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Turning Algal Biomass Waste into a Sustainable Substrate for Oyster Settlement

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in
Biology

By
Ariana Lipat

Under the mentorship of Dr. Jennifer Zettler

ABSTRACT

When an aquatic ecosystem becomes oversaturated with nutrients, algae in the water utilize the excess nitrogen and phosphorus present and grow uncontrollably. This creates algal blooms on the surface of the water that deplete oxygen levels in the water and kill numerous organisms in the process. One method used to solve this issue is through Algal Turf Scrubber (ATS) technology: a natural wastewater treatment process in which water polluted with excess nitrogen and phosphorus is pumped across a flowway to be absorbed by a culture of algae before it flows out of the system. The algal biomass is harvested periodically but can itself be a waste product that is sent to landfills. It was observed that when the biomass dries, it forms a clay-like brick. The purpose of this project was to find a potential use for this dried waste product, namely in making a suitable substrate for oyster settlement and growth. The raw algal biomass was poured into plaster molds to manufacture pre-formed shapes, and were set out on a floating dock along with concrete and tile controls to determine its suitability for settlement. After a period of time, the substrates were collected, and the masses of the settled organisms on each condition were measured and compared. The results indicate that although oysters were not observed in the organism collection, the algal waste biomass can be used to create a suitable substrate for settlement of various aquatic fouling organisms.

Thesis Mentor: *Dr. Jennifer Zettler*
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Introduction

In oceans, lakes, and other bodies of water, the presence of certain nutrients is vital in order for an ecosystem to exist. However, when an ecosystem becomes oversaturated with these nutrients, namely phosphorus and nitrogen, the result is a detrimental effect on the other organisms in the water. Algae in the water utilize these nutrients and grow uncontrollably-- creating algal blooms on the surface and throughout the water column. The overly-abundant algae consume most of the oxygen dissolved in the water resulting in hypoxic conditions that cause the death of various aquatic organisms that depend on oxygen for survival. Furthermore, these algal mats prevent sunlight from penetrating the water's surface and reaching other photosynthetic organisms in the water. One method used to solve this issue is through Algal Turf Scrubber (ATS) technology. ATS is a natural wastewater treatment process in which algae is cultured on the surface of a sloped flowway. Water polluted with excess nitrogen and phosphorus is then pumped across the flowway, allowing algae to uptake nutrients before

water flows out of the system. The algal biomass is harvested periodically but can itself be a waste product that is sent to landfills (Craggs, 2001).

In efforts to find a more sustainable use for the algal biomass, it was observed that when dried, blended, and rehydrated, the algae's texture was similar to that of clay and can be turned into ceramic products when fired in a kiln. The goal of this research was to determine if processed algal biomass has a possibility to be used as a suitable substrate for oyster settlement and subsequent growth of the oyster population in the coastal Georgia area. Oftentimes efforts to restore oyster beds are limited by the availability of suitable substrates. Bagged oysters are ideally used for these efforts, however they require months of drying to ensure that any pathogens are removed before the recycled shells are utilized. Furthermore, concrete reefs that are commercially manufactured use costly synthetic materials. As a result, efforts have been made to determine the ideal alternative substrate for the settlement of larval oysters, known as spat. Of the articles found, none mention the use of an algae-derived substrate, however, porcelain and ceramic tile have been shown to provide favorable sites for spat recruitment and growth. Goelz *et al.* (2020) found that porcelain had recruitment and growth analogous to natural reefs, as well as resistance to degradation as opposed to calcium carbonate structures. A field study in Ghana showed that ceramic tile was the most effective substrate for *Crassostrea tulipa* spat collection (Chuku *et al.*, 2020). Both studies outlined positive results from clay-derived materials in comparison to other substrates tested upon. The algae harvested from the turf scrubbers naturally hardens into a dense clay-like brick as it dehydrates, and resembles the texture of terra-cotta when fired. For this study, the aim was to create a substrate with the dried biomass that takes the least amount of processing

and refining yet results in comparable spat attachment and growth to that on ceramic tile and porcelain materials.

Methods

Harvesting the Algae

The algal biomass was sourced from an ATS system at the SKIO Priest Landing pier in Savannah, Georgia (Figure 1). For collection, the pipe supplying the running ocean water was temporarily turned off, and a squeegee was used to collect the algae into five gallon buckets. It was critical to scrape enough algae for the project, but leave little enough that there was still a culture of algae present to keep the system functioning properly. If properly scraped and left alone, the algae would regrow to its original state within days. The algae was observed under the microscope, and it was noted that there was a significant amount of diatoms present in the culture (Figure 2). It is assumed that the silica cell wall present in diatoms contributes to the clay-like integrity of the ATS algae biomass when dried and fired in a kiln.



Figure 1. Algal Turf Scrubber (ATS) system at Priest's Landing docking site showing the flowways covered in algae and other benthic marine organisms.



Figure 2. ATS algal biomass under the microscope at 100x magnification showing a diversity of unicellular diatoms and filamentous algae in the field of view.

Making the Pucks

Three types of algal pucks were manufactured, each with different levels of processing: pure algae, powdered algae, and powdered algae+clay mixture. The pure algae ‘sludge’ pucks were made by casting unprocessed ATS biomass into a plaster of paris mold with circular depressions created from impressions with standard Petri dishes (95 x 15 mm), then air dried (Figure 3). For the powdered algae, the raw ATS material was evenly spread onto an aluminum tray and set in a low-fire oven at 65 °C until completely dry. The dried biomass was then crushed into small pieces and powdered via a kitchen blender. To form the puck, the powdered algae was rehydrated until a clay-like consistency was reached then pressed into a glass Petri dish and incubated at

37°C until dry (Figure 4). The rehydrated powdered algae was also mixed with commercially-available clay from Lizella, GA in a 1:1 ratio, forming the algae+clay mixture. This mixture was then pressed into the same plaster of paris mold used for the pure algae pucks and allowed to air dry. All pucks, once dry, were fired in an electric ceramic kiln to a cone 06 bisque temperature of 1000°C to result in a solid puck.

For a control, small 5 x 5 cm pieces of ceramic tile as well as pucks crafted from concrete were included since both are typical substrates used for oyster restoration projects (Figure 5). The ceramic tile was commercially purchased (Premium Mosaics™), was pre-sized, and made of primarily limestone. The concrete pucks were made using a 1:1 ratio of QUIKRETE® and water, and pouring it into the same plaster molds used to form the algae pucks.

A preliminary laboratory experiment was run to determine if the pucks would withstand natural oceanic conditions while in suspension. To determine if the ceramic pucks would withstand submersion in field conditions, a 10-gallon salt water tank was set up at a salinity of 30 and pucks were suspended from a wooden dowel (Figure 6) An aquarium bubbler was run to simulate movement of water current. Results from this experiment showed that that little dry mass would be lost in the duration that the pucks would be exposed to field conditions (Figure 6).

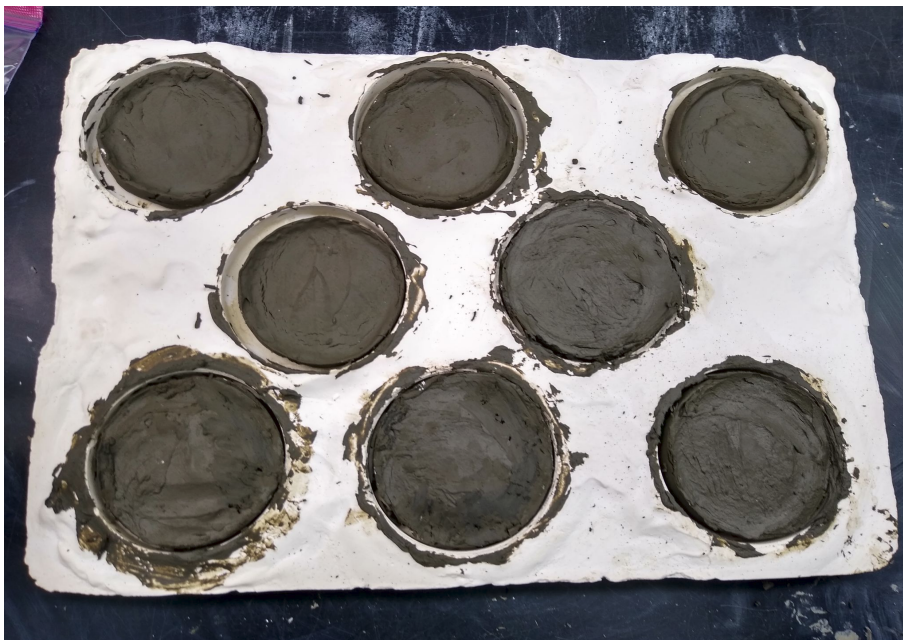


Figure 3. Raw ATS algal biomass poured into plaster of paris mold.



Figure 4. Dried algae that has been pulverized into a fine powder and clay like-ball derived from rehydrating it.



Figure 5. Collection of substrates to be used in field experiments. The top row is limestone tiles, the second row is raw ATS algae pucks that had been dehydrated and fired, and the bottom row is the Quick-crete pucks.

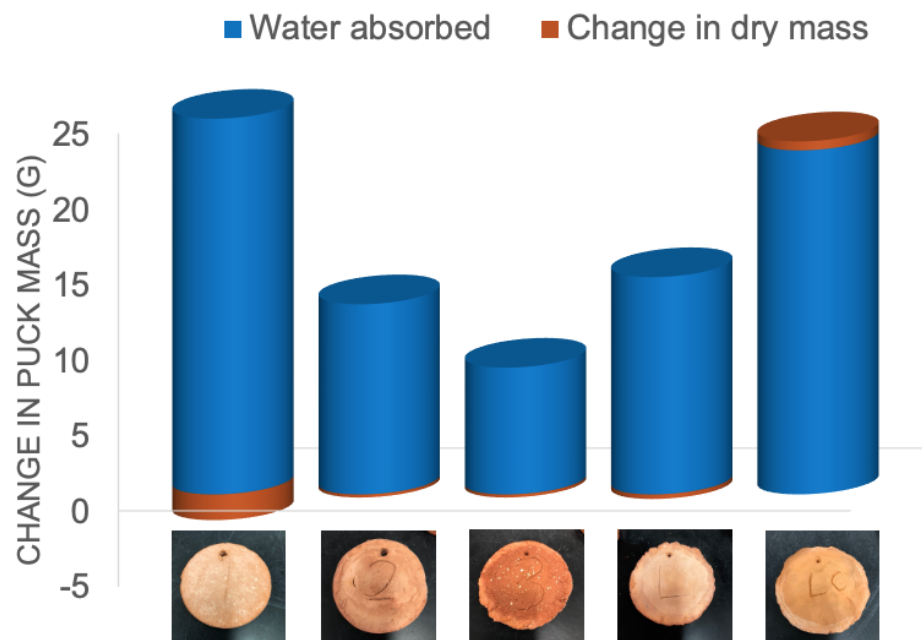


Figure 6. Results showing water absorption and dry mass lost in ceramics after soaking.

Field Set-up and Collection of Data

Five sets of three pucks were set out on a private residential floating dock on the Lincoln River in Richmond Hill, Georgia. Each set had one concrete puck, one pure-algae puck, and one limestone tile in a randomized order. The powdered algae pucks and clay+algae pucks were set out in a pilot study, however, results from that experiment could not be analyzed because most of the pucks were lost when their attachment strings corroded in the weeks they were submerged in the water. In this experiment, solely raw-algae sludge pucks were used because these pucks were made without any processing (i.e. over dying or blending). The suspension set up consisted of nylon rope and zip ties to secure and hang the pucks. Additionally, bird netting (Vigoro brand polypropylene) was wrapped around each puck to create an individual casing for each (Figure 7). The pucks were set out on August 5th, 2022 for approximately 8 weeks, with weekly observations on colonization. The pucks were recovered on September 28th, 2022, and stored in the lab freezer until data collection.



Figure 7. One out of five suspension setups put out on the dock.

Images of the pucks were taken to record diversity of organisms with a noticeable exoskeleton, then all pucks were placed in a low-fire oven to dehydrate. Upon drying, the pucks were weighed, scraped of all attached organisms, then re-weighed. The differences between the pre-scraped and post-scraped masses were calculated to represent the amount of organisms present on each condition. A One-Way ANOVA was initially performed to determine if the mean differences were statistically significant. However it was observed that the data violated the assumption of normality. Consequently the non-parametric version, the Kruskal-Wallis test was run and followed by Bonferroni correction analysis.

Results

Upon observation, all substrates successfully recruited fouling organisms (Figure 8). However, no settled oysters were observed among any of the substrates. The species that colonized the substrates were relatively even among all conditions, mostly consisting of barnacles, mussels, and bryozoans. Although all substrates successfully recruited, it was noticeable to the eye that the algae pucks recruited a greater abundance of organisms than either controls (Figure 8). Results from running the Kruskal-Wallis H test indicated that there is a significant difference in the pre-scraped minus post-scraped puck masses between the different groups, $\chi^2(2) = 7.38, p = .025$, with a mean rank score of 12.25 for Algae, 6.1 for Quick-Crete, and 5.1 for Ceramic Tile. The Post-Hoc Dunn's test using a Bonferroni corrected alpha of 0.017 indicated that the mean rank of the algae condition and the ceramic tile condition is statistically significant (Figure 9).



Figure 8. Images of representative substrates and the recruitment and diversity on each. From left to right: Quick-Crete puck, Algae puck, and Ceramic tile.

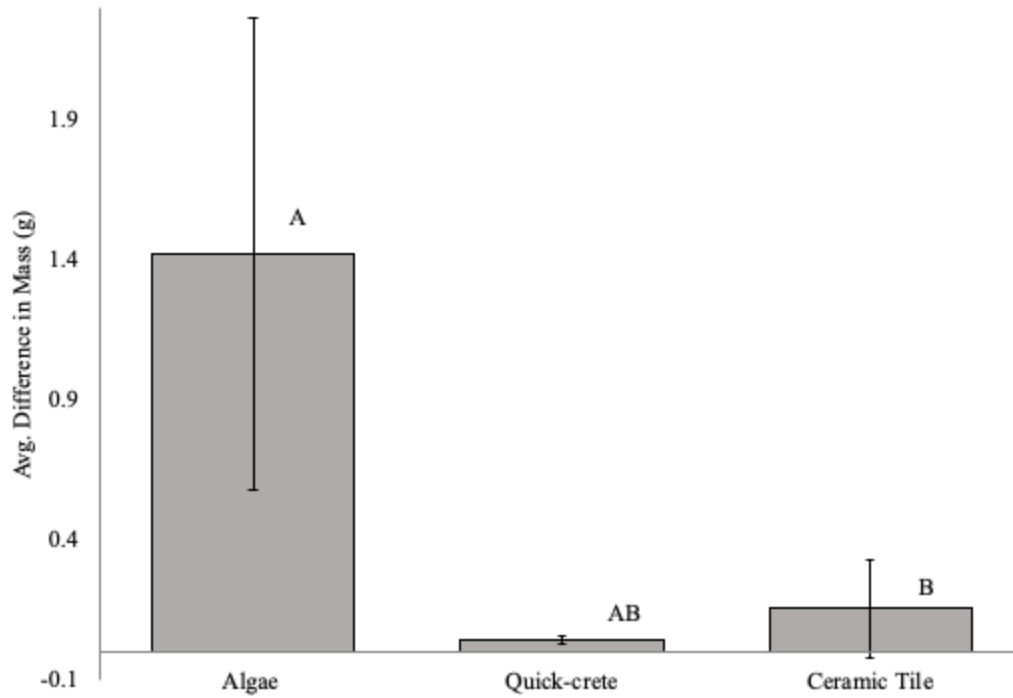


Figure 9. Average mass (g) of fouling organisms scraped from pucks made from algae, Quick-crete, and commercial ceramic tiles. Error bars represent standard error of the means. A Kruskal-Wallis H test indicated that there is a significant difference in the amount of growth on the different substrates ($\chi^2(2) = 7.38$, $p = .025$). A Post-Hoc Dunn's test using a Bonferroni corrected alpha of 0.017 shows significant differences between growth on pucks made from the three substrates. Columns with the same letter represent non-significant differences and those with different letters are significantly different.

Discussion

This project aimed to create a sustainable substrate for oyster recruitment and growth from ATS algal biomass. The results from the field experiments indicate that the algae-derived substrate is not only a suitable material for fouling organisms to settle upon, but also had higher overall colonization success when compared to the concrete and ceramic controls. Upon observation, no oysters were present on any substrate after approximately eight weeks of being submerged. There were frequent downpours during the month of August, and the emergence of Hurricane Ian in September. It is known that oysters prefer brackish and salty water (Chesapeake Bay Company, n.d.), so their lack of prevalence may have been influenced by a salinity change in the water from the abundant rainfall. While surface salinity levels at the site were checked weekly with a refractometer and measured between 32-35 salinity, rainfall events can quickly change salt concentrations in the estuaries. Thus the organisms accounted for were condensed to fouling organisms with a noticeable exoskeleton made out of calcium carbonate or a similar type of structure that could be scraped off to represent its presence. All substrates recruited these types of organisms, but the algae pucks showed a higher density of colonization. The results observed from the Kruskal-Wallis test showed a quantified representation of the same conclusion. The algae substrate was derived from a marine environment, as opposed to the higher processed control substrates. The fouling organisms may have found that the algae-derived pucks were more akin (in chemical nature) to natural reefs and thus were more attracted to settle on them. Another factor could have been the texture of the substrates. The algae pucks were rough and had a fair amount of wrinkles and crevices. The quick-crete pucks were smoother but still had some

crevices, and the ceramic tiles were smoother and did not have any raised surfaces for organisms to hide in. The Bonferroni analysis specified that the significant distinction was between the algae pucks and the ceramic tiles, so this difference in consistency and normalness between the substrates may have contributed to the effectiveness of recruitment and growth.

Ultimately this project outlines how solutions to environmental problems are a good start, but not a stopping point. The ATS system prevents eutrophication, but produces waste that often goes straight to landfills. There is always a potential problem associated with the solution, and a potential solution for the problem. The creation of a substrate from the ATS biomass for restoration projects was the focus of this project, but its potential is diversified. Discussions of future projects include its use in making a proper medium for ceramic art products, either on the wheel or through a 3D-printing machine. There is also the possibility that the biomass can be used in creating artificial reefs to provide shelter, aid in restoration of natural reefs, and essentially create a good foundation necessary for increased biodiversity and a productive aquatic environment. Above all, this project serves as an added foundation for future research in sustainability, and it is hoped that its concepts will extend to major commercial manufacturers.

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