROSITY

The porosity of a reactor is expected to change as biodegradation of the media and accumulation of biomass take place. The porosity affects actual hydraulic residence time as well as the superficial hydraulic velocity.

Porosity was measured by draining water from ports, thereby measuring liquid that is free to flow. This was assumed to be the liquid that was flowing in the reactors while other fluid, absorbed by the media and biomass, was excluded from the measurement. Table 5.2 illustrates how the porosity changed during the experimental period.

Table 5.2 Porosity of the reactors and their sections before and after the denitrification tests.

Section	Kaldnes				wood chips				wheat straw			
	R1	R2	R3	RKM	R4	R5	R6	RWC	R7	R8	R9	RWS
Initial Porosity (%)												
Fourth Quarter + Top	86.22	79.81	81.19	82.41± 3.38	57.69	58.21	55.28	57.06 ± 1.57	57.92	63.73	67.82	63.16 ± 4.97
Third Quarter	77.64	79.50	77.02	78.05±1.29	62.03	51.25	52.80	55.36 ± 5.83	48.13	55.00	55.00	52.71± 3.97
Second Quarter	73.13	75.78	73.75	74.22± 1.39	50.93	51.55	51.57	51.35 ± 0.36	48.75	51.23	51.85	50.61± 1.64
First Quarter + Bottom	90.25	87.60	89.63	89.16± 1.39	68.95	68.70	68.53	68.73 ± 0.21	73.75	73.54	68.90	72.06± 2.74
Whole Reactor	82.87	81.35	81.41	81.88± 0.86	60.77	58.72	58.31	59.27 ± 1.32	58.92	62.58	62.21	61.24± 2.01
Final Porosity (%)												
Fourth Quarter + Top	55.66	56.54	59.60	57.27± 2.07	42.69	43.18	40.20	42.03 ± 1.60	25.54	37.25	24.75	29.18± 7.00
Third Quarter	47.70	45.22	45.96	46.29± 1.27	33.67	27.50	22.36	27.84 ± 5.66	6.25	15.25	3.75	8.42± 6.05
Second Quarter	29.00	34.78	27.50	30.43± 3.85	14.91	14.91	8.55	12.79 ± 3.67	21.25	3.70	4.69	9.88± 9.86
First Quarter + Bottom	15.25	4.46%	28.22	15.98± 11.89	8.23	14.63	8.92	10.59 ± 3.52	45.00	35.95	46.46	42.47± 5.69
Whole Reactor	36.05	33.32	40.10	36.49±3.42	24.05	24.84	19.74	22.88 ± 2.75	26.72	25.39	23.34	25.15± 1.70
Void filled with biomass and/or gases after experiments (%)												
Fourth Quarter + Top	30.56	23.27	21.58	25.14± 4.77	15.00	15.02	15.08	15.03 ± 0.04	32.38	26.47	43.07	33.97± 8.41
Third Quarter	29.94	34.29	31.06	31.76± 2.26	28.35	23.75	30.43	27.51 ± 3.42	41.88	39.75	51.25	44.29± 6.12
Second Quarter	44.13	40.99	46.25	43.79± 2.64	36.02	36.65	43.02	38.56 ± 3.87	27.50	47.53	47.16	40.73± 11.46
First Quarter + Bottom	75.00	83.14	61.41	73.18± 10.98	60.73	54.07	59.60	58.13 ± 3.57	28.75	37.59	22.44	29.59± 7.61
Whole Reactor	46.82	48.03	41.31	45.39± 3.58	36.72	33.88	38.57	36.39 ± 2.36	32.20	37.19	38.87	36.09± 3.47

Generally, approximately half of the original void volume was filled by gas and biomass by the end of the experiments. On average, Kaldnes, wood chips, and wheat straw started at 81.88 ± 0.86%, 59.27 ± 1.32%, and 61.24 ± 2.01%, void space respectively. These values declined to 36.49 ± 3.42%, 22.88 ± 2.75%, and 25.15 ± 1.70%, respectively.

The manner in which the void fractions reduced also differed among reactor sections. Initially, the void volumes were generally higher in the bottom sections than in upper sections. The measurement of void volume on the bottom and uppermost section included the vacant spaces at both ends of the reactors (PVC reducer tees). While these sections had the largest void fraction initially, they ended up having the least void volumes at the end of the study. These observations were found in almost all reactor types except for the wheat straw.

At the end of the study, significant volumes of liquid were extracted from the bottom of wheat straw reactors. Compared to the second and third sections, the volumes were higher at the bottom. The higher porosity at the bottom part of the wheat straw is attributed to degradation of the straw without the collapse of the bed of media. Hollow spaces at the bottom of the media bed, particularly near the screen were observed. While a substantial biomass of bacteria existed near the bottom of the reactors, only a small fraction of the wheat straw was intact or resembled its original state. This will be discussed later.

The low porosity in the second and third sections of wheat straw media were attributed to swelling of the media as well as possible gas and biomass accumulation.

Wheat straw exhibited more swelling than wood chips. Kaldnes, being made of dense plastic, did not swell. Gas bubbles were seen trapped in the interstitial spaces of the wood chips and wheat straw as well as in the spaces in the middle of Kaldnes media. In order to separate solids and gas accumulation, solids were also measured (To be discussed in Section 5.3.)

5.1.3 VISUAL OBSERVATIONS OF THE MEDIA DEGRADATION

Of the two media, wheat straw exhibited more signs of physical change. Figure 5.1 shows a comparison of new and spent (used) wheat straw. It is readily evident that some wheat straws were reduced to strands or fibers. One may assume that what was left of the straw were carbonaceous components (Section 5.2) that were able to withstand anoxic biological decomposition. The spent straw was also darker than the new media. Slight physical changes were also detected in the wood chip media; mainly color changes to darker shades. Additionally, chips were broken into smaller pieces.

Figure 5. 1 A comparison of raw (new) and spent (used) wheat straw media.

5.2 CHANGES IN CHEMICAL CHARACTERISTICS

5.2.1 CHANGES IN CARBON AND NITROGEN CONTENT OF THE MEDIA

The relative composition of carbon and nitrogen of both wheat straw and wood chips were also measured. As shown in Table 5.3, there was enrichment of nitrogen in the both media types. As a result, the media C/N ratio decreased. Low C/N ratios favor biodegradation. Wheat straw reactors had the lowest C/N in the spent media. This

Table 5.3 Carbon and nitrogen content of new and used wood chips and wheat straw.

Media	Reactor	Total dry mass (g)	%C	Calculated mass of carbon (g)	%N	Calculated mass of nitrogen (g)	C/N
New media							
Wood chips	R4	751	39.35[a]	295.55	0.10[a]	0.75	393.54[a]
	R5	719.3		283.07		0.72	
	R6	686.3		270.09		0.69	
	mean			282.90 ±12.73		0.72 ±0.03	
Wheat straw	R7	161.3	43.25 [a]	69.77	0.32[a]	0.52	134.75 [a]
	R8	159.1		68.82		0.51	
	R9	160.3		69.34		0.51	
	mean			69.31± 0.48		0.51 ±0.00	
Used media							
Wood chips	R4	584.3	47.55	277.83	0.211	1.23	225.36
	R5	620.6	47.30	293.54	0.283	1.76	167.14
	R6	599.3	46.77	280.29	0.286	1.71	163.53
	mean		47.21±0.40	283.89 ±8.45	0.26±0.04	1.57 ± 0.29	185.34±34.70
Wheat straw	R7	97.8	44.36	43.38	1.15	1.12	38.57
	R8	96.9	43.58	42.23	1.22	1.18	35.72
	R9	104.8	44.76	46.91	1.23	1.29	36.39
	mean		44.23±0.60	44.17±2.44	1.20±0.04	1.20 ± 0.08	36.90±1.49
Constituent mass reduction							
		Reduction of C mass	**%Change in C mass**	**Mean % Reduction in C**	**Change of N mass**	**%Change in N mass**	**Mean % Reduction in C**
Wood chips	R4	17.71	5.99%	-0.49 ±5.62%	-0.482	-64.16%	-119.36 ±47.88%
	R5	-10.47	-3.70%		-1.037	-144.17%	
	R6	-10.21	-3.78%		-1.028	-149.74%	
Wheat straw	R7	26.38	37.82%	36.27±3.42	-0.607	-117.22%	-133.07 ±16.70%
	R8	26.59	38.64%		-0.671	-131.48%	
	R9	22.43	32.35%		-0.774	-150.51%	

[a]*only one set of samples was analyzed for each organic media*

chemical parameter result supported the physical and visual assessments of media degradation as described in Section 5.1. Conversely, there was an increase in the mass of carbon in R5 and R6; both reactors with wood chip media. The microbial biomass (particularly those on the micropores of the media) may not have been totally removed by washing and brushing with deionized water prior to analysis and the carbon analysis may have erroneously included the microbial biomass carbon as well.

5.2.2 CHANGES IN THE LIGNIN CONTENT OF THE MEDIA

To further assess changes in the carbonaceous components of the media, lignin content was analyzed. This analysis only measured lignin and indirectly estimated hemicellulose and cellulose that are assumed to comprise a major portion of the mass labeled herein as “other constituents”. Table 5.4 shows the concentration of lignin, ash, and other constituents of both media types. Their masses were calculated by multiplying the concentrations (%) by the total mass measured (g) in Section 5.1.1.

The two types of media behaved differently in terms of lignin degradation. As shown in the Table 5.4, the wood chips’ acid-insoluble lignin concentration decreased on average from 27.22% to 25.93%. Conversely, the wheat straw’s acid-insoluble component increased from 22.96% to 24.84%.

Table 5.4 Lignin, ash and other constituents of new and used wood chips (WC) and wheat straw (WS) media.

Reactor (type)	Lignin						Ash		Other Constituents	
	Acid insoluble		Acid soluble		Total					
	% dry basis	calculated mass (g)	% dry basis	calculated mass (g)	% dry basis	calculated weight	% dry basis	calculated mass (g)	% dry basis	calculated mass (g)
New media										
R4(WC)	27.22%	204.45	2.74%	20.61	29.97%	225.05	0.87%	6.54	69.16%	519.41
R5(WC)		195.82		19.74		215.55		6.26		497.48
R6(WC)		186.83		18.83		205.67		5.97		474.66
RWC (mean)		195.70 ±8.81		19.73 ±0.89		215.43 ±9.70		6.26 ±0.28		497.18 ±22.38
R7(WS)	22.96%	37.03	2.36%	3.81	25.32%	40.84	4.15%	6.69	70.53%	113.77
R8(WS)		36.52		3.76		40.28		6.60		112.22
R9(WS)		36.80		3.79		40.59		6.65		113.07
RWS (mean)		36.78 ±0.25		3.79 ±0.03		40.57 ±0.28		6.64 ±0.05		113.02 ±0.78
Used media										
R4(WC)	25.49%	148.94	3.52%	20.59	29.01%	169.53	1.74%	10.18	69.24%	404.59
R5(WC)	25.64%	159.15	3.45%	21.43	29.10%	180.57	1.99%	12.38	68.91%	427.65
R6(WC)	26.65%	159.71	3.52%	21.11	30.17%	180.82	2.09%	12.51	67.74%	405.97
RWC (mean)	25.93 ±0.63%	155.93 ±6.06	3.50 ±0.04%	21.04 ±0.42	29.43 ±0.65%	176.97 ±6.45	1.94 ±0.18%	11.69 ±1.31	68.63 ±0.79%	412.74 ±12.93
R7(WS)	25.07%	24.52	2.28%	2.23	27.35%	26.75	4.92%	4.81	67.73%	66.24
R8(WS)	25.35%	24.56	2.11%	2.05	27.46%	26.61	5.63%	5.46	66.91%	64.83
R9(WS)	24.11%	25.27	2.65%	2.78	26.76%	28.05	6.90%	7.23	66.34%	69.52
RWS (mean)	24.84 ±0.65%	24.78 ±0.42	2.35 ±0.27%	2.35 ±0.38	27.19 ±0.38%	27.14 ±0.79	5.82 ±1.00%	5.83 ±1.25	66.99 ±0.70%	66.86 ±2.40
Constituent mass reduction										
	% mass reduction	mass reduction (g)	% mass reduction	mass reduction (g)	% mass reduction	mass reduction (g)	% mass reduction	mass reduction (g)	% mass reduction	mass reduction (g))
R4(WC)	27.15%	55.50	0.08%	0.02	24.67%	55.52	-55.75%	-3.64	22.11%	114.82
R5(WC)	18.73%	36.67	-8.55%	-1.69	16.23%	34.98	-97.69%	-6.12	14.04%	69.83
R6(WC)	14.52%	27.12	-12.07%	-2.27	12.08%	24.85	-109.38%	-6.53	14.47%	68.69
RWC (mean)	20.13 ±6.43%	39.77 ±14.44	-6.85% ±6.26%	-1.31 ±1.19	17.66% ±6.42%	38.45 ±15.63	-87.61% ±28.20%	-5.43 ±1.56	16.87% ±4.54%	84.45 ±26.31
R7(WS)	33.79%	12.51	41.39%	1.58	34.50%	14.09	28.07%	1.88	41.78%	47.53
R8(WS)	32.75%	11.96	45.57%	1.71	33.94%	13.67	17.28%	1.14	42.23%	47.39
R9(WS)	31.33%	11.53	26.68%	1.01	30.90%	12.54	-8.81%	-0.59	38.51%	43.55
RWS (mean)	32.62% ±1.24%	12.00 ±0.49	37.88% ±9.92%	1.43 ±0.37	33.11% ±1.94%	13.43 ±0.80	12.18% ±18.96%	0.81 ±1.26	40.84% ±2.03%	46.16 ±2.26

However, the wood chips' acid-soluble lignin increased from 2.74% to 3.50% while the wheat straw did not change (2.36% to 2.35%). Analytical results did not show much change in the concentration of total lignin in wood chips although the mass of the wood chips (Figure 5.2) reduced by an average of 38.45 g (17.66% reduction). These results were not expected as we would expect the lignin concentration to increase due to the degradation of hemicellulose and cellulose. But as discussed in Section 5.2.1, there was an increase in the carbon mass of the wood chips which can most likely attributed to the attached microbial biomass. The microbial carbonaceous component being not the majority of lignin apparently reduced the lignin concentration.

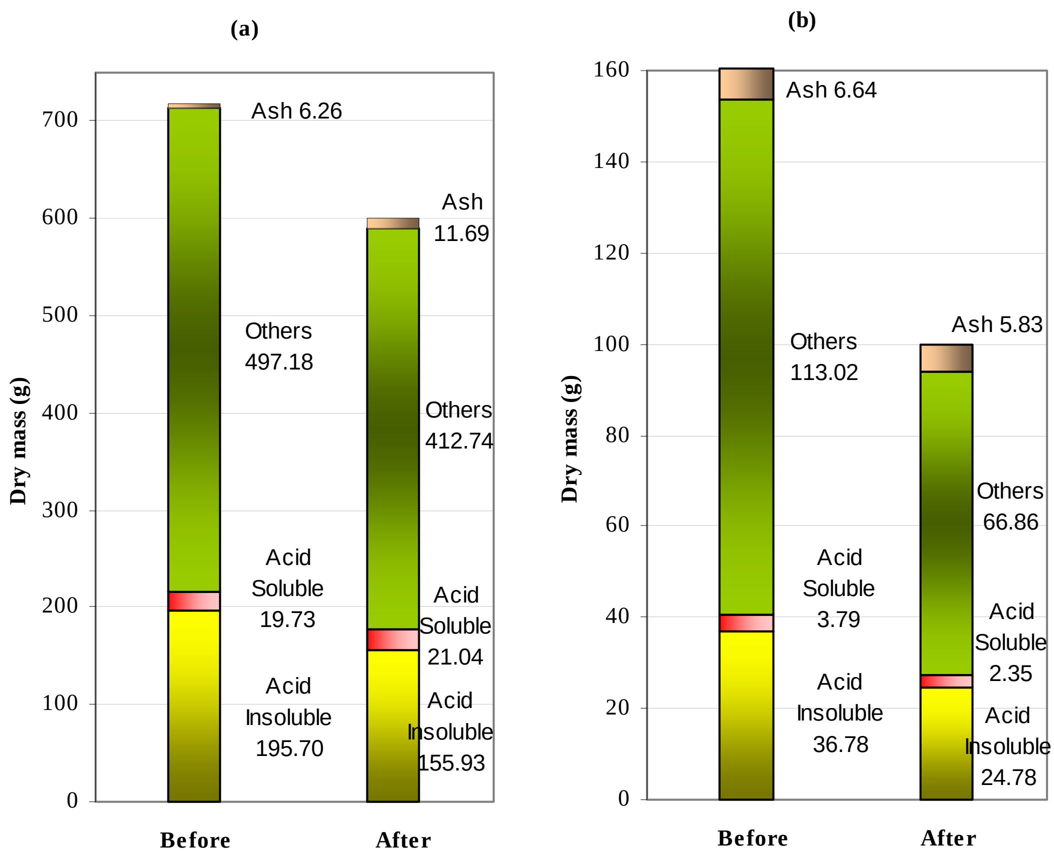

Figure 5. 2 Mean changes of masses of lignin, ash, and other constituents of wood chips and wheat straw before and after the experiments.

The mass of ash in the wood chips increase slightly while not increasing much in the wheat straw. This may be attributed to non-volatile solids that may have attached to the biomass during the tests. Non-volatile solids may be inert particles from microbial decay or the inorganic nutrients added in the synthetic wastewater (trace metal elements) which somehow have been adsorbed on the wood chips' surfaces.

5.3 Biomass development in the Reactors

Table 5.5 summarizes the analysis used to estimate the increase in biomass inside the reactors at the end of the denitrification experiments. In Table 5.5, the column labeled "Initial Volumes" refer to the volume of water taken from the reactor to determine porosity. The column labeled "Diluted Volumes" refer to the liquid volumes summing up the initial volume and the washwater during the cleaning of the media. The assumption was made that the majority of the biomass attached on the media was washed off the media and was captured in buckets where these diluted volumes were measured.

The data in the "Total Suspended Solids" column is from the laboratory analysis and adjusted to fit with the initial volume since TSS values were obtained using the diluted volumes. The same approach was taken in obtaining adjusted volatile suspended solids values. These adjusted TSS and VSS values are used to estimate the solids that were "free" or suspended in the bulk liquid of the reactor. However, it was observed that a majority of the solids were attached on the media. All solids residing in the reactor were collected and quantified to compare between the reactors. VSS are a good indicator of the level of organic solids and were used in these experiments to estimate final microbial biomass.

Table 5.5 Solids Analyses of nine reactors to estimate microbial biomass.

Reactor	Initial Volume (mL)	Diluted Volume (mL)	TSS (mg/L)	Adjusted TSS (mg/L)	Total Suspended solids in the reactor (g)	Average TSS (g)	VSS (mg/L)	Adjusted VSS (mg/L)	Estimated Total Microbial Biomass in the reactor (g VSS)	Average VSS (g)	Diluted Volume VS (% of TS)	Diluted volume TS (% mass)
R1 (KM)	1386	12000	3479	30121	41.75		2577	22312	30.92		63.16	0.38
R2 (KM)	1286	14000	3273	35631	45.82	43.77 ±2.04	2502	27238	35.03	32.93 ±2.05	64.71	0.34
R3 (KM)	1532	12360	3540	28560	43.75		2658	21444	32.85		63.41	0.41
R4 (WC)	932	9632	6200	64076	59.72		5710	59012	55.00		70.42	0.71
R5 (WC)	954	6920	7600	55128	52.59	55.42 ±3.72	5810	42144	40.21	46.21 ±7.78	67.95	0.78
R6 (WC)	760	11084	4900	71463	54.31		3918	57141	43.43		69.81	0.53
R7 (WS)	1018	13390	5670	74578	75.92		4890	64319	65.48		68.63	0.51
R8 (WS)	994	10000	4940	49698	49.40	58.09 ±15.44	4130	41549	41.30	49.55 ±13.39	68.97	0.58
R9 (WS)	908	12000	4080	53921	48.96		3490	46123	41.88		69.77	0.43

Data in Table 5.5 shows that the highest average VSS were in wheat straw (49.55 g); slightly greater than in the wood chips mean (46.21 g). The reactors with Kaldnes media had the lowest microbial biomass content measured as VSS (32.93 g). The increased biomass corresponded with better denitrification rates of the organic media. The reactors containing large quantities of microbial biomass were able to respond quickly to shockloads or sudden increase of nitrate loads as illustrated in Chapter 3.

Wheat straw and wood chips are therefore desirable in the sense that they can develop good biomass for denitrification. It is proposed that bacteria grew better since they could obtain carbon and nitrogen on two fronts – from the organic surface and from the bulk liquid. Aside from that, ammonia, which is preferred by bacteria, was the main nitrogen form obtained from decaying organics. Bacteria can also adhere better to the rougher organic surfaces than the slick inert plastic surface of the Kaldnes media.

5.3.1 BIOMASS GROWTH ALONG THE REACTOR COLUMN HEIGHT

Biomass in each section was not quantified due to the difficulty of removing biomass from one section only. However, visual observations of biomass buildup in each section were conducted. Figure 5.3 showed biomass buildup on the Kaldnes reactors along column height. As shown, biomass was thicker at the bottom and decreased with height. This is also true for wood chips and wheat straw. Biomass growth in the wood chip media can be seen in Figure 5.4 and in the wheat straw media Figure 5.5.

The concentration of biomass in the bottom sections of the reactors contributed to the good nitrate removal in the bottom half of the reactors as discussed in Chapter 3. One can conclude from these results that the biomass in these sections included denitrifiers.

The quantity of biomass in the wheat straw and wood chip reactors, however, may indicate that clogging may become a problem to be considered in reactor future design. While clogging was not experienced in this study, evidence of biomass buildup would indicate possible clogging if the study was undertaken for an extended period of time.

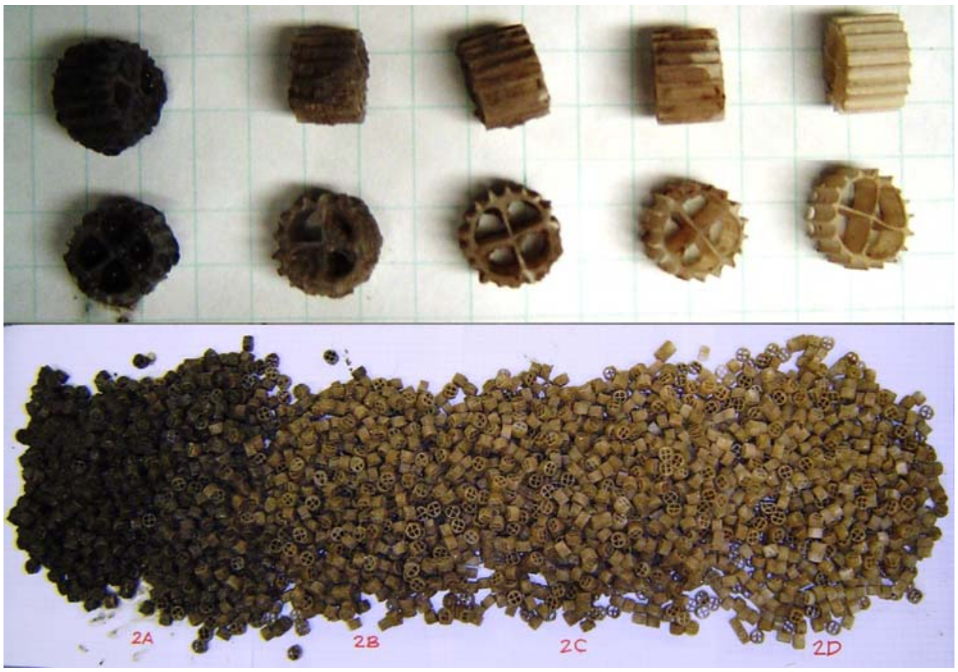

Figure 5. 3. Kaldnes beads taken in each level - 0, 10, 20, 30, 40 cms. (shown above from left to right). The lower portion showed the beads freshly taken out.

Figure 5. 5. Wood chips with biomass attached in varying thickness. Lowest position in biofilter (A) to highest (D).

Figure 5. 5. Biomass on wheat straw from the lowermost portion of the reactor (A) to the uppermost section (D)

5.3.2 BIOMASS BUILDUP WITH TIME

Biomass buildup was also documented during the duration of the study. A photo log was maintained for the 9 reactors. For discussion purposes, only one reactor for each reactor type is presented in this section.

The appearance of Kaldnes reactors started as white going to murky (Figure 5.6). It should be noted that the Kaldnes reactor on 07/20 was darker than 08/10 because of the Fish Barn wastewater. The dark brown colors of wood chips surfaces were later covered with whitish brown (Figure 5.7). These whitish biomass adhering to the wood chip surfaces seem to follow with increasing concentration and age. Straws of wheat started light brown and end up murky black (Figure 5.8). Black colors may also be attributed to sulfide buildup due to a distinct smell.

The photo logs of these three reactors also showed how the colors changed along column height or how biomass growth moved up the reactors. The images on 11/06 seemed to be lighter than the adjacent images due to reflection of light during the taking of pictures.

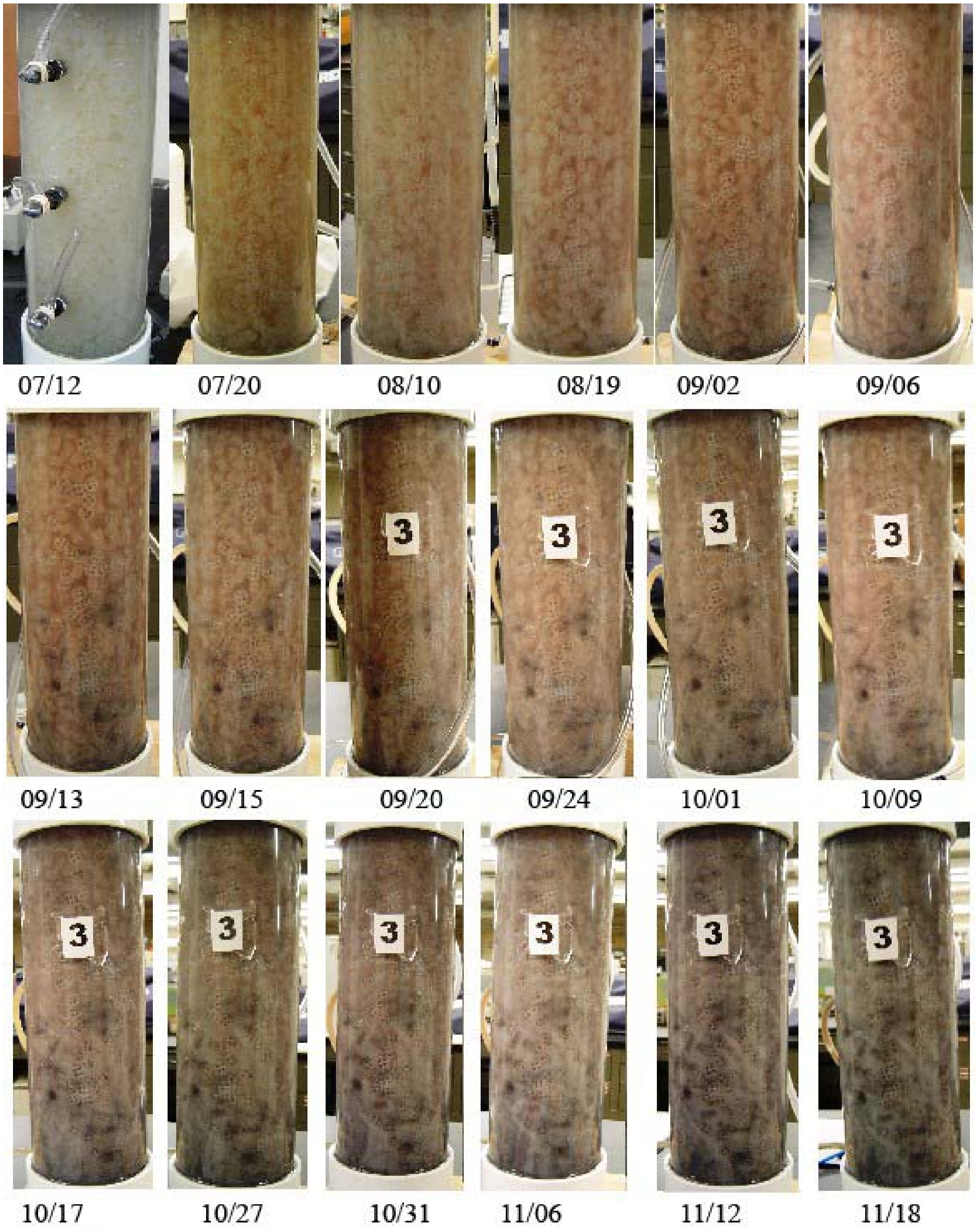

Figure 5. 6 A photo log of the biomass buildup on Reactor 3 – Kaldnes media.

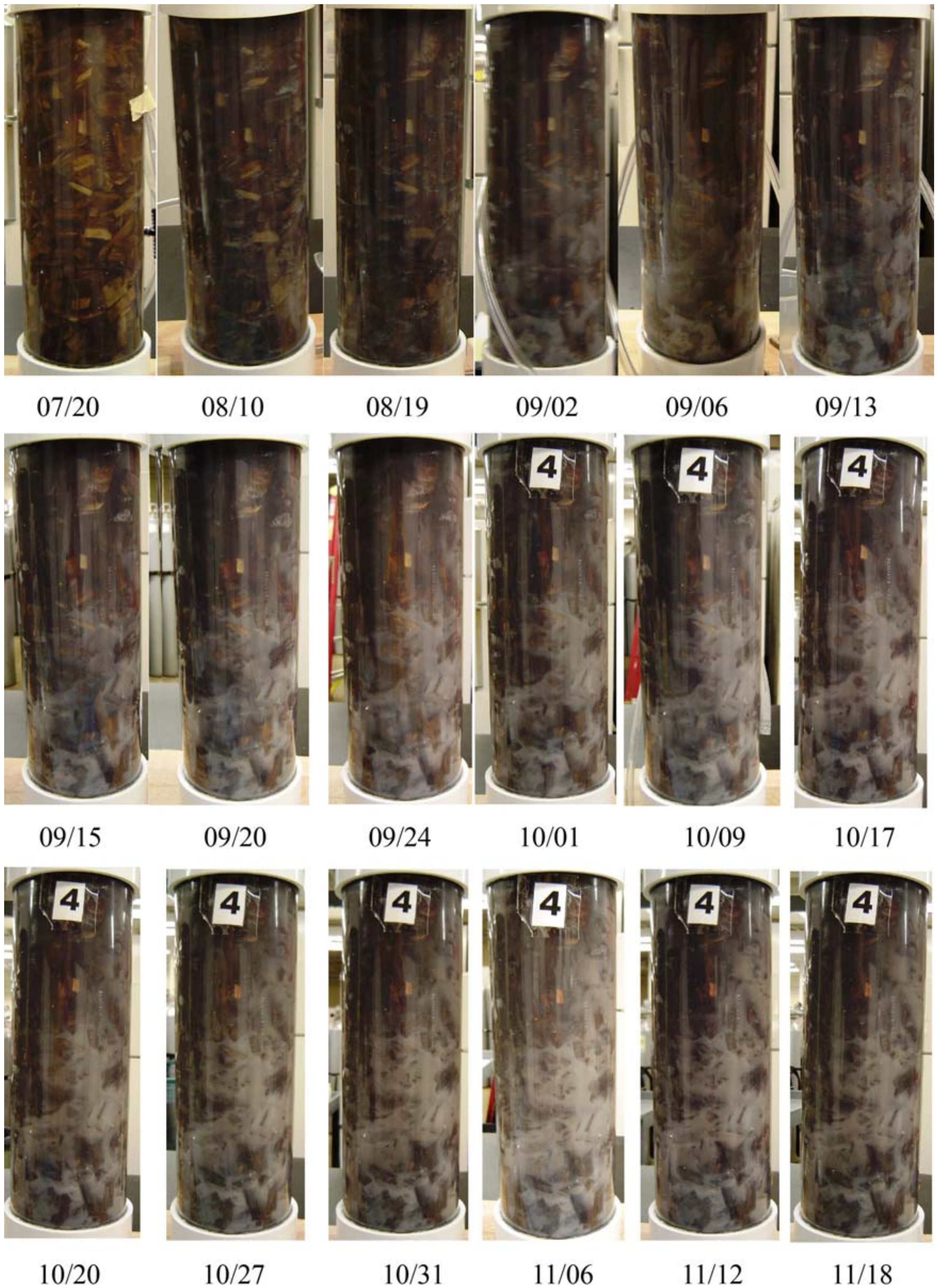

Figure 5. 7. A photo log of biomass buildup on reactor 4 – wood chips.

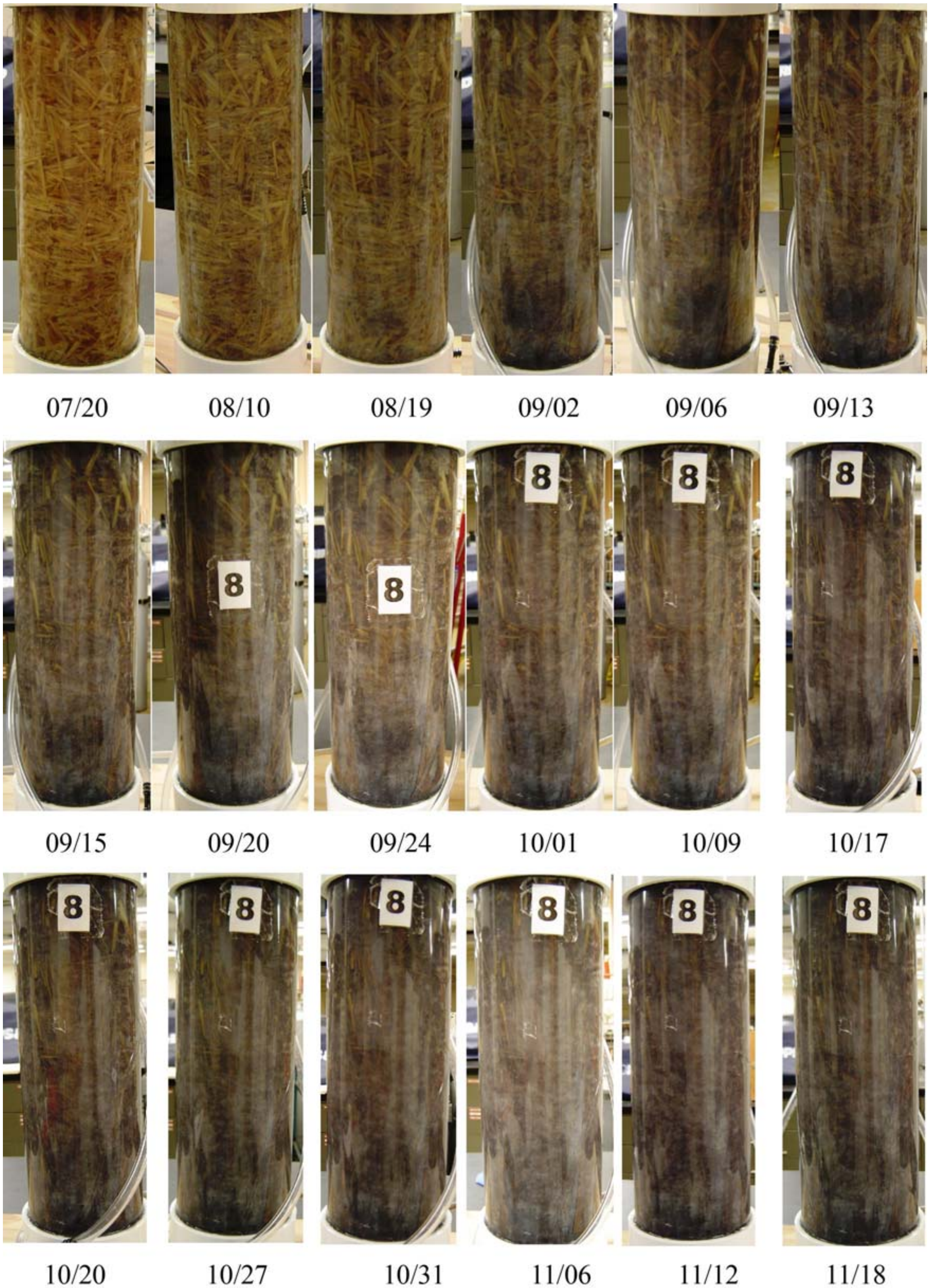

Figure 5. 8 A photo log showing biomass buildup on reactor 8 – wheat straw.

5.4 DIFFERENCES IN THE MICROBIAL COMMUNITY

Results of the Denaturing Gradient Gel Electrophoresis (DGGE) analysis indicated that the reactors maintained a diverse bacterial community. The DGGE gel is shown in Figure 5.9. There were approximately 13 distinct bands representing different ribotypes. Since the procedure used Bacterial primers, the bands represent bacterial deoxyribonucleic acids (DNAs) which were present in the particular reactor environment. Since there was substantial nitrate reduction, we assume that these ribotypes were denitrifying, if these ribotypes survived in the reactors. This analysis can only assess differences in bacterial communities, and no specific organisms were identified.

Figure 5.9 shows the three reactor types and the samples taken from 10, 20, and 30 cm ports (from the bottom of the packed volume) listed as A, B, C, respectively. Gels 1, 2, 3 are from reactor 5 (wood chips) while gels 4, 5, 6 are from reactor 2 (Kaldnes). Gels 7, 8, 9 were from reactor 7 (wheat straw). The arrangement in the photograph did not represent the heights 10, 20, 30 cm in order. The gels were rearranged later to analyze the ribotype difference along height (Section 5.3.1)

Ribotypes common to all three media were labeled as *b, e, g, h, j, k* and *l*. Additional ribotypes identified were: in Kaldnes: *a, m*; wood chips: *d, f, i*; and wheat straw: *a, c, d, i, m*.

Ribotypes *i* and *d* were present in both wheat straw and wood chips possibly indicating that these bacteria were actively growing on organic media. Only wood chips had ribotype *f* while ribotype *c* was only visible in wheat straw. Wheat straw and wood chips were suited as a habitat for those bacteria.

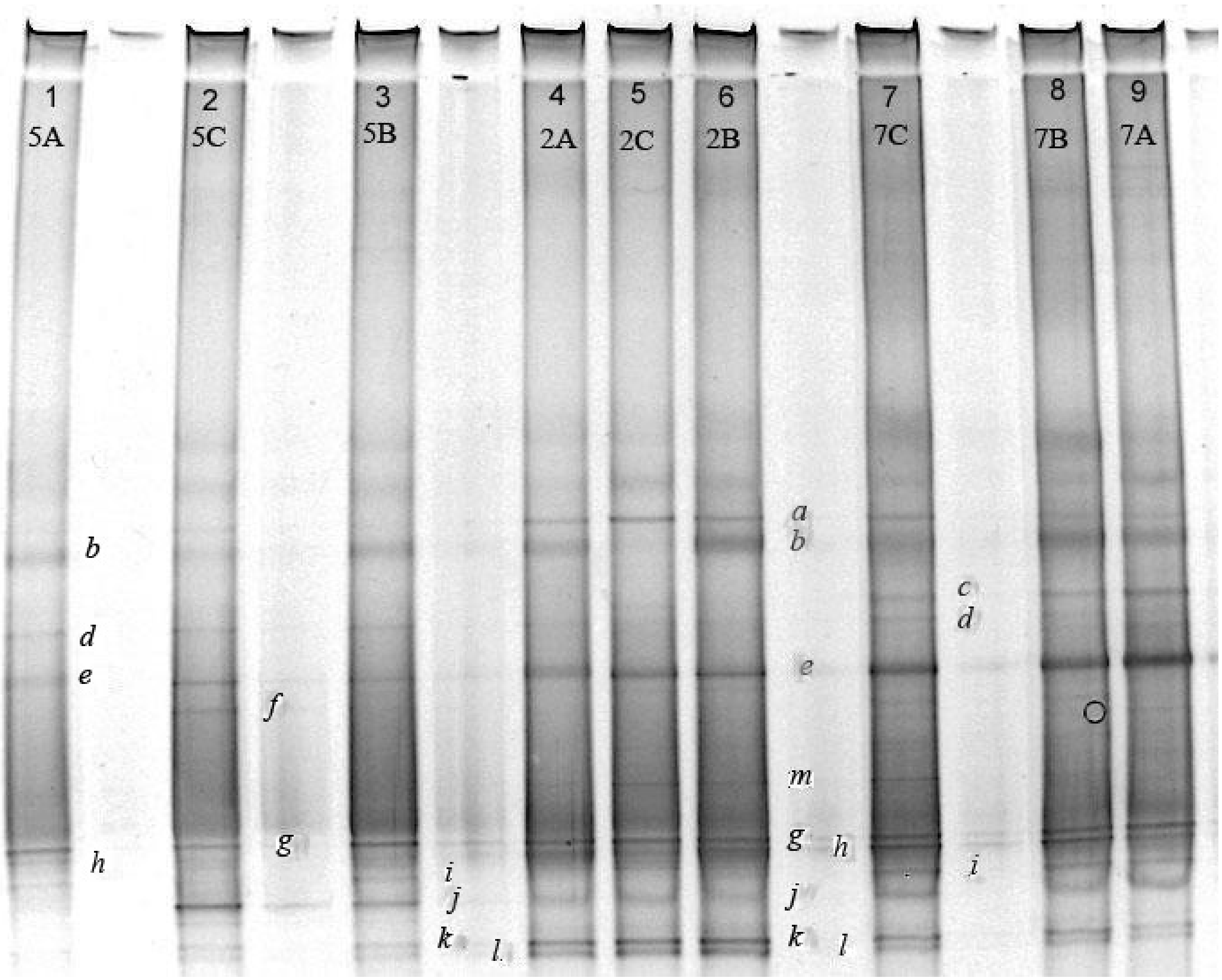

Figure 5. 9 **The DGGE photograph showing nine gels in order (left to right) : 5A, 5C, 5B-(wood chips); 2A, 2C, 2B-(Kaldnes); 7C, 7B, and 7A-(wheat straw).**

Ribotypes *i* and *d* were perhaps responsible for the degradation of the organic media while feeding on hydrolyzed organics which released ammonia.. This may also be true of ribotype *f* for wood chips and ribotype *c* for wheat straw.

Ribotypes common in all three reactors were likely denitrifiers responsible for substantial nitrate reduction. These were possibly inoculated from the Fish Barn wastewater. Bacteria may also have been introduced into the system from wheat straw and wood chips. As time progressed, these bacteria acclimatized to the favorable reactor environments. The differences in the gels (presence/absence of bands) accounted for the selectivity of a bacterial species on a specific environment favorable for its growth.

5.4.1 MICROBIAL COMMUNITY ALONG COLUMN HEIGHT

If ribotype *i* was a denitrifier, it preferred high nitrate concentration since it appeared on port 5A and 5B but faded in port 5C (Figure 5.10). This observation supports the idea that ribotype *i* can feed on hydrolyzed organics since degradation was expected to be higher in the lower sections of these upflow reactor.

Ribotype *f*, which was unique for the wood chips reactor only, showed activity at the 30 cm (5C) port. This bacterium, if a denitrifier, evidently survived on low concentrations of nitrate and is a nitrate scavenger. On the other hand, ribotype *b* possibly belongs to the group that needs high nitrate concentrations. In port 5C, where nitrate concentration is low, bacteria of this type was not present. Nitrate scavengers along with other denitrifiers are important since they have the capability to reduce residual nitrate-nitrogen providing “polishing” for good effluent quality.

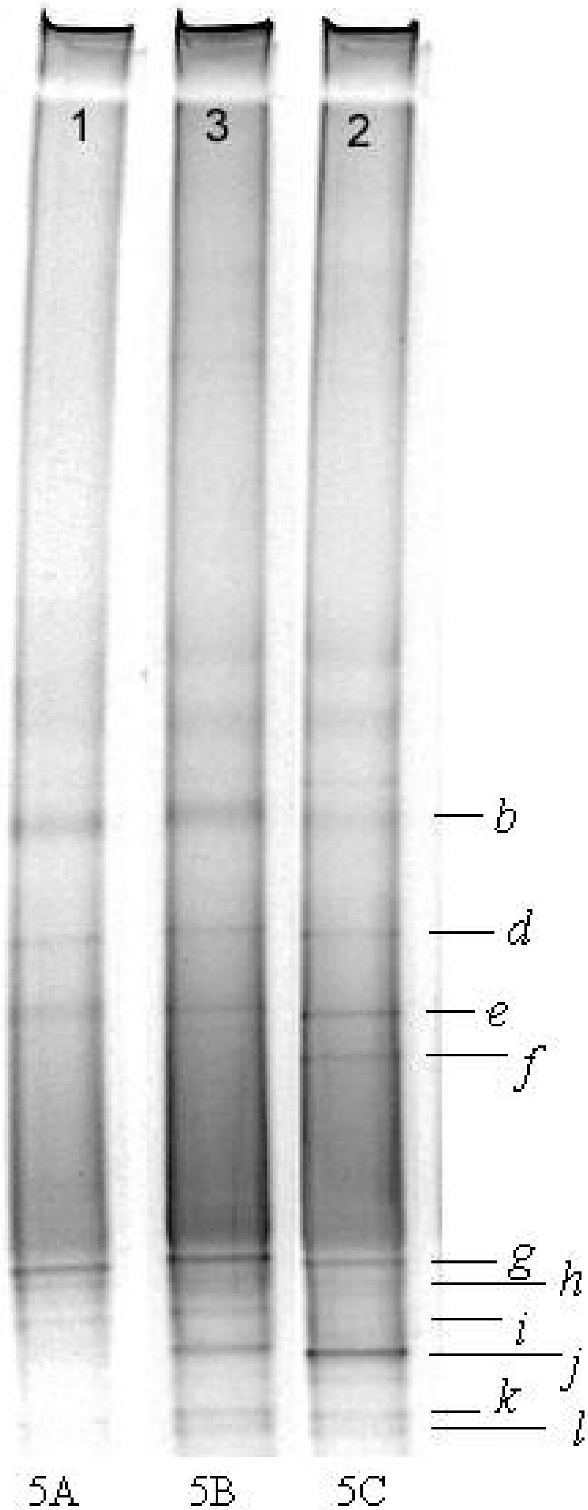

Figure 5. 10 Gels from reactor 5 (wood chips) arranged from port A to C (1, 3, 2).

In Kaldnes media reactors (Figure 5.11), ribotype *b* disappeared at port 2C. This supports the argument that bacterium of this class are favored at higher concentrations of nitrates. Ribotype *m* appears to be another nitrate scavenger and only appeared in port 2C.

Similar to the other media types, ribotype *b* disappeared at port 7C of the wheat straw reactor (Figure 5.12). This is further proof that this bacterium appears to need a higher nitrate concentration. In contrast, ribotype *m* thrives on low nitrate concentration and appeared only at port 7C. Ribotype *i*, described earlier as responsible for the degradation of organic filter media, appeared in all sections sampled. This can be explained by the fact that degradation of wheat straw was not confined to the lower zone. Visual observation showed that wheat straw had exposed strands of fibers, a sign of degradation, on virtually all sections of this particular reactor type.

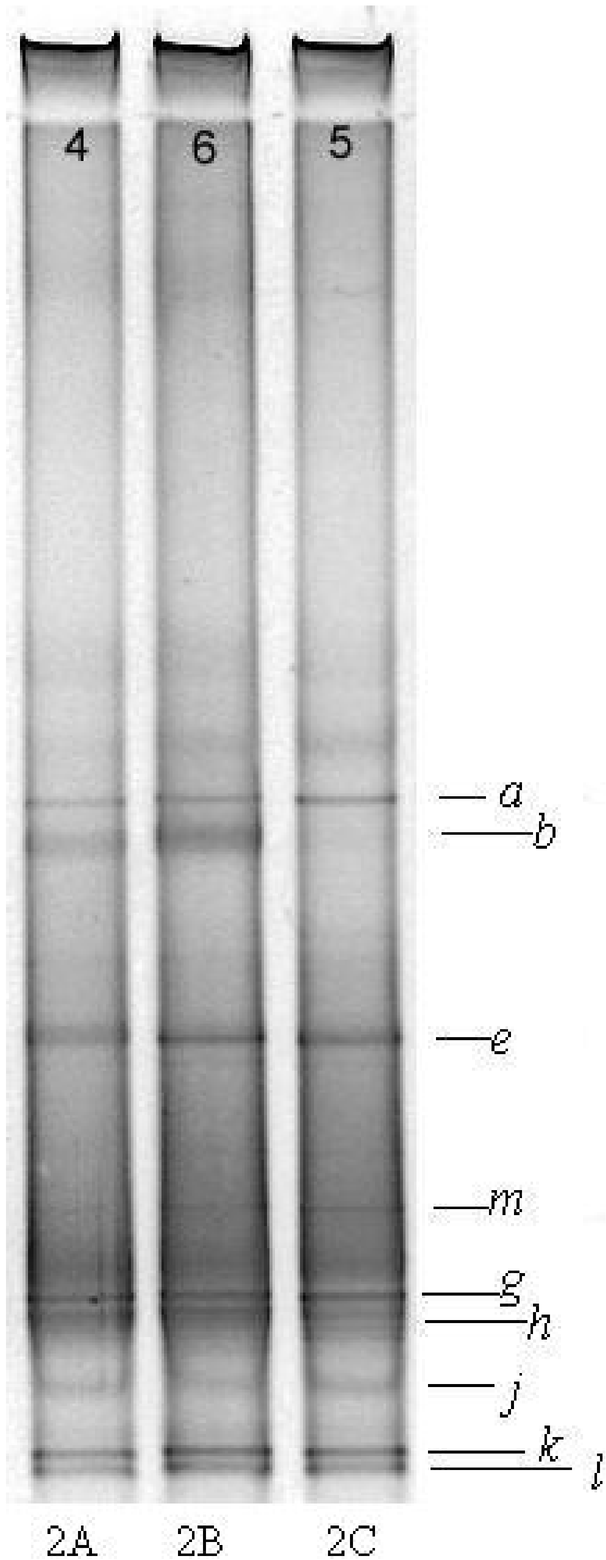

Figure 5. 11Gels from reactor 2 (Kaldnes) arranged from port A to C (4, 6, 5).

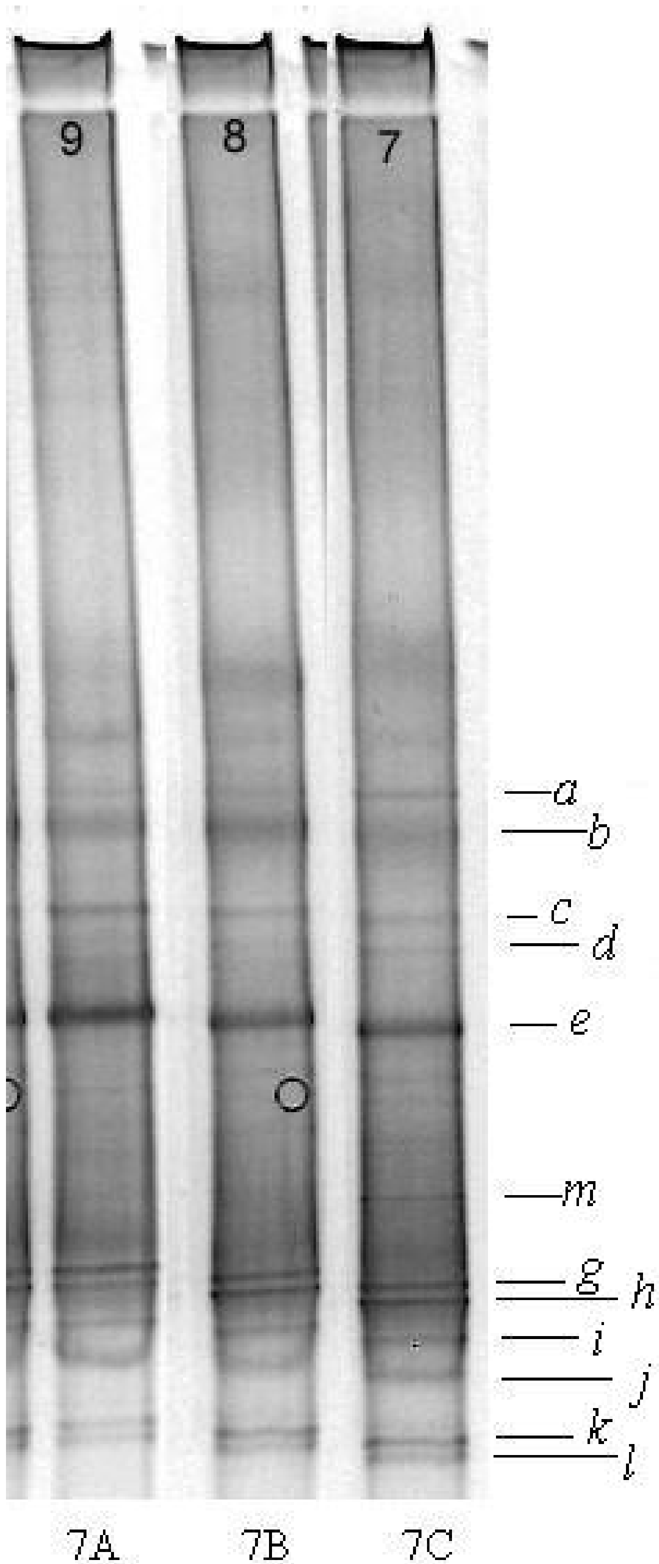

Figure 5. 12 Gels from reactor 7 (wheat straw) arranged from port A to C (9, 8, 7).

Later in the experiments, it was also interesting to see a white-colored biomass layer covering the surfaces of the wood chips (Figure 5.13) and wheat straw (Figure 5.14) before the media was covered by brownish to grayish black biomass. It appears that layers of microbial communities developed on surfaces. This was not evident on plastic media. If the white biomass were fungi, these were not able to be detected as the PCR-DGGE used in this study only targeted bacterial denitrifiers.

Figure 5. 13 Brownish layer of biomass covered the wood chips (leftmost picture). A white grayish layer remained even after washing - sprayed running water (two middle pictures). The rightmost picture was the chip after brushing off the grayish layer.

Figure 5. 14 A straw of wheat showing whitish layer covered by black biomass.

6. SUMMARY AND CONCLUSION

Stringent regulations on nitrogen discharge to the environment have placed recirculating aquaculture systems under the watchful eye of regulators. These systems can produce a highly concentrated waste stream of nutrients, particularly nitrates. A wastewater management system should be developed to control nitrate discharge but it should not be costly so as to reduce the economic viability of these production systems. Previous studies with aquaculture wastewater have shown that volatile fatty acids from sludge tanks can be used as a low cost carbon source but none have investigated the feasibility of finding an alternative to expensive plastic medium for denitrification using biofiltration in aquaculture. This research evaluated wood chips and wheat straw as cheap and abundant resources for biofilter media in denitrification reactors.

Nine 3.6-liter laboratory reactors filled with Kaldnes plastic media, wood chips, and wheat straw were built for treating both synthetic and real aquaculture wastewater in a series of experiments. The synthetic wastewater was formulated for 50, 120, and 200 mg NO_3-N/L. Methanol was added as carbon source with amounts based on the nitrate concentration, while other chemical constituents were determined by characterizing actual wastewater and comparing with previous studies.

The study showed that both wood chips and wheat straw were comparable to Kaldnes in terms of reducing nitrate. Approximately 99 per cent of nitrate (with influent as high as 200 mg/L) was essentially removed at pseudo-steady state. Denitrification rates averaged 1326 ± 74 g NO_3-N /m^3/day for wheat straw, 1365 ± 39 g N/m^3/day for wood chips and 1361 ± 80 g NO_3-N /m^3/day for Kaldnes media. These values were not the maximum potential since the nitrate profile along reactors showed substantially higher rates in the lower half of the reactors. Treatment of real wastewater also showed comparable performance with that at the 50 mg NO_3-N /L synthetic experiments.

Carbon requirement was estimated by determining COD removed per nitrate reduced. COD/NO_3-N ratios were: Kaldnes media (3.41 to 3.95) compared to wood chips (3.34 to 3.64) and wheat straw (3.26 to 3.46). COD/NO_3-N ratios for the real wastewater were low (Kaldnes, 1.90; wood chips, 1.60; wheat straw, 1.44) compared to synthetic experiments.

Effluent ammonia concentrations were near 0 mg NH_3-N/L, and nitrite concentrations on the average were around 2.0 mg NO_2-N/L. There was alkalinity production as a result of denitrification. Synthetic experiments yielded alkalinity/NO_3-N ratios between 3 and 4 while real wastewater had values near 6. Effluent pH ranged on the average from 8 to 9.5 while the oxidation-reduction potential declined to as low as -150 mV.

Wood chips lost 16.2% of its initial mass while wheat straw mass was reduced by 33.7%. There were physical signs of degradation like discoloration and structural transformation (some wood chips easily broke into smaller pieces and wheat straws reduced to fibrous strands). Both wood chips and wheat straw exhibited reduction of C/N ratio. Changes of lignin were also observed for both media.

The reactors also maintained diverse bacterial communities. A denaturing gradient gel electrophoresis (DGGE) showed several ribotypes that differed with media types and with height in each reactor column.

6.1 COST COMPARISON

Based on these findings, the wood chips and wheat straw can treat high levels of nitrate in aquaculture wastewater. For an aquaculture facility with 16 m^3/day discharge at a concentration of 200 mg NO_3-N/L (3200 g NO_3-N/m^3/day), a biofilter can be constructed with the following general specifications and estimates of cost:

Table 6.1 Estimated volume and corresponding economics of constructing denitrification biofilter at a recirculating aquaculture facility.

Reactor/media type	Estimated volume (m^3)	Expected lifetime in years (50% of initial mass)	Initial Cost of media ($)	Total 10-year Cost ($)
Kaldnes[1]	2.41	-	$2,300.00	$2,300.00
Wood chips[2]	2.36	1.2	$45.00	$375.00
Wheat straw[3]	2.35	0.5	$15.00	$300.00

[1]Kaldnes priced at $953/$m^3$; [2]Wood chips priced at $19/ m^3; [3]Wheat straw priced at $6/$m^3$.

From an economic perspective, wood chips and wheat straw were preferable. However, careful consideration should be undertaken in designing biofilters using these media. The denitrification potential was ascertained only using synthetic wastewater and for only 140 days. Performance may differ beyond the time period of this study and with real wastewater. This could result in a higher biofilter volumetric requirement. Nevertheless, the cost ranges presented in Table 5.1 differed significantly, and can offset whatever changes may occur as a result of scaling up.

6.2 FUTURE CHALLENGES

Long term performance of the media on real wastewater under field conditions still needs to be evaluated. Several factors have been controlled during laboratory experiments including temperature. Temperature (seasonal) variations are expected on-site and this should be taken into account when designing prototypes.

Future studies may be undertaken using VFAs from RAS sludge supernatant instead of methanol as carbon source for denitrification. A biofiltration system with wood chips and/or wheat straw as biofilter media and sludge VFAs as carbon source can substantially cut the cost of this end-of-pipe denitrification.

The synthetic wastewater contained less suspended solids and may pose a problem in actual operations. A modified design may be implemented to evenly distribute inflow to the reactors (e.g. step fed) to minimize clogging as well as efficiently utilizing other media surface for denitrification.

The system tested in this study may be able to trap solids-bound phosphorus. Thus, phosphorus removal by solids-separation through filtration may be investigated in the future. Biological removal of soluble phosphorus usually needs an aerated section to arrest phosphorus released during anoxic conditions. A configuration may be designed to facilitate oxygen transfer if phosphorus removal is desired.

Metals and micronutrients primarily from feeds may be adsorbed on the organic surfaces and, thus, should be investigated in the future.

REFERENCES

Aboutboul, Y., R. Arviv, and J. van Rijn. 1995.**Anaerobic treatment of intensive fish culture effluents: volatile fatty acid mediated denitrification**. *Aquaculture* 133: 21-32.

Aesøy, A., H. Ødegaard, K. Bach, R. Pujol and M. Hamon. 1998. **Denitrification in Packed bed biofilm reactor (Biofor) – experiments with different carbon sources**. *Water Research* 32 (5): 1463-1470.

Aslan, S. and A. Turkman. 2003. **Biological denitrification of drinking water using various natural organic solid substrates**. *Water Science and Technology* 48 (11-12): 489-495.

Blowes, D.W., W. D. Robertson, C.J. Ptacek and C. Merkley. 1994. **Removal of agricultural nitrate from tile drainage effluent water using in-line bioreactors**. *Journal of Contaminant Hydrology* 15: 207-221.

Boley, A., W. R. Muller and G. Haider. 2000. **Biodegradable polymers as solid substrate and biofilm carrier for denitrification in recirculated aquaculture systems**. *Aquacultural Engineering* 22: 75-85.

Brazil, B. L. 2000. **Evaluation of an Effluent Treatment Strategy to Control Nitrogen from a Recirculating Aquaculture Facility.** Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. etd-10172001-090020.

Carrera J., T. Vincent and F. J. Lafuente. 2003. **Influence of temperature on denitrification of an industrial high-strength nitrogen wastewater in a two-sludge system**. *Water SA* 29 (1): 11-16.

Chen C. Y. and S. D. Chen. 2000. **Biofilm characteristics in biological denitrification biofilm reactors.** *Water Science and Technology* 41 (4): 147-154.

Chen, S., S. Summerfelt, T. Losordo, and R. Malone. 2002. **Recirculating systems effluents, and treatment.** In *Aquaculture and the Environment in the United States*, ed. J. Tomasso. US Aquaculture Society, Baton Rouge, LA. pp 119-140.

Dussert, B. W. and W. G. Tramposch. 1996. **Impact of support media and properties on the biological treatment of drinking water**. In *Advances in Slow Sand and Alternative Biological Filtration* eds. Graham, N. J. D. and R. Collins; John Wiley: Chichester, New York.

Eiland, F., M. Klamer, A-M Leth, and E. Baath. 2001. **Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw.** *Microbial Ecology* 41: 272-280.

Elefsiniotis, P., D. G. Wareham, M.O. Smith. 2004. **Use of volatile fatty acids from an acid-phase digester for denitrification.** *Journal of Biotechnology* 114: 289-297.

EPA. 1993. **Manual: Nitrogen Control**. EPA/625/R-93/010. Environmental Protection Agency. Washington, DC.

EPA. 2004. **Technical Development Document for the Final Effluent Limitations Guidelines and New Source Performance Standards for the Concentrated Aquatic Animal Production Point Source Category (Revised August 2004).** EPA/821/R-04/012. US Environmental Protection Agency, Washington, DC

FAO. **The State of World Fisheries and Aquaculture – 2002**. Available online: http://www.fao.org/docrep/005/y7300e/y7300e00.htm. Date Accessed: March 21, 2004

Grady, C. P. L., G. T. Daigger, and H. C. Lim. 1999. **Biological Wastewater Treatment**. 2nd edition. Marcel Dekker, Inc., New York.

Henze, M. 2002. **Wastewater Treatment : Biological and Chemical Processes.** 3rd ed. Springer: Berlin.

Kim, H. E., A. Seagren, A. P. Davis. 2003. **Engineered Bioretention for Removal of Nitrate from Stormwater Runoff.** *Water Environment Research* 75: 355-367.

Lee, P. G., R. N. Lea, E. Dohmann, W. Prebilsky, P. E. Turk, H. Ying, and J. L. Whitson. 2000. **Denitrification in aquaculture systems: an example of fuzzy logic control problem.** *Aquacultural Engineering* 23: 37-59.

Losordo, T.M., M.P. Masser, and J. Rakocy. 1998. **Recirculating Aquaculture Tank Production Systems – An Overview of Critical Considerations**. SRAC Publication No. 451. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Losordo, T.M. 2004. **Critical considerations in recirculating aquaculture production system.** A presentation in Aquaculture Recirculation Technology Workshop, North Carolina State University, Raleigh, NC, September 9-11, 2004.

Lowengart, A., S. Diab, M. Kochba, and Y. Avnimelech. 1993. **Development of a biofilter for turbid and nitrogen-rich irrigation water. A: Organic carbon**

degradation and nitrogen removal processes. *Bioresource Technology* 44: 131-135.

McCarty, P.L., L. Beck, and P. St. Amant. 1969. **Biological denitrification of wastewaters by addition of organic materials**. Proceedings of the 24th Purdue Industrial Waste Conference, Purdue University, Lafayette, IN.

Madigan, M.T., J. M. Martinko, and J. Parker. 2003. **Brock Biology of Microorganisms.** Tenth edition. Prentice Hall – Pearson Education, Inc. NJ, USA.

Maier, R.M, I.L. Pepper, and C.P. Gerba. 2000. **Environmental Microbiology.** Academic Press – Elsevier. USA.

Masser, M.P., J. Rakocy and T.M. Losordo. 1999. **Recirculating Aquaculture Tank Production Systems Management of Recirculating Systems**. SRAC Publication No. 452. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Menoud, P., C. H. Wong, H. A. Robinson, A. Farquhar, J. P. Barford and G. W. Barton. 1999. **Simultaneous nitrification and denitrification using Siporax packing**. Water Science and Technology 40 (4-5): 153-160

Nikitin N. 1966. **The Chemistry of Cellulose and Wood.** Academy of Science of USSR. Institute of High Molecular Compounds. Israel Program for Scientific Translation, Jerusalem.

Ødegaard, H., B. Gisvold, and J. Strickland. 2000. **The influence of carrier size and shape of moving bed biofilm process.** *Water Science and Technology* 41 (4-5): 382-391.

Ouyang, C.F., R. J. Chiou and C. T. Lin. 2000. **The characteristics of nitrogen removal by the biofilter system.** *Water Science and Technology* 42 (12): 137-147.

Phillips, Jennifer B. 1998. **Denitrification of Recirculating Aquaculture System Waters Using an Upflow Biofilter and a Fermented Substrate**. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. etd-01298-145427.

Rao, P.V. 1998. **Statistical Research Methods for Life Sciences.** Duxbury Press. CA, USA.

Richard, T. 2004. **The effect of lignin on biodegradability.** Available online: http://www.bulkmsm.com/research/msm/page30.htm Accessed: 12/16/04.

Robertson, W. D., D. W. Blowes, C. J. Ptacek, and J. A. Cherry. **Long-term performance of in situ reactive barriers for nitrate remediation.** *Groundwater* 38 (5): 689-695.

Robertson, W. D., G. I. Ford, and P. S. Lombardo. 2005. **Wood-based filter for nitrate removal in septic systems.** *Transactions of the ASAE* 48 (1): 121-128.

Saka, S. 2001. **Chemical Composition and Distribution.** *In* Wood and Cellulosic Chemistry. Eds. D.N.S. Hon, N. Shiraishi, Marcel-Dekker, Inc. New York. pp 71-72.

Sauthier, N., A. Grasmick and J. P. Blancheton. 1998. **Biological denitrification applied to a marine closed aquaculture system.** *Water Research* 32 (6): 1932-1938.

Shah, S. B., D. K. Bhumbla, T. J. Basden, and L. D. Lawrence. 2002. **Cool temperature performance of a wheat straw biofilter for treating dairy wastewater.** *Journal of Environmental Science and Health Part B – Pesticides, Food Contaminants, and Agricultural Wastes* B37 (5): 493-505.

Soares, M. I. M. and A. Abeliovich. 1998. **Wheat straw as substrate for denitrification**. *Water Research* 32 (12): 3790-3794.

Son, H. J., Y. H. Park, and J. H. Lee. 2004. **Development of supporting materials for microbial immobilization and iron oxidation**. *Applied Biochemistry and Biotechnology* 112: 1-12.

Tal, Y., J. E. M. Watts, S. B. Schreier, K. R. Sowers, and H. J. Schreier. 2003. **Characterization of the microbial community and nitrogen transformation processes associated with moving bed bioreactors in a closed recirculated mariculture system.** *Aquaculture* 215: 187-202

Twarowska, J. G., P. W. Westerman, and T. M. Losordo. 1997. **Water treatment and wastewater characterization evaluation of an intensive recirculation fish production system.** *Aquacultural Engineering* 16 (3): 133-147.

Van Rijn, J., N. Fonarev, and B. Berkowitz. 1995. **Anaerobic treatment of intensive fish culture effluents: digestion of fish feed and release of volatile fatty acids**. *Aquaculture* 133: 9-20.

Van Rijn, J. 1996. **The potential for integrated biological treatment systems in recirculating fish culture – A review**. *Aquaculture* 139: 181-201.

APPENDICES

APPENDIX A
Laboratory procedure and wastewater characterization

A.1. Analytical Procedures followed by the BAE Environmental Analysis Laboratory (Updated April 2004)

NH_3-N (Ammonia) – Ammonia-salicylate method for automated analysis. Emerald green color formed by reaction of ammonia, sodium salicylate, sodium nitroprusside, and sodium hypochlorite in a buffered alkaline medium. References: EPA Method 351.2 (1979) or Standard Methods 4500-NH3 G (1998), with slight modifications including dialysis.

NO_3-N + NO_2-N (Nitrate Nitrogen) - Cadmium reduction method for automated analysis. References: EPA Method 353.2 (1979), Technicon Industrial Method No. 100-70W (1973), or Standards Methods 4500-NO3- E (1998) with slight modifications including dialysis.

TP (Total Phosphorus) – Persulfate digestion, and ascorbic acid method for automated analysis. References: EPA Method (1979) or Standard Method 4500Norg B (1998) with slight modifications including dialysis.

O-PO_4-P (Orthophosphate Phosphorus) – Ascorbic acid method for automated analysis. References: EPA Method 365.1 (1979) or Standard Method 4500-P F (1998) with slight modifications including dialysis.

CL^- (Chloride) – Ferricyanide method for automated analysis. References: EPA Method 325.2 (1979) or Standard Methods 4500-Cl- E (1998) with slight modifications including dialysis.

COD (Chemical Oxygen Demand) – Potassium dichromate – sulfuric acid digestion and colorimetric automated analysis. References: EPA Method 410.4 (1979) with slight modifications or Standard Methods 5220D (1998).

pH – Electrometric Method. Reference: EPA Method 150.1 (1979) or Standard Methods 4500-H+ B pH Value (1998).

Alkalinity - potentiometric titration to end point pH 4.5. Reference: EPA Method 310.1 (Titrimetric, pH 4.5) (1979) or Standard Methods 2320 B (1998).

Residue (Solids) – Gravimetric Method, Reference: EPA Methods 160.1 – 160.4 (1979) or Standard Methods 2540 (1998).

Filterable Residue: Those solids capable of passing through a glass fiber filter and dried to constant weight at 180ºC. Standard Methods 2540C.

FSS – Non-Filterable Residue (Total Suspended Solids): Those solids which are retained by a glass fiber filter and dried to constant weight at 103-105ºC. Standard Methods 2540 D.
%TS – Total Residue (Total Solids): The sum of the homogenous suspended and dissolved materials in a sample.
%VS - Volatile Residue (Volatile Solids): The residue lost from the ignition of total, filterable, and non-filterable residue at 550ºC. in a muffle furnace. StandardMethods 2540E

Metals (Water and Wastewater) – Nitric acid digestion for total metals followed by direct aspiration atomic absorption spectroscopy. Na and K by emission spectroscopy. Reference: EPA (1979) or Standard Methods 3111-B (1998).

TOC - UV - promoted chemical oxidation and IR detection. Reference: EPA Method 415.1 (1979) or Standard Methods 5310 B.

Carbon and Nitrogen (Solids) – Leco C/N 2000 with autoloader. Infrared Radiation Detection for the determination of Carbon. Thermal Conductivity Detection for the determination of Nitrogen. Reference: AOAC Official Method 990.03 (1998).

A.2. A description of Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis (PCR-DGGE)

Polymerase Chain Reaction

PCR amplification steps are (from Maier et al, 2000):

Step 1. DNA containing the target sequence is released from sample to provide the template for the PCR.
Step 2. Template is combined with a PCR mixture in a microcentrifuge tube.
Step 3. Microcentrifuge tube is placed in a thermocycler and subjected to 25-50 PCR cycles. A typical cycle of PCR has three steps. The first step is denaturation of the DNA template. Second, primers are annealed to the single-stranded template and the last step is extension of the primer to make complimentary copy of the DNA template. In a thermocycler, the template is subjected to varying temperatures: denaturation – 92^{o}C; annealing – 55^{o}C; extension – 72^{o}C;

DGGE

After PCR amplification, the 16*S* rDNA sequences from community DNA extractions are analyzed by DGGE. Separation is based on changes in the electrophoretic mobility of different DNA fragments migrating in a gel containing a linearly increasing gradient of DNA denaturants. The fragments remain double stranded until they reach the conditions that cause melting of domains less resistant to denaturants. Once melted, they will position in a gel.

A band in a gel represents a particular ribotype. Its intensity represents abundance in the sample.

A.3. Wastewater characterization

Table A.1 Fish Barn wastewater analyses (samples taken on July 12, 2004) compared with wastewater characteristics of a RAS facility at Virginia Tech (Brazil, 2000). TOC, COD, BOD, and soluble COD samples were taken on December 2, 2004.

Constituents	Fish Barn	Virginia Tech RAS
TP	11.98	-
PO_4-P	-	5.9 $\pm$ 3.1
Na^+	230	232.1 $\pm$ 93.4
K^+	38.3	33.8 $\pm$ 21.0
Ca^{2+}	7	22.6 $\pm$11.1
Mg^{2+}	9.8	9.8 $\pm$ 3.0
Cu	0.03	-
Zn	0.05	-
Fe	0.2	-
Mn	0.09	-
Ni	0	-
Cl^-	60.1	103.4 $\pm$ 59.3
SO_4^{2-}	-	53.4 $\pm$ 17.5
TOC	24.3	
BOD	132	
COD	542	
SCOD	82	

APPENDIX B
Nitrate Data

Table B.1. Data on influent and effluent nitrates and its respective denitrification rates from July to November 2004 with synthetic wastewater as feed.

Source Code	Date	Time	NO_3+ NO_2-N	% reduction	flowrate (mL/min)	packing volume (mL)	NO_3+ NO_2-N removed	DN rate (g/m3/day)	Average DN
Start	12-Jul								
INF	26-Jul	9:30 AM	46.9	ref					
EFF	26-Jul	9:30 AM	43.46	7.33%					
INF	28-Jul	8:08 AM	51.67	ref					
EFF	28-Jul	10:35 AM	47.65	7.78%					
INF	2-Aug	8:30 AM	46.5	ref					
1DKM	Day 21		40.62	12.65%	15.00	3190	5.88	39.81	30.9956
2DKM			44.45	4.41%	15.00	3180	2.05	13.92	
3DKM			40.74	12.39%	15.00	3170	5.76	39.25	
4DWC			17.46	62.45%	15.00	3215	29.04	195.11	247.659
5DWC			5.36	88.47%	15.00	3185	41.14	279.00	
6DWC			6.73	85.53%	15.00	3195	39.77	268.87	
7DWS			31.03	33.27%	15.00	3150	15.47	106.08	162.50
8DWS			16.73	64.02%	15.00	3220	29.77	199.70	
9DWS			19.41	58.26%	15.00	3220	27.09	181.72	
INF	4-Aug	8:30 AM	48.59						
EFF	4-Aug	10:35 AM	22.84	52.99%					
INF	9-Aug	8:55 AM	37.24	ref					
1DKM	Day 28		1.28	96.56%	19.20	3190	35.96	311.70	197.86
2DKM			3.71	90.04%	13.47	3180	33.53	204.54	
3DKM			11.05	70.33%	6.50	3170	26.19	77.33	
4DWC			0.14	99.62%	10.10	3215	37.10	167.77	228.27
5DWC			0	100.00%	23.81	3185	37.24	400.88	
6DWC			0	100.00%	6.92	3195	37.24	116.17	
7DWS			0	100.00%	9.62	3150	37.24	163.70	162.10
8DWS			0	100.00%	6.67	3220	37.24	111.03	
9DWS			0.18	99.52%	12.77	3220	37.06	211.58	
INF	11-Aug	8:00 AM	37.3	ref					
1DKM	Day 30		14.3	61.66%	13.63	3190	23.00	141.51	155.48
2DKM			11.44	69.33%	14.10	3180	25.86	165.10	
3DKM			9.91	73.43%	12.85	3170	27.39	159.83	
4DWC			0	100.00%	11.75	3215	37.30	196.29	197.06
5DWC			3.52	90.56%	14.10	3185	33.78	215.33	
6DWC			1.48	96.03%	11.12	3195	35.82	179.57	
7DWS			0	100.00%	10.81	3150	37.30	184.32	163.46
8DWS			19.12	48.74%	15.04	3220	18.18	122.27	
9DWS			7.49	79.92%	13.79	3220	29.81	183.78	

Table B.1 ***(Continued)***

Source Code	Date	Time	NO_3+ NO_2-N	% reduction	flowrate (mL/min)	packing volume (mL)	NO_3+ NO_2-N removed	DN rate (g/m3/day)	Average DN
INF	16-Aug	7:50 AM	50.86	ref					
1DKM	Day 35		18.03	64.55%	20.80	3190	32.83	308.25	263.62
2DKM			13.14	74.16%	14.00	3180	37.72	239.13	
3DKM			12.3	75.82%	13.90	3170	38.56	243.48	
4DWC			3.43	93.26%	12.50	3215	47.43	265.55	302.09
5DWC			8.02	84.23%	15.40	3185	42.84	298.28	
6DWC			6.94	86.35%	17.30	3195	43.92	342.45	
7DWS			13.17	74.11%	16.90	3150	37.69	291.18	270.97
8DWS			13.32	73.81%	16.70	3220	37.54	280.36	
9DWS			7.68	84.90%	12.50	3220	43.18	241.38	
INF	18-Aug	7:45 AM	51.33	ref					
1DKM	Day 37		18.35	64.25%	21.80	3190	32.98	324.55	316.71
2DKM			8.16	84.10%	15.80	3180	43.17	308.87	
3DKM			0.04	99.92%	0	3170	51.29	pump	Stalled
4DWC			11.49	77.62%	17.40	3215	39.84	310.49	346.46
5DWC			3.95	92.30%	15.20	3185	47.38	325.61	
6DWC			8.72	83.01%	21.00	3195	42.61	403.30	
7DWS			4.82	90.61%	15.40	3150	46.51	327.43	327.35
8DWS			7.7	85.00%	18.00	3220	43.63	351.21	
9DWS			7.84	84.73%	15.60	3220	43.49	303.40	
INF	23-Aug	7:00 AM	46.56	ref					
1DKM	Day 42		6.07	86.96%	15.00	3190	40.49	274.16	269.73
2DKM			6.48	86.08%	14.60	3180	40.08	264.98	
3DKM			6.93	85.12%	15.00	3170	39.63	270.03	
4DWC			0.65	98.60%	13.20	3215	45.91	271.43	292.82
5DWC			0.25	99.46%	15.00	3185	46.31	314.07	
6DWC			0.13	99.72%	14.00	3195	46.43	292.97	
7DWS			0.25	99.46%	12.50	3150	46.31	264.63	268.28
8DWS			0.11	99.76%	11.40	3220	46.45	236.81	
9DWS			0.09	99.81%	14.60	3220	46.47	303.41	
INF	25-Aug	8:15 AM	50.9	ref					
1DKM	Day 44		0.93	98.17%	17.00	3190	49.97	383.47	333.27
2DKM			4.36	91.43%	15.20	3180	46.54	320.34	
3DKM			7.46	85.34%	15.00	3170	43.44	296.00	
4DWC			1.7	96.66%	14.40	3215	49.20	317.33	336.83
5DWC			0.75	98.53%	15.40	3185	50.15	349.18	
6DWC			0.69	98.64%	15.20	3195	50.21	343.98	
7DWS			3.26	93.60%	15.60	3150	47.64	339.74	335.94
8DWS			0.06	99.88%	13.80	3220	50.84	313.76	
9DWS			0.11	99.78%	15.60	3220	50.79	354.33	

Table B.1 ***(Continued)***

Source Code	Date	Time	NO3+ NO2	% reduction	flowrate (mL/min)	packing volume (mL)	NO3+ NO2 removed	DN rate (g/m3/day)	Average DN
INF	30-Aug	8:15 AM	48.7	ref					
1DKM	Day 49		0.81	98.34%	12.47	3190	47.89	269.51	269.558
2DKM			4.94	89.86%	14.67	3180	43.76	290.63	
3DKM			8.47	82.61%	13.60	3170	40.23	248.54	
4DWC			0.7	98.56%	14.00	3215	48.00	300.99	311.128
5DWC			0.44	99.10%	14.93	3185	48.26	325.84	
6DWC			0.8	98.36%	14.20	3195	47.90	306.56	
7DWS			0.86	98.23%	14.40	3150	47.84	314.92	322.92
8DWS			0.31	99.36%	15.13	3220	48.39	327.49	
9DWS			0.05	99.90%	15.00	3220	48.65	326.35	
INF	7-Sep	5:30 PM	61.72	ref					
1DKM	Day 57		0.1	99.84%	11.47	3190	61.62	318.96	375.24
2DKM			1.5	97.57%	14.93	3180	60.22	407.22	
3DKM			2.29	96.29%	14.80	3170	59.43	399.55	
4DWC			0.76	98.77%	11.53	3215	60.96	314.91	375.63
5DWC			0.29	99.53%	15.07	3185	61.43	418.46	
6DWC			0.52	99.16%	14.27	3195	61.20	393.52	
7DWS			0.33	99.47%	13.93	3150	61.39	391.03	385.23
8DWS			0.06	99.90%	12.73	3220	61.66	351.12	
9DWS			0.07	99.89%	15.00	3220	61.65	413.56	
INF	13-Sep	8:00 AM	116	ref					
1DKM	Day 63		32.20	72.24%	12.00	3190	83.80	453.94	475.64
2DKM			50.20	56.72%	15.40	3180	65.80	458.86	
3DKM			50.20	56.72%	17.20	3170	65.80	514.11	
4DWC			7.03	93.94%	11.40	3215	108.97	556.41	650.92
5DWC			16.70	85.60%	16.20	3185	99.30	727.31	
6DWC			15.70	86.47%	14.80	3195	100.30	669.04	
7DWS			15.90	86.29%	15.20	3150	100.10	695.55	655.77
8DWS			11.20	90.34%	14.40	3220	104.80	674.89	
9DWS			35.60	69.31%	16.60	3220	80.40	596.86	
INF	15-Sep	8:00 AM	120	ref					
1DKM	Day 65		27.88	76.77%	12.00	3190	92.12	499.01	547.61
2DKM			37.85	68.46%	15.60	3180	82.15	580.32	
3DKM			39.45	67.13%	15.40	3170	80.55	563.49	
4DWC			8.82	92.65%	11.80	3215	111.18	587.61	715.31
5DWC			11.81	90.16%	16.80	3185	108.19	821.77	
6DWC			11.05	90.79%	15.00	3195	108.95	736.56	
7DWS			9.41	92.16%	15.80	3150	110.59	798.78	764.37
8DWS			9.71	91.91%	14.00	3220	110.29	690.51	
9DWS			16.70	86.08%	17.40	3220	103.30	803.82	

Table B.1 ***(Continued)***

Source Code	Date	Time	NO_3+ NO_2-N	% reduction	flowrate (mL/min)	packing volume (mL)	NO_3+ NO_2-N removed	DN rate (g/m3/day)	Average DN
INF	19-Sep	5:00 PM	129	ref					
1DKM	Day 69		35.38	72.57%	16.18	3190	93.62	683.86	654.67
2DKM			32.43	74.86%	15.40	3180	96.57	673.44	
3DKM			26.26	79.64%	13.00	3170	102.74	606.72	
4DWC			25.25	80.43%	16.55	3215	103.75	768.86	774.65
5DWC			13.09	89.85%	14.40	3185	115.91	754.63	
6DWC			10.60	91.78%	15.00	3195	118.40	800.45	
7DWS			9.64	92.53%	14.18	3150	119.36	773.83	722.84
8DWS			14.53	88.74%	15.00	3220	114.47	767.87	
9DWS			1.58	98.78%	11.00	3220	127.42	626.81	
INF	22-Sep	8:15 AM	115	ref					
1DKM	Day 72		5.76	94.99%	14.40	3190	109.24	710.09	658.56
2DKM			12.08	89.50%	13.40	3180	102.92	624.51	
3DKM			6.44	94.40%	13.00	3170	108.56	641.09	
4DWC			4.71	95.90%	16.60	3215	110.29	820.02	784.42
5DWC			1.49	98.70%	15.00	3185	113.51	769.80	
6DWC			0.55	99.52%	14.80	3195	114.45	763.43	
7DWS			0.73	99.37%	15.60	3150	114.27	814.91	735.38
8DWS			0.03	99.97%	12.00	3220	114.97	616.98	
9DWS			1.10	99.04%	15.20	3220	113.90	774.24	
INF	27-Sep	9:00 AM	116	ref					
1DKM	Day 77		14.58	87.43%	16.20	3190	101.42	741.67	713.08
2DKM			12.78	88.98%	15.80	3180	103.22	738.51	
3DKM			12.37	89.34%	14.00	3170	103.63	659.05	
4DWC			9.94	91.43%	15.80	3215	106.06	750.57	752.299
5DWC			7.30	93.71%	16.20	3185	108.70	796.16	
6DWC			0.14	99.88%	13.60	3195	115.86	710.17	
7DWS			1.46	98.74%	15.20	3150	114.54	795.89	789.24
8DWS			3.39	97.08%	15.40	3220	112.61	775.54	
9DWS			1.86	98.40%	15.60	3220	114.14	796.29	
INF	29-Sep	9:00 AM	120	ref					
1DKM	Day 79		14.05	88.29%	16.00	3190	105.95	765.23	708.76
2DKM			16.72	86.07%	15.00	3180	103.28	701.53	
3DKM			23.21	80.66%	15.00	3170	96.79	659.52	
4DWC			8.06	93.28%	14.00	3215	111.94	701.93	740.20
5DWC			8.94	92.55%	14.60	3185	111.06	733.10	
6DWC			0.62	99.48%	14.60	3195	119.38	785.55	
7DWS			2.16	98.20%	15.00	3150	117.84	808.05	797.57
8DWS			0.03	99.98%	14.00	3220	119.97	751.12	
9DWS			2.03	98.31%	15.80	3220	117.97	833.56	

Table B.1 *(Continued)*

Source Code	Date	Time	NO_3+ NO_2-N	% reduction	flowrate (mL/min)	packing volume (mL)	NO_3+ NO_2-N removed	DN rate (g/m3/day)	Average DN
INF	4-Oct	7:30 AM	134	ref					
1DKM	Day 84		21.09	84.26%	15.40	3190	112.91	784.92	798.331
2DKM			14.03	89.53%	15.60	3180	119.97	847.49	
3DKM			35.25	73.69%	17.00	3170	98.75	762.59	
4DWC			1.42	98.94%	12.00	3215	132.58	712.59	803.18
5DWC			3.97	97.04%	14.20	3185	130.03	834.80	
6DWC			2.98	97.78%	14.60	3195	131.02	862.15	
7DWS			3.87	97.11%	14.20	3150	130.13	844.73	828.09
8DWS			5.69	95.75%	16.20	3220	128.31	929.57	
9DWS			1.70	98.73%	12.00	3220	132.30	709.98	
INF	10-Oct	7:30 PM	108	ref					
1DKM	Day 90		0.02	99.98%	14.40	3190	107.98	701.90	740.53
2DKM			0.17	99.84%	15.00	3180	107.83	732.43	
3DKM			1.02	99.06%	16.20	3170	106.98	787.27	
4DWC			0.64	99.41%	14.60	3215	107.36	702.06	698.00
5DWC			0.17	99.84%	14.40	3185	107.83	702.03	
6DWC			0.20	99.81%	14.20	3195	107.80	689.92	
7DWS			0.73	99.32%	15.80	3150	107.27	774.80	752.66
8DWS			0.05	99.95%	13.80	3220	107.95	666.21	
9DWS			0.54	99.50%	17.00	3220	107.46	816.96	
INF	13-Oct	9:15 AM	124	ref					
1DKM	Day 93		0.59	99.52%	13.00	3190	123.41	724.21	755.44
2DKM			1.38	98.89%	14.20	3180	122.62	788.47	
3DKM			8.79	92.91%	14.40	3170	115.21	753.63	
4DWC			0.94	99.24%	15.00	3215	123.06	826.78	807.69
5DWC			5.83	95.30%	14.20	3185	118.17	758.66	
6DWC			0.10	99.92%	15.00	3195	123.90	837.63	
7DWS			1.01	99.19%	15.40	3150	122.99	865.85	864.34
8DWS			0.19	99.85%	14.40	3220	123.81	797.306	
9DWS			1.69	98.64%	17.00	3220	122.31	929.86	
INF	17-Oct	5:30 PM	220	ref					
1DKM	Day 97		0.97	99.56%	14.00	3190	219.03	1384.21	1300.57
2DKM			45.60	79.27%	16.00	3180	174.40	1263.58	
3DKM			22.83	89.62%	14.00	3170	197.17	1253.93	
4DWC			3.08	98.60%	15.00	3215	216.92	1457.38	1429.18
5DWC			1.51	99.31%	14.60	3185	218.49	1442.24	
6DWC			0.04	99.98%	14.00	3195	219.96	1387.92	
7DWS			24.72	88.76%	15.60	3150	195.28	1392.63	1422.90
8DWS			0.77	99.65%	14.80	3220	219.23	1451.00	
9DWS			7.56	96.56%	15.00	3220	212.44	1425.06	

Table B.1 *(Continued)*

Source Code	Date	Time	NO_3+ NO_2-N	% reduction	flowrate (mL/min)	packing volume (mL)	NO_3+ NO_2-N removed	DN rate (g/m3/day)	Average DN
INF	20-Oct	8:00 AM	200	ref					
1DKM	Day 100		66.30	66.85%	13.00	3190	133.70	784.60	845.62
2DKM			71.67	64.17%	16.60	3180	128.33	964.65	
3DKM			70.61	64.70%	13.40	3170	129.39	787.61	
4DWC			54.86	72.57%	14.80	3215	145.14	962.12	924.90
5DWC			55.81	72.10%	13.00	3185	144.19	847.48	
6DWC			47.05	76.48%	14.00	3195	152.95	965.09	
7DWS			57.19	71.41%	14.80	3150	142.81	966.21	923.66
8DWS			51.35	74.33%	13.00	3220	148.65	864.20	
9DWS			49.77	75.12%	14.00	3220	150.23	940.57	
INF	25-Oct	8:30 AM	199	ref					
1DKM	Day 105		13.65	93.14%	16.00	3190	185.35	1338.7	1194.7
2DKM			1.25	99.37%	13.00	3180	197.75	1164.11	
3DKM			33.70	83.07%	14.40	3170	165.30	1081.28	
4DWC			0.82	99.59%	15.00	3215	198.18	1331.47	1251.44
5DWC			0.30	99.85%	13.00	3185	198.70	1167.87	
6DWC			0.11	99.94%	14.00	3195	198.89	1254.97	
7DWS			8.91	95.52%	15.80	3150	190.09	1372.99	1316.47
8DWS			0.20	99.90%	15.00	3220	198.80	1333.57	
9DWS			0.49	99.75%	14.00	3220	198.51	1242.85	
INF	27-Oct	8:30 AM	208	ref					
1DKM	Day 107		0.30	99.86%	15.00	3190	207.70	1406.37	1285.40
2DKM			0.23	99.89%	13.00	3180	207.77	1223.10	
3DKM			0.27	99.87%	13.00	3170	207.73	1226.72	
4DWC			0.01	100.00%	14.00	3215	207.99	1304.22	1310.01
5DWC			0.51	99.75%	15.00	3185	207.49	1407.15	
6DWC			0.01	100.00%	13.00	3195	207.99	1218.65	
7DWS			0.33	99.84%	16.00	3150	207.67	1518.96	1362.05
8DWS			0.01	100.00%	14.60	3220	207.99	1358.01	
9DWS			0.01	100.00%	13.00	3220	207.99	1209.18	
INF	1-Nov	9:00 AM	190	ref					
1DKM	Day 112		0.04	99.98%	14.00	3190	189.96	1200.50	1239.07
2DKM			0.18	99.91%	14.60	3180	189.82	1254.96	
3DKM			26.61	85.99%	17.00	3170	163.39	1261.76	
4DWC			0.60	99.68%	14.20	3215	189.40	1204.62	1339.27
5DWC			1.01	99.47%	16.00	3185	188.99	1367.14	
6DWC			1.27	99.33%	17.00	3195	188.73	1446.04	
7DWS			0.62	99.67%	14.80	3150	189.38	1281.29	1225.68
8DWS			0.03	99.98%	14.20	3220	189.97	1206.37	
9DWS			0.03	99.98%	14.00	3220	189.97	1189.38	

Table B.1 *(Continued)*

Source Code	Date	Time	NO_3+ NO_2-N	% reduction	flowrate (mL/min)	packing volume (mL)	NO_3+ NO_2-N removed	DN rate (g/m3/day)	Average DN
INF	3-Nov	8:30 AM	203	ref					
1DKM	Day 114		0.04	99.98%	14.00	3190	202.96	1282.66	1276.57
2DKM			0.28	99.86%	14.60	3180	202.72	1340.25	
3DKM			36.96	81.79%	16.00	3170	166.04	1206.80	
4DWC			0.30	99.85%	14.00	3215	202.70	1271.05	1312.56
5DWC			1.29	99.36%	15.00	3185	201.71	1367.95	
6DWC			0.08	99.96%	14.20	3195	202.92	1298.69	
7DWS			0.51	99.75%	15.00	3150	202.49	1388.50	1412.45
8DWS			0.02	99.99%	14.40	3220	202.98	1307.14	
9DWS			0.21	99.90%	17.00	3220	202.79	1541.71	
INF	8-Nov	6:30 AM	203	ref					
1DKM	Day 119		0.15	99.93%	14.40	3190	202.85	1318.59	1314.94
2DKM			0.13	99.94%	15.60	3180	202.87	1433.10	
3DKM			0.96	99.53%	13.00	3170	202.04	1193.12	
4DWC			0.30	99.85%	15.40	3215	202.70	1398.16	1396.14
5DWC			1.94	99.04%	15.00	3185	201.06	1363.55	
6DWC			0.08	99.96%	15.60	3195	202.92	1426.73	
7DWS			0.20	99.90%	14.80	3150	202.80	1372.09	1359.07
8DWS			0.02	99.99%	15.80	3220	202.98	1434.22	
9DWS			0.01	100.00%	14.00	3220	202.99	1270.89	
INF	10-Nov	8:30 AM	187	ref					
1DKM	Day 121		46.56	75.10%	14.00	3190	140.44	887.55	889.98
2DKM			50.11	73.20%	15.20	3180	136.89	942.22	
3DKM			54.89	70.65%	14.00	3170	132.11	840.17	
4DWC			45.08	75.89%	16.80	3215	141.92	1067.91	997.75
5DWC			40.84	78.16%	14.00	3185	146.16	925.15	
6DWC			41.00	78.07%	15.20	3195	146.00	1000.20	
7DWS			42.85	77.09%	15.20	3150	144.15	1001.64	979.31
8DWS			46.62	75.07%	16.00	3220	140.38	1004.46	
9DWS			46.21	75.29%	14.80	3220	140.79	931.84	
INF	15-Nov	9:30 AM	212	ref					
1DKM	Day 126		0.03	99.99%	13.80	3190	211.97	1320.46	1419.04
2DKM			0.13	99.94%	15.80	3180	211.87	1515.87	
3DKM			0.67	99.68%	14.80	3170	211.33	1420.78	
4DWC			0.25	99.88%	14.00	3215	211.75	1327.8	1403.58
5DWC			0.98	99.54%	16.40	3185	211.02	1564.66	
6DWC			0.05	99.98%	13.80	3195	211.95	1318.27	
7DWS			0.32	99.85%	17.00	3150	211.68	1645.06	1426.68
8DWS			0.01	100.00%	13.00	3220	211.99	1232.44	
9DWS			0.09	99.96%	14.80	3220	211.91	1402.55	

Table B.1 ***(Continued)***

Source Code	Date	Time	NO_3+ NO_2-N	% reduction	flowrate (mL/min)	packing volume (mL)	NO_3+ NO_2-N removed	DN rate (g/m3/day)	Average DN
INF	17-Nov	8:00 AM	213	ref					
1DKM	Day 128		0.02	99.99%	14.00	3190	212.98	1345.98	1381.31
2DKM			0.14	99.93%	15.00	3180	212.86	1445.84	
3DKM			0.39	99.82%	14.00	3170	212.61	1352.12	
4DWC			0.09	99.96%	15.00	3215	212.91	1430.44	1373.35
5DWC			0.42	99.80%	15.00	3185	212.58	1441.67	
6DWC			0.01	100.00%	13.00	3195	212.99	1247.94	
7DWS			0.07	99.97%	16.20	3150	212.93	1576.90	1382.9
8DWS			0.01	100.00%	14.00	3220	212.99	1333.50	
9DWS			0.00	100.00%	13.00	3220	213.00	1238.31	

Table B.2. Data on nitrate profile and denitrification rates of reactors at 50 mg/L measured on September 7, 2004.

location	column height		NO_3 $+NO_2$–N (mg/L)	level of reduction	NO_3 $+NO_2$–N reduced per section	column flowrate (mL/min)	Volume of section (mL)	DN rate per quarter (gN/m3/day)	overall reactor DN rate
R1	0	influent	61.72	ref					
1AKM	10	portA	15.92	74.21%	45.8	11.47	785	963.38	
1BKM	20	portB	1.77	97.13%	14.15	11.47	800	292.06	
1CKM	30	portC	0.05	99.92%	1.72	11.47	805	35.28	
1DKM	40	effluent	0.10	99.84%	-0.05	11.47	800	-1.03	318.96
R2	0	influent	61.72	ref					
2AKM	10	portA	30.48	50.62%	31.24	14.93	780	861.26	
2BKM	20	portB	18.82	69.51%	11.66	14.93	805	311.47	
2CKM	30	portC	9.05	85.34%	9.77	14.93	805	260.99	
2DKM	40	effluent	1.50	97.57%	7.55	14.93	790	205.51	407.22
R3	0	influent	61.72	ref					
3AKM	10	portA	23.44	62.02%	38.28	14.80	775	1052.68	
3BKM	20	portB	11.60	81.21%	11.84	14.80	800	315.42	
3CKM	30	portC	3.15	94.90%	8.45	14.80	805	223.71	
3DKM	40	effluent	2.29	96.29%	0.86	14.80	790	23.20	399.55
RKM	0	influent	61.72	ref					
AKM	10	portA	23.28	62.28%	38.44	13.73	780	959.10	
BKM	20	portB	10.73	82.62%	12.55	13.73	802	306.32	
CKM	30	portC	4.08	93.38%	6.65	13.73	805	173.33	
DKM	40	effluent	1.30	97.90%	2.79	13.73	793	75.89	
R4	0	influent	61.72	ref					
4AWC	10	portA	25.46	58.75%	36.26	11.53	795	757.49	
4BWC	20	portB	10.94	82.27%	14.52	11.53	805	299.56	
4CWC	30	portC	3.53	94.28%	7.41	11.53	790	155.78	
4DWC	40	effluent	0.76	98.77%	2.77	11.53	825	55.76	314.91
R5	0	influent	61.72	ref					
5AWC	10	portA	19.62	68.21%	42.1	15.07	785	1163.57	
5BWC	20	portB	4.40	92.87%	15.22	15.07	805	410.20	
5CWC	30	portC	1.02	98.35%	3.38	15.07	800	91.67	
5DWC	40	effluent	0.29	99.53%	0.73	15.07	795	19.92	418.46
R6	0	influent	61.72	ref					
6AWC	10	portA	15.41	75.03%	46.31	14.27	800	1189.24	
6BWC	20	portB	8.28	86.58%	7.13	14.27	795	184.25	
6CWC	30	portC	2.26	96.34%	6.02	14.27	805	153.63	
6DWC	40	effluent	0.52	99.16%	1.74	14.27	795	44.96	393.52

Table B.2 ***(Continued)***

location	column height		NO_3 $+NO_2$–N (mg/L)	level of reduction	NO_3 $+NO_2$–N reduced per section	column flowrate (mL/min)	Volume of section (mL)	DN rate per quarter (gN/m3/day)	Overall reactor DN rate
RWC	0	influent	61.72	ref					
AWC	10	portA	20.16	67.33%	41.56	13.62	793	1036.77	
BWC	20	portB	7.87	87.24%	12.29	13.62	802	298.01	
CWC	30	portC	2.27	96.32%	5.60	13.62	798	133.69	
DWC	40	effluent	0.52	99.15%	1.75	13.62	805	40.22	
R7	0	influent	61.72	ref					
7AWS	10	portA	19.95	67.68%	41.77	13.93	750	1117.43	
7BWS	20	portB	8.73	85.86%	11.22	13.93	800	281.40	
7CWS	30	portC	1.60	97.41%	7.13	13.93	800	178.82	
7DWS	40	effluent	0.33	99.47%	1.27	13.93	800	31.86	391.03
R8	0	influent	61.72	ref					
8AWS	10	portA	12.90	79.10%	48.82	12.73	805	1112.00	
8BWS	20	portB	7.04	88.59%	5.86	12.73	810	132.65	
8CWS	30	portC	0.10	99.84%	6.94	12.73	800	159.06	
8DWS	40	effluent	0.06	99.90%	0.04	12.73	805	0.91	351.12
R9	0	influent	61.72	ref					
9AWS	10	portA	21.90	64.52%	39.82	15.00	800	1075.14	
9BWS	20	portB	6.40	89.63%	15.5	15.00	810	413.33	
9CWS	30	portC	0.74	98.80%	5.66	15.00	800	152.82	
9DWS	40	effluent	0.07	99.89%	0.67	15.00	810	17.87	413.55
RWS	0	influent	61.72	ref					
AWS	10	portA	18.25	70.43%	43.47	13.89	785	1101.52	
BWS	20	portB	7.39	88.03%	10.86	13.89	806.67	275.79	
CWS	30	portC	0.81	98.68%	6.58	13.89	800	163.57	
DWS	40	effluent	0.15	99.75%	0.66	13.89	805	16.88	

Table B.3 Data on nitrate profile and denitrification rates of reactors at 120 mg/L measured on October 4, 2004.

location	column height		$NO_3 + NO_2$–N (mg/L)	level of reduction	$NO_3 + NO_2$–N reduced per section	column flowrate (mL/min)	volume of section (mL)	DN rate per quarter (gN/m3/day)	overall reactor DN rate
R1	0	influent	134.00	ref					
1AKM	10	portA	60.49	54.86%	73.51	15.40	785	2076.63	
1BKM	20	portB	41.60	68.96%	18.89	15.40	800	523.63	
1CKM	30	portC	34.98	73.90%	6.62	15.40	805	182.37	
1DKM	40	effluent	21.09	84.26%	13.89	15.40	800	385.03	784.92
R2	0	influent	134.00	ref					
2AKM	10	portA	62.45	53.40%	71.55	15.60	780	2060.64	
2BKM	20	portB	38.42	71.33%	24.03	15.60	805	670.57	
2CKM	30	portC	28.19	78.96%	10.23	15.60	805	285.47	
2DKM	40	effluent	14.03	89.53%	14.16	15.60	790	402.65	847.49
R3	0	influent	134.00	ref					
3AKM	10	portA	61.97	53.75%	72.03	17.00	775	2275.22	
3BKM	20	portB	52.82	60.58%	9.15	17.00	800	279.99	
3CKM	30	portC	36.40	72.84%	16.42	17.00	805	499.33	
3DKM	40	effluent	35.25	73.69%	1.15	17.00	790	35.64	762.59
RKM	0	influent	134.00	ref					
AKM	10	portA	61.64	54.00%	72.36	16	780	2137.50	
BKM	20	portB	44.28	66.96%	17.36	16	802	491.40	
CKM	30	portC	33.19	75.23%	11.09	16	805	322.39	
DKM	40	effluent	23.46	82.50%	9.73	16	793	274.44	
R4	0	influent	134.00	ref					
4AWC	10	portA	46.89	65.01%	87.11	12.00	795	1893.41	
4BWC	20	portB	25.31	81.11%	21.58	12.00	805	463.23	
4CWC	30	portC	1.26	99.06%	24.05	12.00	790	526.06	
4DWC	40	effluent	1.42	98.94%	-0.16	12.00	825	-3.35	712.59
R5	0	influent	134.00	ref					
5AWC	10	portA	71.60	46.57%	62.4	14.20	785	1625.42	
5BWC	20	portB	20.52	84.69%	51.08	14.20	805	1297.50	
5CWC	30	portC	1.12	99.16%	19.4	14.20	800	495.86	
5DWC	40	effluent	3.97	97.04%	-2.85	14.20	795	-73.30	834.80
R6	0	influent	134.00	ref					
6AWC	10	portA	37.46	72.04%	96.54	14.60	800	2537.07	
6BWC	20	portB	17.03	87.29%	20.43	14.60	795	540.28	
6CWC	30	portC	3.66	97.27%	13.37	14.60	805	349.18	
6DWC	40	effluent	2.98	97.78%	0.68	14.60	795	17.98	862.15

Table B.3 ***(Continued)***

location	column height		NO_3 $+NO_2$–N (mg/L)	level of reduction	NO_3 $+NO_2$–N reduced per section	column flowrate (mL/min)	volume of section (mL)	DN rate per quarter (gN/m3/day)	overall reactor DN rate
RWC	0	influent	134.00	ref					
AWC	10	portA	51.98	61.21%	82.02	13.6	793	2018.63	
BWC	20	portB	20.95	84.36%	31.03	13.6	802	767.00	
CWC	30	portC	2.01	98.50%	18.94	13.6	798	457.03	
DWC	40	effluent	2.79	97.92%	-0.78	13.6	805	-19.56	
R7	0	influent	134.00	ref					
7AWS	10	portA	59.13	55.87%	74.87	14.20	750	2041.26	
7BWS	20	portB	18.63	86.10%	40.5	14.20	800	1035.18	
7CWS	30	portC	6.63	95.05%	12	14.20	800	306.72	
7DWS	40	effluent	3.87	97.11%	2.76	14.20	800	70.55	844.73
R8	0	influent	134.00	ref					
8AWS	10	portA	61.46	54.13%	72.54	16.20	805	2102.13	
8BWS	20	portB	23.93	82.14%	37.53	16.20	810	1080.86	
8CWS	30	portC	7.31	94.54%	16.62	16.20	800	484.64	
8DWS	40	effluent	5.69	95.75%	1.62	16.20	805	46.95	929.57
R9	0	influent	134.00	ref					
9AWS	10	portA	50.59	62.25%	83.41	12.00	800	1801.66	
9BWS	20	portB	18.82	85.96%	31.77	12.00	810	677.76	
9CWS	30	portC	4.57	96.59%	14.25	12.00	800	307.8	
9DWS	40	effluent	1.70	98.73%	2.87	12.00	810	61.23	709.98
RWS	0	influent	134.00	ref					
AWS	10	portA	57.06	57.42%	76.94	14.13	785	1981.68	
BWS	20	portB	20.46	84.73%	36.6	14.13	807	931.27	
CWS	30	portC	6.17	95.40%	14.29	14.13	800	366.39	
DWS	40	effluent	3.75	97.20%	2.41667	14.13	805	59.57	

Table B.4 Data on nitrate profile and denitrification rates of reactors at 200 mg/L measured on November 8, 2004.

location	col height		NO_3+NO_2-N conc. (mg/L)	level of reduction	NO_3 +NO_2-N reduced per section	column flowrate (mL/min)	volume of section (mL)	DN rate per section (gN/m3/day)	Overall reactor DN rate
R1	0	influent	203.00						
1AKM	10	portA	55.06	72.88%	147.94	14.40	785	3907.88	
1BKM	20	portB	10.50	94.83%	44.56	14.40	800	1155.00	
1CKM	30	portC	2.61	98.71%	7.89	14.40	805	203.24	
1DKM	40	effluent	0.15	99.93%	2.46	14.40	800	63.76	1318.59
R2	0	influent	203.00						
2AKM	10	portA	52.72	74.03%	150.28	15.60	780	4328.06	
2BKM	20	portB	16.71	91.77%	36.01	15.60	805	1004.88	
2CKM	30	portC	5.05	97.51%	11.66	15.60	805	325.38	
2DKM	40	effluent	0.13	99.94%	4.92	15.60	790	139.90	1433.10
R3	0	influent	203.00						
3AKM	10	portA	66.81	67.09%	136.19	13.00	775	3289.65	
3BKM	20	portB	28.74	85.84%	38.07	13.00	800	890.84	
3CKM	30	portC	3.47	98.29%	25.27	13.00	805	587.65	
3DKM	40	effluent	0.96	99.53%	2.51	13.00	790	59.48	1193.12
RKM	0	influent	203.00	ref					
AKM	10	portA	58.20	71.33%	144.80	14.33	780	3841.86	
BKM	20	portB	18.65	90.81%	39.55	14.33	802	1016.90	
CKM	30	portC	3.71	98.17%	14.94	14.33	805	372.09	
DKM	40	effluent	0.41	99.80%	3.30	14.33	793	87.71	
R4	0	influent	203.00	ref					
4AWC	10	portA	65.94	67.52%	137.06	15.40	795	3823.20	
4BWC	20	portB	27.94	86.24%	38	15.40	805	1046.82	
4CWC	30	portC	3.82	98.12%	24.12	15.40	790	677.07	
4DWC	40	effluent	0.30	99.85%	3.52	15.40	825	94.6176	1398.16
R5	0	influent	203.00	ref					
5AWC	10	portA	137.00	32.51%	66	15.00	785	1816.05	
5BWC	20	portB	86.48	57.40%	50.52	15.00	805	1355.57	
5CWC	30	portC	12.19	94.00%	74.29	15.00	800	2005.83	
5DWC	40	effluent	1.94	99.04%	10.25	15.00	795	278.49	1363.55
R6	0	influent	203.00	ref					
6AWC	10	portA	80.39	60.40%	122.61	15.60	800	3442.89	
6BWC	20	portB	37.05	81.75%	43.34	15.60	795	1224.64	
6CWC	30	portC	2.35	98.84%	34.7	15.60	805	968.32	
6DWC	40	effluent	0.08	99.96%	2.27	15.60	795	64.14	1426.73

Table B.4 *(Continued)*

location	col height		NO_3+NO_2-N conc. (mg/L)	level of reduction	NO_3 +NO_2-N reduced per section	column flowrate (mL/min)	volume of section (mL)	DN rate per section (gN/m3/day)	Overall reactor DN rate
RWC	0	influent	203.00	ref					
AWC	10	portA	94.44	53.48%	108.56	15.33	793	3027.38	
BWC	20	portB	50.49	75.13%	43.95	15.33	802	1209.01	
CWC	30	portC	6.12	96.99%	44.37	15.33	798	1217.07	
DWC	40	effluent	0.77	99.62%	5.35	15.33	805	145.75	
R7	0	influent	203.00	ref					
7AWS	10	portA	84.59	58.33%	118.41	14.80	750	3364.74	
7BWS	20	portB	35.00	82.76%	49.59	14.80	800	1321.08	
7CWS	30	portC	4.81	97.63%	30.19	14.80	800	804.26	
7DWS	40	effluent	0.20	99.90%	4.61	14.80	800	122.81	1372.09
R8	0	influent	203.00	ref					
8AWS	10	portA	62.46	69.23%	140.54	15.80	805	3972.13	
8BWS	20	portB	19.89	90.20%	42.57	15.80	810	1195.74	
8CWS	30	portC	0.46	99.77%	19.43	15.80	800	552.59	
8DWS	40	effluent	0.02	99.99%	0.44	15.80	805	12.44	1434.22
R9	0	influent	203.00	ref					
9AWS	10	portA	119.00	41.38%	84	14.00	800	2116.80	
9BWS	20	portB	45.39	77.64%	73.61	14.00	810	1832.07	
9CWS	30	portC	3.07	98.49%	42.32	14.00	800	1066.46	
9DWS	40	effluent	0.01	100.00%	3.06	14.00	810	76.16	1270.89
RWS	0	influent	203.00	ref					
AWS	10	portA	88.68	56.31%	114.32	14.87	785	3151.2234	
BWS	20	portB	33.43	83.53%	55.26	14.87	807	1449.6309	
CWS	30	portC	2.78	98.63%	30.65	14.87	800	807.7716	
DWS	40	effluent	0.08	99.96%	2.70	14.87	805	70.468759	

Table B.5. Nitrate effluent and denitrification rates of reactor fed with NCSU Fish Barn waste.

media type	reactor	NO3+ NO2	NO3-N Removed	%reduction (based on Ave-Inf	flowrate (mL/min)	packing volume (mL)	DN rate (g/m3/day) based on AveINF	Average DN rates (g/m3/day)
INF1		55.58						
INF2		46.94						
AveINF		51.26						
KM	R1	11.98	39.28	76.63%	13.00	3190	230.51	232.67
KM	R2	15.04	36.22	70.66%	14.80	3180	242.74	
KM	R3	15.92	35.34	68.94%	14.00	3170	224.75	
WC	R4	15.29	35.97	70.17%	14.80	3215	238.44	242.72
WC	R5	18.92	32.34	63.09%	16.80	3185	245.64	
WC	R6	14.67	36.59	71.38%	14.80	3195	244.07	
WS	R7	1.14	50.12	97.78%	8.40	3150	192.46	242.24
WS	R8	8.72	42.54	82.99%	15.60	3220	296.78	
WS	R9	15.38	35.88	70.00%	14.80	3220	237.48	

APPENDIX C
COD and Alkalinity Data

Table C.1 Synthetic COD raw data

Date	COD (mg/L)												
	INF	**R1**	**R2**	**R3**	**RKM**	**R4**	**R5**	**R6**	**RWC**	**R7**	**R8**	**R9**	**RWS**
28-Jul	255	*COD at sink effluent port: 261 mg/L*											
2-Aug	249	202	228	219	**216.33**	136	104	110	**116.67**	176	152	160	**162.67**
9-Aug	229	73	75	109	**85.67**	91	136	115	**114.00**	93	149	87	**109.67**
16-Aug	237	100	76	92	**89.33**	27	52	56	**45.00**	72	83	62	**72.33**
23-Aug	203	34	39	46	**39.67**	39	38	35	**37.33**	38	53	46	**45.67**
30-Aug	225	16	35	47	**32.67**	38	29	28	**31.67**	42	46	36	**41.33**
7-Sep	269	68	60	64	**64.00**	73	62	66	**67.00**	75	79	66	**73.33**
13-Sep	405	124	203	188	**171.67**	49	77	95	**73.67**	91	64	145	**100.00**
19-Sep	418	103	106	78	**95.67**	88	43	32	**54.33**	28	37	27	**30.67**
27-Sep	453	104	78	90	**90.67**	84	81	50	**71.67**	55	64	57	**58.67**
4-Oct	425	61	30	100	**63.67**	11	14	6	**10.33**	21	11	10	**14.00**
10-Oct	572	221	172	210	**201.00**	206	224	191	**207.00**	228	237	236	**233.67**
17-Oct	810	91	226	155	**157.33**	85	86	69	**80.00**	172	82	99	**117.67**
20-Oct	1282	*Effluent COD not sampled*											
25-Oct	1343	635	600	718	**651.00**	593	597	577	**589.00**	636	590	598	**608.00**
1-Nov	739	51	47	116	**71.33**	55	64	47	**55.33**	57	67	65	**63.00**
8-Nov	823	133	129	150	**137.33**	138	143	134	**138.33**	152	141	142	**145.00**
10-Nov	420	*Effluent COD not sampled*											
15-Nov	835	103	101	112	**105.33**	107	113	119	**113.00**	108	176	115	**133.00**
17-Nov	880	*Effluent COD not sampled*											
LR1	238	82	86	96	**87.94**	67	70	68	**68.61**	83	94	76	**84.17**
LR2	455	123	118	133	**124.53**	88	88	75	**83.40**	85	83	95	**87.40**
LR3	892	203	221	250	**224.47**	196	201	189	**195.13**	225	211	204	**213.33**

Table C.2 COD removed and percentage removal

Date	COD removed (mg/L)											
	R1	R2	R3	RKM	R4	R5	R6	RWC	R7	R8	R9	RWS
2-Aug	47	21	30	**32.67**	113	145	139	**132.33**	73	97	89	**86.33**
9-Aug	156	154	120	**143.33**	138	93	114	**115.00**	136.00	80	142	**119.33**
16-Aug	137	161	145	**147.67**	210	185	181	**192.00**	165.00	154	175	**164.67**
23-Aug	169	164	157	**163.33**	164	165	168	**165.67**	165.00	150	157	**157.33**
30-Aug	209	190	178	**192.33**	187	196	197	**193.33**	183.00	179	189	**183.67**
7-Sep	201	209	205	**205.00**	196	207	203	**202.00**	194.00	190	203	**195.67**
13-Sep	281	202	217	**233.33**	356	328	310	**331.33**	314.00	341	260	**305.00**
19-Sep	315	312	340	**322.33**	330	375	386	**363.67**	390.00	381	391	**387.33**
27-Sep	349	375	363	**362.33**	369	372	403	**381.33**	398.00	389	396	**394.33**
4-Oct	364	395	325	**361.33**	414	411	419	**414.67**	404.00	414	415	**411.00**
10-Oct	351	400	362	**371.00**	366	348	381	**365.00**	344.00	335	336	**338.33**
17-Oct	719	584	655	**652.67**	725	724	741	**730.00**	638.00	728	711	**692.33**
25-Oct	708	743	625	**692.00**	750	746	766	**754.00**	707.00	753	745	**735.00**
1-Nov	688	692	623	**667.67**	684	675	692	**683.67**	682.00	672	674	**676.00**
8-Nov	690	694	673	**685.67**	685	680	689	**684.67**	671.00	682	681	**678.00**
15-Nov	732	734	723	**729.67**	728	722	716	**722.00**	727.00	659	720	**702.00**
LR1	153.17	149.83	139.2	**147.39**	168.0	165.2	167	**166.72**	152.67	141.7	159.2	**151.17**
LR2	332	336.8	321.4	**330.07**	367.0	366.8	379.8	**371.20**	370.00	372	359.6	**367.20**
LR3	707.4	689.4	659.8	**685.53**	714.4	709.4	720.8	**714.87**	685.00	698.8	706.2	**696.67**

Date	Percentage of COD removed (%)											
	R1	R2	R3	RKM	R4	R5	R6	RWC	R7	R8	R9	RWS
2-Aug	18.88%	8.43%	12.05%	**13.12%**	45.38%	58.23%	55.82%	**53.15%**	29.32%	38.96%	35.74%	**34.67%**
9-Aug	68.12%	67.25%	52.40%	**62.59%**	60.26%	40.61%	49.78%	**50.22%**	59.39%	34.93%	62.01%	**52.11%**
16-Aug	57.81%	67.93%	61.18%	**62.31%**	88.61%	78.06%	76.37%	**81.01%**	69.62%	64.98%	73.84%	**69.48%**
23-Aug	83.25%	80.79%	77.34%	**80.46%**	80.79%	81.28%	82.76%	**81.61%**	81.28%	73.89%	77.34%	**77.50%**
30-Aug	92.89%	84.44%	79.11%	**85.48%**	83.11%	87.11%	87.56%	**85.93%**	81.33%	79.56%	84.00%	**81.63%**
7-Sep	74.72%	77.70%	76.21%	**76.21%**	72.86%	76.95%	75.46%	**75.09%**	72.12%	70.63%	75.46%	**72.74%**
13-Sep	69.38%	49.88%	53.58%	**57.61%**	87.90%	80.99%	76.54%	**81.81%**	77.53%	84.20%	64.20%	**75.31%**
19-Sep	75.36%	74.64%	81.34%	**77.11%**	78.95%	89.71%	92.34%	**87.00%**	93.30%	91.15%	93.54%	**92.66%**
27-Sep	77.04%	82.78%	80.13%	**79.99%**	81.46%	82.12%	88.96%	**84.18%**	87.86%	85.87%	87.42%	**87.05%**
4-Oct	85.65%	92.94%	76.47%	**85.02%**	97.41%	96.71%	98.59%	**97.57%**	95.06%	97.41%	97.65%	**96.71%**
10-Oct	61.36%	69.93%	63.29%	**64.86%**	63.99%	60.84%	66.61%	**63.81%**	60.14%	58.57%	58.74%	**59.15%**
17-Oct	88.77%	72.10%	80.86%	**80.58%**	89.51%	89.38%	91.48%	**90.12%**	78.77%	89.88%	87.78%	**85.47%**
25-Oct	52.72%	55.32%	46.54%	**51.53%**	55.85%	55.55%	57.04%	**56.14%**	52.64%	56.07%	55.47%	**54.73%**
1-Nov	93.10%	93.64%	84.30%	**90.35%**	92.56%	91.34%	93.64%	**92.51%**	92.29%	90.93%	91.20%	**91.47%**
8-Nov	83.84%	84.33%	81.77%	**83.31%**	83.23%	82.62%	83.72%	**83.19%**	81.53%	82.87%	82.75%	**82.38%**
15-Nov	87.66%	87.90%	86.59%	**87.39%**	87.19%	86.47%	85.75%	**86.47%**	87.07%	78.92%	86.23%	**84.07%**
LR1	65.94%	64.42%	59.72%	**63.36%**	71.84%	70.37%	71.29%	**71.17%**	65.51%	60.49%	68.07%	**64.69%**
LR2	73.76%	74.03%	70.96%	**72.92%**	81.94%	82.07%	84.61%	**82.87%**	82.78%	83.44%	80.31%	**82.18%**
LR3	81.22%	78.66%	76.01%	**78.63%**	81.67%	81.07%	82.32%	**81.69%**	78.46%	79.73%	80.69%	**79.63%**

Table C.3 Alkalinity measurements at the influent and effluent under synthetic wastewater experiments.

Date	INF	R1	R2	R3	RKM	R4	R5	R6	RWC
9-Aug	20	166	160	144	**156.67**	180	188	192	**186.67**
16-Aug	8	132	130	132	**131.33**	188	188	162	**179.33**
23-Aug	36	200	194	196	**196.67**	208	220	220	**216.00**
30-Aug	22	208	200	192	**200.00**	218	206	210	**211.33**
7-Sep	46	232	234	224	**230.00**	256	246	258	**253.33**
13-Sep	40	336	250	248	**278.00**	412	386	384	**394.00**
19-Sep	44	322	328	358	**336.00**	342	404	416	**387.33**
27-Sep	66	398	418	396	**404.00**	406	420	450	**425.33**
4-Oct	86	428	448	390	**422.00**	506	484	496	**495.33**
10-Oct	170	488	468	486	**480.67**	498	478	488	**488.00**
17-Oct	40	756	602	660	**672.67**	726	740	708	**724.67**
25-Oct	42	686	738	618	**680.67**	766	744	716	**742.00**
1-Nov	50	728	710	634	**690.67**	720	722	738	**726.67**
8-Nov	50	728	710	634	**690.67**	720	722	738	**726.67**
15-Nov	42	760	780	750	**763.33**	760	740	780	**760.00**
LR1 ave	26.40	187.60	183.60	177.60	**182.93**	210.00	209.60	208.40	**209.33**
LR2 ave	81.20	394.40	382.40	375.60	**384.13**	432.80	434.40	446.80	**438.00**
LR3 ave	44.80	731.60	708.00	659.20	**699.60**	738.40	733.60	736.00	**736.00**

Date	INF	R7	R8	R9	RWS
9-Aug	20	170	182	180	**177.33**
16-Aug	8	158	150	164	**157.33**
23-Aug	36	216	226	220	**220.67**
30-Aug	22	212	198	224	**211.33**
7-Sep	46	236	256	238	**243.33**
13-Sep	40	380	406	322	**369.33**
19-Sep	44	394	370	442	**402.00**
27-Sep	66	434	430	438	**434.00**
4-Oct	86	494	496	508	**499.33**
10-Oct	170	492	474	490	**485.33**
17-Oct	40	658	728	688	**691.33**
25-Oct	42	730	728	780	**746.00**
1-Nov	50	710	736	716	**720.67**
8-Nov	50	710	736	716	**720.67**
15-Nov	42	760	760	780	**766.67**
LR1 ave	26.40	198.40	202.40	205.20	**202.00**
LR2 ave	81.20	438.80	435.20	440.00	**438.00**
LR3 ave	44.80	713.60	737.60	736.00	**729.07**

APPENDIX D
Ammonia and Nitrite Raw Data

Table D.1 Ammonia (mg NH_3-N/L) concentrations at the influent and effluent under synthetic wastewater experiments.

Date	INF	R1	R2	R3	RKM	R4	R5	R6	RWC	R7	R8	R9	RWS
2-Aug	0.64	0.00	0.60	0.55	**0.38**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
9-Aug	0.00	0.00	0.00	0.00	**0.00**	0.00	0.13	0.41	**0.18**	0.08	0.61	0.18	**0.29**
16-Aug	0.34	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
23-Aug	0.65	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.08	0.00	**0.03**
30-Aug	0.69	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.08	0.00	**0.03**
7-Sep	0.32	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.05	0.00	**0.02**
13-Sep	0.40	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
19-Sep	0.39	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.12	**0.04**
27-Sep	0.57	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
4-Oct	0.24	0.00	0.00	0.00	**0.00**	0.00	0.00	0.15	**0.05**	0.07	0.02	0.00	**0.03**
10-Oct	0.24	0.15	0.23	0.00	**0.13**	0.00	0.00	0.02	**0.01**	0.14	0.11	0.08	**0.11**
17-Oct	0.80	0.02	0.00	0.00	**0.01**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
25-Oct	0.18	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
1-Nov	0.26	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
8-Nov	0.57	0.00	0.00	0.08	**0.03**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
15-Nov	0.87	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**

Table D.2 Nitrite (mg NO_2-N/L) concentrations at the influent and effluent under synthetic wastewater experiments.

Date	INF	R1	R2	R3	RKM	R4	R5	R6	RWC	R7	R8	R9	RWS
2-Aug	**0**	3.81	1.81	3.34	**2.99**	14.31	4.37	5.83	**8.17**	15.24	15.38	15.8	**15.49**
9-Aug	**1.33**	0.98	2.11	2.13	**1.74**	0.04	0.00	0.00	**0.01**	0.00	0.00	0.00	**0.00**
16-Aug	**0.15**	0.74	1.09	1.00	**0.94**	1.18	1.80	0.99	**1.32**	0.73	0.92	0.87	**0.84**
23-Aug	**0.11**	1.48	1.56	1.95	**1.66**	0.57	0.12	0.04	**0.24**	0.04	0.02	0.02	**0.03**
30-Aug	**0.08**	0.54	2.21	2.35	**1.70**	0.60	0.39	0.52	**0.50**	0.71	0.26	0.04	**0.34**
7-Sep	**0.33**	0.03	0.83	1.06	**0.64**	0.40	0.24	0.32	**0.32**	0.28	0.05	0.06	**0.13**
13-Sep	**0.26**	3.04	4.94	3.17	**3.72**	2.48	3.95	4.68	**3.70**	2.82	4.29	3.56	**3.56**
19-Sep	**1.40**	3.18	2.84	3.54	**3.19**	3.49	4.62	2.37	**3.49**	2.43	4.18	1.37	**2.66**
27-Sep	**1.63**	4.45	2.57	4.01	**3.68**	1.15	2.36	0.10	**1.20**	0.75	1.45	1.07	**1.09**
4-Oct	**4.44**	4.85	3.57	5.10	**4.51**	0.71	1.79	0.84	**1.11**	1.81	1.61	1.36	**1.59**
10-Oct	**13.32**	0.02	0.17	0.67	**0.29**	0.31	0.15	0.13	**0.20**	0.52	0.05	0.45	**0.34**
17-Oct	**0.17**	0.75	3.15	3.82	**2.57**	0.95	0.74	0.05	**0.58**	4.57	0.53	4.02	**3.04**
25-Oct	**0.31**	5.90	0.78	5.95	**4.21**	0.43	0.21	0.10	**0.25**	4.70	0.20	0.44	**1.78**
1-Nov	**2.71**	0.04	0.12	3.53	**1.23**	0.28	0.39	0.50	**0.39**	0.36	0.02	0.02	**0.13**
8-Nov	**5.73**	0.11	0.08	0.39	**0.19**	0.18	0.58	0.06	**0.27**	0.12	0.01	0.00	**0.04**
15-Nov	**0.20**	0.02	0.06	0.27	**0.12**	0.14	0.33	0.04	**0.17**	0.21	0.02	0.06	**0.10**

APPENDIX E
pH, DO, and ORP raw data

Table E.1 pH, DO, and ORP, water temperature under synthetic wastewater.

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
14-Jul	beaker	23.5		1.56		
14-Jul	beaker	23.5		0.32		
15-Jul	beaker	22.5		0.39		
15-Jul	tank	22.5		0.75		
16-Jul	tank	22.5		0.3		
16-Jul	carboy	22.5		0.38		
25-Jul	tank	21		4.32		
25-Jul	effluent	21		3.5		
26-Jul	tank	21.5		7.8		
26-Jul	effluent	21.5		4.2		
2-Aug	influent tap	22.3		4.24		
4-Aug	influent tap	21.4		2.38		
4-Aug	effluent	21.5		0.08		
4-Aug	influent tap	22.3		1.55		
4-Aug	effluent	22.3		0.6		
9-Aug	INF	22		3.23	6.68	
9-Aug	1DKM	22			8.56	
9-Aug	2DKM	22			8.59	
9-Aug	3DKM	22			8.38	
9-Aug	4DWC	22			8.06	
9-Aug	5DWC	22			7.56	
9-Aug	6DWC	22			8.02	
9-Aug	7DWS	22			8.24	
9-Aug	8DWS	22			7.62	
9-Aug	9DWS	22			8.34	
11-Aug	INF	20.9		4.52		
11-Aug	1DKM	20.9				
11-Aug	2DKM	20.9				
11-Aug	3DKM	20.9				
11-Aug	4DWC	20.9				
11-Aug	5DWC	20.9				
11-Aug	6DWC	20.9				
11-Aug	7DWS	20.9				
11-Aug	8DWS	20.9				
11-Aug	9DWS	20.9				
12-Aug	INF	21		2.96		
12-Aug	EFF	21		0.07		

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
16-Aug	INF	20	21.1	1.85	5.95	
16-Aug	1DKM	20	20.4		8	
16-Aug	2DKM	20	19.7		7.19	
16-Aug	3DKM	20	19.2		7.46	
16-Aug	4DWC	20	20		7.83	
16-Aug	5DWC	20	20		8.37	
16-Aug	6DWC	20	19.5		7.49	
16-Aug	7DWS	20	21.1		7.96	
16-Aug	8DWS	20	19.6		7.67	
16-Aug	9DWS	20	19.3		7.68	
16-Aug	EFF	20	21.1	3		
18-Aug	INF	20.7	21.4	2.2		
18-Aug	1DKM	20.7	21.1			
18-Aug	2DKM	20.7	20.6			
18-Aug	3DKM	20.7	20.3			
18-Aug	4DWC	20.7	20.9			
18-Aug	5DWC	20.7	20.8			
18-Aug	6DWC	20.7	20			
18-Aug	7DWS	20.7	20.9			
18-Aug	8DWS	20.7	20.5			
18-Aug	9DWS	20.7	20.4			
21-Aug	INF	24.9		1.49		
21-Aug	1DKM	24.9	25.6			
21-Aug	2DKM	24.9				
21-Aug	3DKM	24.9				
21-Aug	4DWC	24.9				
21-Aug	5DWC	24.9				
21-Aug	6DWC	24.9				
21-Aug	7DWS	24.9				
21-Aug	8DWS	24.9				
21-Aug	9DWS	24.9				
22-Aug	INF	24.9		0.48		
22-Aug	1DKM	24.9				
22-Aug	2DKM	24.9				
22-Aug	3DKM	24.9				
22-Aug	4DWC	24.9				
22-Aug	5DWC	24.9				
22-Aug	6DWC	24.9				
22-Aug	7DWS	24.9				
22-Aug	8DWS	24.9				
22-Aug	9DWS	24.9				

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
23-Aug	INF	20.6	22.5	1.01	6.89	
23-Aug	1DKM	20.6	21.5		8.79	
23-Aug	2DKM	20.6	21.5		8.51	
23-Aug	3DKM	20.6	21		8.86	
23-Aug	4DWC	20.6	21.5		8.08	
23-Aug	5DWC	20.6	21.6		8.68	
23-Aug	6DWC	20.6	20.6		8.69	
23-Aug	7DWS	20.6	21.5		8.74	
23-Aug	8DWS	20.6	21.2		8.24	
23-Aug	9DWS	20.6	21		8.71	
23-Aug	INF	20.6	22.5	1.75		
23-Aug	1DKM	20.6	21.4			
23-Aug	2DKM	20.6	21.3			
23-Aug	3DKM	20.6	20.8			
23-Aug	4DWC	20.6	21.3			
23-Aug	5DWC	20.6	21.3			
23-Aug	6DWC	20.6	20.4			
23-Aug	7DWS	20.6	21.3			
23-Aug	8DWS	20.6	21			
23-Aug	9DWS	20.6	20.9			
25-Aug	INF	20.6	22.5	1.4		
25-Aug	1DKM	20.6	21.1			
25-Aug	2DKM	20.6	21.2			
25-Aug	3DKM	20.6	20.6			
25-Aug	4DWC	20.6	21			
25-Aug	5DWC	20.6	21			
25-Aug	6DWC	20.6	20.4			
25-Aug	7DWS	20.6	20.9			
25-Aug	8DWS	20.6	20.7			
25-Aug	9DWS	20.6	20.6			
26-Aug	INF	21.3		1.99		
26-Aug	1DKM	21.3	21.6			
26-Aug	2DKM	21.3	21.5			
26-Aug	3DKM	21.3	21.4			
26-Aug	4DWC	21.3	21.6			
26-Aug	5DWC	21.3	21.6			
26-Aug	6DWC	21.3	21.3			
26-Aug	7DWS	21.3	21.6			
26-Aug	8DWS	21.3	21.4			
26-Aug	9DWS	21.3	21.4			
26-Aug	INFafter fillup	21.3		1.18		
26-Aug	EFF	21.3		0.75		

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
27-Aug	INF			1.53		
27-Aug	INF after sparging			0.49		
27-Aug	EFF			0.4		
28-Aug	INF	21.8		2.78		
28-Aug	1DKM	21.8				
28-Aug	2DKM	21.8				
28-Aug	3DKM	21.8				
28-Aug	4DWC	21.8				
28-Aug	5DWC	21.8				
28-Aug	6DWC	21.8				
28-Aug	7DWS	21.8				
28-Aug	8DWS	21.8				
28-Aug	9DWS	21.8				
29-Aug	INF			2.42		
29-Aug	INF after sparging			1.65		
30-Aug	INF			1.72	6.73	
30-Aug	1DKM				8.92	
30-Aug	2DKM				8.79	
30-Aug	3DKM				8.85	
30-Aug	4DWC				8.71	
30-Aug	5DWC				8.47	
30-Aug	6DWC				8.67	
30-Aug	7DWS				8.76	
30-Aug	8DWS				7.94	
30-Aug	9DWS				9.03	
31-Aug	INF			2.88		
31-Aug	1DKM					
31-Aug	2DKM					
31-Aug	3DKM					
31-Aug	4DWC					
31-Aug	5DWC					
31-Aug	6DWC					
31-Aug	7DWS					
31-Aug	8DWS					
31-Aug	9DWS					

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
7-Sep	INF	21.3	21.3	2.36	7.07	
7-Sep	1DKM	21.3			8.67	
7-Sep	2DKM	21.3			8.70	
7-Sep	3DKM	21.3			8.71	
7-Sep	4DWC	21.3			8.65	
7-Sep	5DWC	21.3			8.74	
7-Sep	6DWC	21.3			8.72	
7-Sep	7DWS	21.3			8.55	
7-Sep	8DWS	21.3			8.46	
7-Sep	9DWS	21.3			8.76	
13-Sep	INF	20.7	20.7	1.74	7.55	
13-Sep	1DKM	20.7			9.42	
13-Sep	2DKM	20.7			9.25	
13-Sep	3DKM	20.7			9.34	
13-Sep	4DWC	20.7			9.30	
13-Sep	5DWC	20.7			9.32	
13-Sep	6DWC	20.7			9.27	
13-Sep	7DWS	20.7			9.37	
13-Sep	8DWS	20.7			9.35	
13-Sep	9DWS	20.7			9.26	
13-Sep	EFF	20.7		0.68		
20-Sep	INF	22.9		2.11	7.20	
20-Sep	1DKM	22.9			9.35	
20-Sep	2DKM	22.9			9.23	
20-Sep	3DKM	22.9			9.26	
20-Sep	4DWC	22.9			9.24	
20-Sep	5DWC	22.9			9.29	
20-Sep	6DWC	22.9			9.29	
20-Sep	7DWS	22.9			9.31	
20-Sep	8DWS	22.9			9.30	
20-Sep	9DWS	22.9			9.22	
20-Sep	EFF	22.9		0.74		
27-Sep	INF	20.9		2.29	7.56	
27-Sep	1DKM	20.9			9.30	
27-Sep	2DKM	20.9			9.38	
27-Sep	3DKM	20.9			9.35	
27-Sep	4DWC	20.9			9.30	
27-Sep	5DWC	20.9			9.27	
27-Sep	6DWC	20.9			9.33	
27-Sep	7DWS	20.9			9.39	
27-Sep	8DWS	20.9			9.34	
27-Sep	9DWS	20.9			9.37	
27-Sep	EFF	20.9		0.27		

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
4-Oct	INF	19.8		0.48	7.54	
4-Oct	1DKM	19.8			9.31	
4-Oct	2DKM	19.8			9.37	
4-Oct	3DKM	19.8			9.25	
4-Oct	4DWC	19.8			9.30	
4-Oct	5DWC	19.8			9.31	
4-Oct	6DWC	19.8			9.33	
4-Oct	7DWS	19.8			9.36	
4-Oct	8DWS	19.8			9.37	
4-Oct	9DWS	19.8			9.37	
10-Oct	INF	18.8	19.5	0.62	7.53	
10-Oct	1DKM	18.8			9.19	
10-Oct	2DKM	18.8			8.76	
10-Oct	3DKM	18.8			9.02	
10-Oct	4DWC	18.8			9.07	
10-Oct	5DWC	18.8			9.11	
10-Oct	6DWC	18.8			9.06	
10-Oct	7DWS	18.8			9.04	
10-Oct	8DWS	18.8			8.93	
10-Oct	9DWS	18.8			9.67	
10-Oct	EFF	18.8	19.7	0.77		
13-Oct	INF	20.7	21.4	2.32	6.90	4.5
13-Oct	1DKM	20.7	21.4		9.39	-141.3
13-Oct	2DKM	20.7	21.4		9.41	-142.1
13-Oct	3DKM	20.7	21		9.36	-139.3
13-Oct	4DWC	20.7	21.5		9.25	-133.8
13-Oct	5DWC	20.7	21.4		9.32	-137.4
13-Oct	6DWC	20.7	20.8		9.33	-137.6
13-Oct	7DWS	20.7	21.5		9.31	-136.5
13-Oct	8DWS	20.7	21.2		9.30	-135.7
13-Oct	9DWS	20.7	21.2		9.33	-137.8
13-Oct	EFF	20.7	21.4	0.26	9.33	-137.8
16-Oct	INF	19.8	19.3	0.75	6.93	2.9
16-Oct	1DKM	19.8	20.2		9.33	-137.0
16-Oct	2DKM	19.8	19.9		9.36	-139.0
16-Oct	3DKM	19.8	19.5		9.38	-139.6
16-Oct	4DWC	19.8	20.2		9.32	-136.7
16-Oct	5DWC	19.8	20.2		9.33	-137.0
16-Oct	6DWC	19.8	19.3		9.34	-137.4
16-Oct	7DWS	19.8	20.3		9.35	-138.4
16-Oct	8DWS	19.8	19.8		9.35	-137.9
16-Oct	9DWS	19.8	19.6		9.39	-140.4
16-Oct	EFF	19.8		0.2		

Table E.1 ***(Continued)***

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
17-Oct	INF	18.5	19.7	0.74	7.17	-8.8
17-Oct	1DKM	18.5	19.4		9.43	-142.8
17-Oct	2DKM	18.5	19.4		9.47	-144.8
17-Oct	3DKM	18.5	18.5		9.49	-145.7
17-Oct	4DWC	18.5	19.7		9.48	-145.7
17-Oct	5DWC	18.5	19.5		9.48	-145.5
17-Oct	6DWC	18.5	18.3		9.50	-145.9
17-Oct	7DWS	18.5	19.7		9.47	-144.8
17-Oct	8DWS	18.5	19.1		9.51	-147.1
17-Oct	9DWS	18.5	18.8		9.51	-147.1
17-Oct	EFF	18.5		0.25		
18-Oct	INF	21.9	22.1	0.85	7.24	-15.0
18-Oct	1DKM	21.9	22.1		9.40	-142.2
18-Oct	2DKM	21.9	22.5		9.42	-143.3
18-Oct	3DKM	21.9	22.6		9.39	-141.7
18-Oct	4DWC	21.9	22.4		9.40	-142.3
18-Oct	5DWC	21.9	22.3		9.36	-139.8
18-Oct	6DWC	21.9	22.5		9.38	-141.0
18-Oct	7DWS	21.9	22.4		9.39	-141.5
18-Oct	8DWS	21.9	22.5		9.43	-143.9
18-Oct	9DWS	21.9	22.6		9.42	-143.5
18-Oct	EFF	21.9		0.65		
20-Oct	INF	20.8	21.2	1.6	7.88	-52.6
20-Oct	1DKM	20.8	20.8		9.42	-142.5
20-Oct	2DKM	20.8	20.7		9.52	-148.2
20-Oct	3DKM	20.8	20.1		9.48	-145.7
20-Oct	4DWC	20.8	20.9		9.45	-144.3
20-Oct	5DWC	20.8	20.7		9.43	-142.8
20-Oct	6DWC	20.8	19.9		9.46	-144.6
20-Oct	7DWS	20.8	20.6		9.47	-145.3
20-Oct	8DWS	20.8	20.3		9.46	-144.7
20-Oct	9DWS	20.8	20.3		9.45	-144.0
20-Oct	EFF			0.49		

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
25-Oct	INF	17.0	18.3	0.61	7.20	-13.0
25-Oct	1DKM	17.0	18.7		9.55	-149.0
25-Oct	2DKM	17.0	19		9.55	-149.4
25-Oct	3DKM	17.0	18.8		9.51	-146.7
25-Oct	4DWC	17.0	18.7		9.55	-149.4
25-Oct	5DWC	17.0	19.2		9.55	-149.4
25-Oct	6DWC	17.0	18.9		9.57	-150.4
25-Oct	7DWS	17.0	19		9.54	-148.8
25-Oct	8DWS	17.0	18.9		9.60	-151.8
25-Oct	9DWS	17.0	19.2		9.58	-151.3
25-Oct	EFF	17.0		0.06		
27-Oct	INF	22.8	22.1	0.86	7.21	-13.3
27-Oct	1DKM		23.3		9.42	-144.1
27-Oct	2DKM		23.1		9.43	-144.0
27-Oct	3DKM		22.5		9.39	-141.9
27-Oct	4DWC		23.1		9.38	-141.3
27-Oct	5DWC		23.3		9.41	-143.4
27-Oct	6DWC		22.3		9.37	-140.4
27-Oct	7DWS		23.1		9.41	-143.3
27-Oct	8DWS		23.1		9.40	-142.7
27-Oct	9DWS		22.9		9.44	-142.4
27-Oct	EFF			0.45		
1-Nov	INF	20.3	21.1	1.87	6.93	2.9
1-Nov	1DKM		21.4		9.46	-145.5
1-Nov	2DKM		21.3		9.49	-147.0
1-Nov	3DKM		21.2		9.50	-147.5
1-Nov	4DWC		21.5		9.47	-146.5
1-Nov	5DWC		21.2		9.49	-147.0
1-Nov	6DWC		21.1		9.49	-147.0
1-Nov	7DWS		21.8		9.49	-147.0
1-Nov	8DWS		21		9.49	-147.0
1-Nov	9DWS		21.1		9.48	-145.7
1-Nov	EFF			0.75		

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
3-Nov	INF	20.6	21.1	0.47	7.33	-20.7
3-Nov	1DKM		21.6		9.50	-147.1
3-Nov	2DKM		21.4		9.53	-149.3
3-Nov	3DKM		21.2		9.52	-148.4
3-Nov	4DWC		21.5		9.48	-145.7
3-Nov	5DWC		21.7		9.49	-148.1
3-Nov	6DWC		20.6		9.49	-146.5
3-Nov	7DWS		21.7		9.51	-148.3
3-Nov	8DWS		21.1		9.50	-148.0
3-Nov	9DWS		21.5		9.55	-150.8
3-Nov	EFF			0.25		
8-Nov	INF	21.0	22.2	1.47	7.42	-23.2
8-Nov	1DKM		24.1		9.45	-145.1
8-Nov	2DKM		24		9.45	-143.9
8-Nov	3DKM		21.7		9.39	-144.7
8-Nov	4DWC		24.2		9.39	-142.7
8-Nov	5DWC		24.3		9.41	-142.8
8-Nov	6DWC		21.6		9.44	-145.1
8-Nov	7DWS		24.2		9.43	-142.4
8-Nov	8DWS		22		9.46	-145.1
8-Nov	9DWS		24.1		9.38	-143.7
8-Nov	EFF			0.57		
10-Nov	INF	20.6	21.1	0.53	7.33	-21.0
10-Nov	1DKM		21.6		9.21	-130.9
10-Nov	2DKM		21.4		9.22	-131.3
10-Nov	3DKM		21.2		9.25	-132.6
10-Nov	4DWC		21.5		9.19	-129.7
10-Nov	5DWC		21.7		9.16	-128.1
10-Nov	6DWC		20.6		9.20	-129.5
10-Nov	7DWS		21.7		9.17	-128.4
10-Nov	8DWS		21.1		9.19	-129.7
10-Nov	9DWS		21.5		9.21	-130.4
10-Nov	EFF			0.27		

Table E.1*(Continued)*

Date	Location	Amb Temp	Water Temp	DO Reading	pH	ORP (mV)
13-Nov	INF	20.0				
13-Nov	1DKM		21.2		9.63	
13-Nov	2DKM		21.4		9.62	
13-Nov	3DKM		20.5		9.60	
13-Nov	4DWC		21.3		9.58	
13-Nov	5DWC		21.1		9.57	
13-Nov	6DWC		21.3		9.59	
13-Nov	7DWS		21.2		9.59	
13-Nov	8DWS		21.1		9.61	
13-Nov	9DWS		20.6		9.59	
13-Nov	EFF					
15-Nov	INF	22.0	21.5	0.7	7.43	-26.4
15-Nov	1DKM		22.4		9.55	-150.8
15-Nov	2DKM		22.3		9.55	-150.8
15-Nov	3DKM		22.2		9.54	-150.4
15-Nov	4DWC		22.5		9.53	-149.9
15-Nov	5DWC		22.6		9.52	-149.8
15-Nov	6DWC		21.7		9.44	-143.0
15-Nov	7DWS		22.5		9.54	-149.8
15-Nov	8DWS		21.8		9.52	-148.8
15-Nov	9DWS		22.2		9.53	-149.4
15-Nov	EFF			0.35		
17-Nov	INF	21.8	21.9	0.82	7.55	-33.3
17-Nov	1DKM		21.7		9.49	-147.4
17-Nov	2DKM		22.6		9.49	-147.6
17-Nov	3DKM		21.9		9.49	-147.3
17-Nov	4DWC		22.9		9.48	-147.1
17-Nov	5DWC		22.3		9.48	-146.5
17-Nov	6DWC		21.9		9.46	-145.5
17-Nov	7DWS		22.8		9.48	-147.1
17-Nov	8DWS		22.0		9.48	-146.9
17-Nov	9DWS		21.5		9.49	-142.8
17-Nov	EFF		22.7	0.47	9.41	

Table E.2. pH along column height (November 8, 2004).

Reactor	INF	Port A (10 cm)	Port B (20 cm)	Port C (30 cm)	Effluent port (40 cm)
R1	7.42	9.35	9.44	9.45	9.45
R2	7.42	9.37	9.43	9.45	9.45
R3	7.42	9.20	9.39	9.43	9.39
R4	7.42	9.32	9.38	9.42	9.39
R5	7.42	9.17	9.31	9.41	9.41
R6	7.42	9.33	9.37	9.43	9.44
R7	7.42	9.30	9.38	9.39	9.43
R8	7.42	9.34	9.42	9.45	9.46
R9	7.42	9.19	9.35	9.41	9.38
RKM	7.42	9.31	9.42	9.44	9.43
RWC	7.42	9.27	9.35	9.42	9.41
RWS	7.42	9.28	9.38	9.42	9.42

Table E.3. ORP (mV) along column height (November 8, 2004).

Reactor	INF	Port A (10 cm)	Port B (20 cm)	Port C (30 cm)	Effluent port (40 cm)
R1	-23.2	-140.5	-145.4	-145.9	-145.1
R2	-23.2	-141.7	-145.0	-146.1	-143.9
R3	-23.2	-131.1	-142.7	-144.5	-144.7
R4	-23.2	-138.0	-141.9	-144.2	-142.7
R5	-23.2	-129.1	-137.8	-143.7	-142.8
R6	-23.2	-138.6	-141.3	-144.7	-145.1
R7	-23.2	-136.9	-141.5	-142.7	-142.4
R8	-23.2	-139.6	-144.5	-146.1	-145.1
R9	-23.2	-130.5	-139.8	-143.7	-143.7
RKM	-23.2	-137.8	-144.4	-145.5	-144.6
RWC	-23.2	-135.2	-140.3	-144.2	-143.5
RWS	-23.2	-135.7	-141.9	-144.2	-143.7

APPENDIX F
Physical and chemical characteristics of filter media

Table F.1 Acid-insoluble lignin content of wood chips and wheat straw.

Sample	media	Initial weight (g)	equivalent dry weight (g)	Crucible (g)	Crucible + AIL+ash (g)	AIL +ash (g)	Crucible + ash (g)	ash (g)	AIL (g)	%AIL	Aver AIL (%)
IN-1	WS	0.31630	0.29058	31.40250	31.47320	0.07070	31.40630	0.00380	0.06690	23.02	**22.96**
IN-2		0.30480	0.28002	29.98710	30.05510	0.06800	29.99030	0.00320	0.06480	23.14	
IN-3		0.30250	0.27790	31.37430	31.44110	0.06680	31.37800	0.00370	0.06310	22.71	
IN-4	WC	0.30470	0.25529	29.91790	29.98720	0.06930	29.91860	0.00070	0.06860	26.87	**27.22**
IN-5		0.30370	0.25446	29.83710	29.90710	0.07000	29.83730	0.00020	0.06980	27.43	
IN-6		0.30440	0.25504	29.98650	30.05630	0.06980	29.98650	0.00000	0.06980	27.37	
R4-1	wood chips (WC)	0.29450	0.28500	25.17219	25.24451	0.07232	25.17239	0.00020	0.07212	25.31	**25.49**
R4-2		0.30996	0.29996	24.81745	24.89394	0.07649	24.81756	0.00011	0.07638	25.46	
R4-3		0.31182	0.30176	24.59506	24.67283	0.07777	24.59527	0.00021	0.07756	25.70	
R5-1		0.31329	0.30469	24.79723	24.87639	0.07916	24.79777	0.00054	0.07862	25.80	**25.64**
R5-2		0.30908	0.30059	24.95922	25.03643	0.07721	24.95939	0.00017	0.07704	25.63	
R5-3		0.30692	0.29849	24.66306	24.73956	0.07650	24.66345	0.00039	0.07611	25.50	
R6-1		0.30889	0.29406	24.54049	24.63091	0.09042	24.5495	0.00901	0.08141	27.69	**26.65**
R6-2		0.29928	0.28491	25.69025	25.76522	0.07497	25.69034	0.00009	0.07488	26.28	
R6-3		0.30625	0.29154	25.03718	25.11311	0.07593	25.03736	0.00018	0.07575	25.98	
R7-1	Wheat Strarw (WS)	0.29433	0.28203	24.92864	24.99855	0.06991	24.92892	0.00028	0.06963	24.69	**25.07**
R7-2		0.29903	0.28653	24.82663	24.89897	0.07234	24.82668	0.00005	0.07229	25.23	
R7-3		0.29879	0.28630	25.03531	25.10837	0.07306	25.03598	0.00067	0.07239	25.28	
R8-1		0.29490	0.28473	25.06239	25.13506	0.07267	25.06263	0.00024	0.07243	25.44	**25.35**
R8-2		0.29610	0.28589	24.43502	24.50862	0.07360	24.43510	0.00008	0.07352	25.72	
R8-3		0.30128	0.29089	24.42869	24.50430	0.07561	24.43189	0.00320	0.07241	24.89	
R9-1		0.29386	0.28362	25.17869	25.24778	0.06909	25.17876	0.00007	0.06902	24.34	**24.11**
R9-2		0.30655	0.29586	25.08765	25.15759	0.06994	25.08775	0.00010	0.06984	23.61	
R9-3		0.30908	0.29831	25.27950	25.35358	0.07408	25.28080	0.00130	0.07278	24.40	

Table F.2 Acid-soluble lignin content of wood chips and wheat straw.

Sample	media	dry weight (g)	ABS	K*ABS	dilution	%ASL	Ave-ASL (%)
IN-1	WS	0.290583	0.45600	0.45560	20	2.48%	
IN-2	WS	0.280018	0.41600	0.41640	20	2.35%	
IN-3	WS	0.277905	0.39600	0.39620	20	2.26%	**2.36%**
IN-4	WC	0.255294	0.46700	0.46660	20	2.89%	
IN-5	WC	0.254456	0.40800	0.40770	20	2.53%	
IN-6	WC	0.255043	0.45300	0.45250	20	2.81%	**2.74%**
R4-1	WC	0.284996	0.65600	0.65580	20	3.64%	
R4-2	WC	0.299957	0.64300	0.64330	20	3.39%	
R4-3	WC	0.301757	0.67500	0.67520	20	3.54%	**3.52%**
R5-1	WC	0.304685	0.66300	0.66330	20	3.44%	
R5-2	WC	0.300591	0.65900	0.65880	20	3.47%	
R5-3	WC	0.298490	0.65000	0.65040	20	3.45%	**3.45%**
R6-1	WC	0.294056	0.67800	0.67820	20	3.65%	
R6-2	WC	0.284907	0.60800	0.60840	20	3.38%	
R6-3	WC	0.291542	0.65200	0.65230	20	3.54%	**3.52%**
R7-1	WS	0.282026	0.41700	0.41710	20	2.34%	
R7-2	WS	0.286530	0.42200	0.42210	20	2.33%	
R7-3	WS	0.286300	0.39400	0.39490	20	2.18%	**2.28%**
R8-1	WS	0.284727	0.35600	0.35560	20	1.98%	
R8-2	WS	0.285886	0.38400	0.38440	20	2.13%	
R8-3	WS	0.290887	0.41000	0.41040	20	2.23%	**2.11%**
R9-1	WS	0.283616	0.42500	0.42460	20	2.37%	
R9-2	WS	0.295863	0.50400	0.50390	20	2.69%	
R9-3	WS	0.298305	0.54400	0.54430	20	2.89%	**2.65%**

Table F.3 Ash Content of wood chips and wheat straw.

Sample	media	Crucible (g)	Dry sample (g)	Ash + Crucible (g)	Ash (g)	%Ash	**Ave ash %**
IN-1	WS	31.40250	0.98940	31.44350	0.04100	4.14%	
IN-2	WS	29.98650	1.00780	30.02820	0.04170	4.14%	
IN-3	WS	31.37420	1.05300	31.41800	0.04380	4.16%	**4.15%**
IN-4	WC	29.91480	1.00720	29.92450	0.00970	0.96%	
IN-5	WC	29.83350	1.04150	29.84200	0.00850	0.82%	
IN-6	WC	29.98270	0.97350	29.99080	0.00810	0.83%	**0.87%**
R4-1	WC	25.68953	0.99283	25.70679	0.01726	1.74%	
R4-2	WC	25.03664	1.01157	25.05426	0.01762	1.74%	
R4-3	WC	24.54885	1.01195	24.56653	0.01768	1.75%	**1.74%**
R5-1	WC	24.81664	1.04992	24.83750	0.02086	1.99%	
R5-2	WC	24.95820	0.99976	24.97857	0.02037	2.04%	
R5-3	WC	24.79694	0.99854	24.81650	0.01956	1.96%	**1.99%**
R6-1	WC	25.17152	1.01271	25.19242	0.02090	2.06%	
R6-2	WC	24.66255	1.04139	24.68451	0.02196	2.11%	
R6-3	WC	24.59456	0.97723	24.61497	0.02041	2.09%	**2.09%**
R7-1	WS	29.98968	0.99918	30.03630	0.04662	4.67%	
R7-2	WS	29.92088	0.95839	29.97065	0.04977	5.19%	
R7-3	WS	29.92006	0.92821	29.96554	0.04548	4.90%	**4.92%**
R8-1	WS	31.37508	0.99485	31.42593	0.05085	5.11%	
R8-2	WS	29.98775	0.97493	30.04806	0.06031	6.19%	
R8-3	WS	29.98919	0.96023	30.04296	0.05377	5.60%	**5.63%**
R9-1	WS	31.40487	0.96029	31.46873	0.06386	6.65%	
R9-2	WS	29.83928	0.98807	29.90999	0.07071	7.16%	
R9-3	WS	29.83887	0.970440	29.90583	0.06696	6.90%	**6.90%**

Table F.4 Moisture content of wood chips and wheat straw.

Sample	media	weighing dish	initial sample weight	weigh dish + dry samples	dry weight	moisture	%MC	**average**
IN-1	WS	0.98000	3.09840	3.84340	2.86340	0.23500	8.21%	
IN-2	WS	0.98060	3.01300	3.76800	2.78740	0.22560	8.09%	
IN-3	WS	0.97110	3.03120	3.77540	2.80430	0.22690	8.09%	**8.13%**
IN-4	WC	0.97360	3.07720	3.62220	2.64860	0.42860	16.18%	
IN-5	WC	0.97300	3.00150	3.55430	2.58130	0.42020	16.28%	
IN-6	WC	0.97810	3.03900	3.59380	2.61570	0.42330	16.18%	**16.21%**
R4-1	WC	0.80428	3.06835	3.77681	2.97253	0.09582	3.22%	
R4-2	WC	0.79965	3.08573	3.78942	2.98977	0.09596	3.21%	
R4-3	WC	0.81236	3.06768	3.78352	2.97116	0.09652	3.25%	**3.23%**
R5-1	WC	0.79640	3.04955	3.76462	2.96822	0.08133	2.74%	
R5-2	WC	0.79645	3.07013	3.78368	2.98723	0.08290	2.78%	
R5-3	WC	0.80436	3.03397	3.75786	2.95350	0.08047	2.72%	**2.75%**
R6-1	WC	0.80733	3.03942	3.70772	2.90039	0.13903	4.79%	
R6-2	WC	0.81360	3.01942	3.69368	2.88008	0.13934	4.84%	
R6-3	WC	0.81290	3.02336	3.69845	2.88555	0.13781	4.78%	**4.80%**
R7-1	WS	0.81305	1.03758	1.80690	0.99385	0.04373	4.40%	
R7-2	WS	0.80467	0.99432	1.75992	0.95525	0.03907	4.09%	
R7-3	WS	0.79653	0.99332	1.75118	0.95465	0.03867	4.05%	**4.18%**
R8-1	WS	0.79656	1.02520	1.79239	0.99583	0.02937	2.95%	
R8-2	WS	0.80742	1.00360	1.77446	0.96704	0.03656	3.78%	
R8-3	WS	0.81389	1.02047	1.79872	0.98483	0.03564	3.62%	**3.45%**
R9-1	WS	0.81291	0.99724	1.77859	0.96568	0.03156	3.27%	
R9-2	WS	0.79995	1.01968	1.78317	0.98322	0.03646	3.71%	
R9-3	WS	0.80513	1.02980	1.80028	0.99515	0.03465	3.48%	**3.49%**

Table F.5. Volume of water drained (to estimate porosity) from reactors (empty, with media (before and after denitrification)).

Section	Volume occupied with water (mL)								
	R1	R2	R3	R4	R5	R6	R7	R8	R9
Empty Reactors									
Top	180	250	220	215	210	200	210	215	200
Fourth Quarter	800	790	790	825	795	795	800	805	810
Third Quarter	805	805	805	790	800	805	800	800	800
Second Quarter	800	805	800	805	805	795	800	810	810
First Quarter	785	780	775	795	785	800	750	805	800
Bottom (upper space)	220	260	250	245	245	255	250	245	245
Bottom (lower space)	175	170	180	200	200	200	200	235	225
Total Reactor Volume	3765	3860	3820	3875	3840	3850	3810	3915	3890
Packing volume	3190	3180	3170	3215	3185	3195	3150	3220	3220
With Wet media (Initial)									
Top	260	250	220	215	210	200	210	215	200
Fourth Quarter	585	580	600	385	375	350	375	435	485
Third Quarter	625	640	620	490	410	425	385	440	440
Second Quarter	585	610	590	410	415	410	390	415	420
First Quarter and Bottom	1065	1060	1080	855	845	860	885	945	875
Total Volume occupied by water	3120	3140	3110	2355	2255	2245	2245	2450	2420
Water in Packed Volume	2640	2630	2640	1895	1800	1790	1785	1990	1975
Porosity/Void ratio(Initial)									
Fourth Quarter+top	86.22%	79.81%	81.19%	57.69%	58.21%	55.28%	57.92%	63.73%	67.82%
Third Quarter	77.64%	79.50%	77.02%	62.03%	51.25%	52.80%	48.13%	55.00%	55.00%
Second Quarter	73.13%	75.78%	73.75%	50.93%	51.55%	51.57%	48.75%	51.23%	51.85%
First Quarter+bottom	90.25%	87.60%	89.63%	68.95%	68.70%	68.53%	73.75%	73.54%	68.90%
Reactor void	82.87%	81.35%	81.41%	60.77%	58.72%	58.31%	58.92%	62.58%	62.21%

Table F.5 ***(Continued)***

Section	Volume occupied with water (mL)								
	R1	R2	R3	R4	R5	R6	R7	R8	R9
With wet media (Final)									
Fourth Quarter + top	590	588	602	444	434	400	258	380	250
Third Quarter	384	364	370	266	220	180	50	122	30
Second Quarter	232	280	220	120	120	68	170	30	38
First Quarter and bottom space	180	54	340	102	180	112	540	462	590
Total Volume occupied by water	1386	1286	1532	932	954	760	1018	994	908
Porosity/Void ratio(Final)									
Fourth Quarter+top	55.66%	56.54%	59.60%	42.69%	43.18%	40.20%	25.54%	37.25%	24.75%
Third Quarter	47.70%	45.22%	45.96%	33.67%	27.50%	22.36%	6.25%	15.25%	3.75%
Second Quarter	29.00%	34.78%	27.50%	14.91%	14.91%	8.55%	21.25%	3.70%	4.69%
First Quarter+ bottom	15.25%	4.46%	28.22%	8.23%	14.63%	8.92%	45.00%	35.95%	46.46%
Total Void	36.05%	33.32%	40.10%	24.05%	24.84%	19.74%	26.72%	25.39%	23.34%

APPENDIX G
SAS source codes and Partial Output

G.1 Denitrification rates at LR1 (overall)

Source Code

```
data DNR50;
input type$  reactor$ days  inf_N  eff_N  flow  vol;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
cards;
Data set: refer to Table B.1 (Day 21 to 57)
run;
proc mixed method=type3;
   title "Split-plot analysis of DNR at 50";
   class type days reactor;
   model DNR = type|days;
   random reactor(type);
   lsmeans type;
   estimate "DNR KM-WC" type 1 -1 0;
estimate "DNR KM-WS" type 1 0 -1;
estimate "DNR WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of DNR at 50/The Mixed Procedure/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	30840	15420	Var(Residual) + 8.82 Var(reactor(type)) +Q(type,type*days)	0.9985 MS(reactor(type)) + 0.0015 MS(Residual)	6.0082	3.27	0.1093
days	8	450115	56264	Var(Residual) + Q(days,type*days)	MS(Residual)	47	26.55	<.0001
type*days	16	60206	3762.86	Var(Residual) + Q(type*days)	MS(Residual)	47	1.78	0.0645
reactor(type)	6	28293	4715.51	Var(Residual) + 8.8333 Var(reactor(type))	MS(Residual)	47	2.23	0.0569
Residual	47	99591	2118.96	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DNR KM-WC	-47.8686	18.9096	6	-2.53	0.0446
DNR KM-WS	-21.2906	18.9096	6	-1.13	0.3032
DNR WC-WS	26.5780	18.7863	6	1.41	0.2069

Least Squares Means

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM	245.24	13.4577	6	18.22	<.0001
type	WC	293.11	13.2839	6	22.06	<.0001
type	WS	266.53	13.2839	6	20.06	<.0001

G.2 Denitrification rates at LR2 (overall)

Source Code

```
data DNR120;
input type$  reactor$ days  inf_N  eff_N  flow  vol;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
cards;
Data Set: refer to Table B.1 (Day 63 to 93)
run;
proc mixed method=type3;
   title "Split-plot analysis of DNR at 120";
   class type days reactor;
   model DNR = type|days;
   random reactor(type);
   lsmeans type type*days/slice=days;
estimate "DNR KM-WC" type 1 -1 0;
estimate "DNR KM-WS" type 1 0 -1;
estimate "DNR WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of DNR at 120 /The Mixed Procedure/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	135954	67977	Var(Residual) + 9 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	12.99	0.0066
days	8	315370	39421	Var(Residual) + Q(days,type*days)	MS(Residual)	48	11.11	<.0001
type*days	16	95770	5985.63	Var(Residual) + Q(type*days)	MS(Residual)	48	1.69	0.0825
reactor(type)	6	31394	5232.35	Var(Residual) + 9 Var(reactor(type))	MS(Residual)	48	1.47	0.2072
Residual	48	170383	3549.64	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DNR KM-WC	-74.8955	19.6871	6	-3.80	0.0089
DNR KM-WS	-95.2923	19.6871	6	-4.84	0.0029
DNR WC-WS	-20.3968	19.6871	6	-1.04	0.3401

Least Squares Means

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM	672.51	13.9209	6	48.31	<.0001
type	WC	747.41	13.9209	6	53.69	<.0001
type	WS	767.80	13.9209	6	55.15	<.0001

G.3 Denitrification rates at LR3 (overall)

Source Code

```
data DNR200;
input type$  reactor$ days  inf_N  eff_N  flow  vol;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
cards;
Data set: refer to Table B.1 (Day 97 to 128)
run;

proc mixed method=type3;
   title "Split-plot analysis of DNR at 200";
   class type days reactor;
   model DNR = type|days;
   random reactor(type);
   lsmeans type;
estimate "DNR KM-WC" type 1 -1 0;
estimate "DNR KM-WS" type 1 0 -1;
estimate "DNR WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of DNR at 200/ The Mixed Procedure/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	79546	39773	Var(Residual) + 10 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	1.90	0.2300
days	9	2664047	296005	Var(Residual) + Q(days,type*days)	MS(Residual)	54	35.72	<.0001
type*days	18	79538	4418.76	Var(Residual) + Q(type*days)	MS(Residual)	54	0.53	0.9289
reactor(type)	6	125833	20972	Var(Residual) + 10 Var(reactor(type))	MS(Residual)	54	2.53	0.0312
Residual	54	447435	8285.84	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DNR KM-WC	-59.0984	37.3918	6	-1.58	0.1651
DNR KM-WS	-66.3977	37.3918	6	-1.78	0.1261
DNR WC-WS	-7.2993	37.3918	6	-0.20	0.8517

Least Squares Means

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM	1214.72	26.4400	6	45.94	<.0001
type	WC	1273.82	26.4400	6	48.18	<.0001
type	WS	1281.12	26.4400	6	48.45	<.0001

G.4 Denitrification rates at LR1 (steady)

Source Code

```
data DNR50;
input type$  reactor$ days  inf_N  eff_N  flow  vol;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
cards;
Data set: refer to Table B.1 (Day 37 to 57)
run;
proc mixed method=type3;
   title "Split-plot analysis of DNR at 50 (steady)";
   class type days reactor;
   model DNR = type|days;
   random reactor(type);
  lsmeans type;
   estimate "DNR KM-WC" type 1 -1 0;
estimate "DNR KM-WS" type 1 0 -1;
estimate "DNR WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of DNR at 50 (steady)/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	3218.43	1609.21	Var(Residual) + 4.8077 Var(reactor(type)+ Q(type,type*days)	0.9947 MS(reactor(type)) + 0.0053 MS(Residual)	6.0402	1.14	0.3799
days	4	52864	13216	Var(Residual) + Q(days,type*days)	MS(Residual)	23	14.91	<.0001
type*days	8	4307.19	538.40	Var(Residual) + Q(type*days)	MS(Residual)	23	0.61	0.7622
reactor(type)	6	8479.75	1413.29	Var(Residual) + 4.8333 Var(reactor(type))	MS(Residual)	23	1.59	0.1938
Residual	23	20384	886.27	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DNR KM-WC	-19.9504	14.0448	6	-1.42	0.2053
DNR KM-WS	-15.3220	14.0448	6	-1.09	0.3172
DNR WC-WS	4.6284	13.8153	6	0.34	0.7490

Least Squares Means

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM	312.62	10.0909	6	30.98	<.0001
type	WC	332.57	9.7689	6	34.04	<.0001
type	WS	327.94	9.7689	6	33.57	<.0001

G.5 Denitrification rates at LR2 (steady)

```
data DNR120;
input type$  reactor$ days  inf_N  eff_N  flow  vol;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
cards;
Data set: refer to Table B.1 (Day 77 to 93)
run;
proc mixed method=type3;
   title "Split-plot analysis of DNR at 120";
   class type days reactor;
   model DNR = type|days;
   random reactor(type);
   lsmeans type;
estimate "DNR KM-WC" type 1 -1 0;
estimate "DNR KM-WS" type 1 0 -1;
estimate "DNR WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of DNR at 120 (steady)/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	32023	16012	Var(Residual) + 5 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	8.67	0.0170
days	4	49316	12329	Var(Residual) + Q(days,type*days)	MS(Residual)	24	3.62	0.0190
type*days	8	13121	1640.09	Var(Residual) + Q(type*days)	MS(Residual)	24	0.48	0.8567
reactor(type)	6	11080	1846.73	Var(Residual) + 5 Var(reactor(type))	MS(Residual)	24	0.54	0.7703
Residual	24	81630	3401.26	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DNR KM-WC	-17.0480	15.6918	6	-1.09	0.3190
DNR KM-WS	-63.1533	15.6918	6	-4.02	0.0069
DNR WC-WS	-46.1053	15.6918	6	-2.94	0.0260

Least Squares Means

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM	743.23	11.0958	6	66.98	<.0001
type	WC	760.27	11.0958	6	68.52	<.0001
type	WS	806.38	11.0958	6	72.67	<.0001

G.6 Denitrification rates at LR3 (steady)

Source Code

```
data DNR200;
input type$  reactor$ days  inf_N  eff_N  flow  vol  inf_COD  eff_COD;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
cards;
Data Set: refer to Table B.1 (Day 112 to Day 128 except Day 121)
run;
proc mixed method=type3;
   title "Split-plot analysis of DNR at 200 (steady)";
   class type days reactor;
   model DNR = type|days;
   random reactor(type);
   lsmeans type type*days/slice=days;
   estimate "DNR KM-WC" type 1 -1 0;
estimate "DNR KM-WS" type 1 0 -1;
estimate "DNR WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of DNR at 200 (steady)/Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	13776	6887.90	Var(Residual) + 5 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	0.32	0.7355
days	4	110612	27653	Var(Residual) + Q(days,type*days)	MS(Residual)	24	2.87	0.0448
type*days	8	49986	6248.27	Var(Residual) + Q(type*days)	MS(Residual)	24	0.65	0.7299
reactor(type)	6	127781	21297	Var(Residual) + 5 Var(reactor(type))	MS(Residual)	24	2.21	0.0772
Residual	24	231256	9635.65	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
DNR KM-WC	-38.7947	53.2878	6	-0.73	0.4940
DNR KM-WS	-35.1710	53.2878	6	-0.66	0.5337
DNR WC-WS	3.6237	53.2878	6	0.07	0.9480

Least Squares Means

Effect	type	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM	1326.19	37.6801	6	35.20	<.0001
type	WC	1364.98	37.6801	6	36.23	<.0001
type	WS	1361.36	37.6801	6	36.13	<.0001

G.7 COD removed at LR1 (overall)

Source Code

```
data COD;
input type$  reactor$  days inf_COD  eff_COD;
COD = inf_COD-eff_COD;
cards;
Data set:refer to Table C.1 (2-Aug to 7-Sep)
run;
proc mixed method=type3;
   title "Split-plot analysis of COD removed at 50";
   class type days reactor;
   model COD = type|days;
   random reactor(type) ;
   lsmeans type type*days/slice=days;
   estimate "COD KM-WC  overall" type 1 -1 0;
   estimate "COD KM-WS overall" type 1 0 -1;
   estimate "COD WC-WS overall" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD removed at 50/ The Mixed Procedure/ Type 3 Analysis of Variances

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	3780.14	1890.07	Var(Residual) + 6 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	7.06	0.0265
days	5	85120	17024	Var(Residual) + Q(days,type*days)	MS(Residual)	30	88.33	<.0001
type*days	10	15965	1596.51	Var(Residual) + Q(type*days)	MS(Residual)	30	8.28	<.0001
reactor(type)	6	1605.55	267.59	Var(Residual) + 6 Var(reactor(type))	MS(Residual)	30	1.39	0.2515
Residual	30	5781.77	192.72	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD KM-WC overall	-19.3333	5.4528	6	-3.55	0.0121
COD KM-WS overall	-3.7778	5.4528	6	-0.69	0.5143
COD WC-WS overall	15.5556	5.4528	6	2.85	0.0291

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		147.39	3.8557	6	38.23	<.0001
type	WC		166.72	3.8557	6	43.24	<.0001
type	WS		151.17	3.8557	6	39.21	<.0001

G.8 COD removed at LR2 (overall)

Source Code

```
data COD120;
input type$  reactor$  days inf_COD  eff_COD;
COD = inf_COD-eff_COD;
cards;
Data set:refer to Table C.1 (13-sep to 17-Oct)
run;
proc mixed method=type3;
   title "Split-plot analysis of COD removed";
   class type days reactor;
   model COD = type|days;
   random reactor(type) ;
   lsmeans type type*days/slice=days;
   estimate "COD KM-WC  overall" type 1 -1 0;
   estimate "COD KM-WS overall" type 1 0 -1;
   estimate "COD WC-WS overall" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD removed at 120/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	15434	7717.08	Var(Residual) + 5 Var(reactor(type)) + Q(type,type*days)	MS(reactor(type))	6	28.60	0.0009
days	4	58465	14616	Var(Residual) + Q(days,type*days)	MS(Residual)	24	24.62	<.0001
type*days	8	15186	1898.22	Var(Residual) + Q(type*days)	MS(Residual)	24	3.20	0.0129
reactor(type)	6	1618.93	269.82	Var(Residual) + 5 Var(reactor(type))	MS(Residual)	24	0.45	0.8346
Residual	24	14248	593.68	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD KM-WC overall	-41.1333	5.9980	6	-6.86	0.0005
COD KM-WS overall	-37.1333	5.9980	6	-6.19	0.0008
COD WC-WS overall	4.0000	5.9980	6	0.67	0.5296

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		330.07	4.2412	6	77.82	<.0001
type	WC		371.20	4.2412	6	87.52	<.0001
type	WS		367.20	4.2412	6	86.58	<.0001

G.9 COD removed at LR3 (overall)

Source Code

```
data COD200;
input type$  reactor$  days inf_COD  eff_COD;
COD = inf_COD-eff_COD;
cards;
Data set:refer to Table C.1 (25-oct to 15-nov)
run;
proc mixed method=type3;
   title "Split-plot analysis of COD removed at 200";
   class type days reactor;
   model COD = type|days;
   random reactor(type) ;
   lsmeans type type*days/slice=days;
   estimate "COD KM-WC  overall" type 1 -1 0;
   estimate "COD KM-WS overall" type 1 0 -1;
   estimate "COD WC-WS overall" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD removed at 200/The Mixed Procedure/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	6578.17	3289.08	Var(Residual) + 5 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	2.72	0.1444
days	4	17973	4493.24	Var(Residual) + Q(days,type*days)	MS(Residual)	24	4.93	0.0048
type*days	8	10161	1270.14	Var(Residual) + Q(type*days)	MS(Residual)	24	1.39	0.2498
reactor(type)	6	7260.80	1210.13	Var(Residual) + 5 Var(reactor(type))	MS(Residual)	24	1.33	0.2839
Residual	24	21896	912.32	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD KM-WC overall	-29.3333	12.7024	6	-2.31	0.0603
COD KM-WS overall	-11.1333	12.7024	6	-0.88	0.4145
COD WC-WS overall	18.2000	12.7024	6	1.43	0.2019

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		685.53	8.9820	6	76.32	<.0001
type	WC		714.87	8.9820	6	79.59	<.0001
type	WS		696.67	8.9820	6	77.56	<.0001

G.10 COD removed at LR1 (steady)

Source Code

```
data COD50;
input type$  reactor$  N_level  days  inf_N  eff_N  flow  vol  inf_COD  eff_COD;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data set:refer to Table C.1 (23-Aug to 7-sep)
run;
proc mixed method=type3;
   title "Split-plot analysis of COD removed at 50 steady";
   class type days reactor N_level;
   model COD = type|days;
   random reactor(type) ;
   lsmeans type type*days;
   estimate "COD KM-WC  overall" type 1 -1 0;
   estimate "COD KM-WS overall" type 1 0 -1;
   estimate "COD WC-WS overall" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD removed at 50 steady/ The Mixed Procedure/Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	389.40	194.70	Var(Residual) + 3 Var(reactor(type)) + Q(type,type*days)	MS(reactor(type))	6	2.25	0.1862
days	2	7177.85	3588.92	Var(Residual) + Q(days,type*days)	MS(Residual)	12	93.99	<.0001
type*days	4	27.25	6.81	Var(Residual) + Q(type*days)	MS(Residual)	12	0.18	0.9452
reactor(type)	6	518.44	86.40	Var(Residual) + 3 Var(reactor(type))	MS(Residual)	12	2.26	0.1077
Residual	12	458.22	38.18	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD KM-WC overall	-0.1111	4.3820	6	-0.03	0.9806
COD KM-WS overall	8.0000	4.3820	6	1.83	0.1177
COD WC-WS overall	8.1111	4.3820	6	1.85	0.1136

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		186.89	3.0985	6	60.32	<.0001
type	WC		187.00	3.0985	6	60.35	<.0001
type	WS		178.89	3.0985	6	57.73	<.0001

G.11 COD removed at LR2 (steady)

Source Code

```
data COD120;
input type$  reactor$  N_level  days  inf_N  eff_N  flow  vol  inf_COD  eff_COD;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
*if DNR>0;
cards;
Data set:refer to Table C.1 (4-oct to 17-Oct)
run;
proc mixed method=type3;
   title "Split-plot analysis of COD removed 120 steady";
   class type days reactor N_level;
   model COD = type|days;
   random reactor(type) ;
   lsmeans type type*days;
   estimate "COD KM-WC  overall" type 1 -1 0;
   estimate "COD KM-WS overall" type 1 0 -1;
   estimate "COD WC-WS overall" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD removed 120 steady/The Mixed Procedure/Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	2367.18	1183.59	Var(Residual) + 3 Var(reactor(type)) + Q(type,type*days)	MS(reactor(type))	6	1.86	0.2355
days	2	6382.74	3191.37	Var(Residual) + Q(days,type*days)	MS(Residual)	12	21.84	0.0001
type*days	4	6325.70	1581.42	Var(Residual) + Q(type*days)	MS(Residual)	12	10.82	0.0006
reactor(type)	6	3822.44	637.07	Var(Residual) + 3 Var(reactor(type))	MS(Residual)	12	4.36	0.0145
Residual	12	1753.55	146.12	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD KM-WC overall	-22.1111	11.8984	6	-1.86	0.1125
COD KM-WS overall	-16.3333	11.8984	6	-1.37	0.2189
COD WC-WS overall	5.7778	11.8984	6	0.49	0.6445

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		364.89	8.4134	6	43.37	<.0001
type	WC		387.00	8.4134	6	46.00	<.0001
type	WS		381.22	8.4134	6	45.31	<.0001

G.12 COD removed at LR3 (steady)

Source Code

```
data COD200;
input type$  reactor$  N_level  days  inf_N  eff_N  flow  vol  inf_COD  eff_COD;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data set:refer to Table C.1 (1-Nov to 15-nov)
run;
proc mixed method=type3;
   title "Split-plot analysis of COD removed at 200 steady";
   class type days reactor N_level;
   model COD = type|days;
   random reactor(type) ;
   lsmeans type type*days;
   estimate "COD KM-WC  overall" type 1 -1 0;
   estimate "COD KM-WS overall" type 1 0 -1;
   estimate "COD WC-WS overall" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD removed at 200 steady/Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	653.85	326.92	Var(Residual) + 3 Var(reactor(type)) + Q(type,type*days)	MS(reactor(type))	6	0.64	0.5615
days	2	9165.40	4582.70	Var(Residual) + Q(days,type*days)	MS(Residual)	12	16.07	0.0004
type*days	4	1058.81	264.70	Var(Residual) + Q(type*days)	MS(Residual)	12	0.93	0.4796
reactor(type)	6	3082.22	513.70	Var(Residual) + 3 Var(reactor(type))	MS(Residual)	12	1.80	0.1814
Residual	12	3421.11	285.09	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD KM-WC overall	-2.4444	10.6844	6	-0.23	0.8266
COD KM-WS overall	9.0000	10.6844	6	0.84	0.4319
COD WC-WS overall	11.4444	10.6844	6	1.07	0.3253

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		694.33	7.5550	6	91.90	<.0001
type	WC		696.78	7.5550	6	92.23	<.0001
type	WS		685.33	7.5550	6	90.71	<.0001

G.13 COD/NO_3-N ratio at 50 (overall)

Source Code

```
data COD_N50;
input type$  reactor$ days  inf_N  eff_N  inf_COD  eff_COD;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data set: refer to Table B.1 (Day 21 to 57) and corresponding COD in Table C.1
run;
proc mixed method=type3;
   title "Split-plot analysis of COD/N ratio at 50";
class type days reactor;
model COD_Nratio = type|days;
random reactor(type);
lsmeans type type*days/slice=days;
estimate "COD/N ratio KM-WC" type 1 -1 0;
estimate "COD/N ratio KM-WS" type 1 0 -1;
estimate "COD/N ratio WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD/N ratio at 50/ The Mixed Procedure/Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	14.18	7.09	Var(Residual) + 6 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	10.62	0.0107
days	5	17.43	3.48	Var(Residual) + Q(days,type*days)	MS(Residual)	30	7.95	<.0001
type*days	10	24.85	2.48	Var(Residual) + Q(type*days)	MS(Residual)	30	5.67	<.0001
reactor(type)	6	4.00	0.66	Var(Residual) + 6 Var(reactor(type))	MS(Residual)	30	1.52	0.2046
Residual	30	13.15	0.43	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD/N ratio KM-WC	1.0510	0.2724	6	3.86	0.0084
COD/N ratio KM-WS	1.1199	0.2724	6	4.11	0.0063
COD/N ratio WC-WS	0.06888	0.2724	6	0.25	0.8088

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		4.7056	0.1926	6	24.43	<.0001
type	WC		3.6545	0.1926	6	18.97	<.0001
type	WS		3.5856	0.1926	6	18.61	<.0001

G.14 COD/NO_3-N ratio at 120 (overall)

Source Code

```
data COD_N;
input type$  reactor$  N_level  days  inf_N  eff_N  flow  vol  inf_COD  eff_COD;
DNR = inf_N*flow*60*24/vol-eff_N*flow*60*24/vol;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data Set: refer to Table B.1 (Day 63 to 93)and corresponding COD in Table C.1
run;
proc mixed method=type3;
   title "Split-plot analysis of COD/N ratio at 120";
class type days reactor;
model COD_Nratio = type|days;
random reactor(type);
lsmeans type type*days/slice=days;
estimate "COD/N ratio KM-WC" type 1 -1 0;
estimate "COD/N ratio KM-WS" type 1 0 -1;
estimate "COD/N ratio WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD/N ratio at 120

The Mixed Procedure

Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	0.103954	0.05198	Var(Residual) + 5 Var(reactor(type+ Q(type,type*days)	MS(reactor(type))	6	12.66	0.0070
days	4	0.486016	0.12150	Var(Residual) + Q(days,type*days)	MS(Residual)	24	9.60	<.0001
type*days	8	0.096993	0.01212	Var(Residual) + Q(type*days)	MS(Residual)	24	0.96	0.4906
reactor(type)	6	0.024642	0.00411	Var(Residual) + 5Var(reactor(type)	MS(Residual)	24	0.32	0.9176
Residual	24	0.303877	0.01266	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD/N ratio KM-WC	0.06569	0.02340	6	2.81	0.0309
COD/N ratio KM-WS	0.1175	0.02340	6	5.02	0.0024
COD/N ratio WC-WS	0.05177	0.02340	6	2.21	0.0689

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		3.3569	0.01655	6	202.87	<.0001
type	WC		3.2913	0.01655	6	198.90	<.0001
type	WS		3.2395	0.01655	6	195.77	<.0001

G.15 COD/NO_3-N ratio at 200 (overall)

Source Code

```
data COD_N200;
input type$  reactor$  days  inf_N  eff_N  inf_COD  eff_COD;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data set: refer to Table B.1 (Day 97 to 128)and corresponding COD in Table C.1
run;
proc mixed method=type3;
   title "Split-plot analysis of COD/N ratio at 200";
class type days reactor;
model COD_Nratio = type|days;
random reactor(type);
lsmeans type type*days/slice=days;
estimate "COD/N ratio KM-WC" type 1 -1 0;
estimate "COD/N ratio KM-WS" type 1 0 -1;
estimate "COD/N ratio WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD/N ratio at 200/ The Mixed Procedure/Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	0.039813	0.01991	Var(Residual) + 5 Var(reactor(type)) + Q(type,type*days)	MS(reactor(type))	6	8.28	0.0188
days	4	1.375675	0.34392	Var(Residual) + Q(days,type*days)	MS(Residual)	24	79.91	<.0001
type*days	8	0.023266	0.00291	Var(Residual) + Q(type*days)	MS(Residual)	24	0.68	0.7079
reactor(type)	6	0.014420	0.00240	Var(Residual) + 5 Var(reactor(type))	MS(Residual)	24	0.56	0.7587
Residual	24	0.103286	0.00430	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD/N ratio KM-WC	0.01500	0.01790	6	0.84	0.4342
COD/N ratio KM-WS	0.06925	0.01790	6	3.87	0.0083
COD/N ratio WC-WS	0.05425	0.01790	6	3.03	0.0231

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		3.5256	0.01266	6	278.53	<.0001
type	WC		3.5106	0.01266	6	277.34	<.0001
type	WS		3.4564	0.01266	6	273.06	<.0001

G.16 COD/NO_3-N ratio at 50 (steady)

Source Code

```
data COD_N50;
input type$  reactor$ days  inf_N  eff_N inf_COD  eff_COD;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data set: refer to Table B.1 (Day 37 to 57)and corresponding COD in Table C.1
run;
proc mixed method=type3;
   title "Split-plot analysis of COD/N ratio at 50 steady";
class type days reactor;
model COD_Nratio = type|days;
random reactor(type);
lsmeans type type*days/slice=days;
estimate "COD/N ratio KM-WC" type 1 -1 0;
estimate "COD/N ratio KM-WS" type 1 0 -1;
estimate "COD/N ratio WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD/N ratio/ The Mixed Procedure/ Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	1.114921	0.55746	Var(Residual) + 3 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	35.92	0.0005
days	2	2.715759	1.35788	Var(Residual) + Q(days,type*days)	MS(Residual)	12	169.2	<.0001
type*days	4	0.207172	0.05179	Var(Residual) + Q(type*days)	MS(Residual)	12	6.45	0.0052
reactor(type	6	0.093115	0.01552	Var(Residual) + 3 Var(reactor(type))	MS(Residual)	12	1.93	0.1559
Residual	12	0.096313	0.00803	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD/N ratio KM-WC	0.3127	0.05873	6	5.33	0.0018
COD/N ratio KM-WS	0.4917	0.05873	6	8.37	0.0002
COD/N ratio WC-WS	0.1790	0.05873	6	3.05	0.0226

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		3.9489	0.04153	6	95.10	<.0001
type	WC		3.6361	0.04153	6	87.56	<.0001
type	WS		3.4571	0.04153	6	83.25	<.0001

G.17 COD/NO_3-N ratio at 120 (steady)

Source Code

```
data COD_N120;
input type$  reactor$ days  inf_N  eff_N inf_COD  eff_COD;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data set: refer to Table B.1 (Day 77 to 93)and corresponding COD in Table C.1
run;
proc mixed method=type3;
   title "Split-plot analysis of COD/N ratio at 120 steady";
class type days reactor;
model COD_Nratio = type|days;
random reactor(type);
lsmeans type type*days/slice=days;
estimate "COD/N ratio KM-WC" type 1 -1 0;
estimate "COD/N ratio KM-WS" type 1 0 -1;
estimate "COD/N ratio WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD/N ratio at 120 steady
The Mixed Procedure
Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	0.112823	0.05641	Var(Residual) + 3 Var(reactor(type))+ Q(type,type*days)	MS(reactor(type))	6	2.93	0.1298
days	2	0.375938	0.18797	Var(Residual) + Q(days,type*days)	MS(Residual)	12	26.60	<.0001
type*days	4	0.074294	0.01857	Var(Residual) + Q(type*days)	MS(Residual)	12	2.63	0.0872
reactor(type	6	0.115704	0.01928	Var(Residual) + 3 Var(reactor(type))	MS(Residual)	12	2.73	0.0656
Residual	12	0.084803	0.00707	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD/N ratio KM-WC	0.07739	0.06546	6	1.18	0.2819
COD/N ratio KM-WS	0.1583	0.06546	6	2.42	0.0520
COD/N ratio WC-WS	0.08094	0.06546	6	1.24	0.2625

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		3.4143	0.04629	6	73.76	<.0001
type	WC		3.3369	0.04629	6	72.09	<.0001
type	WS		3.2559	0.04629	6	70.34	<.0001

G.17 COD/NO_3-N ratio at 200 (steady)

Source Code

```
data COD_N200;
input type$  reactor$ days  inf_N  eff_N inf_COD  eff_COD;
N_remov = inf_N-eff_N;
COD = inf_COD-eff_COD;
COD_Nratio = COD/N_remov;
cards;
Data Set: refer to Table B.1 (Day 112 to Day 128 except Day 121)and corresponding COD in Table C.1
run;
proc mixed method=type3;
   title "Split-plot analysis of COD/N ratio at 200";
class type days reactor;
model COD_Nratio = type|days;
random reactor(type);
lsmeans type type*days/slice=days;
estimate "COD/N ratio KM-WC" type 1 -1 0;
estimate "COD/N ratio KM-WS" type 1 0 -1;
estimate "COD/N ratio WC-WS" type 0 1 -1;
run;
```

Partial Output

Split-plot analysis of COD/N ratio at 200 steady

The Mixed Procedure

Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
type	2	0.048521	0.024260	Var(Residual) + 3 Var(reactor(type)) + Q(type,type*days)	MS(reactor(type))	6	5.89	0.0384
days	2	0.357457	0.178728	Var(Residual) + Q(days,type*days)	MS(Residual)	12	27.96	<.0001
type*days	4	0.009984	0.002496	Var(Residual) + Q(type*days)	MS(Residual)	12	0.39	0.8116
reactor(type)	6	0.024701	0.004117	Var(Residual) + 3 Var(reactor(type))	MS(Residual)	12	0.64	0.6945
Residual	12	0.076714	0.006393	Var(Residual)				

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
COD/N ratio KM-WC	0.03650	0.03025	6	1.21	0.2729
COD/N ratio KM-WS	0.1024	0.03025	6	3.39	0.0147
COD/N ratio WC-WS	0.06593	0.03025	6	2.18	0.0721

Least Squares Means

Effect	type	days	Estimate	Standard Error	DF	t Value	Pr > |t|
type	KM		3.5081	0.02139	6	164.02	<.0001
type	WC		3.4716	0.02139	6	162.32	<.0001
type	WS		3.4056	0.02139	6	159.24	<.0001