Examination of Nitrogen to Phosphorus Ratio in Nutrient Removal from Wastewater through *Chlorella Vulgaris*

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Background

- Phosphorus and nitrogen are limiting nutrients for algal growth, causing potential for eutrophication the ultimate depletion of oxygen supply for aquatic systems.
- Recent studies show that microalgae has great potential for the removal of nitrogen and phosphorous due to their capacity to assimilate nutrients. Benefits in utilizing this method include low cost of the operation, recycling assimilated nutrients in microalgae as fertilizer.
- *Chlorella Vulgaris* is one of the most extensively used microalgae for nutrient removal as it is a unicellular free-living green alga that is widely distributed around the world. Many researchers have investigated the nutrient removal of this algae and developed techniques to increase its capacity to do so. However, it has not been determined if utilizing *Chlorella Vulgaris* is suitable for all cases.
- Research conducted at the University of Arkansas seeks to establish the relationship between nitrogen to phosphorus ratio in wastewater effluent and the level of nutrient reduction using *Chlorella Vulgaris.* This will provide further information to establish the feasibility of using this microalgae as a widespread treatment option for nutrient removal.

Methods and Materials

- Chlorella Vulgaris was cultivated in an environmental growth chamber at 25 ± 1° C. The relative humidity was kept at 50 ± 1%. The culture was maintained in an incubator kept at 25 ± 1° C. The culture was exposed to 4 foot cool-white fluorescent light tubes mounted approximately 10 inches above the culture. During the life of the culture, it was not aerated or agitated. The algae was grown in Bristol Medium ($NaNO_3$ (25 mg/L), $CaCl_2.H_2O$(2.5 mg/L), $MgSO_4.7H_2O$(7.5 mg/L), K_2HPO_4 (7.5 mg/L), KH_2PO_4(17.5 mg/L), NaCl(2.5 mg/L)).
- An aliquot of the culture suspension is inoculated into fresh medium when needed. The newly transferred culture was placed on a lighted shelf and allowed to grow for seven days.
- Four jars were prepared with 1 liter of Bristol Medium where the nitrogen to phosphorus ratios were changed to 30:24, 21:20, 6:20, and 2:20 in the forms of Nitrate and Phosphate (mg/L) , and innoculated with 2 mL of extracted *Chlorella Vulgaris* growing in Bristol Medium. A 25:5 jar was used for the control. For a period of eight days, two 8 mL samples were filtered using a syringe filter. Algal density was also checked daily at a 680 nm. A similar experiment was also conducted with 3 identical 1 L jars of 1:2 N:P Bristol Medium with the same sampling method.

Materials and Methods (cont.)

- The nutrient content of each 8 mL sample was determined on an ion chromatography system (Metrohm USA, Riverview, FL).
- Light microscopy was conducted using a Nikon Eclipse Ni-E 100x microscope (Nikon, Melville, New York).

Figure 1. Innoculated *Chlorella Vulgaris* in 1L Bristol Medium on the 8th day of sampling.

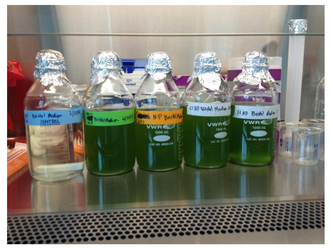


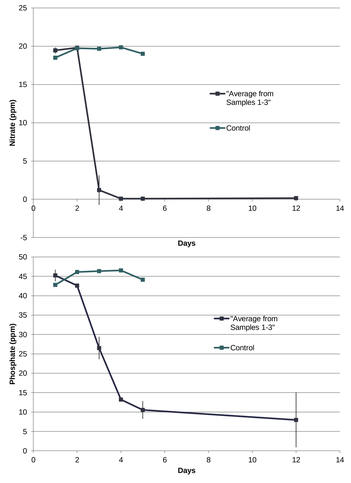
Figure 2. Innoculated *Chlorella Vulgaris* in 1L Bristol Medium on the 8th day of sampling.

Results and Discussions

Figures 3 and 4. Photos of Chlorella Vulgaris taken from a Nikon Eclipse Ni-E 100x microscope

Nitrate and Phosphate Reduction

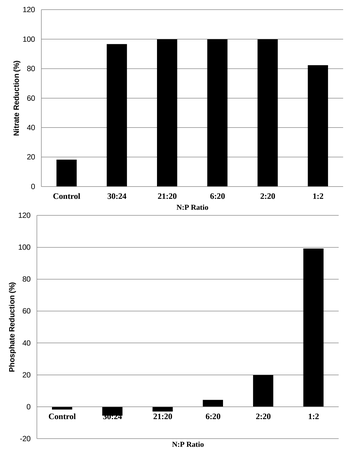
- *Chlorella Vulgaris* growing in Bristol Medium with a N:P ratio of 1:2 showed an average 99% and 82% percent reduction of nitrate and phosphate, respectively, with standard deviations displayed in Figures 5-6.
- Although this experiment showed sufficient removal of both nitrate and phosphate, results from a second experiment only showed phosphate reduction as displayed in Figures 7-8.



Figures 5-6. Nitrate and Phosphorus Reduction of Chlorella Vulgaris in Bristol Medum (N:P 1:2)

Results and Discussions (cont.)

- The percent reduction of nitrate and phosphate from growth of *Chlorella Vulgaris* in Bristol Medium with varying N:P ratios is displayed in Figures 7 and 8, respectively.
- For N:P ratios of 30:24, 21:20, 6:20, 2:20, and 1:2 phosphate was reduced to a minimum 97 percent while nitrate concentration remained the same, indicating that nitrogen is not the limiting nutrient.



Figures 7-8. Nitrate and Phosphorus Reduction of Chlorella Vulgaris in Bristol Medum (N:P 1:2)

Table 1: Nutrient Data from Previous Work (Wang et al., 2012)

Total Nitrogen (mg/L)		Total Phosphorus (mg/L)		N:P Ratio	Percent Reduction	
Intital Value	Terminal Value	Intital Value	Terminal Value		Total Nitrogen	Total Phosporus
38.03	15.65	1.83	0.053	38:2	58.8	97.1
29.45	16.81	0.57	0.046	29:0.5	42.9	91.9
19.01	13.79	0.92	0.065	19:1	27.5	92.9
14.73	12.22	0.29	0.05	15:0.5	17.0	82.8

- It can be seen from a previous study that phosphorus yielded 82-97 percent reduction while the reduction of Nitrogen ranged from 17-59 percent. It can also be observed that the amount of nitrogen and phosphorus in wastewater affects the removal efficiency using *Chlorella Vulgaris.*

Conclusion & Future Work

- Results suggest that when N:P ratio is among 30:24, 21:20, 6:20 and 2:20 in wastewater effluent, nitrogen will likely not be the limiting nutrient during removal. Sufficient nitrogen removal could be achieved when N:P ratio is 1:2.
- Future tests will be done on other N:P ratios and real secondary wastewater effluent to determine what wastewater effluent characteristics provide for the most efficient removal using *Chlorella Vulgaris*.

Acknowledgements

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