

DEVELOPMENT OF ALGAE-BASED NITROGEN REMOVAL TECHNOLOGIES

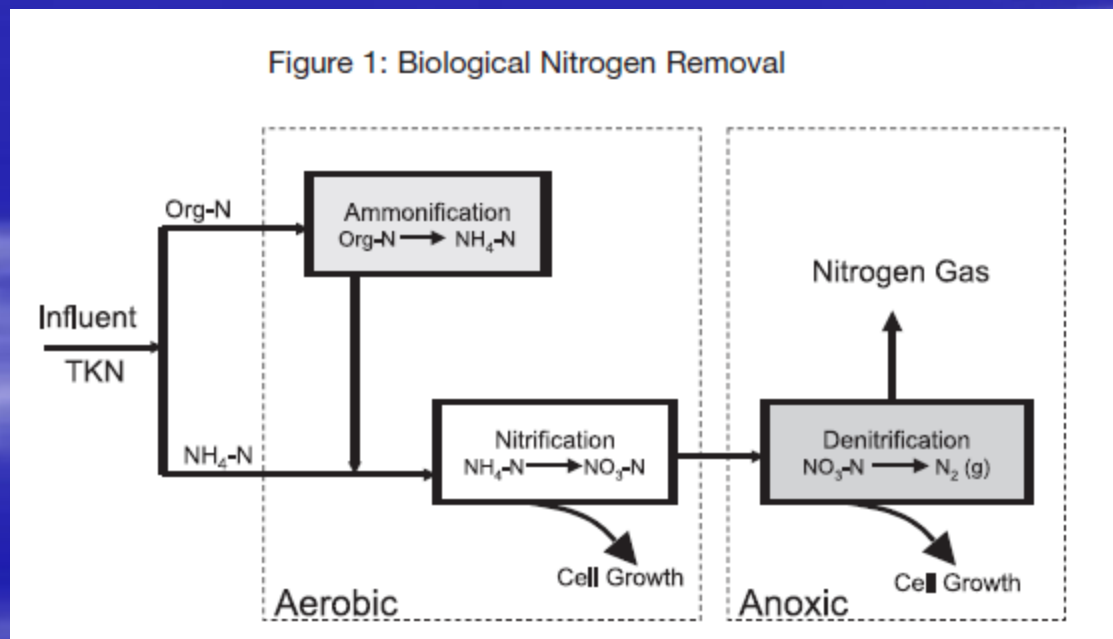
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Old Dominion University*



Outline of talk

- Phycoremediation –
 - pros and cons, state of technology, potential solutions
- Approach & Experimental design
- Results
- Conclusions
- Future Work

Current technologies for low TN in WWTP effluent commonly involve biological nutrient removal (BNR) using bacteria

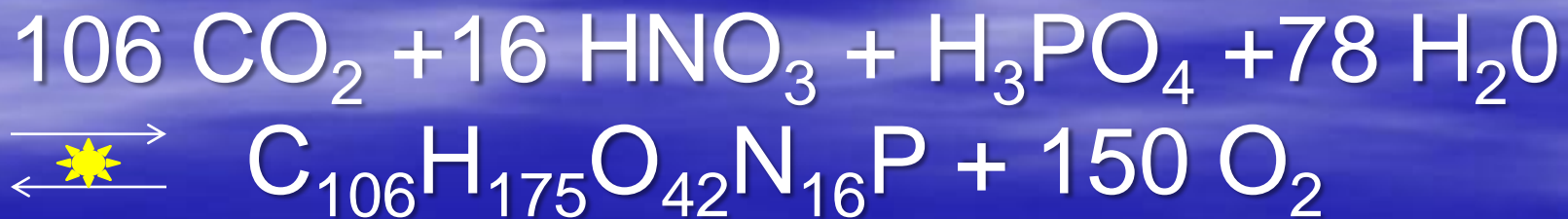


Algae-based Nitrogen Removal

- Phycoremediation – the use of algae to remove or reduce nutrients
- Potential replacement for BNR or post-BNR polishing
- Certain sectors have already capitalized on the ability of algae to take up a diverse suite of N:
 - Aquaculture, agriculture, livestock, and small community wastewater facilities
 - There are many bioreactor designs that achieve nutrient removal using algae

Phycoremediation - Pros

- N rapidly converted to biomass that can be removed and used
- No need for supplemental carbon (C) additions (e.g. methanol) – need to control pH/aerate
- Algae also remove phosphorus (P) during their growth – could reduce P removal costs
- No gaseous N intermediates (e.g., N₂O)
- Inexpensive, simple, and environmentally friendly



Phycoremediation - Cons

- Requires light
- Separation of algae from treated wastewater stream
- Continuous flow – chemostat reactors
 - Short in-plant hydraulic residence times (HRTs) and high flow rates – need fast-growing algae!
 - Balance conversion of N to biomass and wash out
- Space – large surface area required to provide access to “free” light
 - Requires large footprint
 - Existing WWTP reactors use less space

Current state of technology

- Phycoremediation technologies using algae have been developed, primarily outside of the US or where space is not limiting
- While various phycoremediation techniques have been described, none have been designed for use in large WWTP applications (>1-3 MGD) for plants with short HRTs (< 4-8 hours)
- Algal nutrient removal has focused on dissolved inorganic P (DIP) as PO_4^{3-} and N primarily as ammonium (NH_4^+)

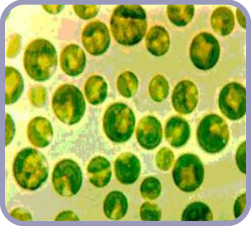
Potential solutions

- Separation problem - Immobilize algae so they can be easily removed
 - Natural polymers – sodium alginate
 - Embed or apply as a biofilm
- Light problem - Increase light penetration
 - Submerged light sources
 - Side-emitting fiber optics
 - Solar/light collectors
 - Wavelength specific light sources

Our approach

Algal Selection

- Mixed algal suspension from WWTP
- *Chlorella* spp.
- *Desmodesmus* spp.



Algal Immobilization

- Embed as beads, strands, or layers in sodium alginate
- Attach to biofilm carriers



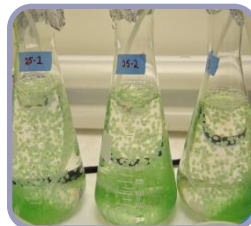
Nutrient Removal

- Polishing step for final effluent from wastewater treatment process
- N removal: NO_3^- , NH_4^+ , DON
- Evaluate P requirements for algae and P removal



Optimization

- Temperature - 15, 20, 25, 30°C
- Light supply – surface, surround, side-emitting fiber optics
- Mixing/aeration
- CO_2 concentrations



- Biological endpoints – Chl a, fluorescence, cell counts, productivity
- Nutrient concentrations – TDN, $\text{NO}_x\text{-N}$, NH_4^+ , DON, PO_4^{3-}
- pH, DIC

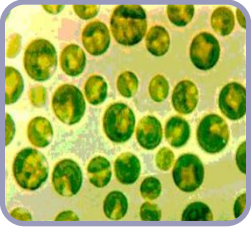
Measurements



Our approach

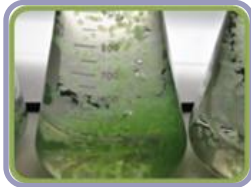
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- Mixed algal s
- *Chlorella* spp
- *Desmodesm*



Algal I

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- Attach to bio



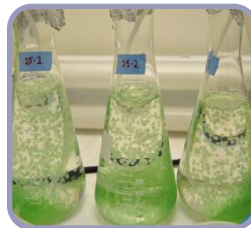
Nutrie

- Polishing ste
- treatment pro
- N removal: N
- Evaluate P re



Opt

- Temperature
- Light supply
- fiber optics
- Mixing/aerati
- CO₂ concentr



biological – Chlorophyll a,
fluorescence, cell counts,
conductivity
nutrient concentrations –
N, NO_x-N, NH₄⁺, DON,
PO₄³⁻,
H₂, DIC

measurements



Experimental Design

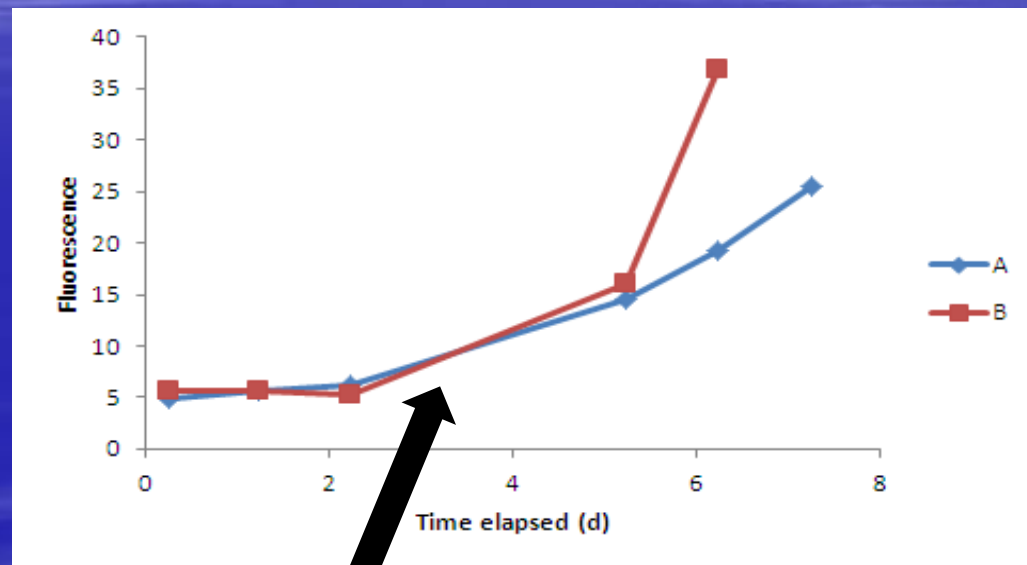
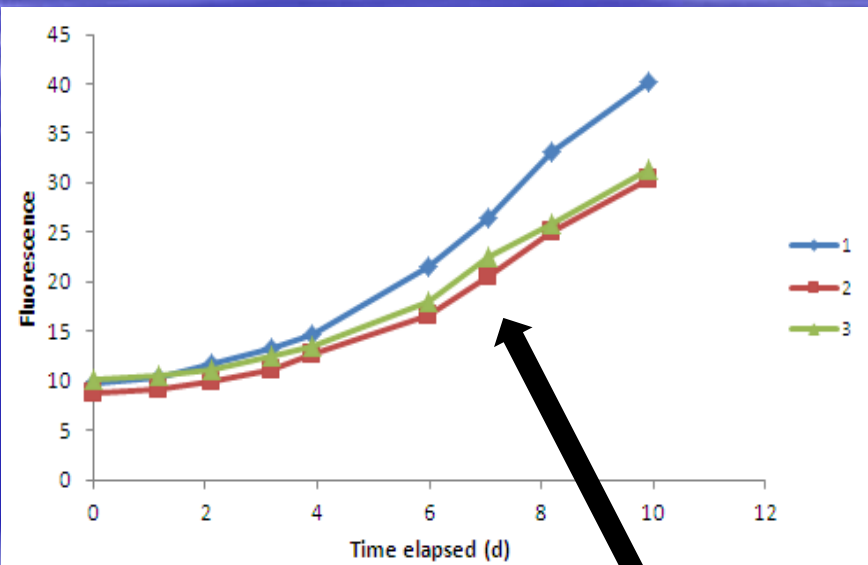
1. Free-floating algal growth in effluent amended with P
2. Small-scale (<1L) encapsulation (alginate beads) in batch mode, light penetration from all sides
 - a) Altered aeration/mixing
 - b) Altered temperature
3. Larger scale (5L) encapsulation
 - a) Batch vs continuous flow
 - b) pH effects
 - c) Varied light source

Experimental constants

- All experiments start with HRSD's Virginia Initiative Plant treated effluent (fully nitrifying/partially denitrifying)
 - $\text{NH}_4^+ < 1 \text{ mg/L}$
 - $\text{NO}_x\text{-N} = 5 - 7 \text{ mg/L}$
 - $\text{OP} < 0.1 - 0.3 \text{ mg/L}$
 - $\text{TP} < 0.5 \text{ mg/L}$
- All experiments conducted using 24 h light
- P always added as 16 N:1 P (algal molar N:P requirement)
- All bioreactors either mixed or aerated
- Growth rates calculated as doubling times

Results - Free algae

- Algae like to grow in wastewater



Desmodesmus sp. and *Chlorella v.*
– common freshwater algae, grow well in effluent

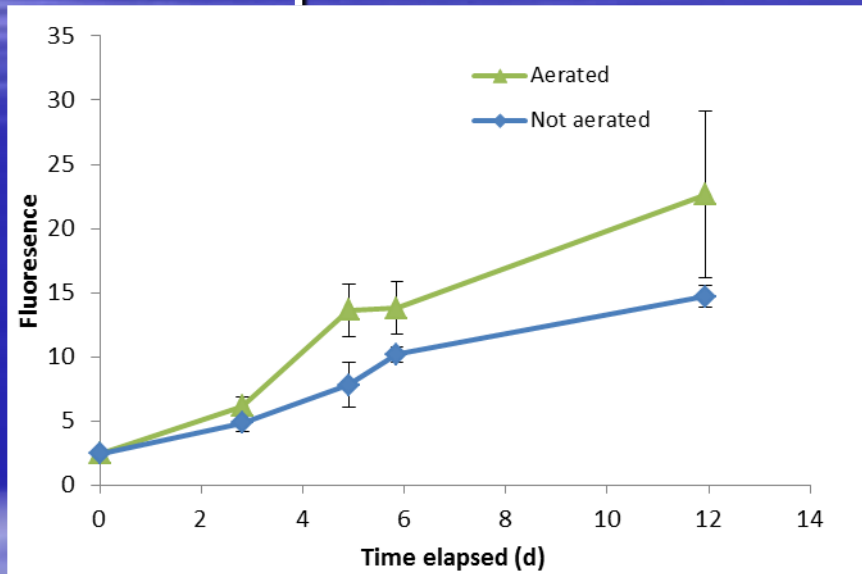
Algal Immobilization - embedding

- Sodium alginate
 - Simple and cost-effective natural polymers, derived from algae, form rigid beads when dropped into an ionic solution.
 - When mixed with suspended algae, beads encapsulate algae that can grow within the polymer, allowing nutrients from effluent to diffuse into the beads

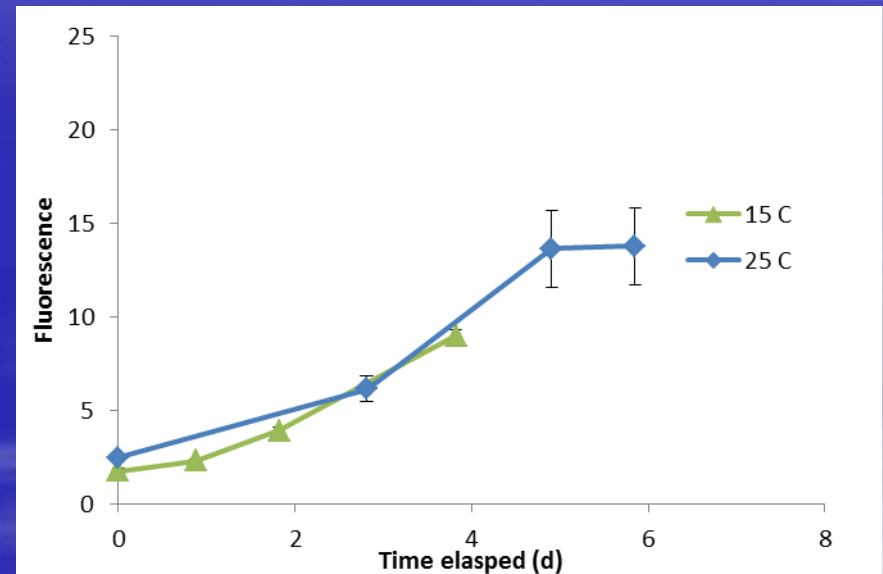


Results – encapsulated algae

- Algae like to grow in wastewater while encapsulated



Chlorella v.
doubling time
greater in aerated
bioreactor



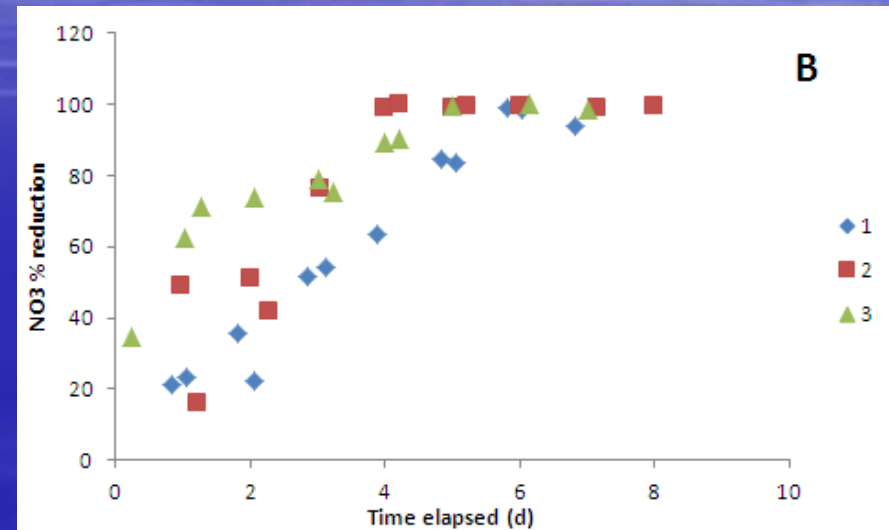
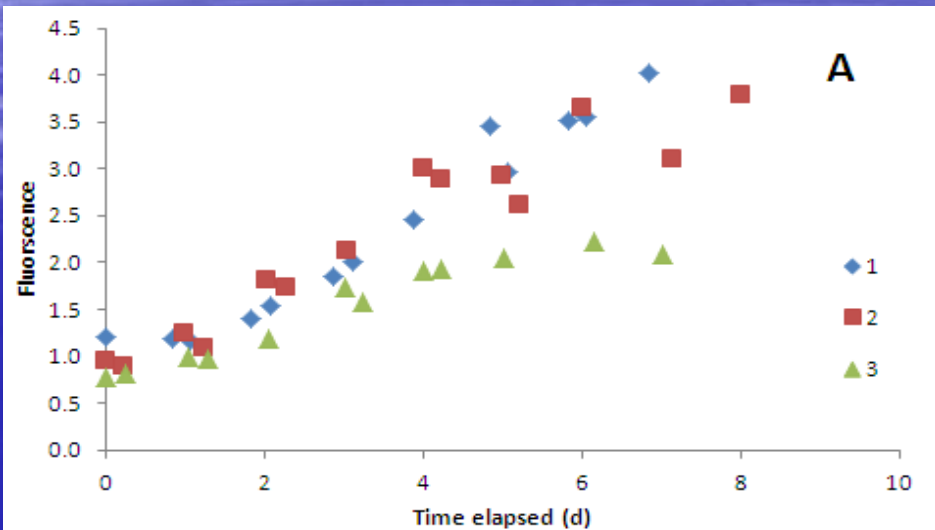
Chlorella v. doubling
times similar in
different temperature
bioreactors

Results – Nutrient reduction

- Algae can remove N and P

Exp. #	Type of algae/ free or embedded	Batch or flow through	Doubling time (d)	NO _x -N removal efficiency	P removal efficiency
1	Desmodesmus/free	Batch	5.1 ± 0.6	N/A	N/A
2	Desmodesmus/free	FT ¹ (0.2 mL/min)	0	20%	N/A
3	Desmodesmus/free	Batch	4.9 ± 0.5	<40%	N/A
4	Synechococcus/free	Batch	0	N/A	N/A
5	Chlorella/free	Batch	2.5 ± 0.4	N/A	N/A
6	Chlorella/embedded	Batch	4.7 ± 0.3	N/A	N/A
7	Chlorella/embedded	Batch	4.0 ± 0.5	N/A	N/A
8	Chlorella/embedded	Batch	1.6 ± 0.1	100% in 4d	90% in 4d
9	Chlorella/embedded	Batch	4.0 ± 0.5	100% in 6d	90% in 12d

Results – large- scale encapsulated algae

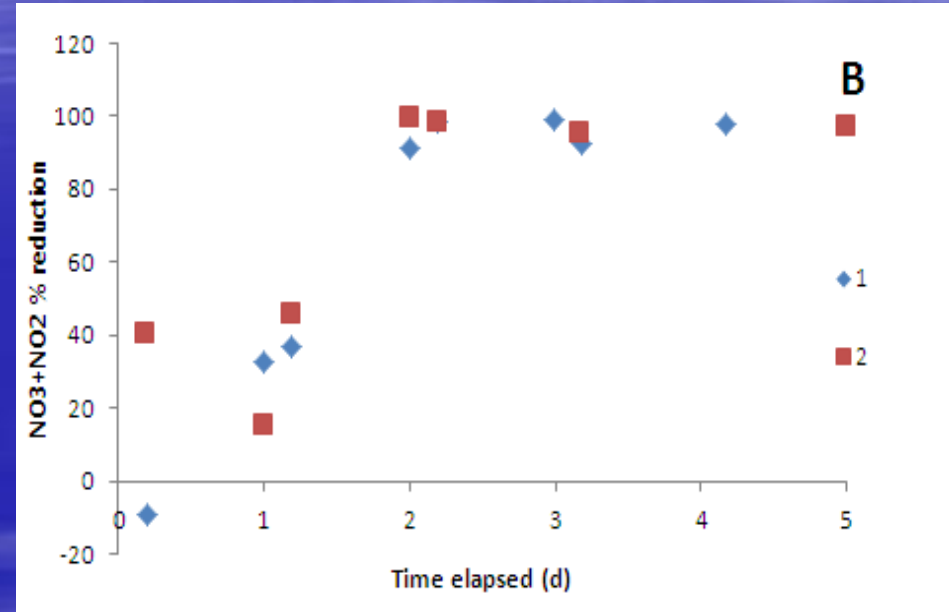
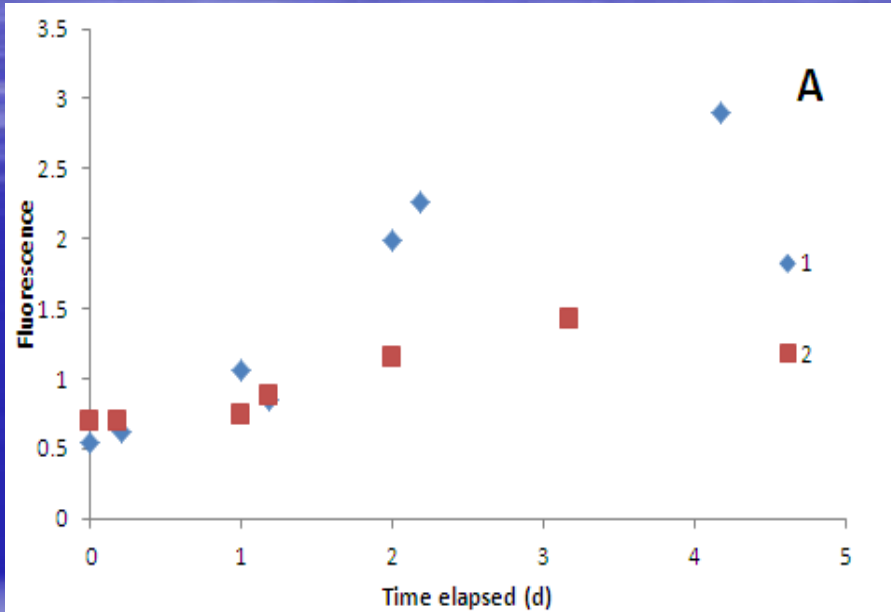


Doubling time: 3.8 ± 0.5 d

100% NO_x-N
reduction in 4 days

* 30° C bioreactor,
overhead fluorescent light

Results – Increased light



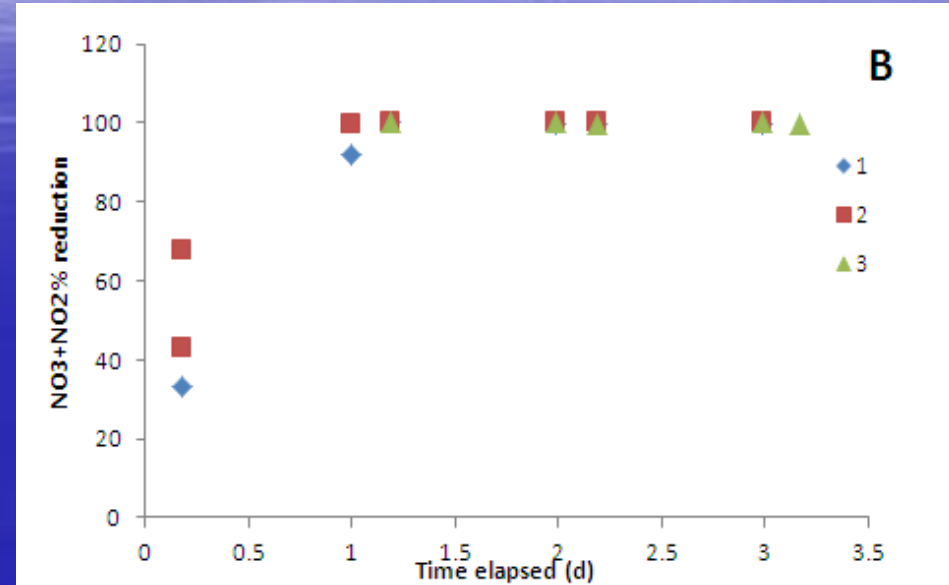
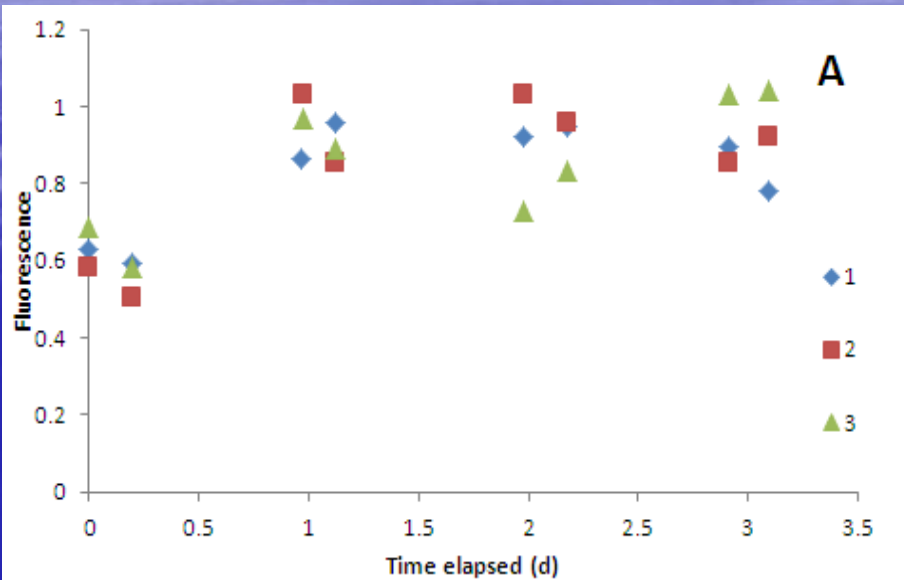
Doubling time: 2.2 ± 0.8 d

100% NO_x-N
reduction in 2 days

* 30° C bioreactor,
increased light by 23%

Results – Controlled pH

Prevent C limitation



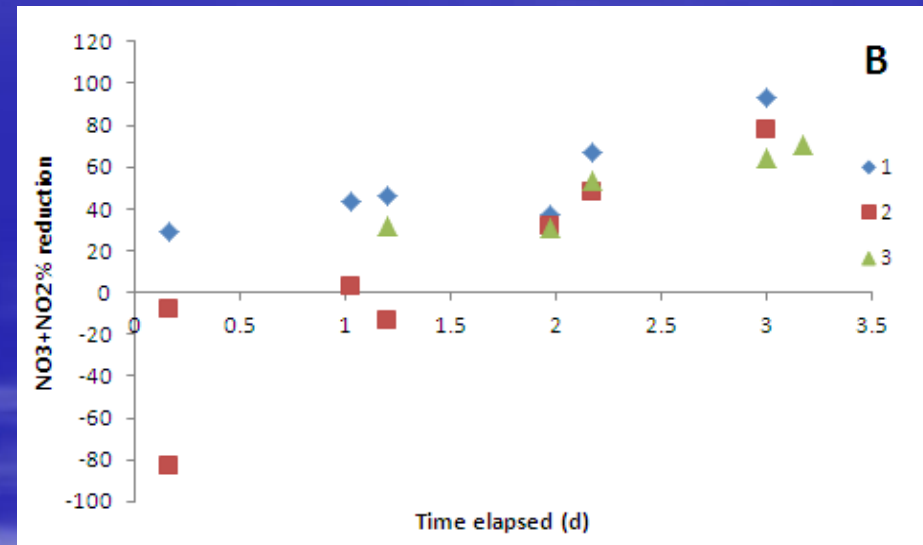
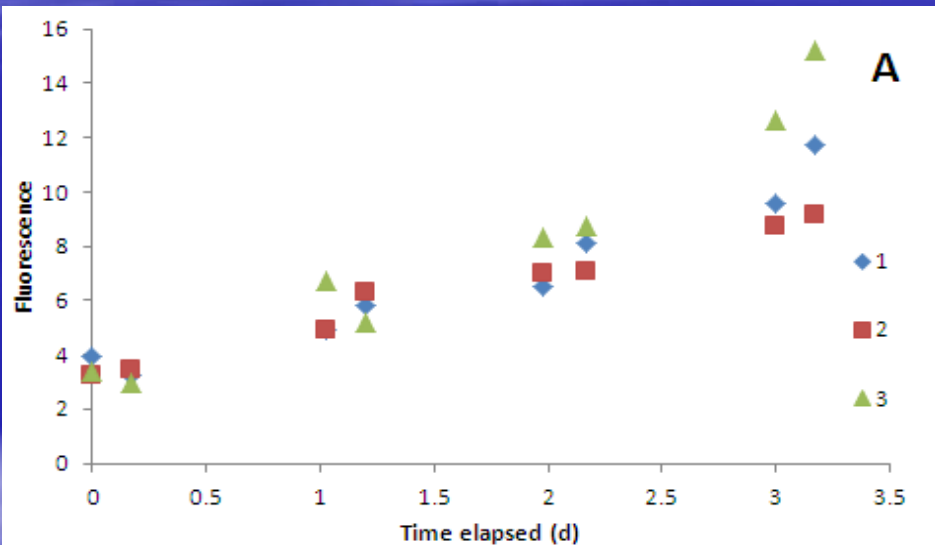
Doubling time : 1.4 ± 0.3 d

100% NO_x-N
reduction in 1 day

* 30° C bioreactor, increased
light by 23%, pH = 7-7.5

Results – Flow through system

- 5L bioreactors (3.6L effluent), stirred, 30°C, 23% increased light, 5 mL/min (12 h HRT)

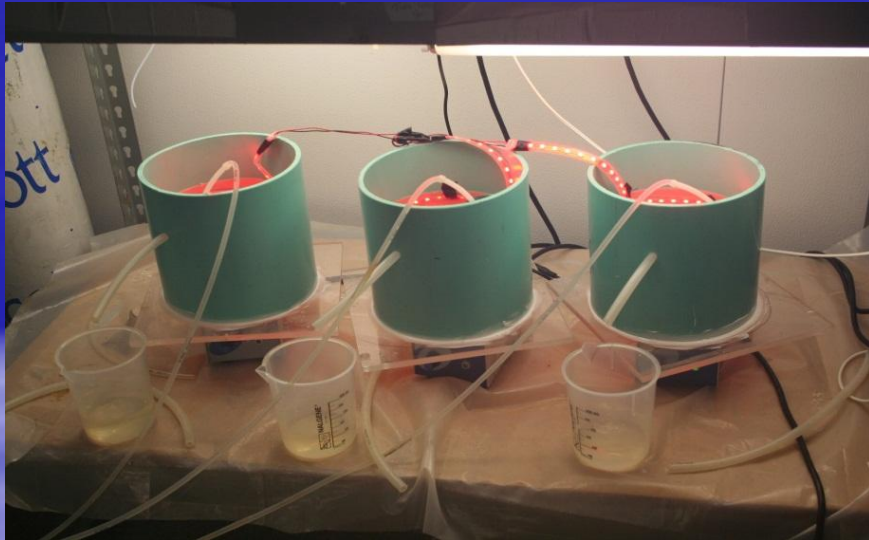


Doubling time : 1.8 ± 0.4 d
DT > HRT

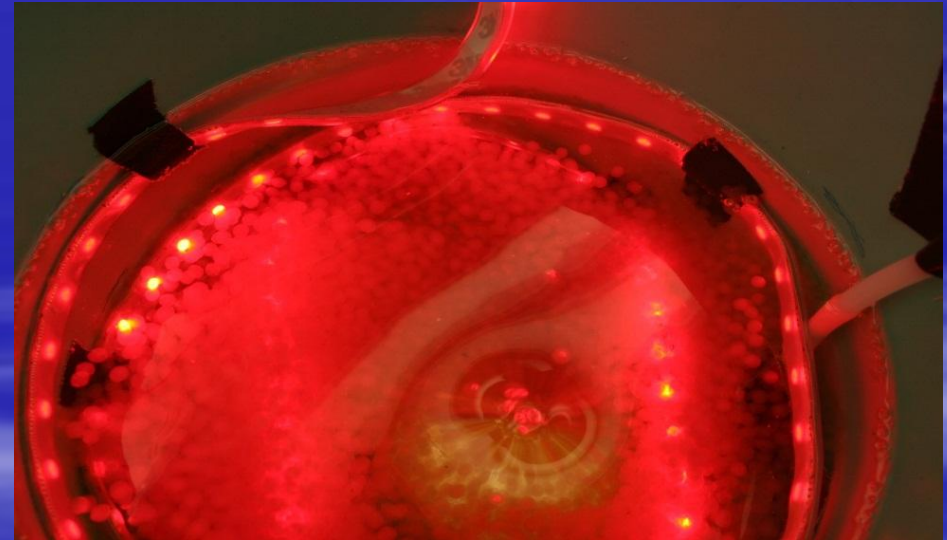
<100% NO_x-N
reduction in 3 days

Results – Flow through system

- 5L bioreactors, stirred, 30°C, 23% increased light, 5 mL/min (12 h HRT), **submersible wavelength specific LEDs (623 nm)**



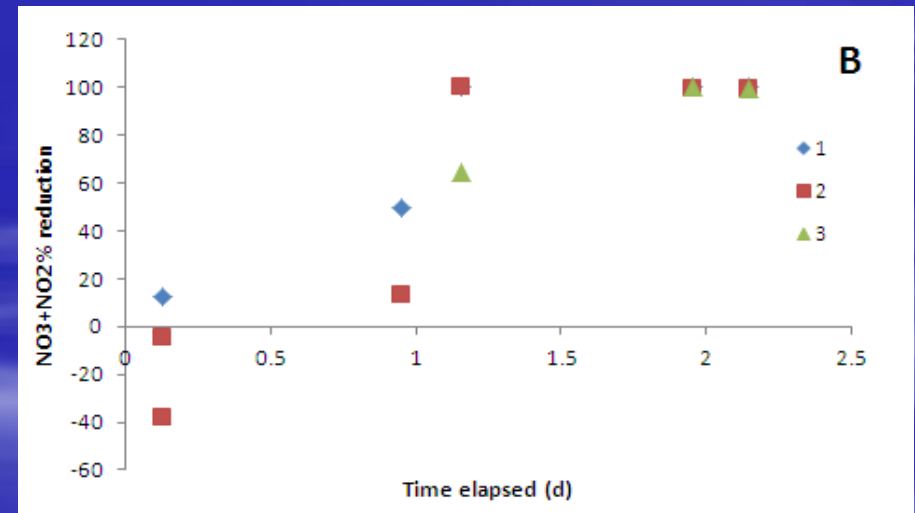
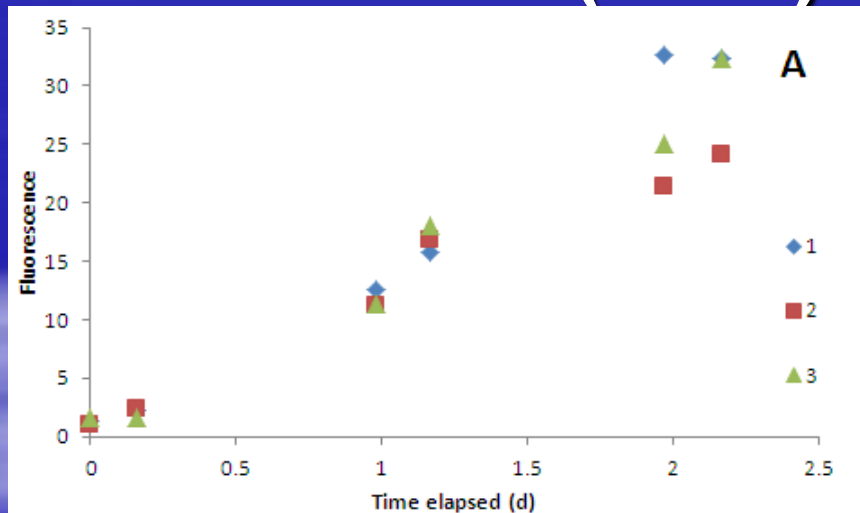
Doubling time : 0.89 ± 0.04 d
DT > HRT



Only 50% NO_x-N
reduction in 3 days

Results – Flow through system

- 5L bioreactors, stirred, 30°C, 23% increased light, 5 mL/min (12 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5)

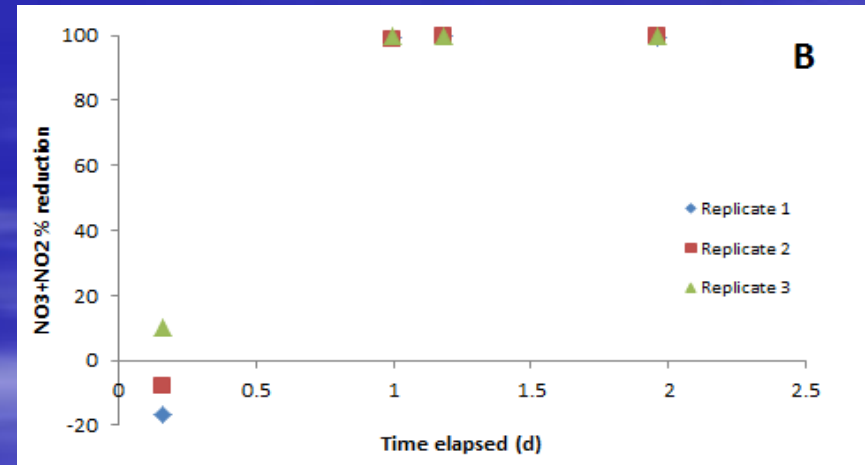
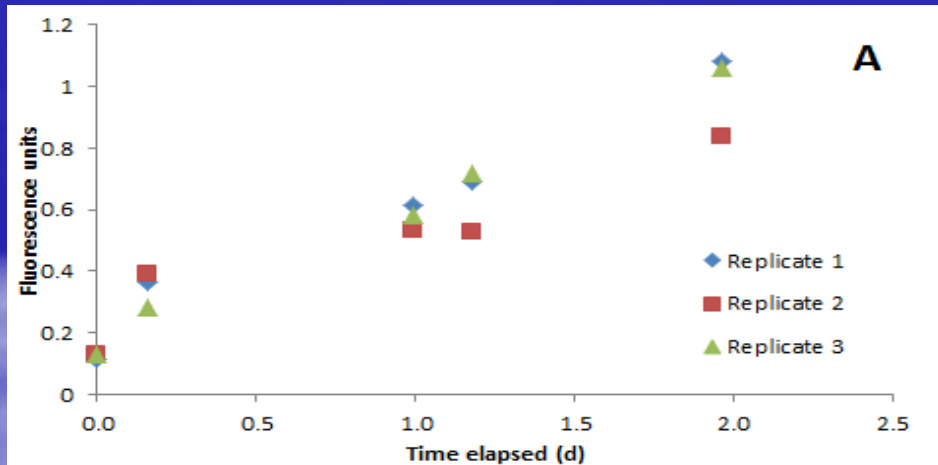


Doubling time : 0.48 ± 0.01 d
Now about equal to HRT!

100% NO_x-N reduction in 1 day (2/3 replicates)

Results – Flow through system

- 5L bioreactors, stirred, 30°C, 23% increased light, 8.5 mL/min (6.5 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5)

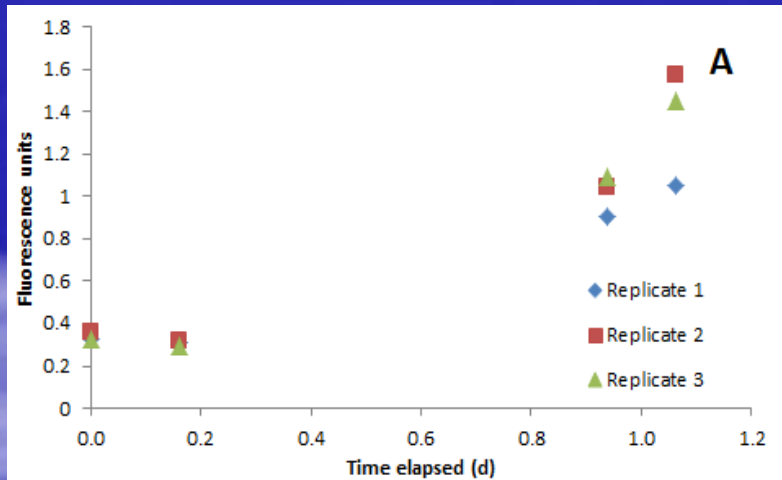


Doubling time : 0.72 ± 0.01 d

100% NO_x-N reduction in 1 day

Results – Flow through system

- 5L bioreactors, stirred, 20°C, 23% increased light, 8.5 mL/min (6.5 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5)

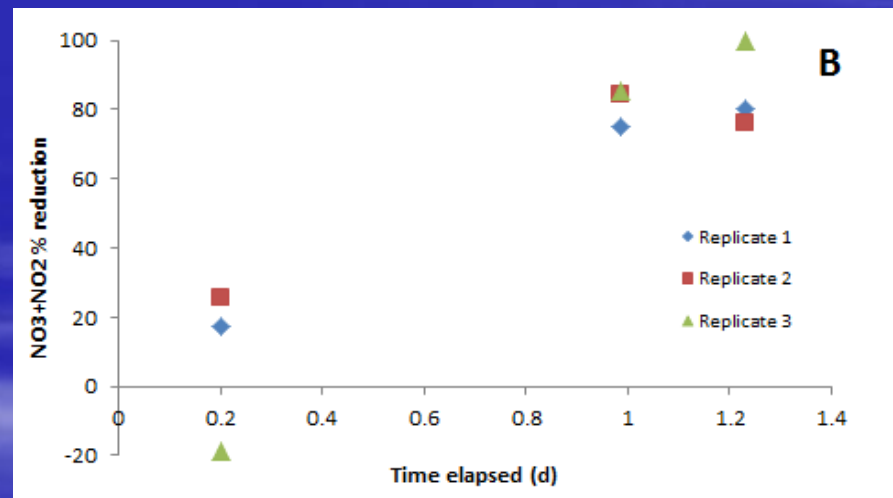
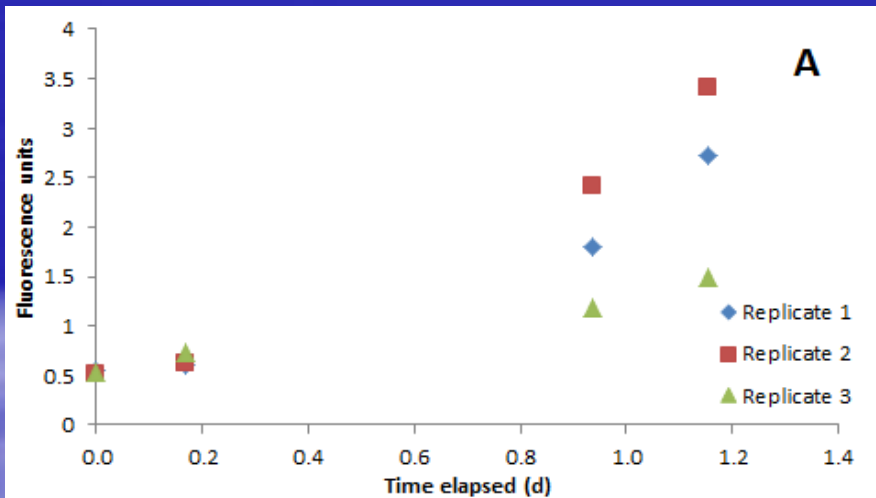


NO_x-N produced but VIP effluent was uncharacteristically dominated by NH₄⁺ which was depleted to 0 within 24 h

Doubling time : 0.52 ± 0.08 d

Results – Flow through system

- 5L bioreactors, stirred, 20°C, 23% increased light, 8.5 mL/min (6.5 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5), bead/effluent = 10% (v/v)

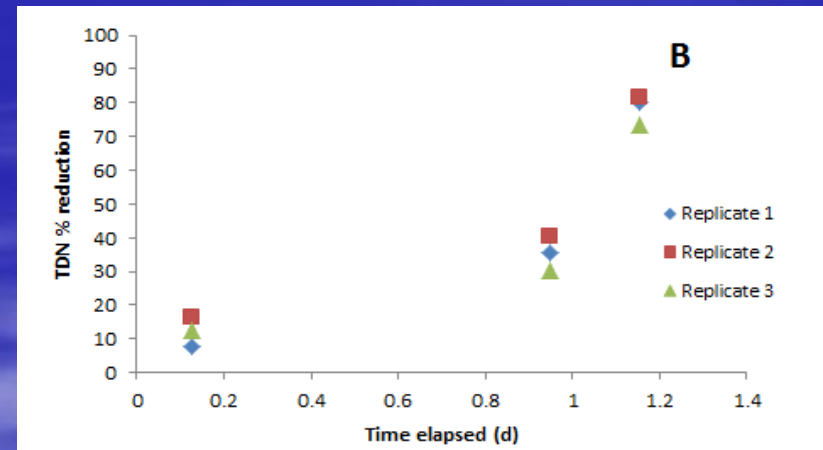
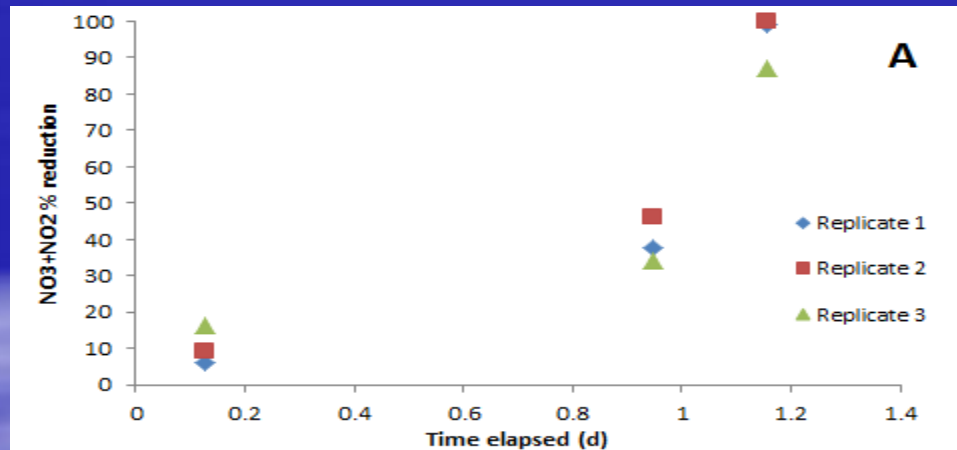


Doubling time : 0.68 ± 0.26 d

80% NO_x-N reduction in 1 day

Results – Flow through system

- 5L bioreactors, stirred, 20°C, 23% increased light, 5 mL/min (12 h HRT), submersible wavelength specific LEDs (red; 623 nm), pH maintained (7-7.5), coated biofilm carriers



100% NO_x-N reduction in 28 h

80 – 90% TDN reduction in 28 h

Results – Summary

Exp. #	Type of algae/ free or embedded	Batch or flow through	Light type	Temp. (°C)	pH regulated	Doubling time (d)	NOx-N removal efficiency	P removal efficiency
10	Chlorella/embedded	Batch + P	Fluor. 24h	25	N/A	3.8 ± 0.5	100% in 4d	70% in 8d
11	Chlorella/embedded	Batch + P	Fluor. 24h +23%	25	N/A	2.2 ± 0.8	100% in 2d	100% in 2d
12	Chlorella/embedded	Batch + P	Fluor. 24h +23%	25	7 – 7.5	1.4 ± 0.3	100% in 1d	100% in 1d
13	Chlorella/embedded	FT (5 mL/min) + P	Fluor. 24h +23%	30	N/A	1.8 ± 0.4	100% in 2d	100% in 2d
14	Chlorella/embedded	FT (5 mL/min) + P	Fluor. 24h + red LEDs	30	N/A	0.89 ± 0.4	50% in 1d	90% in 1d
15	Chlorella /embedded	FT (5 mL/min) + P	Fluor. 24h + red LEDs	30	7 – 7.5	0.48 ± 0.4	100% in 1d	100% in 1d
16	Chlorella /embedded	FT (8.5 mL/min) + P	Fluor. 24h + red LEDs	30	7 – 7.5	0.472± 0.01	100% in 1d	100% in 1d
17	Chlorella /embedded	FT (8.5 mL/min) - P	Fluor. 24h + red LEDs	30	7 – 7.5	1.25 ± 0.25	100% in 1d	N/A
18	Chlorella /embedded	FT (8.5 mL/min) - P	Fluor. 24h + red LEDs	20	7 – 7.5	0.52 ± 0.08	0	N/A
19	Chlorella /embedded (re-used)	FT (8.5 mL/min) - P	Fluor. 24h + red LEDs	20	7 – 7.5	1.3 ± 0.1	30-50% in 1d	N/A
20	Chlorella /embedded (10% v/v)	FT (8.5 mL/min) - P	Fluor. 24h + red LEDs	20	7 – 7.5	0.68 ± 0.26	80% in 1d	N/A
21	Chlorella /embedded (10% v/v)	FT (8.5 mL/min) - P	Fluor. 24h + blue LEDs	20	7 – 7.5	1.9 ± 0.9	30% in 2d	N/A
22	Chlorella /embedded (10% v/v; re-used)	FT (8.5 mL/min) - P	Fluor. 24h + blue LEDs	20	7 – 7.5	0.77 ± 0.13	30% in 2d	N/A
23	Chlorella /embedded (plastic carriers)	FT (5 mL/min) + P	Fluor. 24h + red LEDs	30	7 – 7.5	N/A	100% in 1.2d	0

Conclusions

- Phycoremediation strategies - successful at HRTs of 6.5 and 12 h
- 10% bead to effluent (v/v) efficient at N removal, reduce more?
- Coated biofilm carriers proved promising

Conclusions

- Effluent 'type' will effect results, NH_4^+ preferentially removed over NO_x and organics
- Significant $\text{NO}_x\text{-N}$ removal was obtained, steady state within 24 h
- Wavelength specific submersible LEDs increase growth rates, red > blue
- Maintaining pH increases growth rates and N and P removal efficiencies because it alleviates C limitation of photosynthesis, could be a good use of plant CO_2

Reality check = Costs

- Lights and alginate are greatest expense
- Costs ~\$0.03/m to use submersible red LEDs for 1 day - Need to scale down amount of lights used per L effluent
- Need to find cheaper source for large-scale alginate purchases, beads can be used for ~ 2 weeks and still maintain integrity and efficiency
- Other chemical costs – may be offset by recycling CO₂ and not removing PO₄³⁻

Future work

- Decrease HRTs further
- Scale up
- Perform experiments in a series
- ID robust algal communities for plant setting
- Determine optimal N:P ratios
- More work into biofilm carriers like that used for bacteria in moving bed biofilm reactors (MBBR)
- Potential for algal and polymer recycle streams (However, algal beads dry rapidly)

