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MERCENARIA MERCENARIA

ABSTRACT

Algal harvesting is one of the costliest portions of algal biofuels production and finding an alternative is critical to bringing this type of biofuel to the market. This research tested the viability of using the hard clam *Mercenaria mercenaria* as an alternative algal harvesting technique which converts the algal cells to a partially digested excrement called biodeposits. It was found that increasing the clam density increased the biodeposit rate. Algal density had an impact on the biodeposit rate, but no maximum was found. The presence of ammonia increased the biodeposit production rate within the four hour test period. The mineral composition shows the clams sequester metals, CNHS does not differ from the original algae, ash content and lipid content increased 59% and 63%, respectively, in biodeposits relative to original algae. This is needed in assessing the potential in using biodeposits as a feedstock for further downstream processes.

ALTERNATIVE ALGAL HARVESTING TECHNIQUE WITH THE USE OF THE
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by

ERIC ROY MYERS

(Under the Direction of K.C. Das)

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Algal harvesting is one of the costliest portions of algal biofuels production and finding an alternative is critical to bringing this type of biofuel to the market. This research tested the viability of using the hard clam *Mercenaria mercenaria* as an alternative algal harvesting technique which converts the algal cells to a partially digested excrement called biodeposits. It was found that increasing the clam density increased the biodeposit rate. Algal density had an impact on the biodeposit rate, but no maximum was found. The presence of ammonia increased the biodeposit production rate within the four hour test period. The mineral composition shows the clams sequester metals, CNHS does not differ from the original algae, ash content and lipid content increased 59% and 63%, respectively, in biodeposits relative to original algae. This is needed in assessing the potential in using biodeposits as a feedstock for further downstream processes.

INDEX WORDS: Biodeposit Harvesting Algae Biofuels

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DEDICATION

This thesis is dedicated to my family and friends that helped make this journey possible.

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CHAPTER 1

INTRODUCTION

1. Microalgal biofuels

Microalgal biofuels have become a significant topic because of algae's ability to sequester carbon dioxide and grow in any type of water containing nitrogen and phosphorus. With its fast growing ability, algae can be turned into bio-oil and used in place of petroleum or used in an anaerobic digester to produce bio-methane (Dębowski et al., 2013; Frigon et al., 2013; López Barreiro et al., 2013; Sawayama et al., 1999). The amount of algal oil that could be produced vastly outperforms the next three best oil crops of oil palm, coconut, and jatropha (Chisti, 2007). Algae are also advantageous because of their ability to grow on non-arable land hence not competing with food crops. Diverse taxa of algae can grow in freshwater, saltwater, brackish water, or any sort of waste water. These versatile advantages make algal biofuels an attractive option for the future.

1.2 Algal harvesting techniques

Typically in large scale cultivation, microalgae grow to densities of 500-800 mg L⁻¹. There are multiple ways to harvest algae, and although new methods are being researched and tested, the most commonly used methods are centrifugation, gravity sedimentation, filtration, dissolved air flotation, and flocculation (Brennan and Owende, 2010; Milledge and Heaven, 2013; Molina Grima et al., 2003). With these methods there are some drawbacks resulting from the large volume of water being processed and the small size of algal cells. Drawbacks of these methods are that they are capital intensive, energy intensive, can easily be clogged (in filters),

require a lot of time to harvest, and the need for additional chemicals (Ives and Bernhardt, 1995; Nurdogan and Oswald, 1996; Sukenik et al., 1988; Zhang et al., 2010).

Algal species commonly used for biofuels, such as *Nannochloropsis oculata*, *Chlorella vulgaris*, and *Dunaliella tertiolecta* range in size between two and 18 microns and their small size makes it difficult to harvest biomass efficiently (Butcher, 1959; de Grooth BG, 1985). Harvesting something this small from large volumes of accounts for 20% to 30% of the total production cost of algae (Gudin and Therpenier, 1986). The need for an alternative method to alleviate the drawbacks of the current ways to harvest algal cells is well recognized.

1.3 Novel harvesting technique

A novel harvesting technique was developed using the hard clam *Mercenaria mercenaria* that consumes the algal cells and converts them into larger size pseudofeces that are easier to harvest. Clams are natural filter feeders, siphoning water for food (e.g. algal cells) to satisfy their metabolic processes. During filter feeding they expel out of their siphon both digested (feces) and undigested (pseudofeces) materials as a flocculated material that is typically much larger than the single algal cells. This flocculated material is referred to as “biodeposits”. These biodeposits could then be easily removed from the culture and used in downstream processes such as anaerobic digestion, pyrolysis, and hydrothermal liquefaction.

1.4 Ammonia toxicity

Algal growth requires nitrogen as a key macronutrient, in the form of ammonia or nitrate, but ammonia is the preferred form due to its lower cost and availability. Temperature and pH control the equilibrium between ionized ammonium and the more toxic unionized NH_3 (Emerson et al., 1975). Many studies have been conducted on the toxicity of ammonia at different temperatures and pH on different freshwater species. Unionized ammonia is very toxic to

freshwater clams, ranging from 0.11 mg L⁻¹ for *V. iris* to 0.8 mg L⁻¹ for *C. fluminea* (Cherry et al., 2005). Marine species have much higher tolerances to ammonia due to salinity, temperature, and pH, which is evident with *M. mercenaria* being able to withstand 9.3 mg L⁻¹ of unionized ammonia (Boardman et al., 2004; Bower and Bidwell, 1978). The only true fixed production variable would be salinity, as temperature fluctuates on diel cycles and pH increase in response to daytime peaks in algal photosynthesis and decreases from algal respiration. Ammonia toxicity is a primary concern for algal harvesting using clams because these daily increases in pH and temperature fluctuating easily in outdoor cultivation shift the ammonia to the more toxic unionized form.

This work is built on the preliminary design and evaluation of bivalve based algal harvesting that is ongoing at the University of Georgia. Specifically, in this thesis, we conducted experiments to document the performance of the clam-harvesting system under different operating conditions so as to understand the relationships between operating conditions and biodeposit production rates. We had the following four goals:

Research Objectives

1. Determine the impact of clam densities on biodeposit production rates from *N. oculata*.
2. Determine the impact of algal densities on biodeposit production rates.
3. Determine the impact of the presence of ammonia on biodeposit production rates.
4. Determine the chemical and biochemical composition of the biodeposits produced from *N. oculata* including C, N, H, S, minerals and micronutrients, and lipid and ash contents.

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CHAPTER 2

LITERATURE REVIEW

1. Methods of harvesting algae from culture vessels

There are currently many ways to harvest algae, such as centrifugation, gravity sedimentation, filtration, dissolved air flotation, and flocculation (Amin, 2009; Brennan and Owende, 2010; Milledge and Heaven, 2013; Molina Grima et al., 2003; Muñoz and Guieysse, 2006; Shelef et al., 1984). A new method, albeit with small volumes, that is currently being researched is ultrasound, or using high frequency waves to agglomerate the biomass (Bosma et al., 2003). While this list is not exhaustive, these are currently the main ways that algae is harvested on a large scale. Unfortunately, each method has its own drawbacks. For example, centrifugation is capital and energy intensive, requiring constant feeding with large volumes of rather dilute concentrations of algae (Molina Grima et al., 2003; Sander and Murthy, 2010). Gravity sedimentation can take hours or days depending on algal culture density (Nurdogan and Oswald, 1996). Filtration screens can get clogged with high algal densities or can be ineffective if the algal culture is too dilute, along with the increase in energy consumption to force water through partially clogged screens. (Zhang et al., 2010). Dissolved air flotation (DAF) is also energy intensive as it uses compressors and pumps to create small air bubbles to coagulate algal particles and bring them to the water surface so they can be skimmed off the top (Ives and Bernhardt, 1995). Lastly, flocculation uses chemicals to flocculate the particles and let them settle at the bottom of the reactor. High saline environments have not been conducive to

flocculants due to the ionic strengths of seawater interfering with the chemicals needed to coagulate the algal cells (Sukenik et al., 1988).

1.2 Impact of clam and algal densities on harvesting rates

Clam densities can vary widely with some species being found having densities of more than 2000 clams m^{-2} (Werner and Rothhaupt, 2007). Sometimes these high clam densities can have impacts on the behavior of clams from different locations. If the clams are stacked on top of each other, and depending on the flow velocity, density of algae, and the thickness of the stack, clams on the bottom and the middle will not have the same filtration and clearance rates as the clams on top (Tuchman et al., 2004; Yu and Culver, 1999; Zaiko and Daunys, 2012).

An experiment conducted by Haven and Morales-Alamo (1967) related the weight of the oyster, (a close relative of the clam) to the amount of pseudofeces (biodeposits) produced and showed that the more an oyster weighed the more biodeposits it produced (Haven and Morales-Alamo, 1967). The study also showed that as algal density increased the biodeposit production increased. After collecting and measuring the biodeposits it was determined that their sizes ranged between one to five mm (Haven and Morales-Alamo, 1967). Based on their numbers, this represents at least a 200 times size increase between algal cells and the biodeposits with *Dunaliella tertiolecta* and *Dunaliella bardawil*, and at least a 400 times size increase for *Nannochloropsis oculata*. A study by Widdows (1979) showed that in *Mytilus edulis* increasing shell lengths (larger clams) resulted in higher clearance rates at algal concentrations greater than 100 mg L^{-1} (Widdows et al., 1979).

This was the only article located that evaluated clam behavior and biodeposit production in an algal concentration of 100 mg L^{-1} or greater. Many of the papers do not use concentrations that produce biodeposits or even algal concentrations larger than 100 mg L^{-1} . For example,

Roditi (1996) found a clearance rate of 115 mL mussel⁻¹ hr⁻¹, but that was with an algal concentration of less than 1 mg L⁻¹ and included inorganic particulate matter in the water that the mussel was filtering (Roditi et al., 1996). Loyaza-Muro (2007) found clearance rates for the mussel *Anodontites trapesialis* between 700 and 1000 mL mussel⁻¹ hr⁻¹ and Sylvester (2005) found rates for the mussel *Limnoperna fortunei* between 125 and 350 mL mussel⁻¹ hr⁻¹, but both of these researchers report algal concentrations in cells mL⁻¹, which cannot be converted to an effective concentration of mg L⁻¹ (Loayza-Muro and Elías-Letts, 2007; Sylvester et al., 2005).

1.3 Impact of clam densities on clearance and filtration rates

There have been multiple studies on the clearance and filtration rates of different species of clams. To clarify terminology, some literature papers use clearance and filtration rates interchangeably even though technically they have two different meanings, and in this thesis they mean two different things with different units of measurement. The clearance rate is the amount of water that flows through the clam in a given time, calculated by the equation

$$(\text{Volume of culture}/\text{Time}) * \ln(C_0/C_t)$$

expressed in units of L hr⁻¹ (Coughlan, 1969). The variables for the equation are as follows: volume of culture is in liters, time is in hours, C₀ is the initial concentration, and C_t is the final concentration. The filtration rate is the amount of algae consumed by the clam, expressed in units of g hr⁻¹. In a study with the scallop *Chlamys farreri*, a close relative of clams, the authors found the filtration rates to range from 7.90 to 5.13 mg clam⁻¹ day⁻¹ for a density of 10 and 40 clams m⁻², respectively (Zhou et al., 2006). This research showed a decreasing relationship between filtration rate and the scallop density (Zhou et al., 2006). The researchers reasoned that the increased competition for the algae at higher clam densities decreased the amount of algae available for other individuals around them while the flow rates for all the experiments were kept

constant (Zhou et al., 2006). But increasing the flow rates does not necessarily translate to higher filtration rates, as flow rates resulting in a surface velocity greater than 10 cm/s are growth inhibiting for the clams and filtration rates decrease as the flow rates increase (Newell et al., 2001; Wildish et al., 1987; Wildish et al., 1992; Wildish and Miyares, 1990).

1.4 Characterization of biodeposits

Clams bring in water through their siphons and filter the algal particles with their gill, where some algae are taken into their gut for metabolic and essential life function purposes while the rest is passed through the digestive tract partially digested. Clams excrete two types of waste: feces, which is digested algae, and pseudofeces, which is undigested algae. For the purposes of this research, we combine the two excretions and define it as biodeposits. The biodeposits are coated in mucus and expelled forcefully through their siphon. After an exhaustive search for biodeposit composition, to date there has not been much research done on the analysis of the biodeposits from the clams. Jaramillo (1992) had found that in the clams *Choromytilus chorus* and *Mytilus chilensis*, the biodeposits averaged 6.4% carbon, 0.3% nitrogen and 6.0% carbon, 0.4% nitrogen, respectively (Jaramillo et al., 1992). While Jaramillo (1992) had analyzed carbon and nitrogen percentages from different species, no analyses on ash content, lipid content, hydrogen and sulfur percentages, nor mineral characterization, has been found. There is critical knowledge gaps for this valuable information, especially if the biodeposits are to be used for potential downstream processes, it is extremely useful to understand their physical and chemical characteristics.

1.5 Impact of water parameters on ammonia prevalence and the toxic nature to biological organisms

Nutrient toxicity is prevalent in any biological system as the system cannot function properly if it has an increased amount of toxic chemicals in or around it (Boardman et al., 2004; Chen et al., 1990; Mummert et al., 2003). Many studies have been conducted on the toxicity of ammonia on different freshwater species due to the low tolerance many freshwater species have based on a combination of temperature and pH, which controls the toxic percentage of NH_3 (Emerson et al., 1975). Marine species, however, have higher tolerances to ammonia due to the third additional element of salinity, along with temperature and pH (Bower and Bidwell, 1978). Another clam, *Lampsilis siliquoidea*, survived for 28 days in a water-only study that was exposed to 0.36 mg L^{-1} of NH_3 (Wang et al., 2011). The 96 hour experiment using *Corbicula fluminea* found that an adult's toxicity level to NH_3 is between 0.79 and 0.99 mg L^{-1} of ammonia, while a juvenile's threshold to NH_3 at seven days old is between 0.07 and 0.21 mg L^{-1} (Cherry et al., 2005). The study confirmed mortality by visually seeing gaping clams or no response of the adductor. Another 96-hr experiment found the toxic NH_3 concentration of the freshwater clam *Villosa iris* to be 0.11 mg L^{-1} and 0.26 mg L^{-1} for *Lampsilis fasciola*. This study also determined mortality by visually observing a gaping shell or no internal movement when viewed through a microscope. All of these studies used mortality as the key factor in determining the toxicity level for the clams. They did not look at sublethal effects and use a decrease in clearance or filtration rate to determine if a clam is stressed. Epifanio and Srna (1975) tested impact on the clearance rate for adult *M. mercenaria* when exposed to ammonium chloride (0.43 to 4.3 g L^{-1} , pH 7.70 to 7.96) that showed no decrease in clearance rate over the experimental test range of 20 hours (Epifanio and Srna, 1975). These values are relevant in this research as they bracket the concentration of ammonia that is currently utilized in raceway ponds.

Additional research on marine clam culture would clarify the interaction of temperature, pH, ammonia, and there has been no research that discusses the impact of ammonia during the first few hours of exposure and the effect it has on the biodeposit production rate.

1.6 Impact of temperature on biodeposit production rate and survivability

Temperature was not a controlled experimental variable in this research but is needed to properly care for the clams. It is also worth noting that temperature will play a factor in a production scale model due to sunlight increasing the temperature of the water, so timing of harvesting could be critical. The temperature range that clams can survive and grow ranges between 2 and 32 °C (Chandra, 2008; Lei et al., 1996). As for the highest clearance and filtration rates, though, there are a few articles that suggest different temperatures, albeit in a narrow range. Lei and Eversole (2008) suggest that the optimal temperature is 22 °C for *Dreissena polymorpha* and *Elliptio complanata*, while Sylvester et al. (2005) and Pestana et al. (2009), suggest that the optimal temperature for *L. fortune* is 25 °C and 30 °C, respectively (Eversole et al., 2008; Lei et al., 1996; Pestana et al., 2009; Sylvester et al., 2005). The optimal temperature range for maximal clearance and filtration rates is 20 to 30 °C. It should be noted that these conclusions are based on tests conducted at low algal concentrations.

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CHAPTER 3

INFLUENCE OF CLAM AND ALGAL DENSITIES ON BIODEPOSIT PRODUCTION

RATES AND BIODEPOSIT CHARACTERIZATION

Abstract

A study on the potential use of harvesting algae through the hard clam *Mercenaria mercenaria* was conducted to quantify biodeposit production rates across different clam and algal densities, along with characterization of the biodeposits for potential downstream processing. The highest clam density, 6369 clams m⁻² had the highest biodeposit rates. While the wide range of algal densities (149 to 602 mg L⁻¹) used had a varied effect at different algal densities on the biodeposit rate, no maximum was found. Characterization of biodeposits showed that the clams can sequester large amounts of metals; the ultimate analysis differed very little from the source algae, and the lipid and ash contents increased 63% and 59%, respectively. Understanding the characterization of the biodeposits is essential to assess the potential for further downstream processing.

1. Introduction

Microalgal biofuels have become a significant topic because of algae's ability to grow rapidly (relative to terrestrial plants), sequester carbon dioxide, and grow in water that has key macronutrients. Algae can be converted into bio-oil through thermal processing and used in place of petroleum or converted in an anaerobic digester to bio-methane (Dębowski et al., 2013; Frigon et al., 2013; López Barreiro et al., 2013; Sawayama et al., 1999). Multiple species, such as *Nannochloropsis oculata*, *Chlorella vulgaris*, *Dunaliella tertiolecta*, and *Dunaliella bardawil*, have been used for biofuel application. These species range in size between two and 18 microns and this small size makes it difficult to harvest them efficiently (Butcher, 1959; Converti et al., 2009; Das et al., 2011; de Grooth BG, 1985; Kandilian et al., 2013). This small size helps to account for 20% to 30% of the total production cost of algae (Gudin and Therpenier, 1986).

1.1 Methods of harvesting

There are currently many ways to harvest algae including centrifugation, gravity sedimentation, filtration, dissolved air flotation, and flocculation (Brennan and Owende, 2010; Milledge and Heaven, 2013; Molina Grima et al., 2003). A new method, albeit with small volumes, that is currently being researched is ultrasound treatment that used high frequency waves to agglomerate the biomass (Bosma et al., 2003).

Unfortunately, each method has economic and logistic limitations for industrial implementation. Centrifugation is capital and energy intensive, with it needing to be constantly fed with large volumes of rather dilute concentrations of algae, requiring constant feeding with large volumes of water with low algal concentrations (Molina Grima et al., 2003; Sander and Murthy, 2010). Gravity sedimentation can take hours or days depending on algal culture density (Nurdogan and Oswald, 1996). Filtration screens can get clogged with high algal densities or can be ineffective if the algal culture is too dilute. It also requires larger amounts of energy to force water through screens that have partially collected algal biomass (Zhang et al., 2010). Dissolved air flotation (DAF) is also energy intensive by using compressors and pumps to create small air bubbles to coagulate algal particles and bring them to the water surface so they can be skimmed off the top (Ives and Bernhardt, 1995). Lastly, flocculation uses chemicals to flocculate the particles and let them settle at the bottom of the reactor. High saline environments have not been conducive to flocculants due to the ionic strengths of seawater interfering with the chemicals needed to coagulate the algal cells (Sukenic et al., 1988).

1.2 Utilization of clams

Mercenaria mercenaria is a hard clam found in the coastal waters of the eastern seaboard of the U.S. all the way to the Gulf of Mexico (FAO, 2014). Clams are natural filter feeders, consuming algae through their siphon, moving it through their body cavity, and then expelling

them through the siphon into the water where it settles to the bottom. This excretion includes both digested and undigested algal particles, and will subsequently be combined and defined biodeposits in this study. These biodeposits can range in size from one to five mm: much larger than the original algae that was in suspension representing a 400-fold or greater size increase (Haven and Morales-Alamo, 1967). Converting the algae into biodeposits could make the harvesting process much easier, with no direct energy inputs or additional chemicals needed.

1.3 Biodeposit and *N. oculata* characterization

In order for this harvesting system to work, the harvested “biodeposits” needs to be further analyzed to consider if this is an appropriate feedstock for downstream processes. Currently not much research has been completed on the characterization of biodeposits with the exception of reports on carbon and nitrogen percentages for freshwater bivalve. *N. oculata* has a lipid content ranging from 32% to 60% and an ash content of 24% (Rodolfi et al., 2009; Sukarni et al., 2014). Research is lacking on how algal composition might affect the composition of the biodeposits produced.

1.4 Experiment rationale

In order for algae to be a viable biofuel source, alternative solutions for harvesting that minimizes the energy and cost inputs while still removing biomass from the reactor at rates that are comparable to conventional methods. Using a biological system such as clams to harvest algae for biofuel production could potentially decrease harvesting costs and the overall cost of biofuel production. This paper’s aim was to quantify the impact of clam densities and algal densities on the biodeposit production rate. Additionally, we quantify the carbon, nitrogen, hydrogen, and sulfur percentages, ash content, total lipid content, and mineral content of biodeposits.

2. Materials and Methods

2.1 Holding facility for clams

All clams used in the experiments were purchased from the Aquaculture Research Corporation (Dennis, MA, USA). Sizes ranged from 10 to 15 mm and weighed on average 0.54 g. They were held in a recirculating saltwater tank most commonly used for fish culture rearing and fed twice a week with *N. oculata*. Recirculation mode was stopped for 24 hours immediately after addition of food-algae so that clams could feed on the algae without it being removed through recirculation filters. Salinity in the clam storage tank at the nursery was held at 26 parts per thousand (ppt) with temperature between 20 and 22 C.

2.2 Algae growth for experiments

N. oculata was obtained from the sterile culture bank and scaled up to a 200 L reactor. All media additions are standard BG-11 growth media with Red Sea Coral Pro salt at 26 ppt. Algae was grown under continuous lighting and a 1% CO₂ concentration at the rate of 2 L min⁻¹ for 15 minutes every hour.

2.3 Clam and algal density impact

Clams were taken from the recirculation tank where salinity and temperature of the water were recorded and placed into a holding container with fresh saltwater at the same temperature and salinity of the recirculation tank. Clams in the holding container siphoned in clean saltwater for 90 minutes to purge any remaining algae or waste material that was present in their digestive system.

After 90 minutes of siphoning, clams were removed, patted dry as best as possible on a paper towel and placed in the 1-L beaker experimental vessel. The beakers filled with clams

were weighed and randomly placed on the lab table. Four treatments of 0, 10, 30, and 50 clams per beaker resulting in clam densities of 0, 1247, 3822, and 6369 clams m^{-2} , respectively, with four replicates each were used.

Algae was first screened through a 153- μ mesh to remove any large debris, then poured into each beaker using a 1000-mL graduated cylinder. Algae was poured from the 200-L reactor into a 22-L bucket and mixed for 15 minutes. A glass stir rod was used to evenly spread the clams on the bottom of the beaker. After all beakers were filled, a 50-mL sample was taken from each beaker to measure initial algal concentration. Temperature and pH readings were recorded, and salinity measurements were obtained using a salinity probe (Ohaus, Parsippany, NJ).

At the end of the four hours, contents of each beaker was poured through a filtering apparatus with the filtrate going into another beaker. The filtering apparatus, shown in Figure 3.1, was a 2 inch pipe cut in half, 14 inches long, with a Nitex 100 micron mesh screen attached alongside the inner walls, with an angle of declination of 12 degrees while resting on the beaker.



Figure 3.1: Biodeposit harvesting apparatus.

After 900 mL were poured out of the beaker, it was gently swirled to remove any biodeposits that remained adhered to the glass or the clam's outer shell. A 50-mL sample from the filtrate was immediately taken after pouring out the beaker, and used to measure final algal concentration. De-ionized water from a 1-gallon industrial sprayer was sprayed on the mesh that collected the biodeposits and the liquid with biodeposits was collected in a 250-mL flask.

The initial and final 50-mL samples were centrifuged at 4700 rpm for 5 minutes (ThermoFisher Scientific Sorvall ST 40, Waltham, MA, USA). Whatman G/A (GE, CT, USA) filters dried for two hours prior to the experiment in the 105 °C oven. The 50-mL samples were vacuum filtered through a Whatman G/A 1.6- μ 47 mm filter. The 250-mL flask samples that

contain the biodeposits were vacuum filtered through a Whatman G/A 1.6- μ 70 mm filter. Samples were placed in the 105 °C oven for 90 minutes, weighed, and recorded.

2.4 Biodeposit production for characterization

To produce sufficient biodeposits for analyses, thirty-two 32 1-L beakers were filled with 50 clams each and allowed to consume algae for 12 hours. When finished, biodeposits were harvested using filtering apparatus as explained in section 2.3 earlier. Biodeposits were sprayed off with an industrial one gallon sprayer into a 2-L flask and stored at 4 °C. This process was repeated several times to generate sufficient biodeposits for analyses. The biodeposits were centrifuged at 4700 rpm for 10 minutes, supernatant was decanted, and the pellet was used for further analyses. Biodeposits were lyophilized and stored at -4 °C until further use. To compare biodeposits composition to algal composition, *N. oculata* was grown according to methods in section 2.2 and 60 L was sent through a process centrifuge (AML Industries, Hatboro, PA, USA) at 3252 rpm at 300 mL min⁻¹. The concentrated algae was further centrifuged on a bench scale centrifuge at 4700 rpm for 10 minutes. After centrifugation the algae was lyophilized and stored at -4 °C.

2.5 Biodeposit and *N. oculata* characterization

Samples were removed from the -4 °C freezer and 800 mg biodeposit and algae were put into their respectively labelled individual 10-mL centrifuge tubes to satisfy the minimum sample requirement. The samples were sent to the University of Georgia Agricultural and Environmental Services Laboratory for analysis.

2.6 Ash, CHNS, and lipid content of biodeposit and *N. oculata*

Lipid analysis was performed by an automated soxhlet machine with hexane as the solvent (ANKOM XT 10, Macedon, NY, USA). Ash content was determined by ASTM standard number D5142 in a TGA701 proximate analyzer (LECO, St. Joseph, MI, USA). Carbon, hydrogen, nitrogen, and sulfur content were measured using a CHNS932 with VTF900 furnace option (LECO, St. Joseph, MI, USA).

3. Results/Discussion

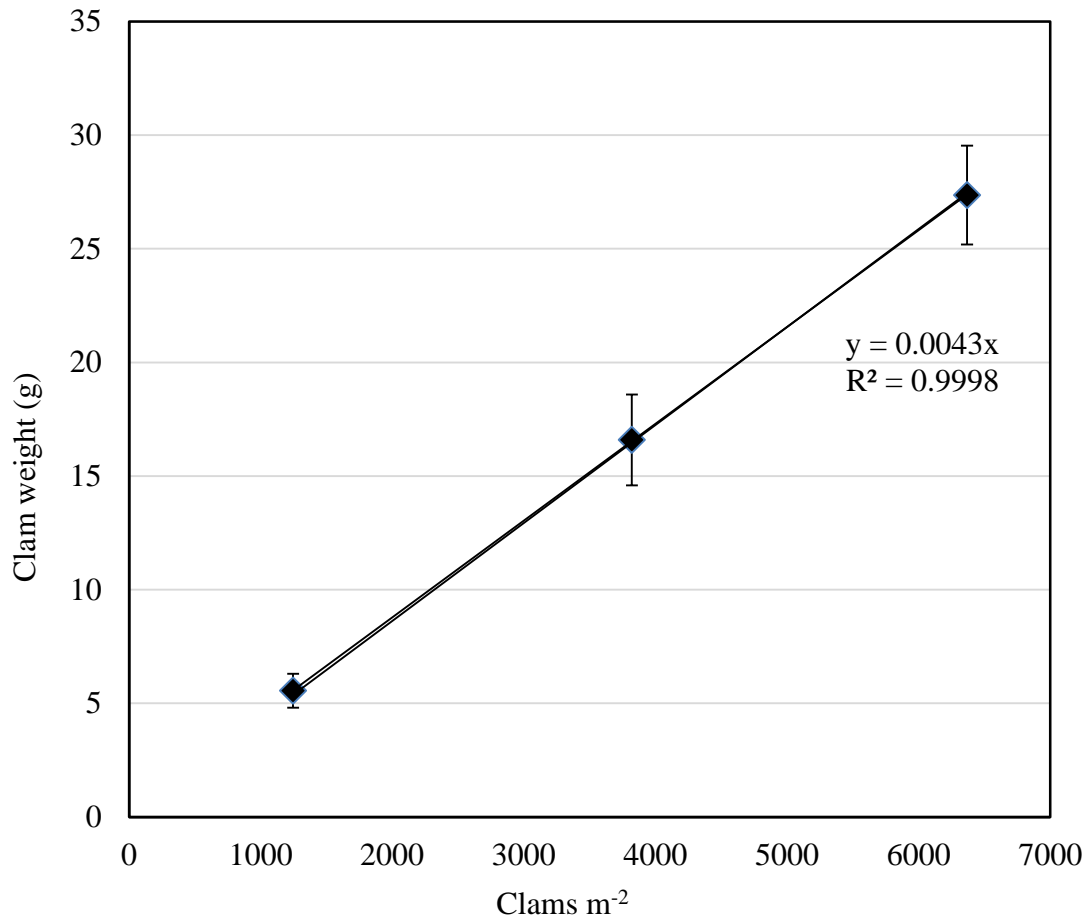


Figure 3.2: Clam weight in relationship to the number of clams. Each data point represents the average of 72 individual flasks and error bar represents \pm one standard deviation.

3.1 Consistency of clam weight

Figure 3.2 shows the relationship between the clam densities and the corresponding weights of each group of clams. Each data point represents 72 beakers from the 18 experiments from each density. Clam weights did not vary widely and a relationship was established between each density and the weight. This is important for a production facility due to the thousands of clams needed for a large scale operation in order to get an accurate density based on the weights

of the clams. Furthermore, most research has been conducted on individual weights of clams and their performance, whether it be biodeposit production, fecal production, clearance rate, or filtration rate, but almost none has been conducted on the total group mass of a collection of similarly sized clams.

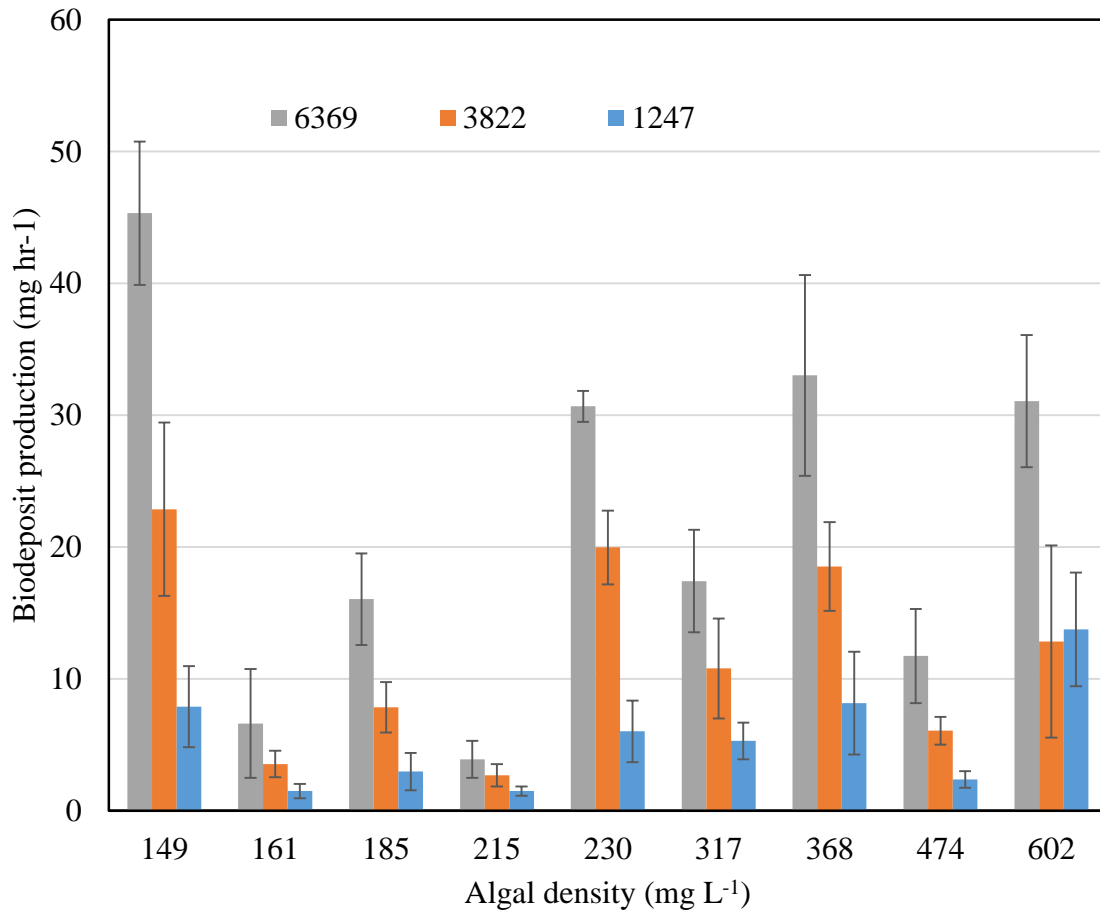


Figure 3.3: Biodeposit production rate in March over a range of algal and clam densities. Tests at algal concentrations of 161, 215, 317, and 474 mg L⁻¹ contained clams that were starved 1 day prior to experiment. All other densities were starved 3 days prior. Legend indicates the three levels of clam packing densities (clams m⁻²).

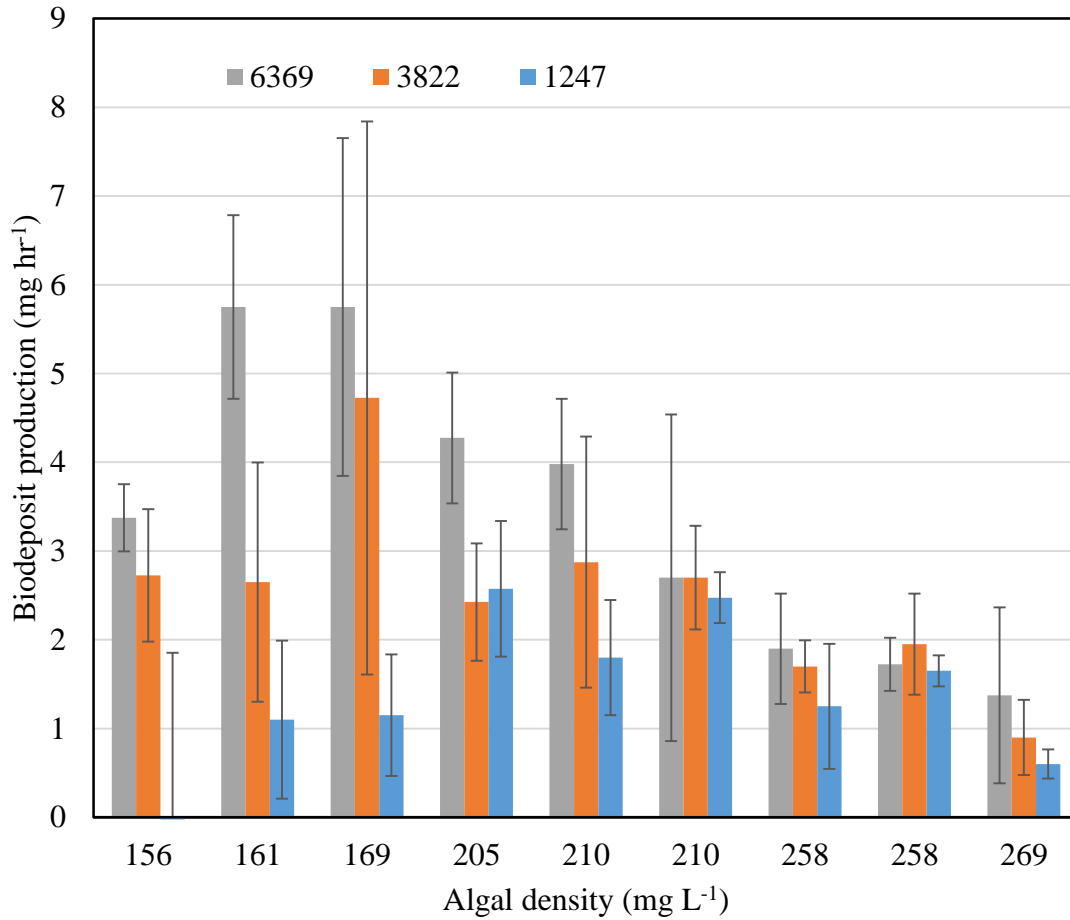


Figure 3.4: Biodeposit rate in May over a range of algal and clam (clams m⁻²) densities. Algal concentrations 156 to 169 mg L⁻¹ had clams that were starved for 3 days, algal concentrations 205 to 210 mg L⁻¹ had clams that were starved for 6 days, and algal concentrations 258 to 269 mg L⁻¹ had clams that were starved for 10 days. Error bar represents \pm one standard deviation.

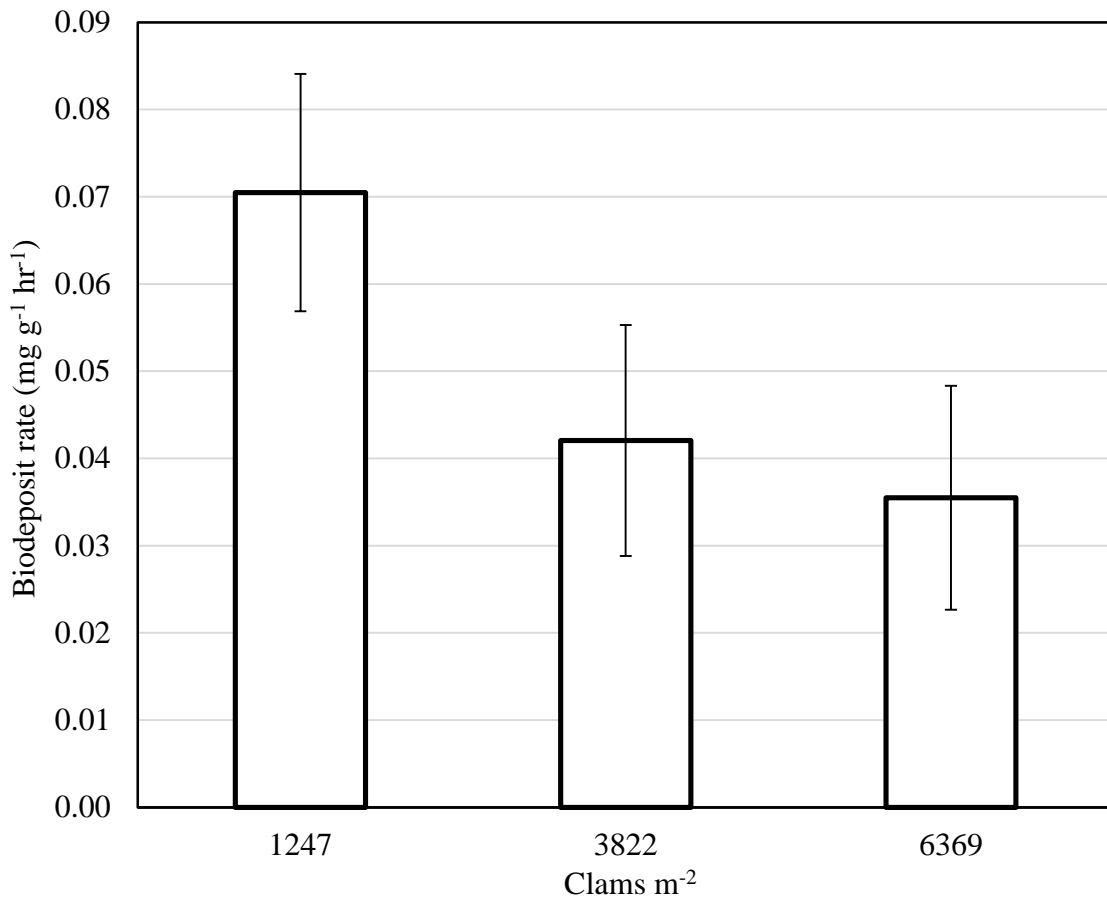


Figure 3.5: Representative trend from one experimental trial in March with an algal cell density of 149 mg L^{-1} . The decrease in efficiency, represented by $\text{mg g}^{-1} \text{ hr}^{-1}$, was a trend noted over all experimental trials. Error bars indicate \pm one standard deviation.

3.2 Clam and algal density impact on biodeposit rate

Experiments were performed at two different times, nine experiments in March and nine in May. The nine completed in March included a wide range of algal densities to test the effect of clam and algal densities on biodeposit rates. As noted in the figure caption, there were four experiments that the clams were starved for one day prior to the experiment and five experiments that were starved for three days prior to the experiment. The May experiments were done with three algal densities in order to test the effect of three, six, and ten day starvation of clams prior to testing and how that affects the biodeposit production rate.

The clam densities that were tested were the same in both sets of experiments and showed that as the clam densities increased the biodeposit rate increased, as seen in Figure 3.3 and 3.4, with the highest biodeposit rate occurring at the highest clam density of 6369 clams m⁻². This was to be expected as the more clams there are the more biodeposits are produced, but further research is needed to find the maximum clam density for the optimal biodeposit production rate. It served to quantify the rates of biodeposition to determine if this could be a viable harvesting alternative. In finding the optimal density, the dissolved oxygen levels in the system should be monitored since the oxygen levels could drop rapidly as more clams consume oxygen in the volume of water.

A range of algal densities that could be seen in an algal raceway pond were used for the March experiments, ranging from 149 to 602 mg L⁻¹. As the algal densities increased, statistically the algal density had an effect on the biodeposit rate, but as seen in Figures 3.3 and 3.4 there is no discernible maximum. There has not been a lot of research with clams at these algal densities because these concentrations are normally not found in nature, nor has there been any effort to optimize the biodeposit production rate. The data has shown that clams could be operated over a wide range of algal densities, which from a production standpoint is critical because the clams can remove part of the raceway algal biomass while leaving the young and fast growing algal cells for maximum growth.

The experiments conducted in May were over a narrower range of algal densities to test the effect of starvation on the clams to better optimize the production system. In Figure 3.4 the first three algal densities had clams that were starved three days, the next three were starved six days, and the last three were starved for ten days. There was actually two variables present, namely algal density and starvation period. From previous experiments (March data), we

established that within the ranges tested algal density had no distinguishable effect on the biodeposit production rate. Figure 3.4 showed that with increasing starvation the biodeposit production rate decreases and the trend with the highest clam density still producing the most biodeposits is evident in this May data. Since the first three algal densities were starved for three days, it should have been comparable to the production rates in the March data for the similar algal densities, but the production rates are almost five times lower in May than in March. This could be for a number of reasons: the health of the clam, the age of the clam, the water temperature of the nursery increasing by 2 °C, or the algae could have been slightly different even though it was the same species.

It should be noted that as the clam density increased, the efficiency of the clams, defined as $\text{mg g}^{-1} \text{hr}^{-1}$, decreases and is shown in Figure 3.5. This drop in efficiency could be explained by having less competition for the limited algae resources in the beaker (Tuchman et al., 2004; Yu and Culver, 1999; Zaiko and Daunys, 2012). Less competition allowed the clams to freely siphon and deposit as much algae as possible. The densities used in this experiment are not normally found in nature, leading to the clams being overcrowded at the middle and highest densities thus reducing their efficiency (Werner and Rothhaupt, 2007). Even though the efficiency drops and the production rate increases, further work is needed to determine the maximum biodeposit rate at the optimal clam density.

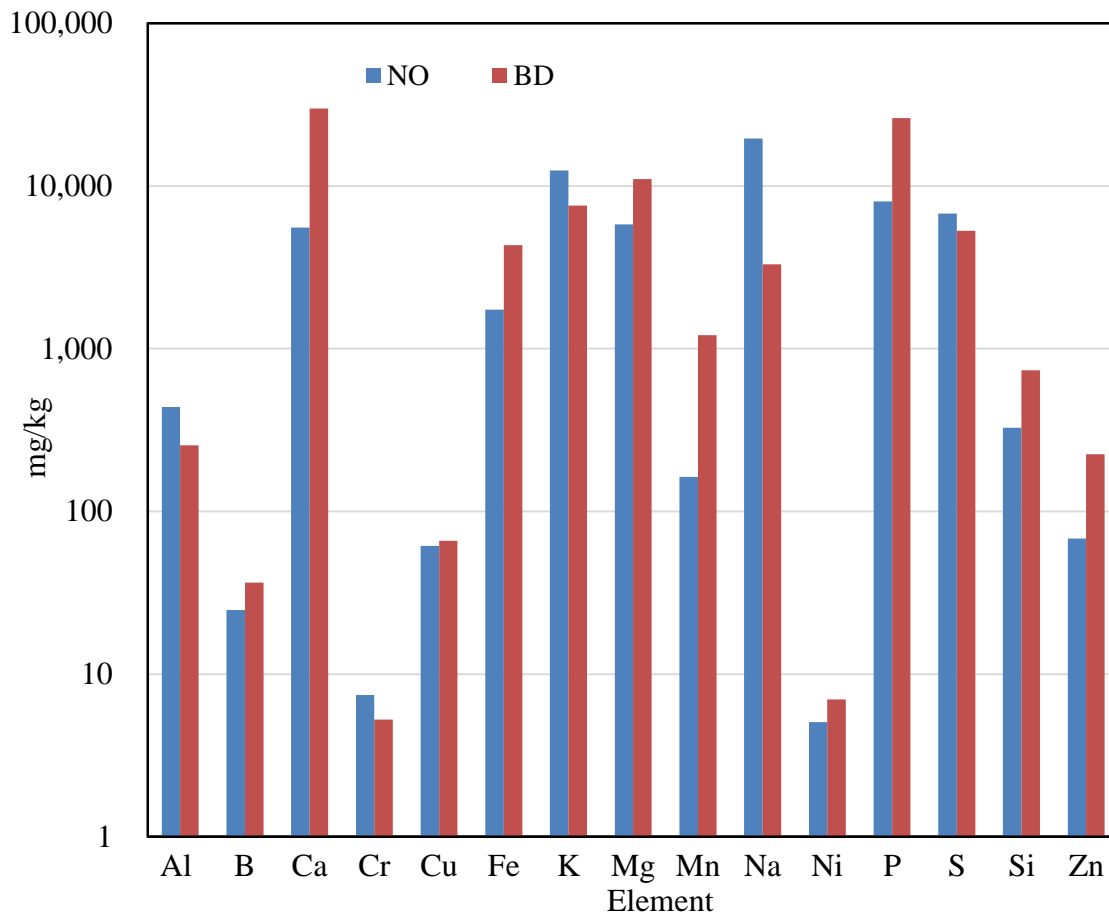


Figure 3.6: Log scale plot of the elemental composition of *N. oculata* (NO) and biodeposits (BD) produced from it.

3.3 Biodeposit characterization

3.3.1 Mineral content

The mineral content of both *N. oculata* and the biodeposits are shown in Figure 3.6. The biodeposits compared to the algae show that biodeposits have a higher concentration of metals, such as zinc, iron, and manganese. This shows that clams have the ability to concentrate certain metals, which could be important in detecting pollution and could be used to remediate polluted waters (Sivalingam and Bhaskaran, 1980). The biodeposits also concentrate phosphorus, a key

fertilizer ingredient, by over 326%. This could make the biodeposits useful as a nutrient rich fertilizer.

Table 3.1: Lipid content percentage comparison between *N. oculata* and biodeposits

	Average
<i>N. oculata</i>	8.9 ± 1.4
Biodeposits	14.5 ± 4.0

3.3.2 Lipid analysis

The lipid content shown in Table 3.1 indicated lipid content in the biodeposits increased 63% when compared to the algae used in the research. Although the test did not show what specific lipids were concentrated, it showed that the lipid profile can increase after being processed by the clam. Future studies could show which lipids were concentrated in the biodeposits and can be tested by first stressing the algae and then passing it through the clam to see how much more concentrated the lipids can get.

Table 3.2: Comparison of CNHS values of *N. oculata* and biodeposits. Values reported are average of four percentage based samples ± one standard deviation.

	C	N	H	S
<i>N. oculata</i>	48.67 ± 0.28	11.47 ± 0.47	6.88 ± 0.06	0.18 ± 0.21
Biodeposits	49.44 ± 0.19	12.10 ± 1.04	7.32 ± 0.16	0.14 ± 0.09

3.3.3 CNHS content

The carbon, nitrogen, hydrogen, and sulfur content, shown in Table 3.2, between the biodeposits and *N. oculata* do not differ, meaning biodeposits could be a suitable downstream feedstock. The only drawback the biodeposits have is the increase in nitrogen content, albeit a very small amount. This would require further processing to reduce the nitrogen, adding further expenses and energy requirements to the already expensive harvesting and processing phase of algal cultivation.

Table 3.3: Comparison of ash content of *N. oculata* and biodeposits. Values reported are averages of four percentage based samples \pm one standard deviation.

	Ash dry
<i>N. oculata</i>	11.40 \pm 0.17
Biodeposits	18.18 \pm 0.24

3.3.4. Ash content

Biodeposits have 59% more ash content than *N. oculata*, as shown in Table 3.3. The increased ash content in the biodeposits seemed to be a result of the increase in metals concentration found in the mineral characterization portion of this research.

4. Statistical analysis

A 2-way ANOVA was used to determine statistically significant differences between treatments. The following table sets up the p-values at 95% confidence intervals for the various algal and clam density experiments.

Table 3.4: P-values of March trials with 1 and 3 day prior feeding regimen along with May 3, 6, and 10 day prior feeding regimen. ANOVA 2-way with $\alpha < 0.05$ was used. March 1 day n = 4, March 3 day n = 5, May n = 9.

P-value	March		May
	1 day prior (n = 4)	3 day prior (n = 5)	3,6,10 day prior (n = 9)
Clams	1.28E-09	3.00E-20	1.16E-10
Algal	5.13E-10	6.00E-10	3.38E-08
Interaction	9.00E-03	7.00E-05	2.07E-04

5. Conclusions

This research was conducted to evaluate if using clams to harvest algae could be a viable alternative to the current methods of harvesting. It was shown over all the experimental trials that clam density has an effect on the biodeposit rate with the highest clam density of 6369 clams m⁻² producing the most biodeposits, but further research needs to be conducted in order to find the maximum clam density for the optimal biodeposit production rate. When the clam density increased, the biodeposit production rate per clam, or the efficiency, decreased. This efficiency

decrease was to be expected due to increased competition for the algal resources from the clams. Algal density was tested over a wide range and the biodeposit rate was not affected nor was an optimal algal density found.

The starvation of the clams was also tested in the May data and it was found that three day starvation works the best under this experimental protocol, but more research would be needed to determine if the optimal starvation period is between one and three days or three and six days. The mineral composition showed that the biodeposits concentrate some metals quite extensively, potentially making it a good bioremediation option. The lipid profile in the biodeposits increased 63%, leading to a potential way to increase the amount of lipids that can be used a biofuel feedstock. Carbon, hydrogen, nitrogen, and sulfur content of the biodeposits do not differ from the algae, with the only drawback coming from a slight increase in nitrogen content and potential processing of it for downstream end products. The ash content of the biodeposits increases 59% over that of the algae, but this was expected due to the increase in concentration of minerals. There still needs to be more research in order to scale up this potential algal harvesting technique to make it feasible.

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CHAPTER 4

INFLUENCE OF AMMONIA ON THE BIODEPOSIT PRODUCTION RATE OF THE HARD CLAM *MERCENARIA MERCENARIA*

Abstract

Ammonia toxicity is prevalent in both marine and freshwater clam species. It is also the main nitrogen source used for algal cultivation. Using clams as an alternative harvesting technique is an attractive concept and understanding how ammonia affects the biodeposit production rate is critical in knowing how to operate the facility. This study was designed to test a range of ammonia concentrations (0 to 100 mg-N L⁻¹) in the culture where the clams were kept over a four-hour time period, and determine the impact of the presence of ammonia on the biodeposit production rate of *Mercenaria mercenaria*. Increasing amounts of ammonia nitrogen at different temperatures, pH, and salinities showed that the biodeposit production rate stayed the same or increased over the experimental time frame. Understanding the impact of the ammonia nitrogen concentrations on the biodeposit production rate can allow the harvesting of biodeposits to occur within the range of tested ammonia nitrogen concentrations and the given time frame.

1. Introduction

Microalgal biofuels have become a significant topic because of its ability to grow rapidly (relative to terrestrial plants), sequester carbon dioxide, and grow in water that has key macronutrients present. Algae can be converted into bio-oil through thermal processing and used in place of petroleum or converted in an anaerobic digester to bio-methane (Dębowski et al., 2013; Frigon et al., 2013; López Barreiro et al., 2013; Sawayama et al., 1999).

1.1 Current methods and Drawbacks

There are a few ways to harvest algae, and although new methods are being researched and tested, currently the main ways to harvest algae are centrifugation, gravity sedimentation, filtration, dissolved air flotation, and flocculation (Brennan and Owende, 2010; Milledge and Heaven, 2013; Molina Grima et al., 2003). There is a need for an alternative harvesting method

due to the drawbacks of the current ways to harvest algae. Some of the drawbacks include capital intensive, energy intensive, clogging of machinery, length of time to harvest, and additional chemicals (Ives and Bernhardt, 1995; Nurdogan and Oswald, 1996; Sander and Murthy, 2010; Sukenik et al., 1988; Zhang et al., 2010).

1.2 Novel harvesting technique

A novel harvesting technique was developed to mitigate the aforementioned drawbacks by using the hard clam *Mercenaria mercenaria* to increase the harvestable size of the algae by its own natural filter feeding ability. Clams are natural filter feeders, siphoning the water for algal cells to satisfy their metabolic processes. They then expel out of their siphon both digested (feces) and undigested (pseudofeces) biomass as a flocculated material that is much larger (over 400-times) than single algal cells. The clam siphoning process is attractive because there are no additional chemicals needed to flocculate the algae, nor any direct energy inputs in making the clam perform this process. This flocculated material containing both fecal and pseudofecal matter is referred to as biodeposits in this paper. These biodeposits can then be easily separated from the culture medium and used in downstream processes such as anaerobic digestion, pyrolysis, and hydrothermal liquefaction.

1.3 Sources of nitrogen

Algal growth requires nitrogen as a key macronutrient, in the form of ammonia or nitrate, with ammonia being the preferred form due to its lower cost and easier availability. But, even though it is preferred for its low cost and availability, small amounts of ammonia is toxic to most biological systems, both in marine and freshwater clam species (Boardman et al., 2004; Chen et al., 1990; Mummert et al., 2003). Many studies have been conducted on the toxicity of ammonia on different freshwater species due to the very low tolerance many freshwater species have based

on a combination of temperature and pH, which controls the toxic NH_3 (Emerson et al., 1975). Marine species, however, have higher tolerances to ammonia due to the third additional element of salinity along with temperature and pH (Bower and Bidwell, 1978). Unionized ammonia increases with an increase in temperature and pH, and ammonia decreases with increasing salinity levels as long as temperature and pH remain fixed. The only true fixed production variable would be salinity, as temperature fluctuates based on the times of day and the pH fluctuates based on the chemistry of the culture, with it being high with high algal growth rates (and depleting CO_2 concentrations) and low during algal respiration. Ammonia toxicity is one of the main hurdles that could hinder algal harvesting using clams.

1.4 Gap in knowledge on ammonia toxicity

Most toxicity studies have done 96-hr lethality tests to determine mortality in the presence of toxicity, with only one study having documented how ammonia affects clearance rates. There currently has been no research conducted on the initial impact of ammonia on the biodeposit production rate. It is useful to understand how ammonia affects the biodeposit production rate in bivalve-based harvesting as added nitrogen can be modulated to maintain active algal growth without negatively impacting the biodeposit production rate of the clam. By understanding the impact of ammonia on the biodeposit production rate it can help determine operating parameters for potential scale up. In this work, we determine the optimal concentration of ammonia in the algal culture system without affecting the biodeposit rate over the course of the experimental time frame.

2. Materials and Methods

2.1 Holding facility for clams

All clams used in the experiments were purchased from the Aquaculture Research Corporation, Dennis, MA. Sizes ranged from 10-15 mm and weighed on average 0.54 g. They were held in a recirculating saltwater tank most commonly used for fish culture rearing and fed twice a week with *N. oculata*. Recirculation mode was stopped for 24 hours immediately after addition of food-algae so that clams could feed on the algae without it being removed through recirculation filters. Salinity in the clam storage tank at the nursery was held at 26 parts per thousand (ppt) with temperature between 20 and 22 °C.

2.2 Algae growth for experiments

N. oculata was obtained from the sterile culture bank and scaled up to a 200 L reactor. All media additions are standard BG-11 growth media with Red Sea Coral Pro salt at 26 ppt. Algae was grown under continuous lighting and a 1% CO₂ concentration at the rate of 2 L min⁻¹ for 15 minutes every hour.

2.3 Ammonia impact on biodeposit production

Clams were taken from the fisheries tank where salinity and temperature of the water recorded and placed into a holding container with fresh saltwater at the same temperature and salinity of the recirculation tank. Clams in the holding container siphoned in clean saltwater for 90 minutes to purge any remaining algae or waste material that was present in their digestive system. Four 4-L flasks of algae were mixed with five treatment concentrations of ammonium chloride with four replicates to achieve NH₄ concentrations of 0, 25, 50, 75, and 100 mg L⁻¹. Each individual 4-L flask of algae was mixed for 5 minutes at 300 rpm on a magnetic stir plate.

After 90 minutes of siphoning in the holding container, clams were patted dry as best as possible with paper towel and each group of 30 clams, density 3822 clams m⁻², with four

replicates, were placed in a 1-L beaker, weighed, and randomly placed on the lab bench. Algae were screened through a 153- μ mesh to remove any large debris then poured into each beaker with a 1000-mL graduated cylinder. A glass stir rod was used to evenly spread the clams on the bottom of the beaker. A 50-mL sample was taken from each beaker for the initial concentration. Temperature and pH readings were recorded, and salinity measurements were obtained by a salinity probe (Ohaus, Parsippany, NJ). Unionized ammonia percentage was calculated from the EPA document 440/5-88-004 (EPA, 1989a).

At the end of four hours, contents of each beaker was poured through a filtering apparatus with the filtrate going into another beaker. The filtering apparatus, shown in Figure 4.1, was a 2 inch pipe cut in half, 14 inches long, with a Nitex 100- μ mesh screen attached alongside the inner walls, with an angle of declination of 12 degrees while resting on the beaker.



Figure 4.1: Biodeposit harvesting apparatus

After 900 mL were poured out of the beaker, it was gently swirled to remove any biodeposits that remained adhered to the glass or clam's outer shell. A 50-mL sample from the filtrate for the final concentration was immediately taken after pouring out the beaker, and used to measure final algal concentration. De-ionized water from a 1-gallon industrial sprayer was sprayed on the mesh that collected the biodeposits and the liquid with biodeposits was collected in a 250-mL flask. The initial and final 50-mL samples were centrifuged at 4700 rpm for 5 minutes (ThermoFisher Scientific Sorvall ST 40, Waltham, MA, USA). Whatman G/A (GE, CT, USA) filters dried for two hours prior in the 105 °C oven. The 50-mL samples were vacuum filtered through a Whatman G/A 1.6- μ 47 mm filter. The 250-mL flask samples that contain the

biodeposits were vacuum filtered through a Whatman G/A 1.6- μ 70 mm filter. Samples were placed in the 105 °C oven for 90 minutes, weighed, and recorded.

3. Results

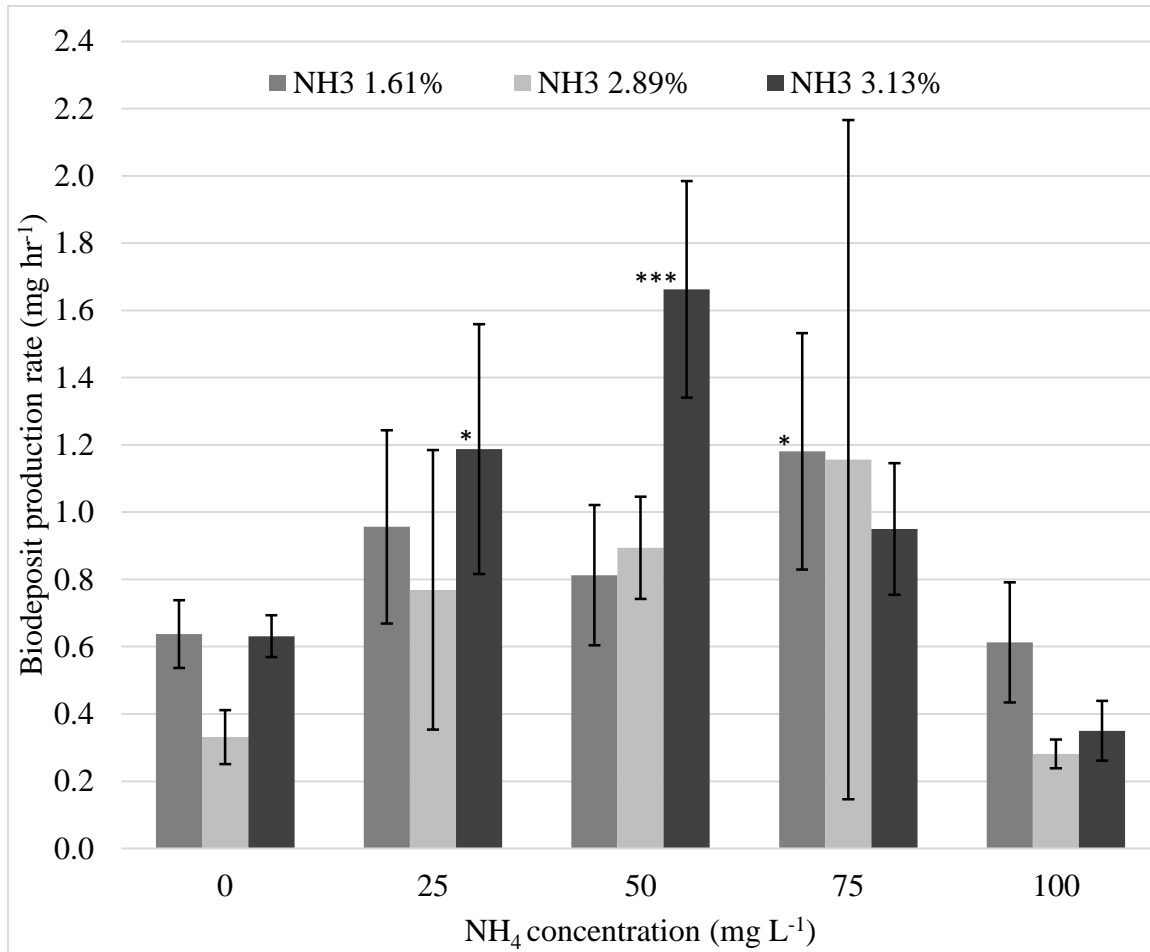


Figure 4.2: Biodeposit production rate of *M. mercenaria* in different concentrations of ammonia-nitrogen. NH₃:2.89% experimental conditions were temperature 24.1 °C, pH 7.80, salinity 22.2 ppt, algal density 214 mg L⁻¹, and clam density 3822 clams m⁻². NH₃: 3.13% experimental conditions were temperature 24.3 °C, pH 7.83, salinity 22.4 ppt, algal density 234 mg L⁻¹, and clam density 3822 clams m⁻². NH₃: 1.61% experimental conditions were temperature 22.9 °C, pH 7.58, salinity 22.9 ppt, algal density 241 mg L⁻¹, and clam density 3822 clams m⁻². * = p<0.05, *** = p<0.0001.

Not a lot of research has been done on the impact of ammonia on the biodeposit production rate in hard clams, specifically *M. mercenaria*, and especially on the effect of ammonia within the first few hours of exposure. Several runs were completed with an intent to keep pH, temperature, and algal concentration as constant as possible, and the results shown in Figure 4.2 are the actual experimental variability across the three experiments. Experimental protocols were followed exactly as explained in section 2.3. In Figure 4.2, the biodeposit production rate was not affected by the increase in ammonia concentrations. All three experiments were done at different pH and temperature which affects the amount of unionized ammonia in the system. A Duncan analysis was conducted to determine which concentrations were different from the control. Previous studies mainly looked at mortality rates over 96-hr to calculate death rates and clearance rate reduction for clams. This study was not meant to kill the clams but rather determine if the presence of high ammonium had an effect on the biodeposit production rate within the first four hours of exposure. A study by Epifanio and Srna (1975) found that the clearance rate over the first 20 hours did not change at ammonium chloride concentrations of 53.5 and 107 mg L⁻¹ (Epifanio and Srna, 1975). In all three of the current experiments the biodeposit production rate either was not statistically different from the control (no ammonia) or increased with increasing amounts of ammonia over the four hour experimental period. The clearance rate in the Epifanio study was not affected by the low concentrations of ammonium chloride, where by their definition the clearance rate determines the rate of algal cell removed from the water. Unionized ammonia percentages ranged from 1.61% to 3.13%, or 1.61 to 3.13 mg L⁻¹ in the three experimental trials.

1 4. Statistical analysis

2 An ANOVA single factor test and a Duncan analysis were performed on the data
3 collected. The single factor ANOVA resulted in two statistically different trials and one
4 non-significant trial. Therefore, a Duncan test was performed to determine which
5 treatments were different from the control mean.

6 Table 4.1: ANOVA single factor test for all three experimental trials. The 214 mg L⁻¹
7 trial was not statistically different from the control mean, whereas the 234 and 241 mg L-
8 1 trials were statistically significant.

Algal density (mg L ⁻¹)	214	234	241
P-value	0.1074	0.00002	0.0244

9
10 Table 4.1 shows that at a confidence level of $\alpha < 0.05$ that two of the trials, 234 and 241
11 mg L⁻¹, are significantly different, while the 214 mg L⁻¹ trial is statistically insignificant.

12
13 5. Discussion

14 5.1 Explanation for increase in biodeposit production rate

15 The increase in the biodeposit production rate is counter-intuitive as we expected
16 of ammonia's toxicity to biological organisms to result in lower clam activity and
17 subsequently lower biodeposit production. The observed increase could be the result of
18 the clams initially siphoning the water while not yet detecting the ammonia in solution.
19 The short time period of the experiment could be the reason the clams didn't stop
20 siphoning as the amount of ammonia was not absorbed and accumulated in their system.
21 The increase in biodeposit production could also be likened to that of an irritant, while
22 the clam's natural reaction is to try to rid itself of that irritant and siphon more algae and
23 water, leading the clam to expel more material in the form of biodeposits. In our tests, the
24 complete duration of the analysis was only four hours. It is likely that the negative effects

25 of ammonia toxicity become symptomatic after a longer period and may have long
26 lasting effects on the clams, something that was not documented in this work.

27 While it is interesting that the biodeposit production remains unchanged or
28 increases with increasing ammonia concentrations, future work could determine how the
29 presence of ammonia is regulated in the metabolic pathways that signal to the clam
30 ammonia is passing through the tissue. Lethality values for unionized ammonia for the 48
31 and 96 hour LC₅₀ are 216 and 36.6 mg L⁻¹, respectively, so there is an endpoint to the
32 amount of ammonia that could be added to the system before killing the clams
33 (Boardman et al., 2004). Since the experiment only lasted four hours, it is undetermined
34 what happens to the biodeposit production rate between four and 48 hours. The optimal
35 ammonia level for the maximum biodeposit production may be identified between those
36 two points in time.

37 5.2 Impact on algal harvesting for biofuels production

38 Using clams as an alternative tool to harvest algae for biofuels production was the
39 intent of this research. Specifically, we wanted to establish a baseline for the exposure to
40 ammonia. Ammonia is one of the low cost nitrogen inputs for algal growth. This study
41 showed that under short exposure times, biodeposit production was not reduced. This
42 knowledge gained is critical in knowing how long clams can be exposed to levels of
43 ammonia found in commercial algal cultivation systems. Further work is needed to find
44 the maximum amount of ammonia that the clams can be exposed to without decreasing
45 biodeposit production. The concentrations of ammonia used in this experiment are similar
46 to concentrations that would be used in large-scale production of algae. The study also
47 identified preferred operating and harvesting conditions for algal harvesting, such as

48 performing the harvest under lower temperatures and lower pH to decrease the unionized
49 portion of ammonia that is the most toxic to clams. This would suggest that the best time
50 to harvest would be night time, as algae would be respiring, therefore having lower pH
51 and temperature. Another critical component in this system would be to determine the
52 maximum length of time the clam can be exposed to ammonia without the decrease in the
53 biodeposit rate. Clams were not removed from the experimental system to test mortality
54 after the four hours. Instead they were removed and put back in a group holding
55 container. Thus, another question that will need to be answered is how multiple
56 exposures will affect the biodeposit production rate and how many days of clean
57 siphoning the clam will need in order to remain healthy and keep the same biodeposit
58 production rates.

59 6. Conclusions

60 This research was conducted in order to see how different concentrations of
61 ammonia affect the biodeposit production rate over the course of the 4-hr experimental
62 time frame. It was shown that clams were either not affected (no change in biodeposit
63 production rate) or showed an increase in biodeposit rates across a range of 0 to 100 mg
64 L⁻¹ of ammonium, or unionized ammonia ranging from 1.61 to 3.13 mg L⁻¹.

65 Additional experimental work is needed to determine the maximum amount of
66 ammonia exposure and length of exposure that is acceptable while continuing to produce
67 the optimal levels of biodeposits. Further research would be needed on the biological side
68 to determine when the clams sense ammonia why they do not immediately stop
69 siphoning, and why they continue to siphon even though higher amounts of ammonia are
70 present in the water.

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218 study in semi-in situ flow-through systems. *Journal of Marine Systems* 59, 143-158.

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CHAPTER 5

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CONSIDERATIONS FOR IMPROVEMENT FOR LARGE SCALE

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IMPLEMENTATION

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Before this novel harvesting technique is tested in a large-scale system, additional experimental findings need to be completed. First, finding the optimal clam density that produces the most biodeposits will need to be done in the laboratory. The experiments ran in this research only tested three clam densities to determine if this would even be a viable harvesting method. These results show that increasing clam density within the range tested continued to increase biodeposit production with no clear maxima. While simultaneously running the experiments to find the optimal clam density it would be imperative to monitor the concentration of dissolved oxygen in the system and evaluate the health of the clams. The health of the clam would be better monitored by a biologist that has experience with clam health, specifically marine clams.

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Aside from monitoring the health, predicting the death rates of the clams is also needed to be examined to determine how often fresh clams need to be introduced into the system in order to compensate for loss of production. This will ensure that the harvesting system will keep the same biodeposit production rates with no downtime.

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The experiments reported in this thesis were conducted with stagnant water to obtain biodeposit production rates. In a large-scale system, however, there will be flowing water which will need to be taken into consideration. Clams may siphon and

249 produce biodeposits at different rates under different flow rates, so finding that optimal
250 flow rate while connected to the raceway will be critical in scaling this method up.

251 It would be important to evaluate different species of algae to determine if the
252 biodeposit production rate is influenced by algal species. *N. oculata* was used in these
253 experiments but it is also one of the most nutritious that the clams can eat hence possibly
254 reducing the biodeposit production rate. If using another species of algae that is not as
255 nutritionally valuable could provide greater biodeposit production, this would be useful in
256 improving performance of the scaled up system.

257 In preliminary experiments with a continuous flow system, it was observed that
258 both algae and biodeposits adhered to the walls of the container and the pipes through
259 which flow occurred. This system was abandoned because it was not possible to get
260 accurate measurements of the final concentration of algae nor biodeposits produced. It
261 was observed that the biodeposits had to have a certain distance to get out of the flow of
262 the water in order to settle to the bottom. This process could be likened to that of waste
263 water sludge treatment with a clarifier, slowing the water down enough to let the heavier
264 solids fall to the bottom. Getting the biodeposits to settle out in order to efficiently
265 remove them is one of the main engineering concerns to get this harvesting method to
266 work.

267 The large-scale operation would depend on the type of algal cultivation system
268 that the algae growing facility will use (e.g. raceway pond or tubular reactors).

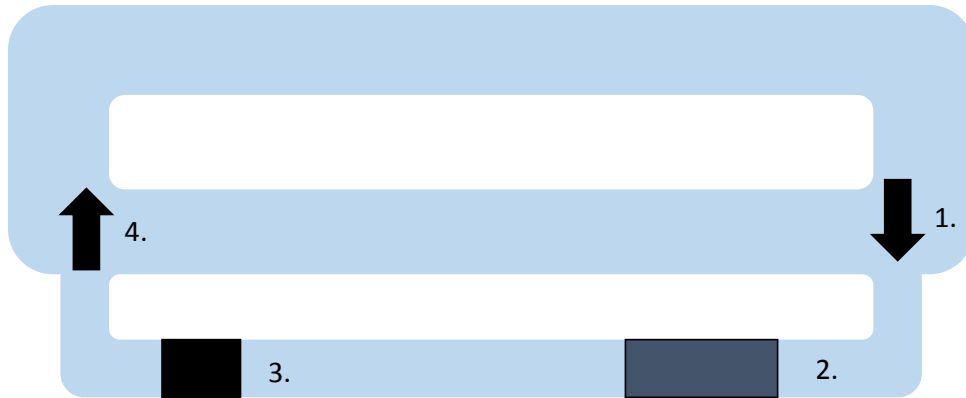
269 Conceptual renditions of both cultivation systems and an associated clam harvesting
270 system are shown in Figures 5.1 and 5.2 below.

271 In Figure 5.1, a raceway pond would be dug into the ground with the appropriate
272 paddlewheels. Next, another small pathway extending from one of the long sides of the
273 pond will come out and form its own detoured pathway until it reconnects back to the
274 pond. The arrow (1) is the direction of the water flow, where it then enters into the side
275 channel with the clams. The box (2) is where the clams would rest on a metal grate that
276 will not allow them to fall through. Further down, with the trench (3), a trench will be
277 dug into the newly created pathway to allow the biodeposits to settle into it and create a
278 way to siphon them off the bottom more efficiently. The arrow (4) is the direction the
279 water would flow after leaving the small pathway where the clams are. Additional
280 research is needed to determine the effects of different flow rates and determine how far
281 down the trench should be dug in order to obtain the maximum amount of biodeposits in
282 the trench.

283 Figure 5.2, depicts how the clam harvesting would work if the facility used a
284 tubular reactor system. The facility would grow the algae in the tubes, then the culture
285 medium is transferred to a large conical holding tank where the clams would be held (1).
286 Multiple layers of clams would be suspended on a metal grate that has holes small
287 enough that will not allow the clams to fall through but can allow algae and the
288 biodeposits to flow freely (2). Algae will flow out the bottom of the first conical tube and
289 into the top of a second conical tank with a much larger volume (3). Algae will flow into
290 the top of the second conical tank (4) and the flow rate will decrease with the help of the
291 baffle (5). Exact volumes will need to be experimented with in order to get the settling
292 rates correct, but the biodeposits should settle slowly to the bottom of the second tank
293 into the conical bottom. The algae and biodeposits settle to the bottom of the conical tank

294 where a valve can be opened every so often to remove the settled material. This will pass
295 through the piping (6) on to further processing.

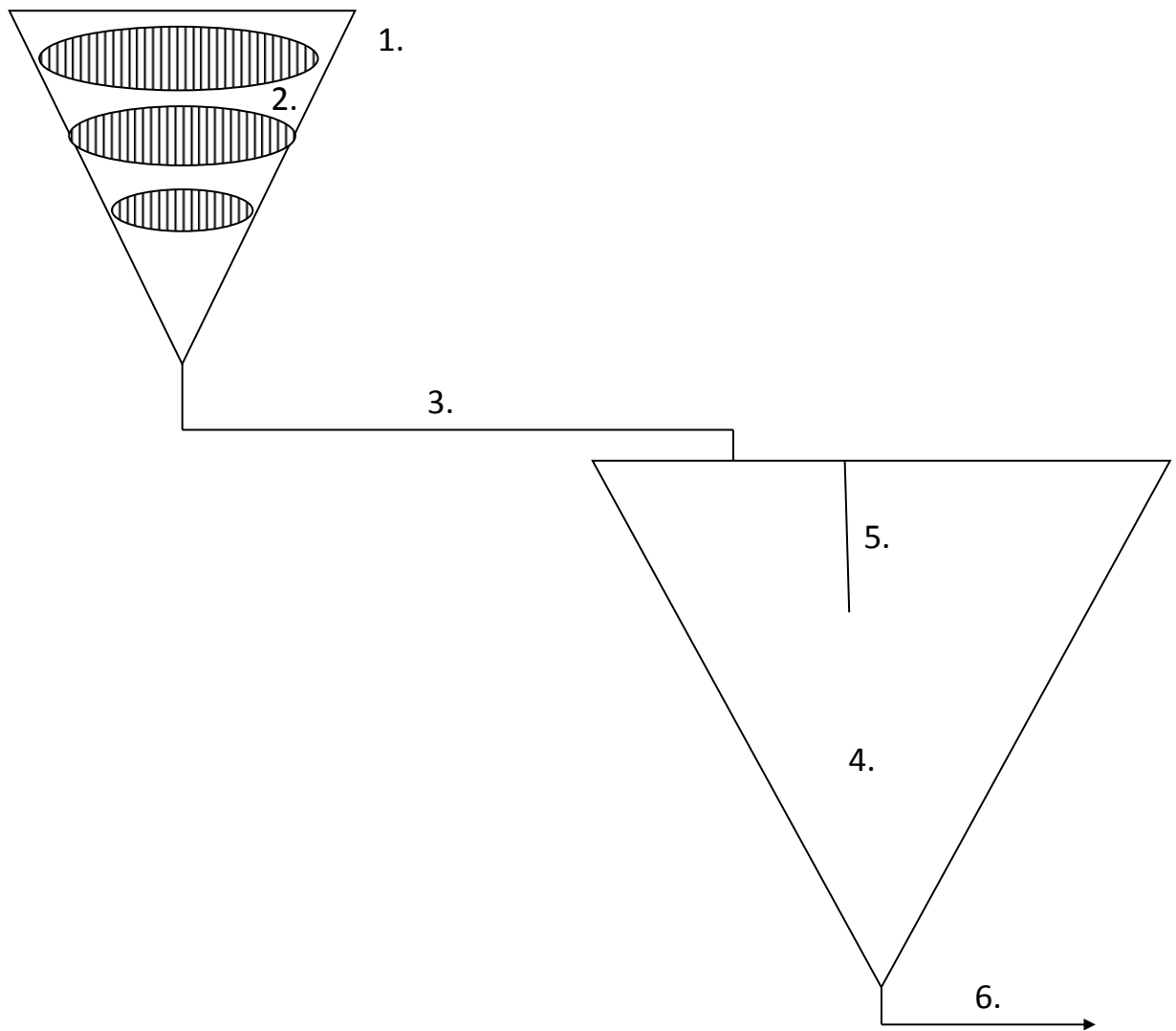
296 These figures are initial conceptual ideas and more detailed design and additional
297 work would need to be completed in order to fully recognize the potential of using clams
298 as an alternative harvesting technique.



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300 Figure 5.1: Conceptual rendition of an algal raceway pond with side pathway to allow
301 clams to harvest the algae.

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Figure 5.2: Conceptual rendition of a clam harvesting system based on a tubular algal growing system.

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CHAPTER 6

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CONCLUSIONS

312 This research was done to determine if it was possible to harvest algae with the
313 use of clams and to complete the four objectives stated in the introduction. These
314 objectives included the impact of algal and clam density on biodeposit production rate,
315 the impact of ammonia concentration on biodeposit production rate, and a physical and
316 chemical characterization of the produced biodeposits.

317 The impact of algal density on biodeposit rate is statistically different across the
318 wide range of algal densities tested, but there seemed to be no maximum algal density
319 that leads to a maximum biodeposit production rate. Over the course of all the
320 experiments it was shown that clam density does have an effect on the biodeposit
321 production rate. This effect over the three densities that were used in this experiment was
322 shown at the highest clam density of 6369 clams m^{-2} , which is intuitive because as more
323 clams are added, the higher the rate should be. There was no maximum among the three
324 densities tested. It was also shown that the effect on the length of starvation does impact
325 the biodeposit production rate, mainly in magnitude of production. The biodeposit
326 production rate efficiency, in $mg\ g^{-1}\ hr^{-1}$, decreased as the clam density increased due to
327 possible dissolved oxygen limitations or increased competition for the same algal
328 resources.

329 The amount of ammonia present in the culture, within tested concentration limits,
330 did not impact the biodeposit rate in one trial and actually increased the biodeposit
331 production rate in two other trials.

332 The mineral composition of the biodeposits suggests that the clams have the
333 ability to sequester different metals and salts. The lipid content of the biodeposits
334 increased compared to the algal cells, a potential benefit for biofuel applications. The
335 ultimate analysis suggests that the biodeposit composition differs very little from that of
336 the reference algae. The ash content of the biodeposits is 59% higher than that of the
337 algae.

338 Additional research into optimizing the clam harvesting apparatus and comparing
339 the harvesting rates in percent solids to that of conventional harvesting techniques is
340 needed.

341 This research was performed to improve our understanding of clam behavior in a
342 harvesting application. The work showed that significant variability existed within the
343 system, a result of having two biological systems (algae and clam) working in unison
344 with each other. Complications arise just in growing the algae, such as varying growth
345 rates based on sunlight and carbon dioxide, supplying enough nutrients in the
346 concentrations needed, and pest control management so the algal culture does not crash in
347 the ponds. Clams bring their own complications with keeping salinity, temperature, and
348 pH at stable levels in order for the clams to survive. Additionally, during operation it is
349 not possible to ensure (or even know) if all the clams are siphoning and producing
350 biodeposits during the time needed for harvesting.

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