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The Effect of Algal Wastewater Treatment Systems on Microbial Communities

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THE EFFECT OF ALGAL WASTEWATER TREATMENT SYSTEMS
ON MICROBIAL COMMUNITIES

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HONORS SENIOR PROJECT
ACCEPTANCE AND APPROVAL FORM

Alexis Leach
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The Effect Algal Wastewater Treatment Systems have on the Microbial Community
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This senior project has been reviewed by the faculty of the NEIU Honors Program and is found to be in good order in content, style, and mechanical accuracy. It is accepted in partial fulfillment of the requirements of the NEIU Honors Program and graduation with honors.

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ABSTRACT

In collaboration with the Metropolitan Water Reclamation District of Greater Chicago and Gross-Wen Technologies, I examined the microbial communities in experimental tertiary wastewater treatment systems employing algal phytoremediation. Three algal phytoremediation systems were examined: two vertical conveyor belt systems, known as the 10-foot belt system and the series 4 system, and a third system lacking a converter belt in which planktonic algae grew in a control pond. I used EcoPlates to measure carbon substrate utilization by microorganisms in each system. EcoPlates contain thirty-one different carbon substrates, with a dye that indicates microbial use of each substrate. I conducted a Principal Component Analysis which indicated differences in microbial community function in each of the three algal systems. There was higher utilization of polymers in the vertical algal systems than in the control pond. Increased polymer utilization by microorganisms may be indicative of their ability to remove larger organic compounds from wastewater. Understanding the microbial use of carbon substrates can help us further understand the symbiotic relationships between algae and the microbial community in wastewater treatment systems.

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INTRODUCTION

Water reclamation plants use physical[1] and biological[2] treatment processes to decompose organic waste. However, the resulting effluent releases too many nutrients into our natural water systems and could thus change the microbiome of the recipient waters (Taziki et al. 2015, Drury et al. 2013). The physical treatment process involves metal grates or screens that remove large particulate matter but allow smaller, organic waste matter to pass through (Abel 1996). Physical treatment processes are typically used in the primary treatment of wastewater. The primary treatment is responsible for removing inorganic material from the water using a grit chamber and primary settling tank (Abel 1996). The grit chamber collects inorganic sediment at the bottom of the chamber and allows for water to flow into the primary settling tank, where organic matter settles (Abel 1996). The wastewater then undergoes secondary treatment, which involves biological treatment (Abel 1996). The biological treatment used for the secondary treatment involves the activated sludge process which aerates the wastewater to allow for microorganisms to aerobically decompose organic matter (Abel 1996). The remaining wastewater is passed through settling tanks to further remove particulate matter (Abel 1996). The liquid effluent is then released into a nearby water system (Abel 1996).

In Chicago, effluent from the Metropolitan Water Reclamation District (MWRD) treatment plants account for 70% of the yearly flow of the Chicago River, including the North Shore Channel (Drury et al. 2013). This effluent increases the ammonium, nitrate, and phosphate concentrations of water in the North Shore Channel. Downstream of the O'Brien Water Reclamation Plant, ammonium concentrations increased by 171%, nitrate concentrations increased by 2,024%, and phosphate concentrations increased by 13,667%

(Drury et al. 2013). Wastewater effluent also affects bacterial communities in sediment. For example, wastewater effluent caused a decline in *Chloroflexi*, a phylum of anoxygenic phototrophic bacteria, in the sediment of the North Shore Channel (Drury et al. 2013). The decrease in the relative abundance of *Chloroflexi* suggests that these organisms may be sensitive to some component of the effluent since they should be thriving in environments with high nitrogen and phosphorus. Overall, bacterial species richness and cell density decreased downstream, but the bacteria that survived had increased metabolic rates (Drury et al. 2013).

The increased levels of nutrients in rivers can also cause algal blooms. After the algae die, decomposition of this excess organic matter depletes the dissolved oxygen from the water. The dwindling oxygen levels can cause aquatic animals to suffocate due to lack of oxygen (Taziki et al. 2015), leading to eutrophication. Because the Chicago River eventually flows into the Mississippi River, which flows into the Gulf of Mexico. In the Gulf of Mexico, the eutrophication[3] ultimately creates a hypoxic area, also known as a dead zone (US Department of Commerce 2019).

One way excess nutrients can be removed from wastewater is algal phytoremediation.[4] Although algal blooms pose a threat to natural water systems, algae can be used to benefit sewage treatment processes. Through the process of algal phytoremediation, pollutants are naturally absorbed out of the wastewater by freshwater algae. The algae remove dissolved nutrients, like nitrogen and phosphorus, from the water as they grow (Cai et al. 2013). Algal-bacterial relationships are also important. For example, bacteria can convert inorganic nitrite, which algae cannot use, into nitrate and ammonium, which algae are able to take up (Cai et al. 2013). My research objective was to determine the impact of algal phytoremediation on microbial communities. Specifically, I determined which types of carbon substrates were utilized the most by microbial communities exposed to different types of algal phytoremediation treatments.

METHODS

In the greenhouse of the O'Brien Water Reclamation Plant, a Skokie, Illinois facility that is part of the Metropolitan Water Reclamation District of Greater Chicago (MWRD), two Revolving Algal Biofilm (RAB) systems, known as the 10-foot system and the series 4 system, were used for phytoremediation of wastewater. The RAB systems consist of vertical belts on which benthic algae[5] grow. The algae grow attached to the belts and receive nutrients when submerged in the wastewater and receive carbon dioxide when exposed to air (Gross et al. 2015). Motors rotate the belts that the algae are attached to in and out of the water. The 10-foot system has a vertical 10-foot belt that rotates through a single holding tank that contains wastewater. The series 4 system, in which wastewater moves from one tank to the next, contains four holding tanks, each with its own rotating belt. The greenhouse also has a control pond, which is a tank that does not have a rotating belt and in which planktonic algae[6] grow suspended in the water column, rather than attached to a belt.

Water samples were collected from each of the three algal systems every two weeks from January 2021 through July 2021. 10-mL samples of the incoming water (influent) and the water leaving each algal system (effluent) were taken to the lab at Northeastern Illinois University, where the microbial communities in the water samples were analyzed with Biolog™ EcoPlates. EcoPlates contain 31 wells, each with a different carbon substrate, such as a carbohydrate or amino acid (Table 1). Each well also contains tetrazolium dye, which develops color as bacteria utilize a carbon substrate. The darker the dye appears, the higher the microbial metabolism of that specific carbon substrate. After 96 hours, the color well development was measured with a spectrophotometer to indicate which carbon substrates were utilized by microbial communities within each algal phytoremediation system. A Principal Component Analysis (PCA) was performed to analyze the differences in microbial community functions based on the utilized carbon substrates.

Calculations of Average Well Color Development (AWCD) were completed using the formula **AWCD = [Σ (R - C)] / n,** where **C** represents the absorbance value of control wells, and **R** represents the mean absorbance of the response wells. AWCD was used to determine which algal phytoremediation system supported the highest metabolism of carbon substrates in microbial communities. An analysis of variance (ANOVA) and t-tests were used to indicate if there were significant differences in AWCD in the influent sample and each of the three effluent samples. Tests were completed using R and Excel. P-values of less than 0.05 indicated statistical significance.

Table 1: Carbon substrates of Biolog™ EcoPlates wells (Poyraz & Mutlu 2017).

Well	C-source	Group	Well	C-source	Group
A1	Water	-	**A3**	D-Galactonic acid-γ-lactone	Carboxylic and Acetic acids
B1	Pyruvic acid methyl ester	Carbohydrates	**B3**	D-Galacturonic acid	Carboxylic and Acetic acids
C1	Tween 40	Polymers	**C3**	2-Hydroxybenzoic acid	Carboxylic and Acetic acids
D1	Tween 80	Polymers	**D3**	4-Hydroxybenzoic acid	Carboxylic and Acetic acids
E1	α- Cyclodextrin	Polymers	**E3**	γ-Hydroxybutyric acid	Carboxylic and Acetic acids
F1	Glycogen	Polymers	**F3**	Itaconic acid	Carboxylic and Acetic acids
G1	D-Cellobiose	Carbohydrates	**G3**	α-Ketobutyric acid	Carboxylic and Acetic acids
H1	α-D-Lactose	Carbohydrates	**H3**	D-Malic acid	Carboxylic and Acetic acids
A2	β-Methyl-D-glucoside	Carbohydrates	**A4**	L-Arginine	Amino acids
B2	D-Xylose	Carbohydrates	**B4**	L-Asparagine	Amino acids
C2	i-Erythritol	Carbohydrates	**C4**	L-Phenylalanine	Amino acids
D2	D-Mannitol	Carbohydrates	**D4**	L-Serine	Amino acids
E2	N-Acetyl-D-glucosamine	Carbohydrates	**E4**	L-Threonine	Amino acids
F2	D-Glucosaminic acid	Carboxylic & Acetic acids	**F4**	Glycyl-L-glutamic acid	Amino acids
G2	Glucose-1-phosphate	Carbohydrates	**G4**	Phenylethylamine	Amines and amides
H2	D, L-α-Glycerol phosphate	Carbohydrates	**H4**	Putrescine	Amines and amides

RESULTS

The EcoPlate data, which indicate use of carbon substrates by microbes, showed differences in the effluent leaving each algal treatment system (Figure 1). In the Principal Component Analysis (PCA), each point on the graph represents a water sample collected from the effluent leaving the algal treatment systems. Points that are closer together indicate samples with microbial communities that are more similar in their carbon substrate utilization. On the other hand, points that are far away from each other mean that the communities have fewer or no similarities. The communities are distinct from each of the algal systems since the data points from the 10-foot system are mostly located on the right side of the graph, and data points from the control pond and the series 4 system are located mostly on the left side of the graph (Figure 1). Furthermore, most data points for the series 4 system are located at the top of the graph, whereas data points from the 10-foot system and the control pond are located at the bottom of the graph (Figure 1). In summary, the microbial communities from the same algal system utilized similar carbon substrates, and microbial communities from different algal systems had fewer similarities in carbon substrate utilization.

Polymers were utilized more in all three systems when compared to carbohydrates, carboxylic/acetic acids, amino acids, and amines/amides (Figure 2). The data indicate that the RAB systems affected the polymer usage by microbes. Polymer utilization was significantly greater in the effluent of the 10-foot and series 4 systems than in the influent (Figure 3, $p = 0.0034$). There was not a significant difference in polymer usage between the influent and the effluent of the control pond.

DISCUSSION

In this study, I investigated the impact algal phytoremediation has on microbial communities in human wastewater. The microbes convert unusable nutrients into nutrients that the algae can use, and they can also remove nutrients out of wastewater themselves. According to research by Zhao et. al (2018), the RAB systems were significantly more effective at removing nitrogen and phosphorus from wastewater when compared to the control pond, removing 60-90% of nutrients from wastewater at the O'Brien Water Reclamation Plant. The RAB systems may also remove heavy metals from wastewater and store heavy metals in algal and microbial biomass. Trace amounts of cadmium and arsenic were found in the biomass of the RAB systems (Zhao et al. 2018). Microbial communities may be contributing to removal of both nutrients and heavy metals from wastewater.

To better understand the microbial communities in the RAB systems, I used EcoPlates to examine the types of carbon substrates utilized by the microbes. The microbial communities utilized polymers more than carbohydrates, carboxylic/acetic acids, amino acids, and amines/amides (Figure 2). In contrast, Poyraz and Mutlu (2017) found that carbohydrates were the most utilized substrate by microbes. They used Ecoplates to examine microbial function at several steps of the sewage treatment process, including a fat/sand removal unit, activated sludge unit, secondary clarifiers unit, return-activated sludge pump, and the anaerobic digester unit (Poyraz & Mutlu 2017). My results may have differed from theirs because the RAB systems I studied utilized algae. I found that the microbial communities had high polymer utilization, rather than high carbohydrate utilization.

The EcoPlates included several types of polymer substrates (Table 1). Some of those polymers are natural, such as glycogen, and others are synthetic and potentially toxic to the aquatic ecosystem, such as Tween 40, which functions as an emulsifier (Oxford n.d.). Tween 40 is known to affect some species of fish, 50% lethality (LC-50) at doses greater than 1g (Oxford n.d.). If microbes are consuming toxic carbon substrates in wastewater effluent, this may help mitigate water pollution in water systems receiving the treated sewage effluent.

The principal component analysis revealed that the microbial communities in the 10-foot system, series 4 system, and the control pond differed from one another based on carbon substrate utilization (Figure 1). Use of polymers may have contributed to the differences in the microbial communities across all phytoremediation systems. Microbes in the effluent of both the 10-foot and series 4 systems utilized significantly more polymers than microbes in the influent (Figure 3). There was not a significant difference in polymer utilization between the control pond and the influent.

EcoPlates measure utilization of carbon substrates by microorganisms, but cannot be used to identify particular taxa. Drury et al. (2013) found that proteobacteria had the highest overall abundance in wastewater, accounting for over 50% of variation in the microbial community. Through direct fluorescence counts, *Bacteroidetes* were found to be the most common proteobacteria found in the water samples. *Bacteroidetes* are common in freshwater ecosystems and are known to degrade organic compounds, including petroleum hydrocarbons (Drury et al. 2013). It is also possible that carbon substrate utilization measured by EcoPlates could be due to fungi growing within the phytoremediation systems. In a study performed by Gałązka and Grządziel (2018), carbon substrate utilization by both bacteria and fungi found in soil were assessed using EcoPlates. Fungi may play an important role in wastewater treatment. According to Pini and Geddes (2020) mycelia can remove over 99% of *Escherichia coli* from water systems.

My study was the first to look at the microbial community functions of algal phytoremediation systems. Although we know that there are different microbial communities found in each of the systems as they pertain to carbon substrate metabolism, more research is needed to determine the microbial taxa. Identifying the taxa may help us understand the relationship between algae and microorganisms, which we can use to further remove toxins, such as synthetic medications and heavy metals, from wastewater. Future research could also examine the relationships between algae, bacteria, and fungi in the algal biomass when using RAB systems to remove pollutants from wastewater.

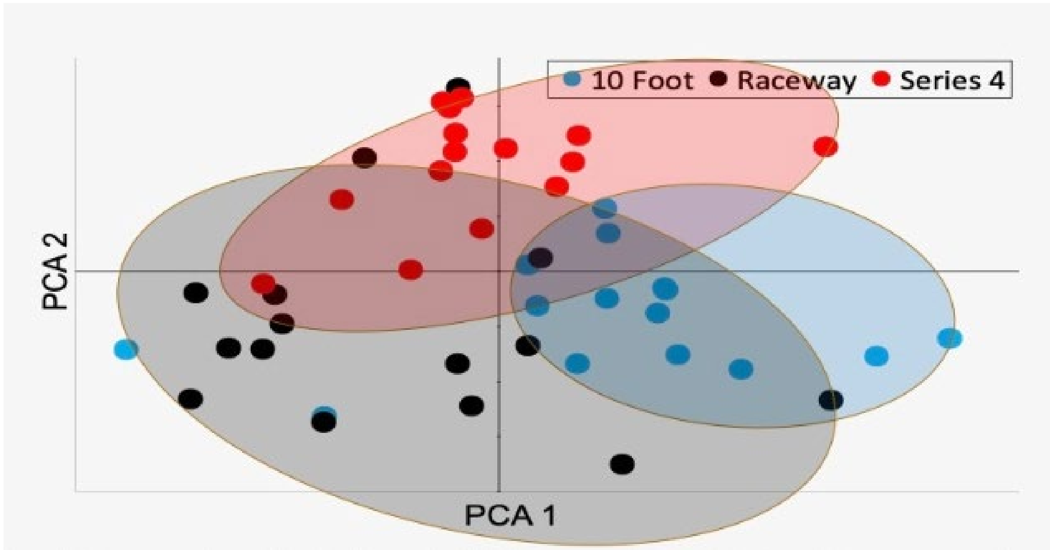

Figure 1: Principal Component Analysis (PCA) of water samples from the 10 foot, series 4, and control (raceway) pond systems by analyzing carbon substrate utilization using EcoPlates (Table 1). The closer points are to each other, the more similar the sample is. There are distinct microbial communities found in each of the algal systems. PCA 1, located on the x-axis, represents 38% of variation in the data. PCA 2, located on the y-axis, represents 10.6% of variation in the data.

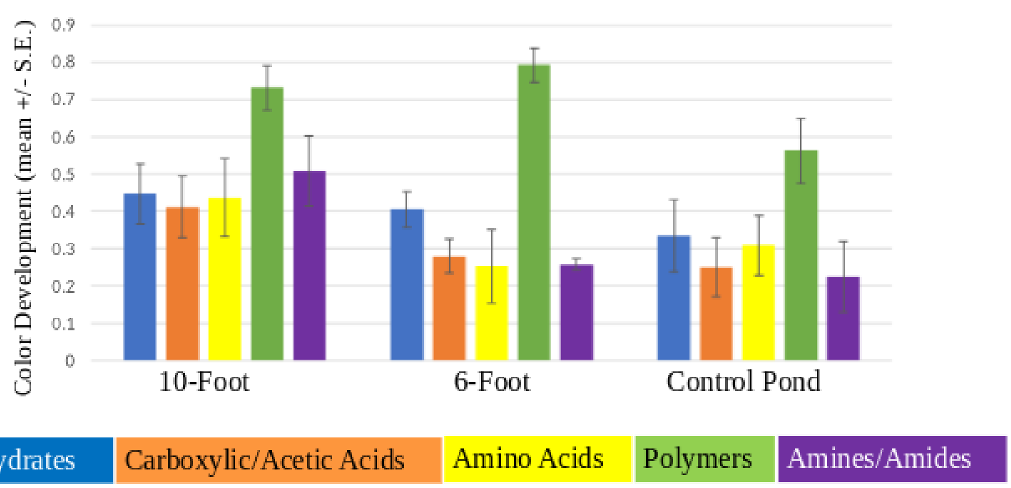

Figure 2: Average well color development (AWCD) of carbon substrates in the categories of carbohydrates, carboxylic/acetic acids, amino acids, polymers, and amines/amides from EcoPlates in each wastewater treatment system (Table 1). Color development is measured on the y-axis, and sample locations are located on the x-axis.

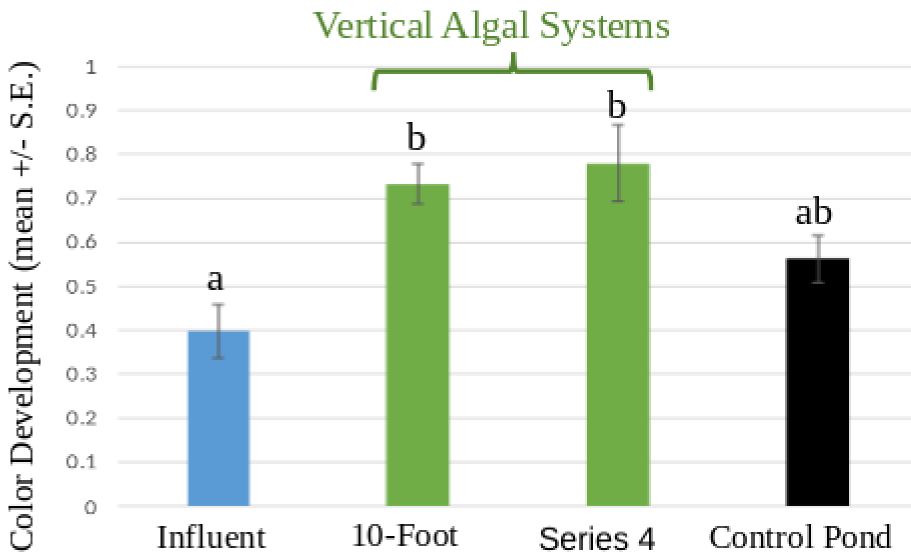

Figure 3: Polymer utilization as measured by color development in EcoPlates of water samples obtained from the influent, 10-foot system, series 4 system, and control pond with color development measured on the y-axis and sample locations located on the x-axis. There was a significant increase in polymer utilization in the 10-foot system and series 4 system when compared to the influent ($p = 0.0034$).

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DEFINITIONS

[1]physical treatments: a primary wastewater treatment method that involves filtering out solids from the wastewater by the use of screens.

[2]biological treatments: a secondary wastewater treatment method that primarily relies on bacteria to break down organic waste. This process is often coupled with aerating the water to promote microbial cellular respiration.

[3]eutrophic: enriched in dissolved nutrients, which stimulates excessive plant growth and removes dissolved oxygen from water systems.

[4]algal phytoremediation: the process of decontaminating water using algae.

[5]benthic algae: plant-like algae that commonly grow on hard surfaces, such as rocks.

[6]planktonic algae: microscopic algae that is free-floating in water systems.