Short communication

Algae Biofilm Produced from Anaerobic Digester Wastewater Demonstrated as Biofertilizer for Dwarf Wheat

ABSTRACT

Aims*:* To correlate yield of dwarf wheat fertilized with algae biofilm produced using anaerobic digester effluent (digestate) from a municipal water resource recovery facility as nutrient source and compare with yield using non-algae biofilm based fertilizers and a negative control .

Study design: Greenhouse testing for measurement of biomass yields and statistical analysis of wheat yield data results.

Place and Duration of Study: Department of Biological Engineering Algae Processing and Products Facility greenhouse between March and June, 2024.

Methodology: Yield of a dwarf cultivar of red spring wheat was measured as a function of four treatments including three fertilizers and a negative control: (1) algae biofilm cultivated on anaerobic digester effluent containing, (2) positive control of Osmocote commercial slow release fertilizer, **(**3) anaerobic digester biosolids, and (4) negative control with no fertilizer addition

Results*:* Average yield values (gm) for the four treatments were: (1) 1.25, (2) 0.99, (3) 0.72, and (4) 0.52, respectively. Algae biofilm fertilizer performed significantly better than Osmocote with $P = .002$, anaerobic digester biosolids with $P = .05$, and no fertilizer addition with $P = .05$. Struvite, a slow release fertilizer, was observed to be associated with the algae biofilm as a precipitate of the high concentrations of nutrients within the anaerobic digester water. Struvite ($MgNH_4PO_4 6H_2O$) releases plant available nitrogen and phosphorus over time and is biologically precipitated due to the increase in solution pH as a result of photosynthesis by the algae biofilm.

Conclusion: Algae biofilm cultivated on anaerobic digester wastewater can serve as a biofertilizer for recycling nutrients within the biosphere for sustainable nutrient management.

Keywords*: algae biofilm; struvite; biofertilizer; anaerobic digestate; biosolids*

1. INTRODUCTION

Water resource reclamation facilities (WRRF) across the United States must adhere to strict concentration limits of nitrogen and phosphorus in the water that they release to curtail potential toxic algal blooms in downstream receiver systems. The Sustainable Waste to Bio-Products Engineering Center (SWBEC) at Utah State University has been collaborating with the Central Valley Water Reclamation Facility (CVWRF), the largest WRRF in the state of Utah at 62 million gallons of water per day, and WesTech Engineering in Salt Lake City, Utah, U.S.A. on a demonstration pilot project sponsored by the US Department of Energy Bioenergy Technology Office (BETO). The goal of the project is to determine the feasibility of using Rotating Algae Biofilm Reactors (RABRs) to reduce the nitrogen and phosphorus content of anaerobic digester effluent through the cultivation of algae biofilms. These biofilms are manually harvested for use in producing bioproducts including biofertilizer, bioplastic, and biofuel (Christenson and Sims, 2012; Adeniyi et al., 2018; Gimondo, et al. 2019; Guo, et al. 2020; Onen et al., 2020). During the process of treating anaerobic digester effluent with biofilm microalgae, the spontaneous formation of the crystalline mineral struvite has been observed to precipitate within the algae biofilm structure (Hillman and Sims, 2020; Goldsberry et al., 2023). Struvite is a precipitate of nitrogen, phosphorus, and magnesium (one to one molar ratio) that acts as a slow release fertilizer (Goldsberry, et al., 2023). Other algae biofilm technologies have demonstrated fertilizer potential for biofilm algae cultivated on non-anaerobic digester municipal wastewater (Lindsey et al., 2021). The interaction between plant growth and struvite rich algae biofilm cultivated on anaerobic digester effluent from WRRFs that was identified in this study has not been previously reported in the refereed literature and was the primary goal of this study with potential applications at WRRFs within the U.S. and globally to sustainably recycle nutrients within the biosphere.

The RABR isan outdoor continuously operating system consisting of a set of shelves rotating at approximately 1 rpm on a shaft through nutrient rich wastewater and into the atmosphere to utilize CO_2 and natural diurnal sunlight as an energy source for cultivating microalgae as a biofilm (Goldsberry, et al, 2023). Each shelf has attached a substratum of recycled outdoor corrugated plastic to allow the algae a foothold to grow while the shelves rotate through nutrient rich anaerobic digester effluent. RABRs have been used in the treatment of municipal wastewater (Christenson and Sims, 2012), dairy farm wastewater (Fica and Sims, 2016), water from hydraulic fracking (Wood et al., 2015, 2022), and petrochemical water (Peterson and Sims, 2021; Hodges et al., 2017). Nutrient removal from wastewater can reach as high as 1.2g of nitrogen and 0.21g of phosphorus per square meter of area (Christenson and Sims, 2012). The pilot RABR used in this outdoor two-year demonstration project was a steel tank constructed with a volume of 11,000 liters, 72 m^2 of substratum surface area, a hydraulic retention time of 2 days, and rotation rate of 1 rpm (Goldsberry, et al, 2023) with average phosphorus and ammonia nitrogen concentrations of 50 mg/L and 500 mg/L, respectively.

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Struvite ($NH_4MgPO_4 \cdot 6H_2O$) is a common precipitate in WRRFs with anaerobic digestion treatment that can cause mechanical damage and clogged pipes, often leading to a reduction in capacity in a facility (Ohlinger et al., 1998). Struvite has been used as an effective slow release fertilizer (Kumar and Pal, 2015), and has been shown to be resistant to nutrient leaching (Rahman et al., 2014). Over the timecourse of the SWBEC RABR pilot testing at the CVWRF, struvite was observed to precipitate spontaneously within the mixed culture algae biofilm matrix due to the increased pH within the biofilm due to photosynthesis, low humidity in the atmosphere causing evaporation of water and increase in concentration of NH_3, PO_4^-, and Mg^{+2}, and nucleation sites provided by the corrugated substratum, without the need for chemical addition or physical CO_2-stripping (Goldsberry and Sims, 2023).

2. MATERIALS AND METHODS

2.1 Fertilizers.

Biomass yield experiments were conducted in 2024 at the Utah State University Biological Engineering Department Sustainable Waste-to-Bioproducts Engineering Center Algae Processing and Products Facility greenhouse in Logan, Utah, U.S.A. Four experimental groups were designated: (1) algae biofilm with precipitated struvite harvested from the pilot RABR at the Central Valley Water Reclamation Facility (CVWRF) located in Salt Lake City, Utah (N:P:K 7.6-1.6-0.9); (2) dewatered biosolids from the CVWRF anaerobic digester without algae; (3) (N:P:K 6.1-1.8-0.9); (3) a control of Osmocote commercial slow release fertilizer (N:P:K 14-14-14): and (4) a control with no added fertilizer. The pilot RABR at the CVWRF was inoculated with a naturally occurring mixed culture biofilm microalgae harvested from onsite trickling filters. The biosolids were taken from CVWRF anaerobic digesters and are utilized for land application systems in other parts of the state of Utah. The commercial Osmocote positive control was applied to manufacturer specifications. The Struvite rich algae and dewatered biosolids were applied at a rate to match the nitrogen content of the Osmocote. A negative control was established with no fertilizer addition.

2.2 *Wheat biomass.* Apogee Dwarf Wheat:

Apogee wheat is a dwarf cultivar of red spring wheat developed by the Utah Agricultural Experiment Station at Utah State University. (Bugbee et al., 1999; Bugbee and Koerner, 1999). It was chosen for this experiment because of its low height and growing footprint requirements and resistance to calcium-induced leaf tip necrosis that occurs often in controlled environments. (Bugbee et al., 1999). Apogee Dwarf wheat was established from seed and grown in the SWBEC Algae Processing and Products Facility greenhouse for 10 weeks, separated from the growth media, and dried for 72 hours at 50^{o}C. Yield data was recorded as the mass (gm) of dried biomass for statistical analysis to compare the algae biofilm fertilizer with the other three fertilizer treatments.

2.3 ***Growth media*.**

Growth media consisted of a blend of peat moss, perlite, and soil pep (composted wood bark, leaves and other organic materials) in a 1:1:0.5 ratio to ensure proper water retention, adequate drainage, and appropriate organic matter in the growing media. Additionally, lime was incorporated into the media to ensure a starting pH of 6.5 to ensure nutrient availability. The synthetic soil was mixed and homogenized before treatments were incorporated.

2.4 Statistical analysis of dried biomass data

Dry biomass yield data for each treatment group was measured, including average, maximum, minimum, and the standard deviation for each group was calculated. A Bartlett's Test was conducted to determine if equal variance between each group could be assumed. If the hypothesis were confirmed, then a one way ANOVA test would be conducted. If not, a Kruskal-Wallis test would be conducted to determine if there were significant differences among the yield data sets comparing algae biofilm with each alternative. Then a Welch's T-Test would be conducted comparing the biofilm algae fertilizer against each alternative.

3. RESULTS AND DISCUSSION

Results for dry biomass yield data for each treatment group are presented in Table 1. Average yield values demonstrated that the order of yield from most to least was as follows: algae biofilm > Osmocote > biosolids > no fertilizer addition.

Table 1. Wheat yields as a function of fertilizer application

Metric	Algal Biofilm	Osmocote	Biosolids	No Fertilizer
Nol. of plants	145	145	123	154
Avg. Yield (gm)	**1.25**	**0.99**	**0.72**	**0.52**
Max Yield (gm)	4.46	3.0	1.9	0.97
Min Yield (gm)	0.17	0.01	0.1	0.14
SD	0.80	0.53	0.28	0.15

Bartlett's Test result showed *P= .001*, indicating that variances were not equal between the treatment groups, the hypothesis of equal variances was rejected, and therefore a one way ANOVA test was not employed. Rather, the Kruskal-Wallis Test was employed and indicated a chi square distribution value of 1 indicating that results differed from expected (hypothesis) therefore there were significantly different results among the treatments. Then multiple independent 2-sample Welch's T-Tests were conducted comparing yield values for the algae biofilm fertilizer with each of the three alternatives. Comparison of results of wheat yield using Welch's T-test are presented in Table 2. Results demonstrated that the yield was statistically superior for the algae biofilm fertilizer compared to each of the other three treatments.

Table 2. Statistical analysis of wheat yield results using Welch's T-Test

Treatments Compared	T-Value	Degrees of Freedom	P-Value
Algae Biofilm and CVWRF Biosolids	7.4	184.71	0.05
Algae Biofilm and Osmocote	3.2	251.48	0.002

Algae Biofilm and No Fertilizer Control	10.7	153.07	0.05

Anaerobic digester effluent is conventionally returned to the headworks of a WRRF that adds to the treatment burden for nutrient removal and therefore does not take advantage of transforming a nutrient rich resource into bioproducts. The interaction between plant cultivation as struvite rich algae biofilm cultivated on anaerobic digester effluent as identified in this study has not been previously reported in the refereed literature. The algae biofilm harvested from the pilot RABR and tested as a fertilizer statistically improved biomass yield compared with a commercial fertilizer and compared with conventional biosolids derived from the CVWRF WRRF and therefore can serve as a bioproduct/biofertilizer for recycling nutrients originating from wastewater within the biosphere. The natural precipitation of struvite within the biofilm matrix due to microalgae photosynthesis increasing the pH within the biofilm (Goldsberry et al, 2023) augments both the fertilizer value, as demonstrated in this study, and the technoeconomic value (Watkins, et al., 2023) of the biofilm algae that has been transformed into bioplastic as a bioproduct while decreasing energy costs associated with conventional struvite precipitation from wastewater.

Areas for future research include how to optimize precipitation of struvite within the biofilm as a function of moisture content of the algae biofilm and type of wastewater treated, and also testing different types of vegetation for recreation areas, while potential limitations could include the presence of toxic chemicals (algicides) in industrial wastes and potential accumulation of PFAS chemicals from wastewater into the algae biofilm.

With regards to the broader implications of using biofertilizers in large-scale agriculture for global food security and food safety, biofertilizers are cost-effective and ecofriendly in nature, and their continuous usage enhances soil fertility. Biofertilizers have also been shown to increase crop yield approximately 10–40% by increasing protein contents, essential amino acids and vitamins, and through nitrogen fixation (Daniel, et al., 2022). Also, struvite (MgNH4PO46H2O) releases plant available nitrogen and phosphorus over time (Bhuiyan, et al., 2008).

4. CONCLUSION

Algae biofilm produced from anaerobic digester effluent treating municipal wastewater and cultivated on a rotating algae biofilm reactor was demonstrated as a biofertilizer for cultivation of biomass as dwarf wheat. The algae biofilm fertilizer demonstrated statistically significantly enhanced biomass yield compared with alternatives including a commercial fertilizer, biosolids derived from anaerobic wastewater treatment without algae, and a control of no fertilizer. These results demonstrate the potential for recycling nutrients within the biosphere using biofilm algae cultivated on anaerobic digestate for sustainable nutrient management.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

AUTHORS' CONTRIBUTIONS

Author PW set up the physical testing in the greenhouse, identified the. control fertilizer, conducted the testing, and analyzed the data. Author RCS was the principal investigator on the U.S. Department of Energy project, initiated the concept for the hypothesis and experimental testing approach, and collaborated with PW in the interpretation of the results. Both authors collaborated on the writing, and read and approved the final manuscript.

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