USDA SBIR FUNDED DENITRIFICATION RESEARCH AND TECHNOLOGY USING BIODEGRADABLE BIOPOLYMER (PHA's)

Paul G. Hightower
Research Associate

James M. Ebeling, Ph.D.
Research Engineer

Douglas Drennan
Managing Member

Aquaculture Systems Technologies, LLC
108 Industrial Ave.
New Orleans, LA 70121

Introduction

Overview

- Fundamentals of Denitrification
    - Stoichiometry & Kinetics
- Phase I USDA SBIR Grant

(Small Business Innovative Research)

    - polyhydroxyalkanoates (PHAs)
    - lab-scale PolyGeyser® denitrification unit
- Phase II of the SBIR
    - critical kinetic constants

Introduction

Increased Density + Higher Nitrates

- Nitrate Toxicity (Marine Systems)
- Nitrate – Environmental Impact

Change thinking from conversion of nitrogen to removal of nitrogen!

Denitrification

(Bacteria widespread in the environment)

- Facultative aerobic bacteria
  - Shift to NO_3^- or NO_2^- when O_2 limited
- Chemotrophic
  - Heterotrophic – organic electron donor
  - Autotrophic – H_2 or reduced sulfur

Denitrification

Nitrate (NO_3^-) $\Rightarrow$ Nitrite (NO_2^-) $\Rightarrow$
Nitric Oxide (NO) $\Rightarrow$ Nitrous Oxide (N_2) $\Rightarrow$
Nitrogen Gas (N_2)

$$NO_3 + \frac{5}{6}CH_3OH \rightarrow \frac{1}{2}N_2 + \frac{5}{6}CO_2 + \frac{2}{3}H_2O + OH^-$$

Denitrification

Optimal Conditions

- Low redox potential
  - ORP > -200 mV incomplete denitrification NO_2^-, NO_2 and N_2O
  - ORP < 400 mV production of hydrogen sulfide (H_2S)
- Low Oxygen Levels (anoxic)
  - High DO $\Rightarrow$ accumulation of intermediates, NO_2^-, NO_2 and N_2O
- pH: 7 to 8
- Alkalinity 3.57 g $CaCO_3$/ g NO_3—N consumed

USDA Phase I SBIR

Passive Self-Regulating Denitrification Technology for Aquaculture

Phase I Technical Objectives

- Characterize the volumetric denitrification capacity of floating PHA pellets in AST's PolyGeyser® configuration
- Determine the effect of hydraulic loading rates on denitrification performance
- Conduct basic reactor modeling to project PolyGeyser® denitrification performances for a variety of applications

PolyGeyser® Bead Filter

Anoxic Reactor

NO_3-N → VSS

Denitrification

$NO_3^- \Rightarrow N_2$

carbon from PHA's

→ Low BOD

High TAN

Low NO_3-N

Polyhydroxyalkanotes (PHAs)

Family of bioplastic polymers, produced from sugar fermentation

- Low Maintenance
- Cost effective
- Carbon source
- Substrate for Bacteria

Denitrification – PHA Stoichiometry

$$NO_3^- + 0.39\ C_4H_6O_2 \longrightarrow 0.088\ C_5H_7O_2N + 0.456\ N_2$$

$$+ HCO_3^- + 0.121\ CO_2 + 0.363\ H_2O$$

Polyhydroxyalkanotes (PHAs)

- excessive biofloc formation at high Nitrate-nitrogen loading & high BOD

Small-Scale Experimental PolyGeyser®

Water Quality Sampling

Parameter	Method / Range
DO / Temperature	YSI Model 58 Dissolved Oxygen Meter
Salinity / Conductivity	YSI Model 33 S-C-T Meter
Nitrogen – Ammonia	Hach Method 8038 Nessler Method 0 – 2.50 mg/L NH_3-N
Nitrogen –Nitrite	Hach Method 8507 Diazotization Method 0 – 0.300 mg/L NO_2^- -N
Nitrogen -Nitrate	Hach Method 8039 Cadmium Reduction Method 0.0 – 10.0 mg/L NO_3^- - N
Alkalinity	Standard Methods 2320B as $CaCO_3$

Mass Balance Analysis – Overall System

$$VNR = \frac{dC_F}{dt} = \frac{V_{Sump}}{V_{media}} \frac{dC_{Sump}}{dt} * \frac{1g}{10^3\,mg}$$

C_{Sump} = concentration of nitrate-nitrogen in the reservoir [mg/L]

V_{Sump} = volume of the sump [L]
C_F = concentration of nitrate-nitrogen in the biofilter [mg/L]
V_{media} = volume of the biofilters [L]
t = time [day]

Mass Balance Analysis – Across BioReactor

$$VNR = \frac{dC_F}{dt} = \frac{(C_i - C_e)}{V_{media}/Q} * 1440\frac{\min}{day} * \frac{1L}{10^3\ ml}$$

dC_F = change in nitrate-nitrogen across biofilter [mg/L]

C_i = nitrate-nitrogen concentration in influent to biofilter [mg/L]

C_e = nitrate-nitrogen concentration in effluent from biofilter [mg/L]

V_{media} = volume of media in the biofilter [L]

Q = flow rate through biofilter [Lpm]

t = time [day]

Monod Format – Satuation Equation

$$VNR = VNR_{max}\left[\frac{C_{NO_3-N}}{C_{NO_3-N} + K_{1/2}}\right]$$

VNR = reaction rate [kg/m^3 day]

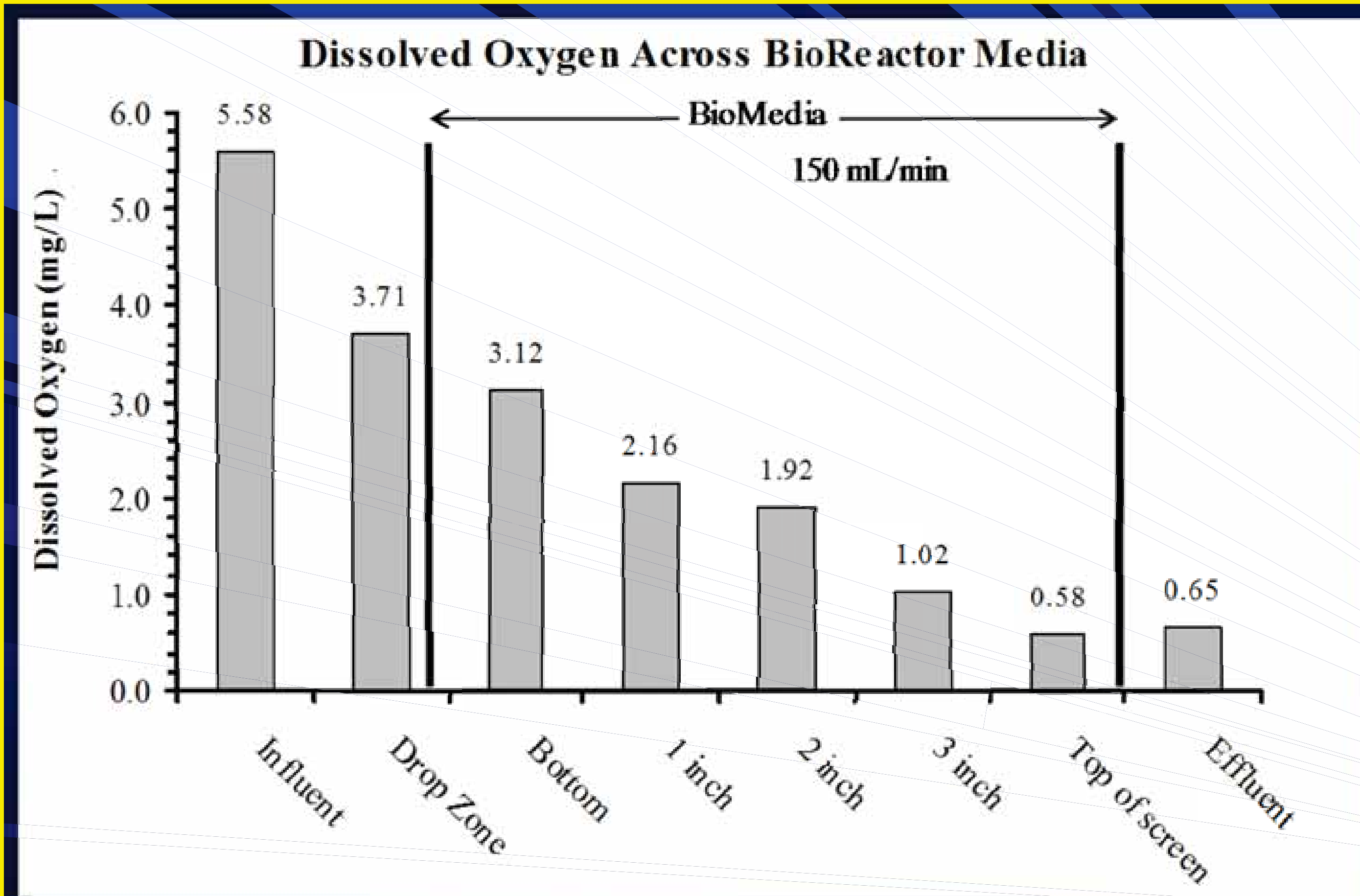
VNR_{max} = maximum reaction rate [kg/m^3 day]

$K_{1/2}$ = half-saturation coefficient for Nitrate [mg NO_3-N /L]

C_{NO3-N} = nitrate-nitrogen [mg NO_3-N]

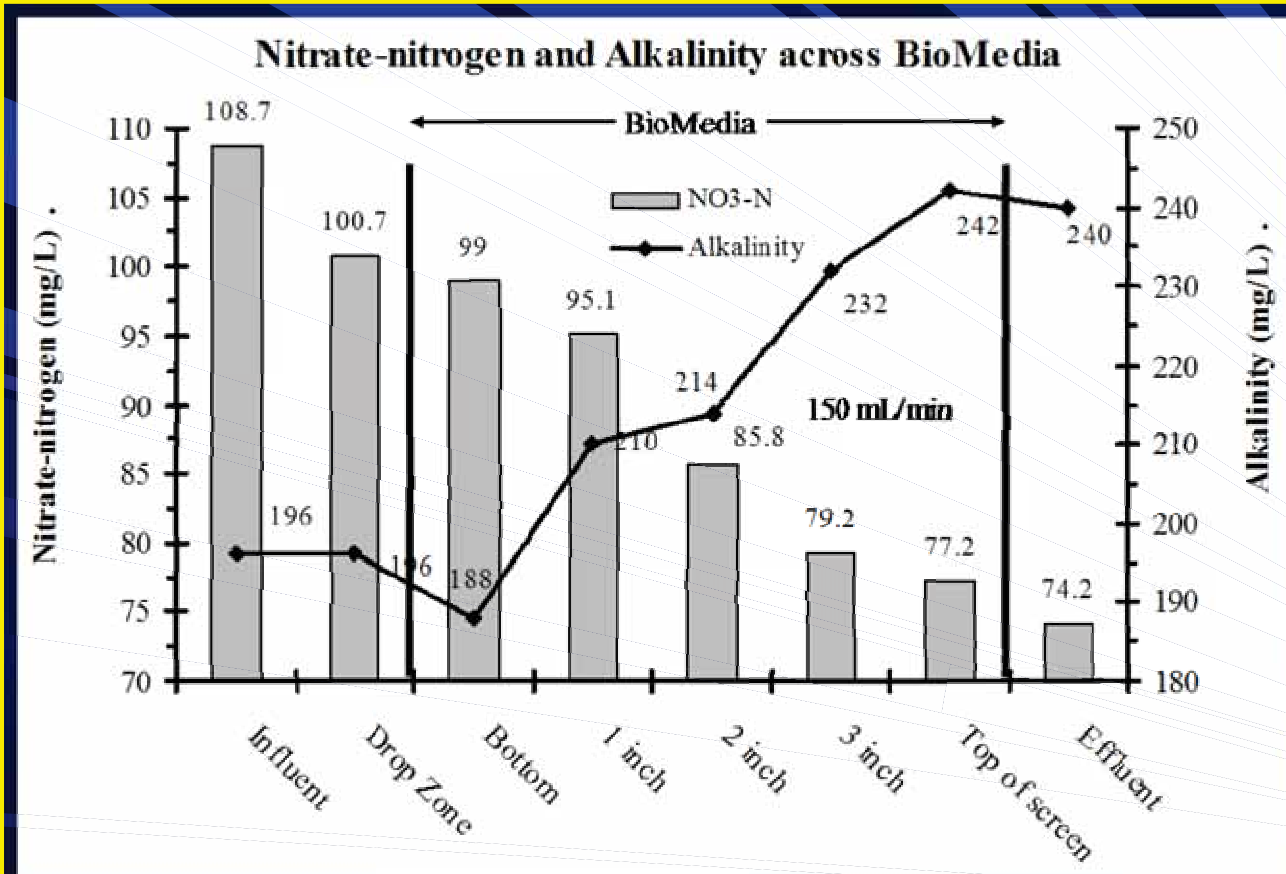
Lab-Scale Experimental PolyGeyser®

Water Quality across Bed



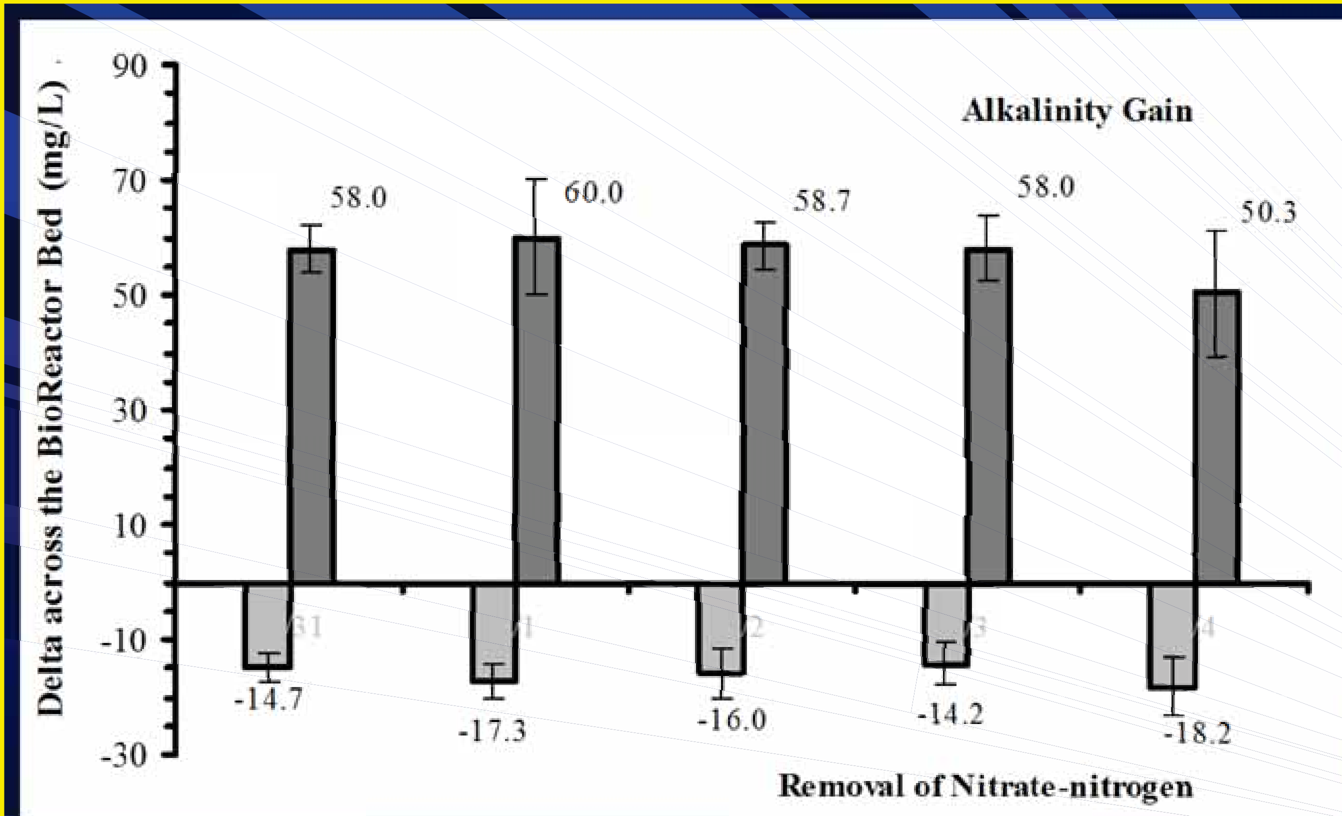
Change in dissolved oxygen across the PolyGeyser® denitrification bioreactor with influent flow rate of 150 mL/min.

Water Quality across Bed



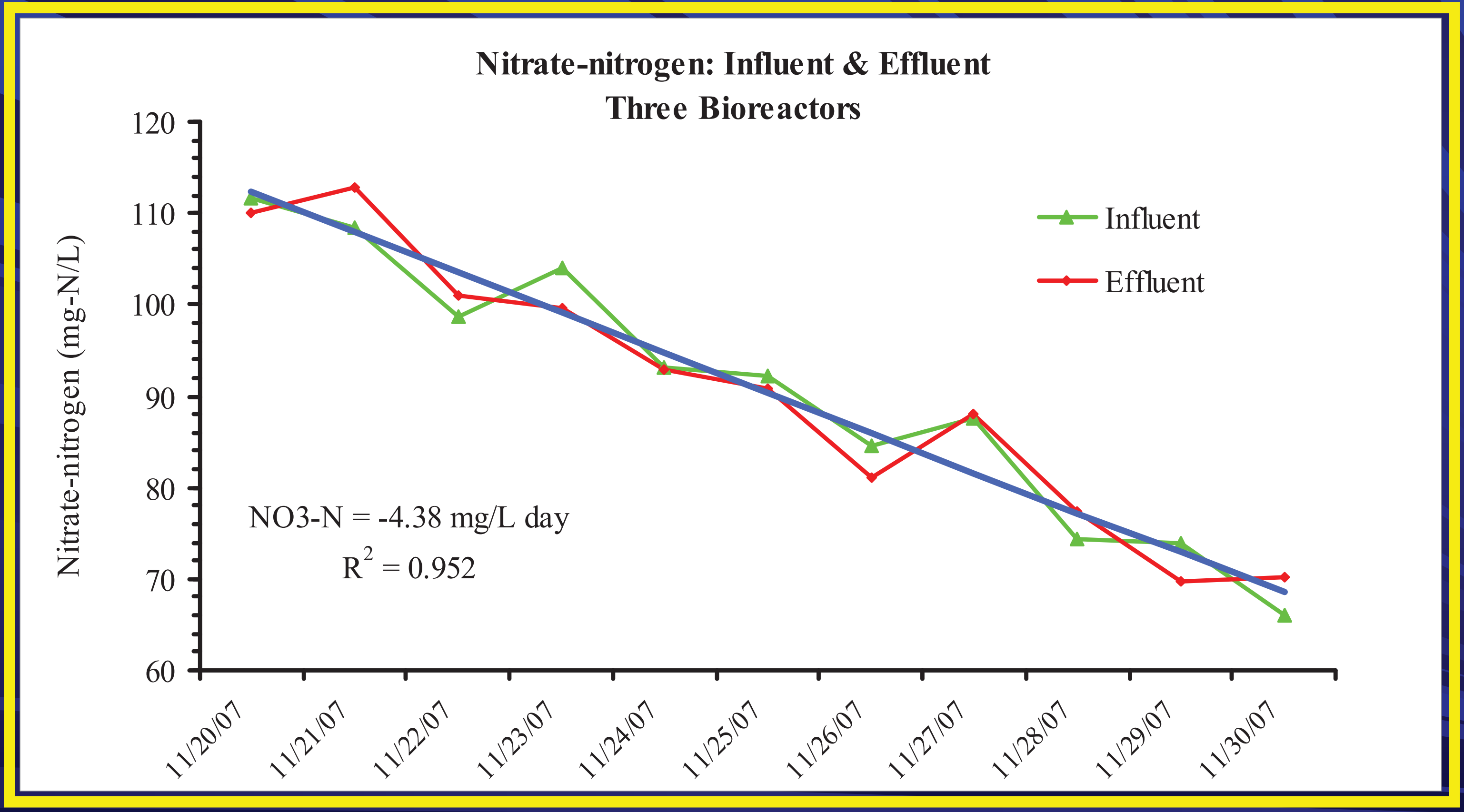
Change in nitrate-nitrogen and alkalinity across the PolyGeyser® denitrification bioreactor with influent flow rate of 150 mL/min.

Water Quality across Bed



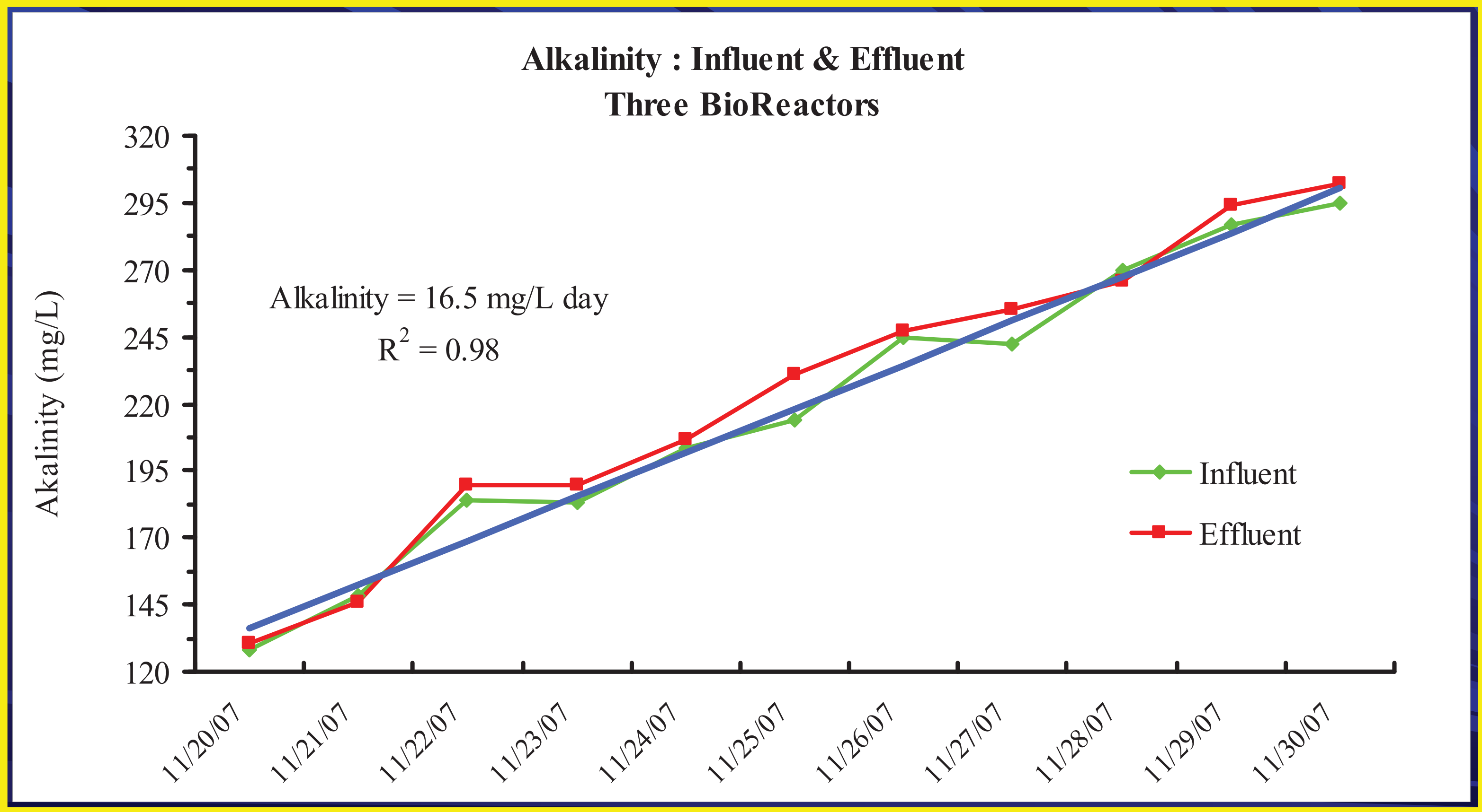
Change in nitrate-nitrogen and alkalinity across the PolyGeyser® denitrification bioreactor with influent nitrate-nitrogen concentration of 80 to 85 mg/l-N and flow rate of 100 mL/min.

Acclimation of Experimental Denitrification Units at High Nitrate-nitrogen



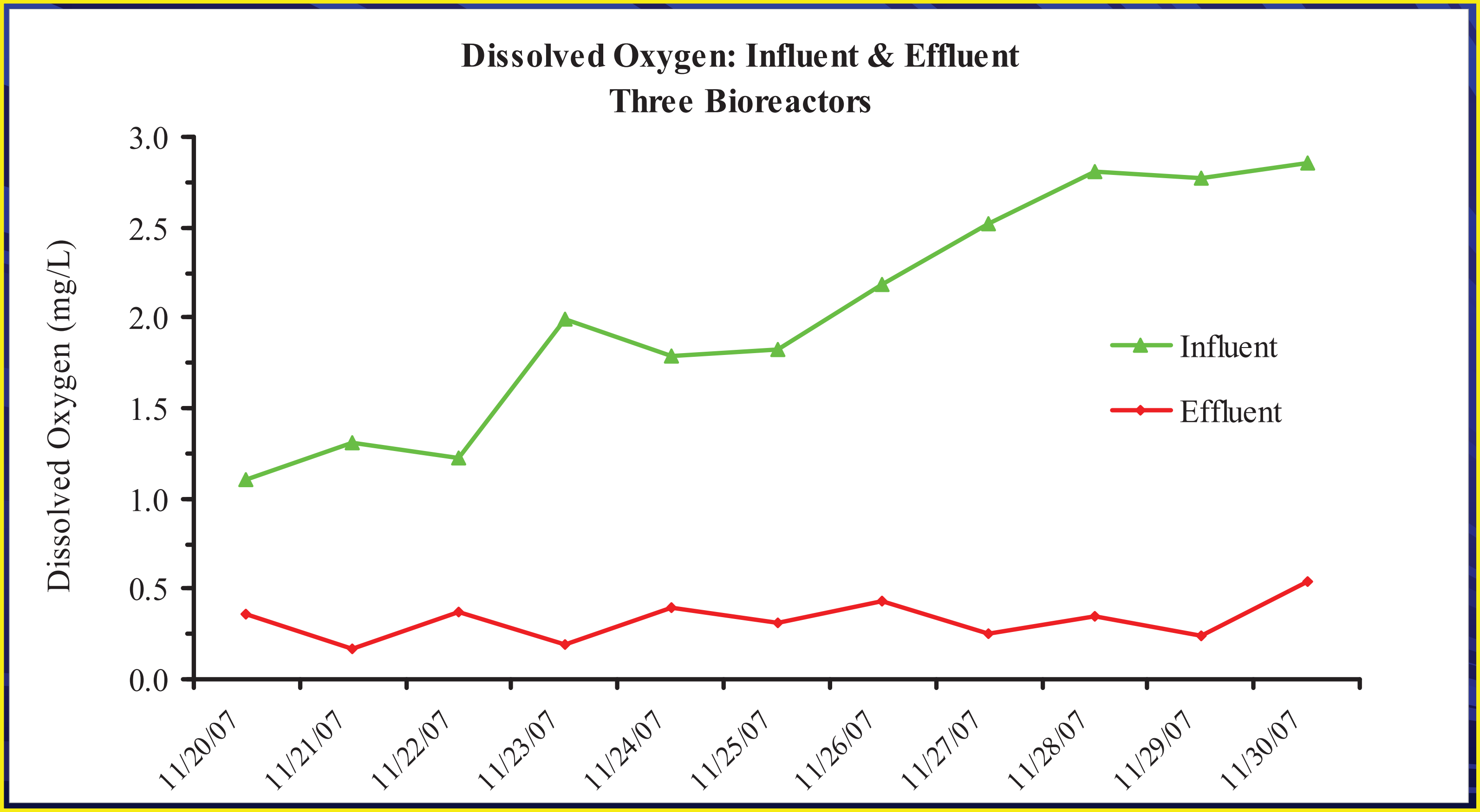
150 mL/min Flow Rate

Acclimation of Experimental Denitrification Units at High Nitrate-nitrogen



Alkalinity / NO_3-N = 3.77

Acclimation of Experimental Denitrification Units at High Nitrate-nitrogen



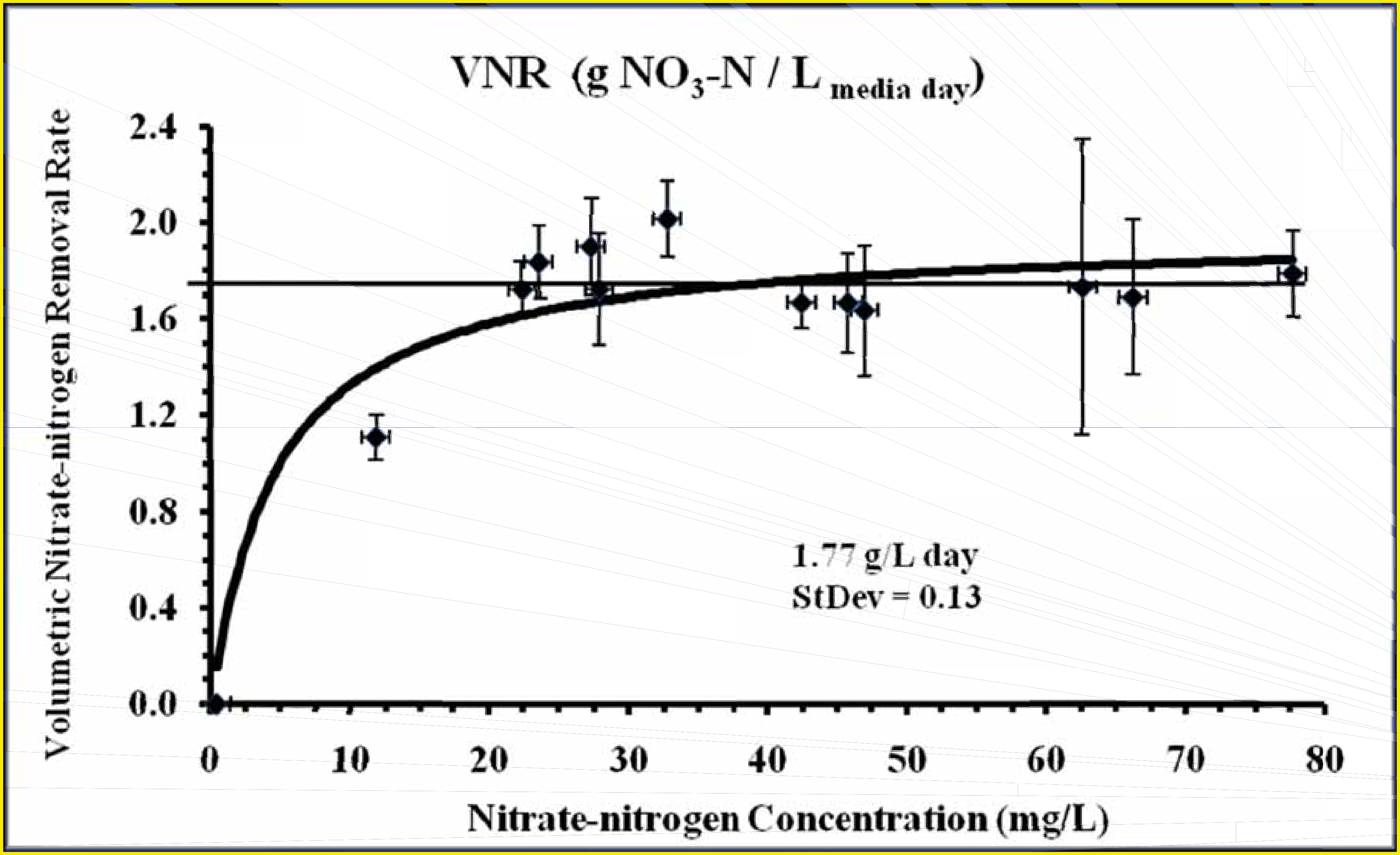
Denitrification - PHAs

Flow Rate (# of samples)	ΔNO_3-N (mg/L-N)	NO_3-N (kg/m^3 day)	ΔAlk (mg/L)	Alkalinity (kg/m^3 day)	ΔNO_3-N/ ΔAlk
100 mL/min (15)	-16.0 ± 3.6	1.15 ± 0.26	56.8 ± 7.0	4.09 ± 0.6	3.69 ± 0.8
150 mL/min (15)	-19.1 ± 4.9	2.06 ± 0.53	66.8 ± 15.9	7.2 ± 1.7	3.28 ± 1.09
220 mL/min (16)	-10.9 ± 2.4	1.73 ± 0.38	36.4 ± 8.5	5.77 ± 1.35	3.52 ± 1.22

Marine Lab-Scale Packed-Bed BioReactors

- Salinity – 10 to 32 ppt
- Low BOD
- Low TSS
- Nitrate – 50 to 250 mg/L-N

VNR – Monod Relationship



USDA Phase II SBIR

Passive Self-Regulating Denitrification Technology for Aquaculture

Phase II Primary Technical Objectives

- Characterize the PHAs mass consumption rate of dissolved oxygen in AST's PolyGeyser® configuration.
- Characterize the PHAs mass consumption rate for nitrate-nitrogen in AST's PolyGeyser® configuration.
- Characterize the PHAs nitrate-nitrogen conversion rate in AST's PolyGeyser® configuration at several trophic levels.

Commercial "*virgin*" Mirel™ PHA Media

Three formulations of PHA media obtained from Mirel ™ Bioplastics.

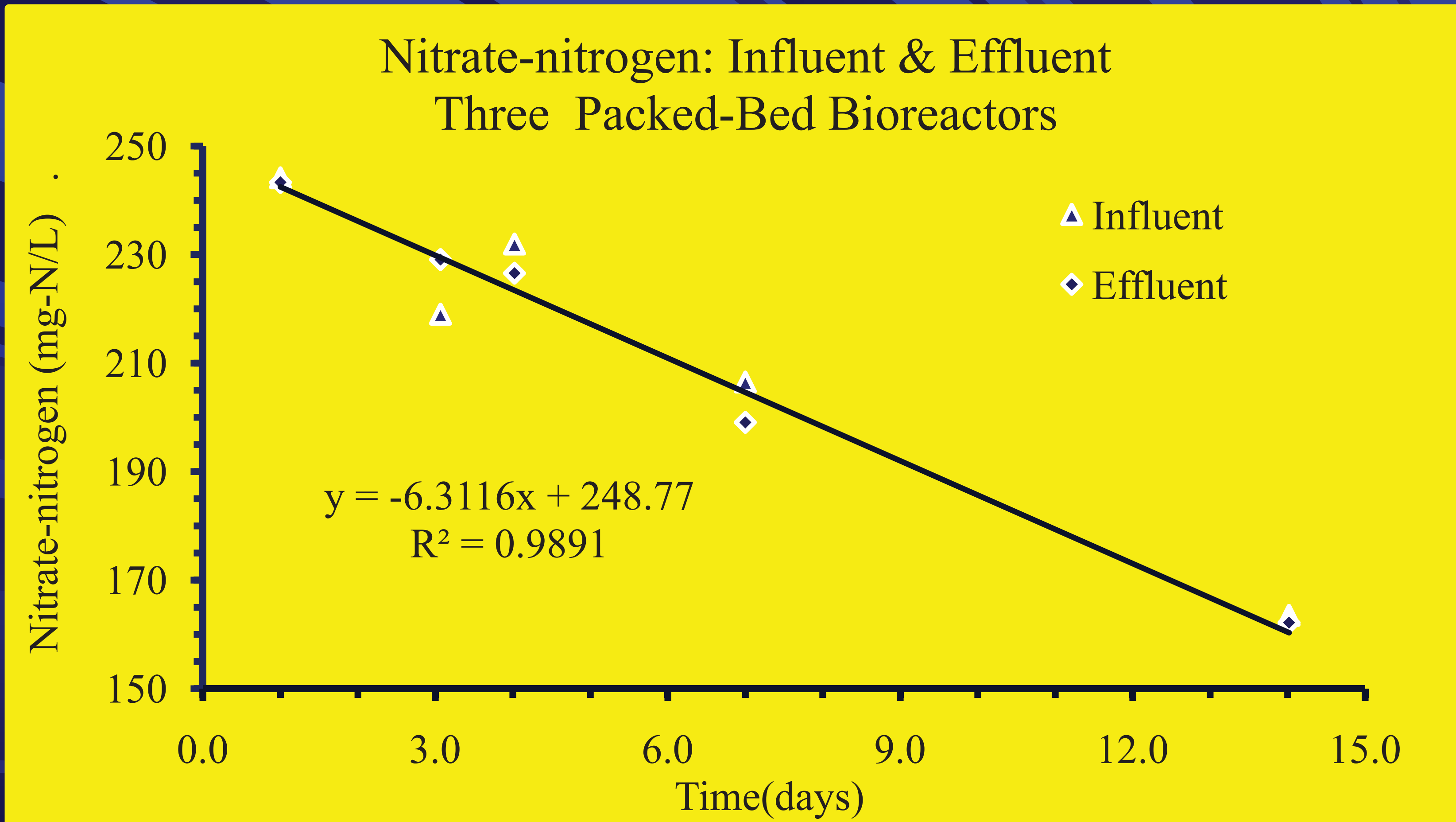
The PHA base polymer in Mirel ™ is made through a patented process for microbial fermentation of plant-derived sugar. c

Marine Lab-Scale Packed-Bed Bioreactors

Representative of an Aquarium/Zoo or
Marine Recirculating Aquaculture System

- 4” Dia clear PVC Pipe
- 18” Tall
- Sampling Ports @ 4” Intervals
- MasterFlex Pump
- 366 gallon sump
- 50 to 250 mg/L NO_3-N

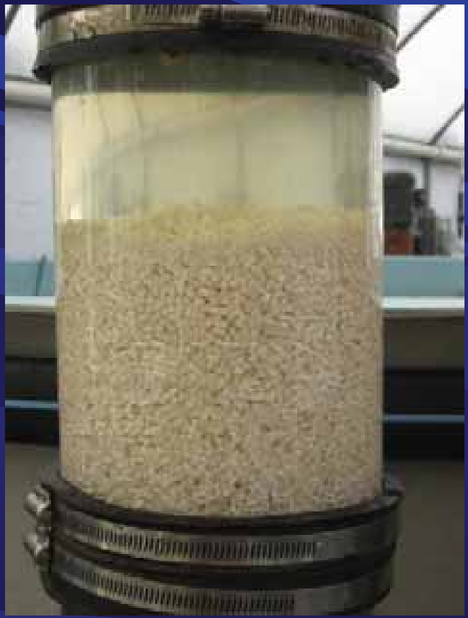
Research Data Packed-Bed BioReactors



0.81 g NO_3-N/ L_{media} day

Research PolyGeyser™ BioReactors

Representative of a Freshwater Intensive
Recirculating Aquaculture System



PHA media obtained
from Mirel ™ Bioplastics

Three replicated BioReactors

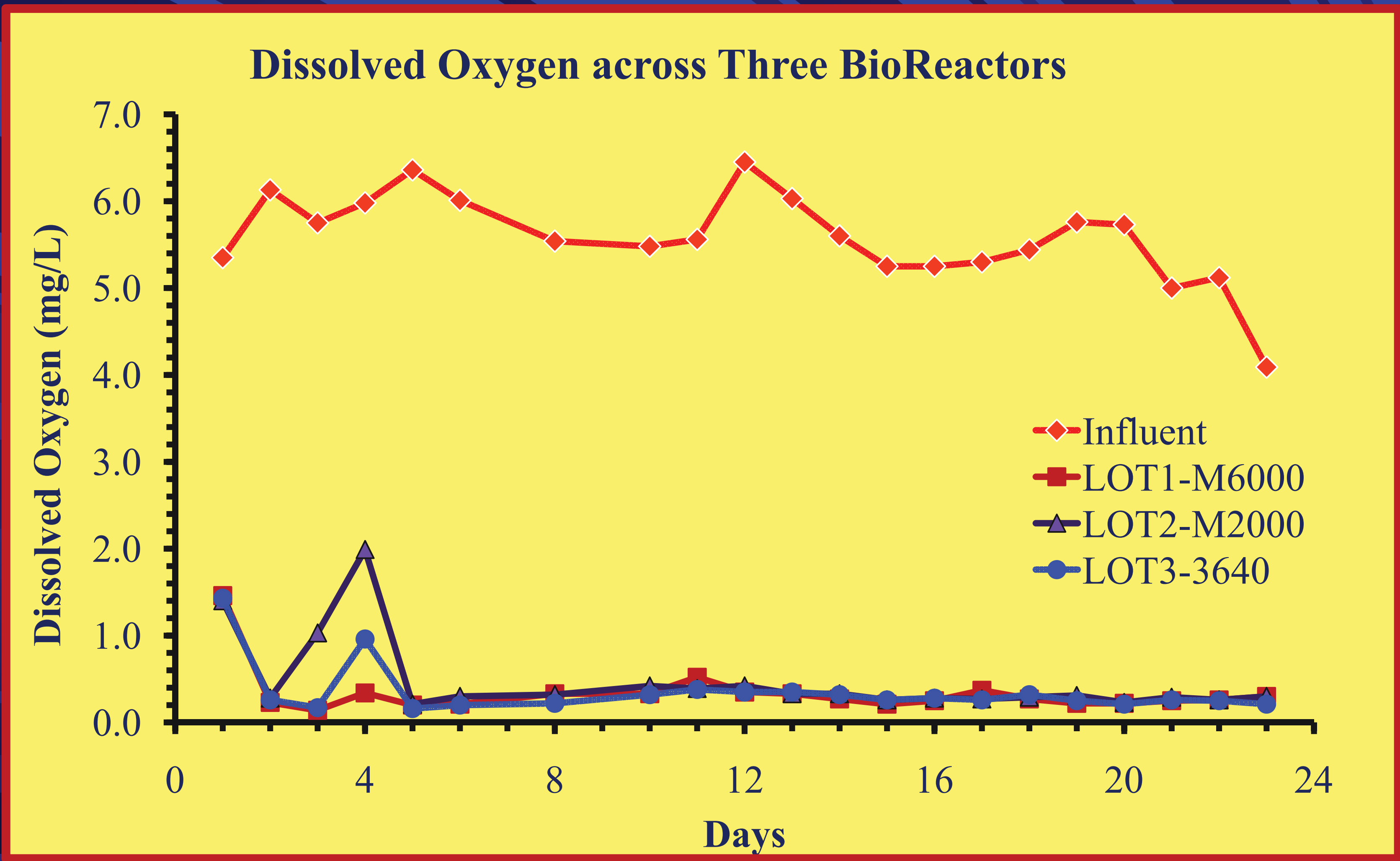
Metered Backwash Air Supply

Research Data BioReactors

Denitrification BioReactors Performance Analysis **150 mL/min** 7/12/2009

Date:		DO (mg/L)	Temp (Deg C)	pH	ORP (mV)	TAN (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	Alkalinity (mg/L)
07/16/09									
Influent	Column #1	5.55	29.1	7.71		1.54	0.232	52.5	191
	Column #2								
Time:	Column #3								
10:19 AM									
Effluent	Column #1	0.47		7.21		2.26	0.073	3.5	442
	Column #2	0.44		7.22		2.00	0.120	4.0	470
	Column #3	0.43		7.30		2.23	0.119	7.0	446

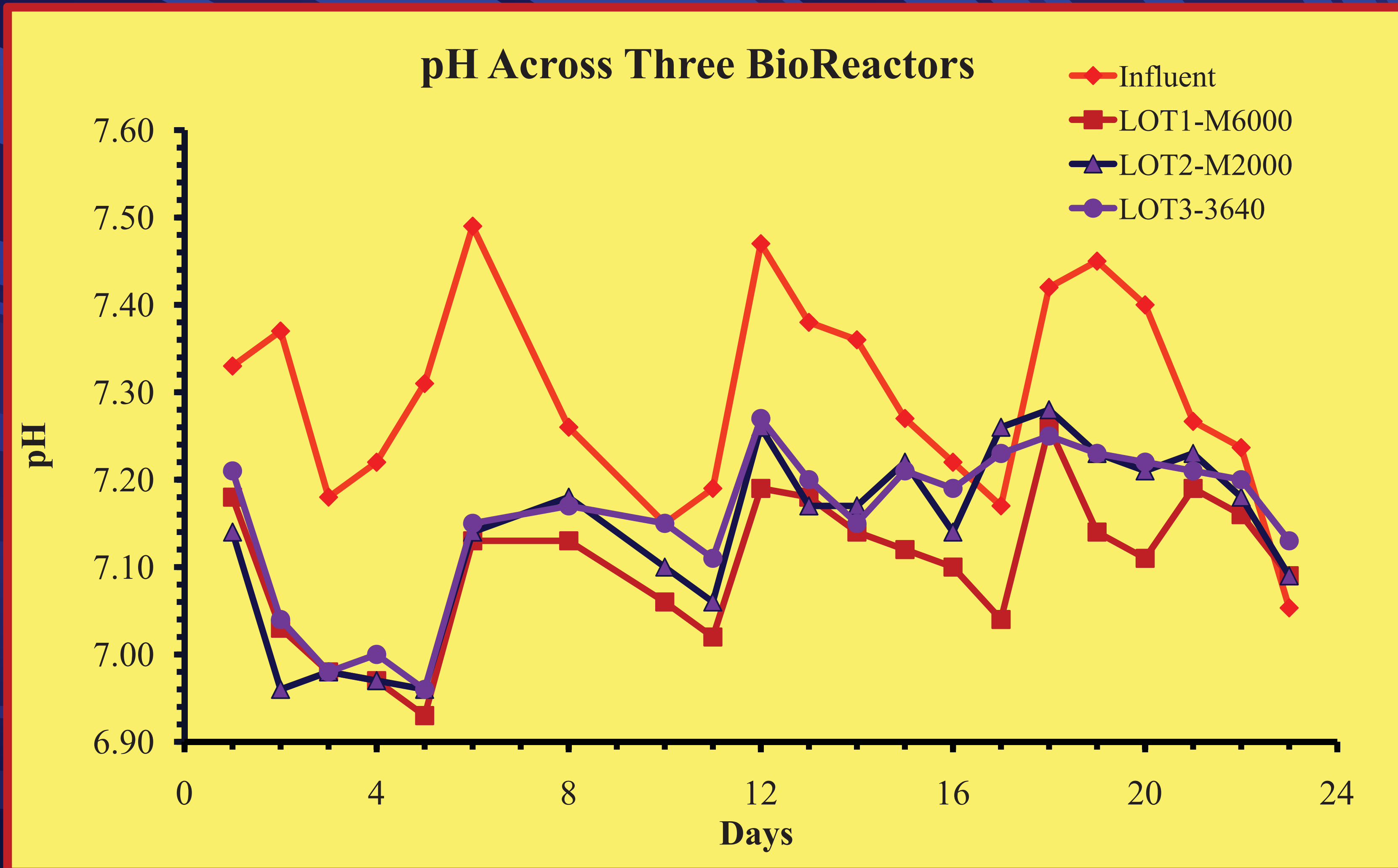
Research Results – Three Lots



Mirel ™ Bioplastics PHA media

Aquaculture Systems Technologies, LLC

Research Results – Three Lots



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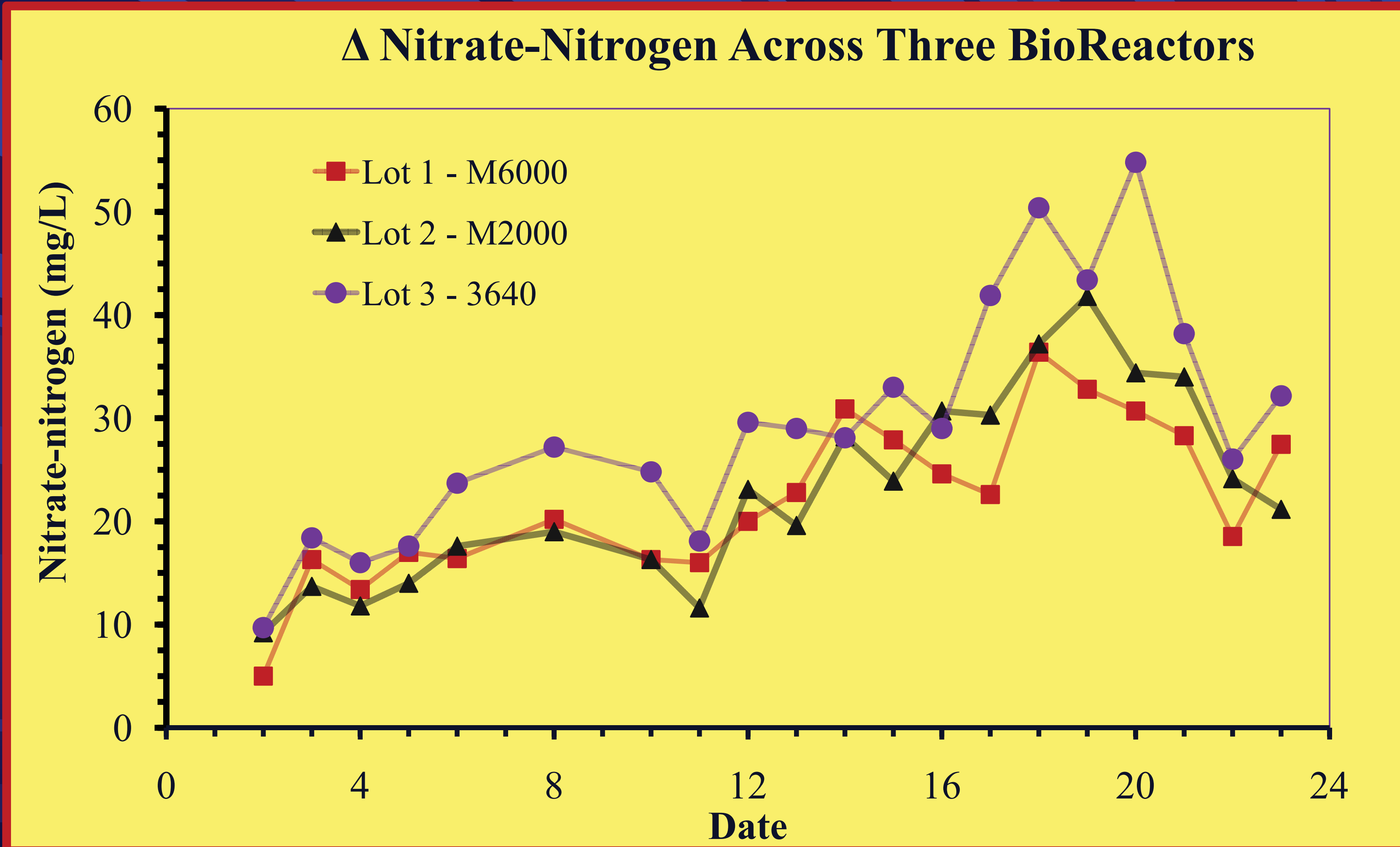

Mirel ™ Bioplastics PHA media

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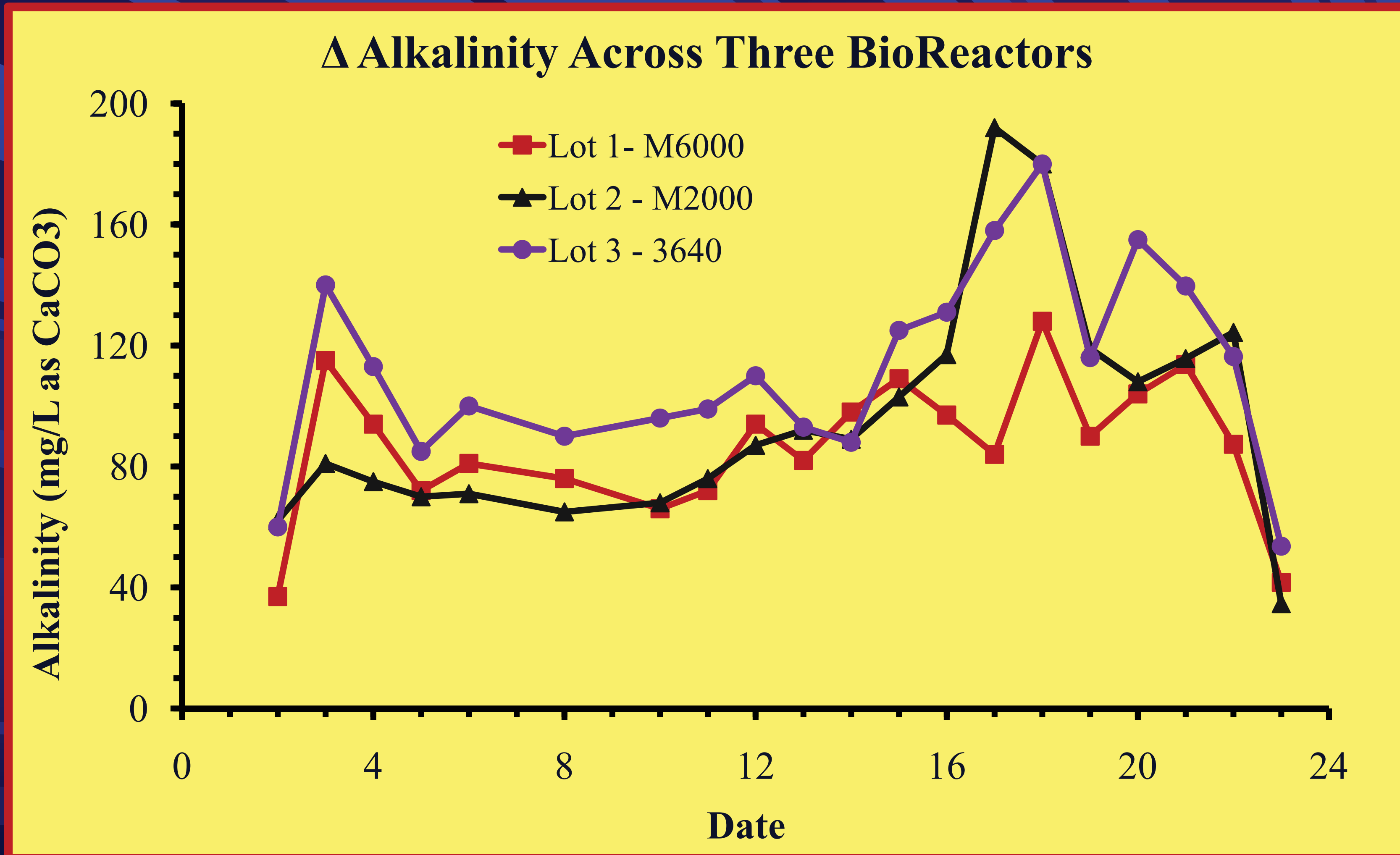
Research Results – Three Lots



Mirel ™ Bioplastics PHA media

Aquaculture Systems Technologies, LLC

Research Results – Three Lots

Nitrate Consumption		
Sample	(g/L media day)	
LOT1-M6000	1.66	0.49
LOT2-M2000	1.71	0.65
LOT3-3640	2.20	0.79

Stoichiometry		
Sample	Alk/Nitrate	
LOT1-M6000	4.13	1.3
LOT2-M2000	4.34	1.3
LOT3-3640	4.07	1.4

Stoichiometry: 3.57 g Alk/ g Nitrate

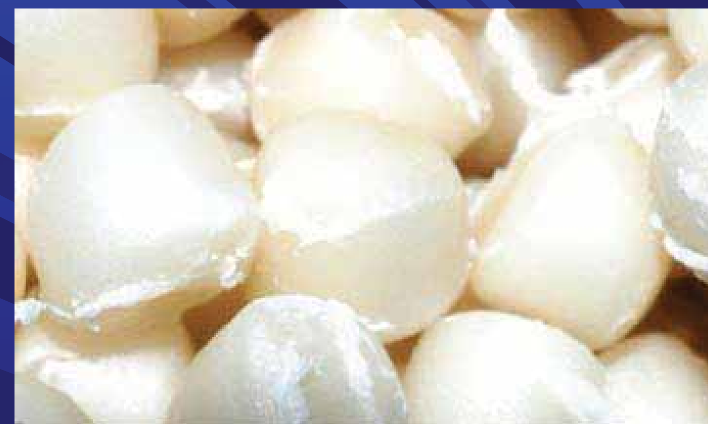
Commercial Floating PHA Media Mirel

Mirel ™ Bioplastics PHA media

Commercial PHA Media - Aquariums

NpX-Bio Beads

The finest in Nitrate and Phosphate control



NP Bio Pellets are a solid organic carbon polymer that provides a substrate and food for bacteria.

Prices for the NPX-Bio Beads are $110 for 1100m

Current Research PolyGeyser™ BioReactors

Prefilter for DO
Removal - EN Meida

Three replicated BioReactors

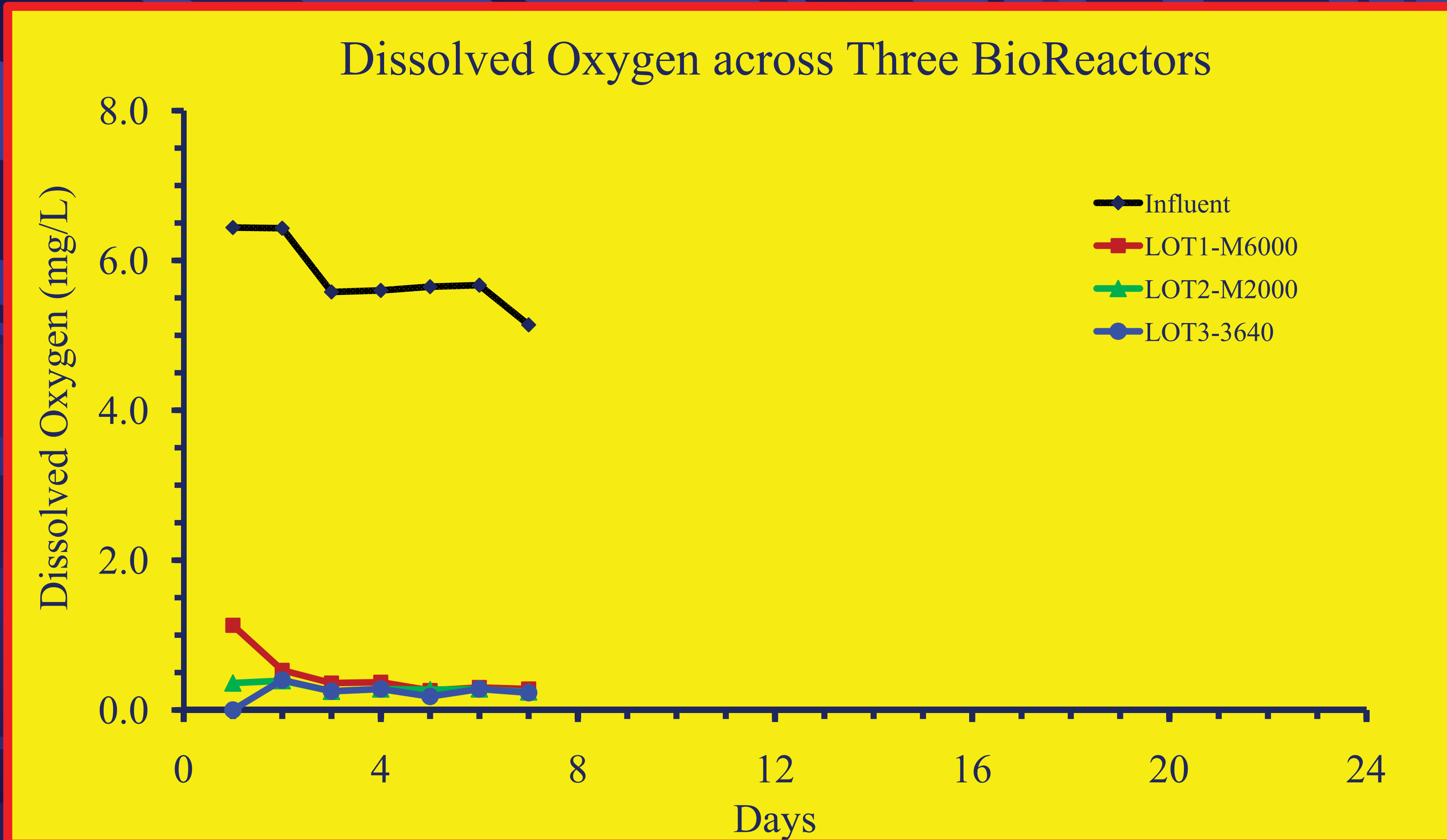
Metered Backwash Air Supply

Research Data BioReactors

Denitrification BioReactors Performance Analysis **200 mL/min**

		DO (mg/L)	Temp (Deg C)	pH	ORP (mV)	TAN (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	Alkalinity (mg/L)
Date: **05/01/10**									
Influent	Column #1	5.14	26.7	7.45		0.53	0.221	50.7	188
	Column #2			7.46		0.57	0.226	53.6	188
Time:	Column #3			7.46		0.56	0.285	80.9	187
9:19 AM									
Effluent	Column #1	0.28	26.8	7.11		1.17	0.144	29.9	297
	Column #2	0.24	26.7	7.13		0.61	0.053	36.4	264
	Column #3	0.23	26.8	7.12		0.95	0.055	30.0	291

Research Results – Three Lots



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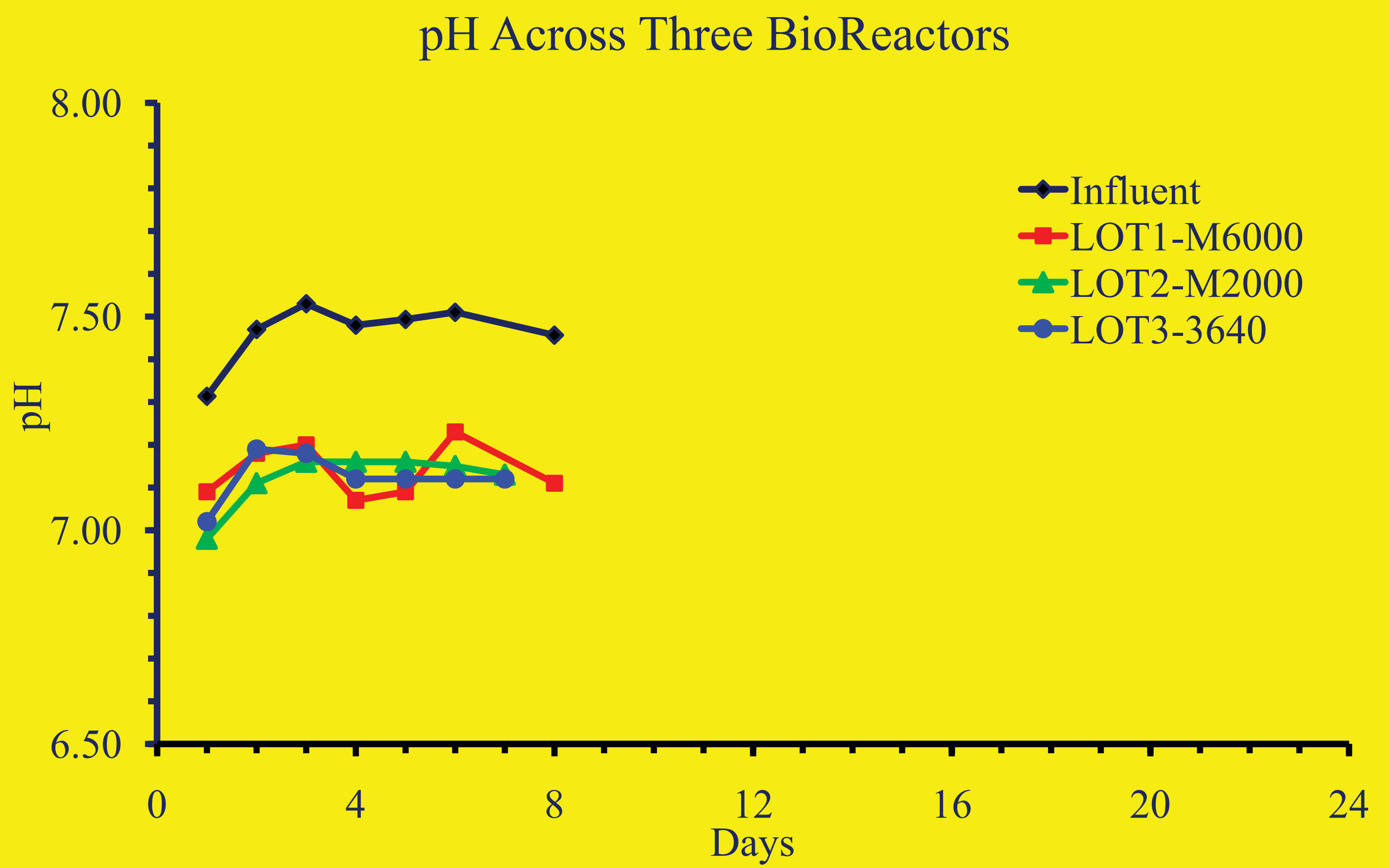

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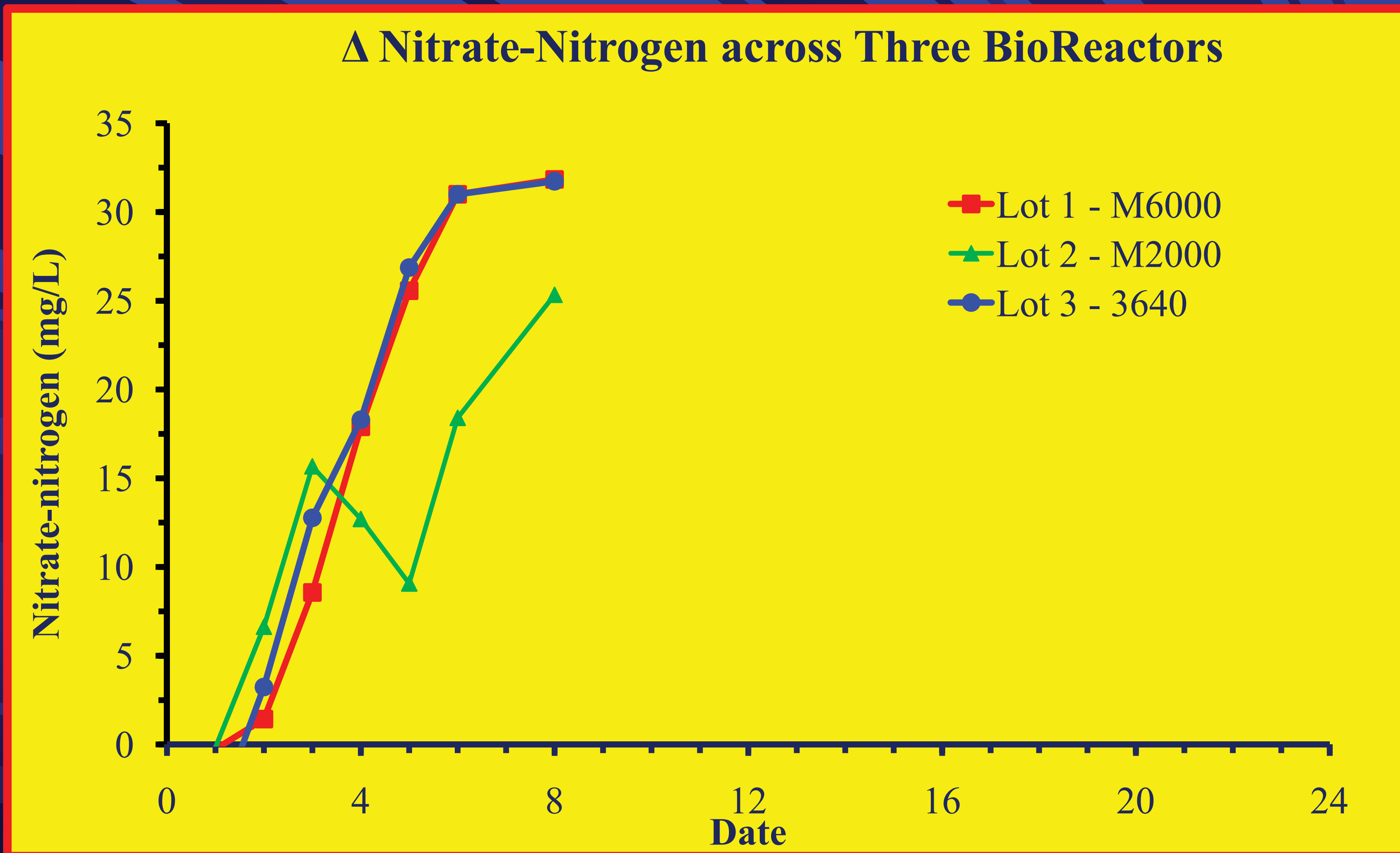

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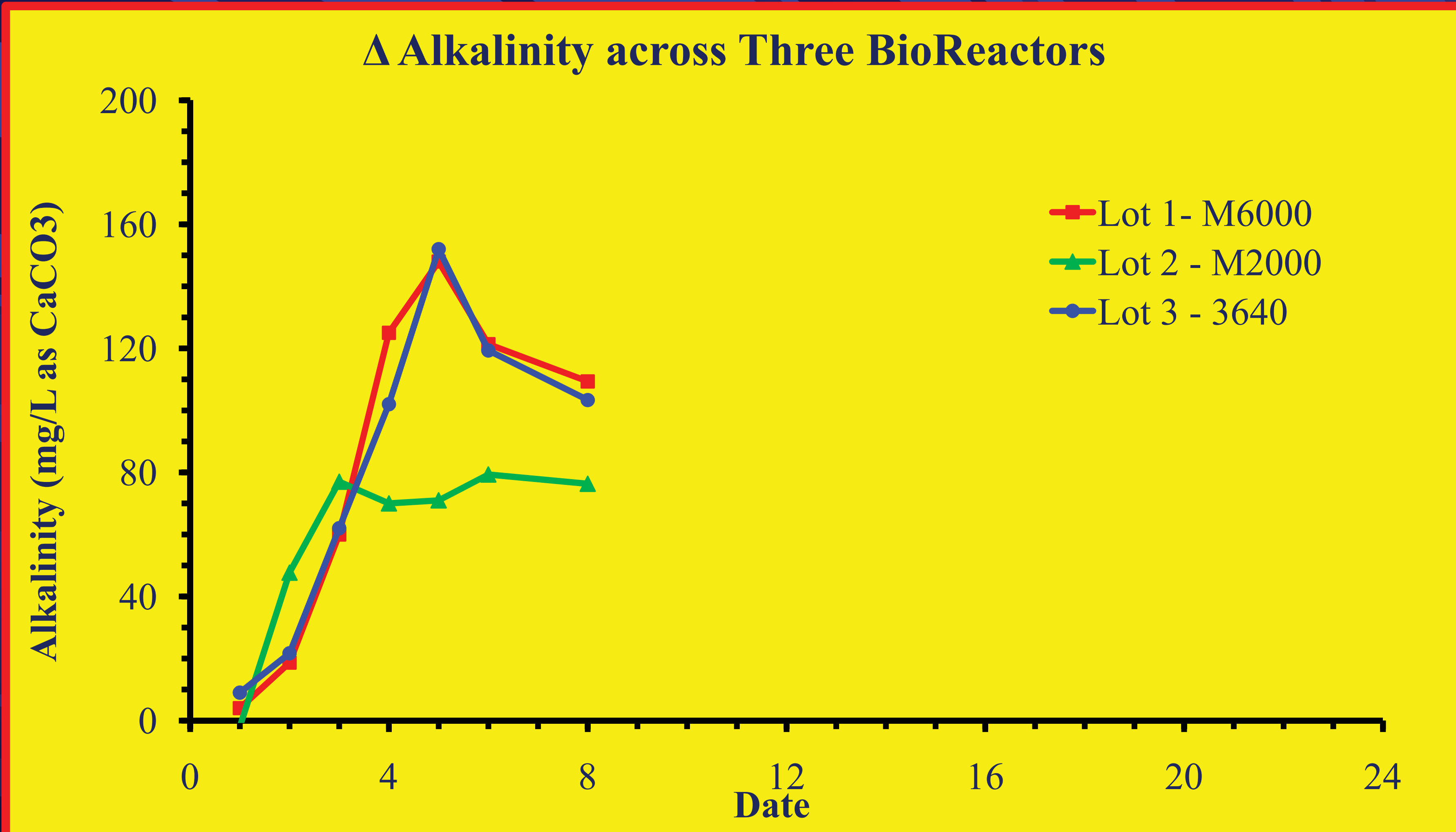

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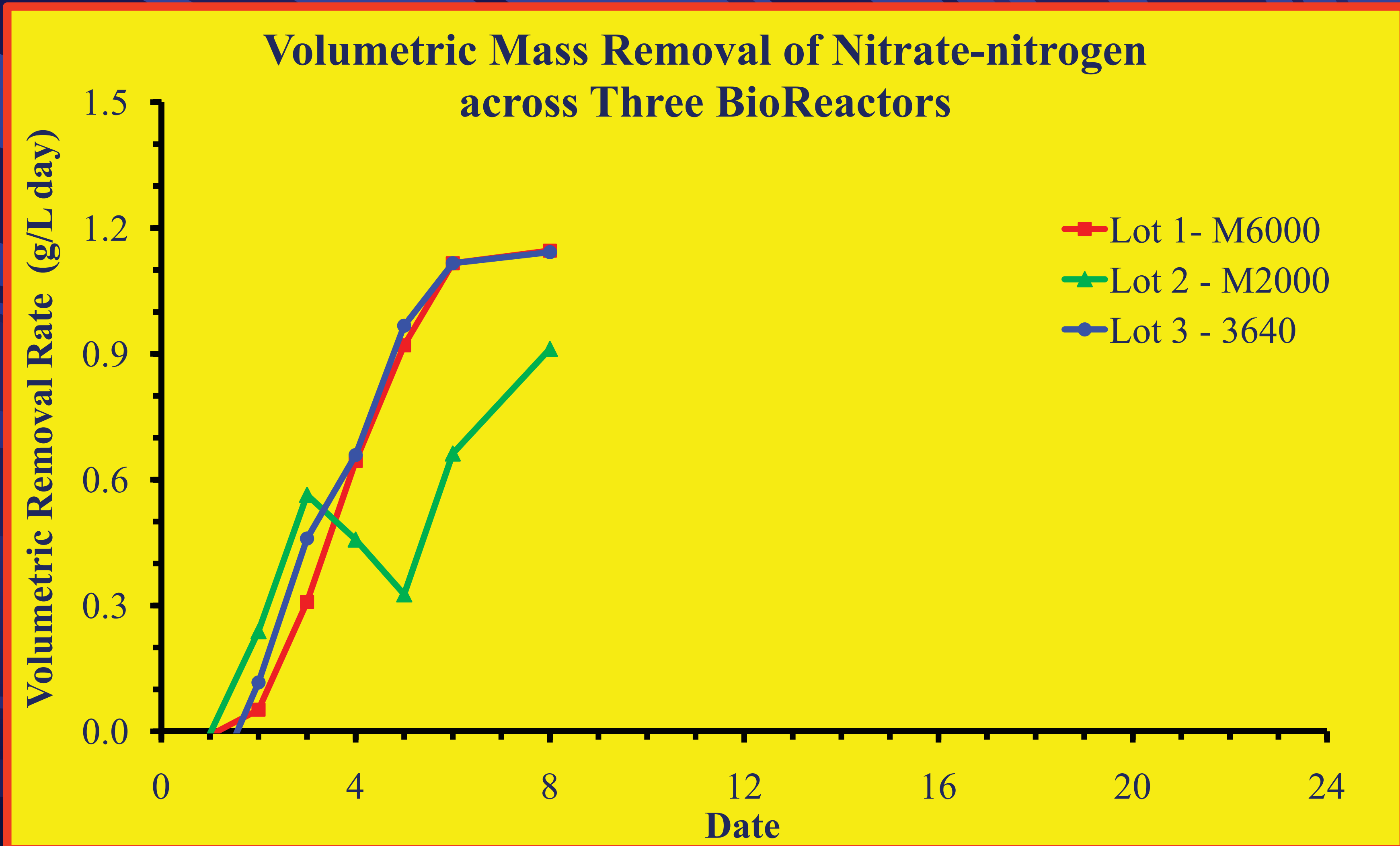


Mirel ™ Bioplastics PHA media

Aquaculture Systems Technologies, LLC

Research Results – Three Lots

Nitrate Consumption

Sample	(g/L media day)
LOT1-M6000	1.15
LOT2-M2000	0.94
LOT3-3640	1.14

Stoichiometry

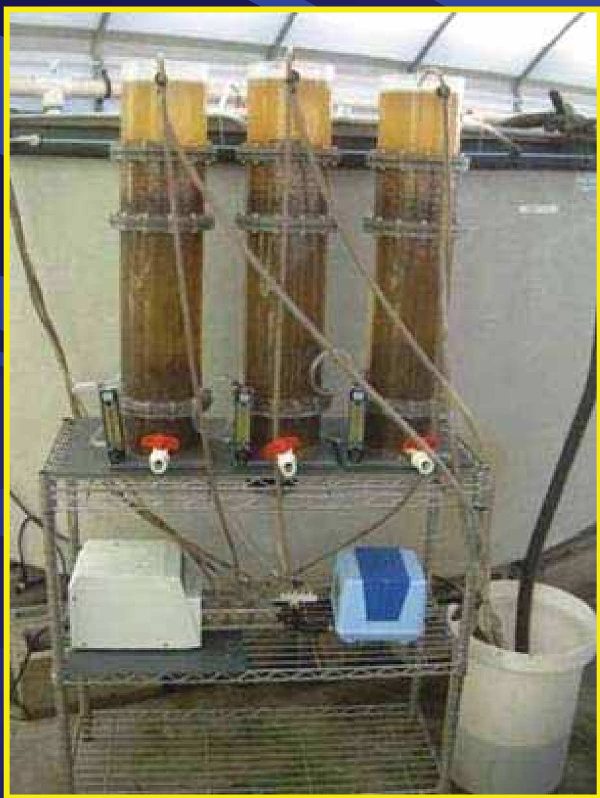
Sample	Alk/Nitrate
LOT1-M6000	3.4
LOT2-M2000	3.0
LOT3-3640	3.3

Stoichiometry: 3.57 g Alk/ g Nitrate

Conclusions

- lab-scale PolyGeyser® denitrification unit was designed constructed
- bioreactor units surpassed 2.0 kg/m^3-day nitrate removal
- no clogging or short circuiting of the media bed
- excess biofloc settled quickly to the bottom

Questions?



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