The Potential of Coastal Marine Filtration

As a Feedstock Source for Biodiesel

by

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ABSTRACT

Second-generation biofuel feedstocks are currently grown in land-based systems that use valuable resources like water, electricity and fertilizer. This study investigates the potential of near-shore marine (ocean) seawater filtration as a source of planktonic biomass for biofuel production. Mixed marine organisms in the size range of 20μm to 500μm were isolated from the University of California, Santa Barbara (UCSB) seawater filtration system during weekly backwash events between the months of April and August, 2011. The quantity of organic material produced was determined by sample combustion and calculation of ash-free dry weights. Qualitative investigation required density gradient separation with the heavy liquid sodium metatungstate followed by direct transesterification and gas chromatography with mass spectrometry (GC-MS) of the fatty acid methyl esters (FAME) produced. A maximum of 0.083g/L of dried organic material was produced in a single backwash event and a study average of 0.036g/L was calculated. This equates to an average weekly value of 7,674.75g of dried organic material produced from the filtration of approximately 24,417,792 liters of seawater. Temporal variations were limited. Organic quantities decreased over the course of the study. Bio-fouling effects from mussel overgrowth inexplicably increased production values when compared to un-fouled seawater supply lines. FAMEs (biodiesel) averaged 0.004% of the dried organic material with 0.36ml of biodiesel produced per week, on average. C16:0 and C22:6n3 fatty acids comprised the majority of the fatty acids in the samples. Saturated fatty acids made up 30.71% to 44.09% and unsaturated forms

comprised 55.90% to 66.32% of the total chemical composition. Both quantities

and qualities of organics and FAMEs were unrealistic for use as biodiesel but

sample size limitations, system design, geographic and temporal factors may have

impacted study results.

My life is indebted to the love and sacrifice of my family: Kristin, Mom, Dad, Brian, Denise, Evan, Jenna, the Pedrazzis and the Dolans, who have all given so much of themselves to help see me through this goal.

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Introduction

Background

The concept of sustainability emerged over forty years ago as a necessary strategy to battle the environmental destruction that has occurred since the industrial revolution. Our global energy dependence has created a demand for fossil fuels that produce detrimental environmental side effects; the most notable being the production and emission of carbon dioxide. Those concerned about the environment commonly agree that air pollution, ocean acidification and global warming threaten current and future generations. Many understand that these environmental problems are caused by increased levels of atmospheric carbon dioxide created by the combustion of fossil fuels. In addition to this destruction, depleted oil supplies make these fuels unsustainable for future generations. Combustible fuels represent 70% of our global energy requirements (Kita et al., 2010). This fact combined with rising populations creates a likelihood that our global energy demand will only increase in the foreseeable future. In order to minimize environmental effects in the future, an alternative source of energy is needed that meets the global energy demand and reduces carbon dioxide emissions while concurrently maintaining economic and social viability.

Renewable energy in the form of solar, wind, geothermal and ocean energy have all been proposed and are currently used throughout the world as viable fossil fuel alternatives. Biomass is another possibility that provides a solution to several of our global environmental problems. Biologically based fuels present a sustainable alternative that is carbon neutral. Biofuels come in

many forms including biodiesel, bio-hydrogen, bio-gasoline, bio-ethanol, bio-methane and even solid biofuels. Currently first-generation biofuels are made from oil derived from vegetables and animal fats. These feedstocks pose their own sustainability issues by requiring arable land needed for food crop production, however, studies show that this relationship is more complicated than originally thought (Ajanovic, 2011; Mueller, Anderson, & Wallington, 2011; Rathmann, Szklo, & Schaeffer, 2010). Regardless, as global populations continue to increase food availability will become even more important. In order to avoid this conflict of interest, second-generation biofuels, -- such as microalgae -- are currently being developed. Microalgae-based biofuels have the potential to become a viable technological contributor to the global energy market (Demirbas, 2010).

Algae exist in macro and micro forms both of which have been researched for suitability as a biofuel feedstock. Currently only microalgae possess the properties needed for an economically viable fuel (Chisti, 2007). Microalgae are photosynthetic, unicellular eukaryotes that take carbon dioxide from the atmosphere and utilize sunlight to convert it into stored biomass, some in the form of lipids. They are found all over the world in fresh, salt and brackish water and some species can survive extreme living conditions. The most important microalgae from a biofuel perspective are the diatoms, green algae, blue-green algae and golden algae. Some golden microalgae have the ability to accumulate up to 80% of their dry cell weight in the form of lipids (Chisti, 2007)., A considerable portion of the total lipids accumulated in algal cells are in the form

of triglycerides and neutral lipids, which are ideal building blocks for biodiesel fuel (Tonon, Harvey, Larson, & Graham, 2002). Various strains of microalgae can be made to increase lipid production via external stimulus, giving them increased potential as feedstock for fuel. Yields from microalgae average 10 to 20 times higher than land-based crop oils (Chisti, 2007). Growth rates of microalgae are also much faster than land-based crops leading to faster harvests and increased production. Current microalgae production is used for bioethanol, bio-oil, biodiesel and bio-hydrogen (Demirbas, 2010). Along with high yields and rich lipid content, microalgae biofuel serves as a carbon-neutral fuel. These fuels help to slow global warming by not adding any new carbon dioxide to the atmosphere, and reduce new environmental issues such as ocean acidification. Their use decreases emissions of carbon monoxide, airborne particulates, sulfur oxides and hydrocarbons (Gouveia & Oliveira, 2008). Algal biomass can be used to scrub coal-fired power plant flue gases or decontaminate municipal and industrial wastewater (Aslan & Kapdan, 2006; Mata, Martins, & Caetano, 2010; Yang et al., 2011). Furthermore, this feedstock does not compete spatially for arable lands needed for crops, and some can grow in contaminated water supplies. The benefits of algal-based biofuel are hard to deny, yet several factors have contributed to their slow maturation in the energy market.

There are many reasons microalgae-based fuels have not skyrocketed to the top of the world energy scene. Due to the small size of algal cells, scaling up operations to produce large enough quantities of microalgae oil that satisfy our global demand for fuel is difficult. Once large scale cultures of single or mixed

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strains of microalgae have been grown, they must be dewatered before extracting the lipid-rich oils. The dewatering and oil extraction process is energy-intensive; much (90%) of the total energy used in algal harvesting is needed in the drying step of the process (Lardon, Helias, Sialve, Steyer, & Bernard, 2009). Along with harvesting and extraction, the life cycles of these tiny aquatic organisms also pose challenges to large scale production. Many microalgae require specific conditions for optimal growth. These conditions include temperature, pH, nutrient availability, access to sunlight and agitation. Microalgae farmers face significant risk of catastrophic failure as they balance the strict growth conditions required and external stress stimulus needed to produce extra quantities of lipids.

The global demand for energy is second only to our demand for freshwater. Only a small percentage of our global water supply is in the form of useable fresh water. Due to the shortage of this resource, any operation that requires potable or freshwater for its success could be deemed unsustainable. Large-scale land-based microalgae production requires large amounts of water. For this reason, it is important for researchers to look to other high-quantity and high-quality sources of microalgae that conform to the sustainable strategy needed for future fuels.

Traditionally microalgae fuels have been cultivated in land-based systems that isolate individual strains or groups of strains for optimal production of lipid-rich oils. These systems are either open or closed depending on the growth requirements of the specific strains and the size and scale of the production. Designs ranging from "raceway" style to photo-bioreactors have been developed

to speed growth. Some of these designs have dual purposes of growing microalgae while also scrubbing factory emissions or cleaning wastewater effluent. The energy-intensive harvest of algal cells has been accomplished by several methods including filtration, centrifugation, flotation, flocculation and ultrasonic means (Demirbas, 2010). Post-harvest processing typically involves the drying, extraction and transesterification of fatty acids to produce fatty acid methyl esters (FAME) which are the basis of biodiesel. Microalgae rich in neutral lipids as well as di- and triglycerides are best for biodiesel production (Tonon et al, 2002). Many procedures have been developed for the extraction of lipids from tissue including the Bligh and Dyer (1959) and Folch (1957) methods. Direct transesterification of algal biomass has proven to be a viable option for the production of FAME without the intermediate step of lipid extraction (Lepage & Roy, 1984; Sheng, Vannela, & Rittmann, 2011; Tran, Hong, & Lee, 2009; Wen & Johnson, 2009). Significant research has been done to develop current microalgae-based biofuel technologies; however, little work has focused on the exploration of new feedstocks for biologically based fuel.

Our world's oceans provide a natural, sustainable and potentially economically viable source of biomass-based fuels. Microalgae, zooplankton and cyanobacteria all float freely in this liquid medium waiting to be harvested for use as a carbon neutral solution to our energy crisis. These organisms are found in broad spatial distributions and are affected by the same nutrient and temperature limitations as land-based systems. Marine plankton communities show large variances in population assemblages based on depth, location, temperature, light,

season and prevailing currents (Anderson, Siegel, Brzezinski, & Guillocheau, 2008; Barnett & Jahn, 1987; Keister, Di Lorenzo, Morgan, Combes, & Peterson, 2011). It can only be assumed that for these reasons, the biofuel potential of this source has not been examined in as much detail as have land-based systems. Though spatially and temporally variable, the quantity and quality of marine-based biomass should not be disregarded. As previously discussed, the lipid fractions of some microalgae can be as high as 80% dry weight depending on the species and growth parameters. Zooplankton -- which include holoplankton (ex. diatoms, ctenophores, dinoflagellates, etc.) and meroplankton (ex. larvae) -- feed on microalgae and accumulate lipid fractions in the form of neutral, di- and triglycerides as well as wax esters. Free fatty acid values between 5% and 25% of total lipids have been found in marine zooplankton (Lee, Hirota, & Barnett, 1971). Triglycerides can be found in the majority of marine phytoplankton and zooplankton as well as the sea surface microlayer, sea foam and particulate matter. The lipid fraction of cyanobacteria -- which is also common in the marine environment -- is mainly in the form of diglycerides (Parrish, 1988). Wax esters can account for up to 92% of total lipids in marine copepods, and have been reported in high quantities in other zooplankton (Cripps & Tarling, 1997). These numbers all illustrate this potential source of biofuel that has yet to be explored. Data on the temporal availability of this resource in accessible near shore environments is integral to a better understanding of the feasibility of the supply.

Problem Statement

A data gap exists in regard to the suitability of marine-based biofuels. More information is needed to determine if seawater filtration can provide the quantity and quality of lipid-rich organisms needed for biofuel production. This study serves to partially bridge this gap and bring a better understanding of the biofuel potential of marine filtration. Utilizing a system already in place, this study will help to determine the natural availability of marine-based feedstocks and the quality of the fatty acids they produce.   The University of California, Santa Barbara (UCSB) sits next to the Pacific Ocean and maintains an open-flow seawater system for use in classes and research. Seawater from a depth of 55 feet is siphoned on-shore where it undergoes gravity filtration through a sand/rock/gravel filtration bed. Particles greater than 20 microns are removed from the water and remain in the filters. The filters are cleaned three times a week by "backwashing" them with filtered seawater and discarding the suspended solids to the UCSB lagoon. This study seeks to capture the suspended solids removed from the system and analyze them as a potential source of lipid-rich compounds for biofuel. Quantitative and qualitative analysis of the solids is necessary to determine viability. Characterization of this system will provide background data for future exploration and comparison. The seawater system at UCSB uses less energy than comparable land-based systems, and due to the location it has the potential to be powered by non-polluting wave energy. Valuable freshwater resources are not required to produce biomass, which adds to its attraction as a sustainable source. Similar infrastructure already exists globally

in the form of power plants, desalinization facilities, public utilities, wastewater treatment plants, aquaculture farms, aquaria and educational institutions. Recovery of suspended solids -- if proven viable -- could be added to systems currently in place, which would potentially create broader implications to fishing and aquaculture communities around the world. Marine filtration has the potential to provide a sustainable, environmentally friendly and economically affordable alternative to land-based second-generation biofuel feedstocks.

Study Objectives

- Recover and quantify the organic material that is collected by the UCSB seawater filters.

- Isolate and purify the recovered solids to remove inorganic contaminants.

- Convert the purified organics into biodiesel.

- Qualitative analysis of the biodiesel to determine suitability as biofuel.

This study does not seek to identify the individual components of the recovered organic material nor will the full spectrum of organisms be targeted. Due to the design of the seawater system and scope of the study a sample size range limitation of 20μm to 500μm is necessary. A variety of geographic and physical limitations prevents a detailed analysis of feedstock potential at varying locations and depths. For these reasons only mixed microalgae and zooplankton that happened to be present on the coast of Santa Barbara, next to UCSB, were captured for analysis. Alternately, qualitative analysis was conducted on the biodiesel produced from the system and did not focus on other potential biofuel products. In addition, no economic evaluation will be done on the production system to determine overall costs or feasibility.

Literature Review

Background

This study focuses on the necessity and current status of the development of second-generation biofuels, biodiesel in particular. The first internal combustion engines developed in the early 1900s were originally meant to run on biologically-derived fuels such as plant oils and ethanol. Detailed scientific study of biological fuels began in the 1950s (Barnwal & Sharma, 2005; Demirbas, 2005; Felizardo et al., 2006; Fukuda, Kondo, & Noda, 2001; Gerpen, 2005). Study results indicate that common biofuels such as biodiesel and bioethanol have the ability to replace petroleum diesel and gasoline in modern-day vehicles without any modification of current technology (Mata et al., 2010). The potential to blend biofuels with current fossil fuels also presents a means of emissions reduction. Burning biofuels leads to a decrease in the emission of carbon monoxide, hydrocarbons, particulate matter and sulfur oxides as compared to fossil fuels (Girard & Fallot, 2006; Gouveia & Oliveira, 2008; Mutanda et al., 2011). Biofuel production has the ability to remove carbon dioxide from the atmosphere and can be used to capture and convert carbon dioxide that is emitted from fossil fuel combustion sources -- such as coal-fired power plants -- into biomass (Amin, 2009; Wang, Li, Wu, & Lan, 2008). Biodiesel has properties similar to those of fossil derived diesel fuel but possesses higher cetane numbers and better lubrication properties which aid in lowering emissions compared to petroleum (Girard & Fallot, 2006).

First-generation biofuels were derived from soybeans, sugar cane, sugar beets, maize, rapeseed, palm, coconut, sunflower, Jatropha as well as used cooking oils from vegetables and animal fats (Khan, Rashmi, Hussain, Prasad, & Banerjee, 2009). Though these fuels provided a good alternative to fossil fuels, further development of strategies that do not require arable land and potable water was needed. Second-generation biofuels were developed and currently focus on microalgae as a main source of lipid-rich material. Chisti (2007) and others have gone as far as to say that microalgae are the only feedstock of biofuel that has any potential for economic viability. Between 1978 and 1996 significant microalgae research was done by the United States National Renewable Energy Laboratory in their Aquatic Species Program (Sheehan, Dunahay, Benemann, & Roessler, 1998). These studies focused on individual algae strain lipid production, culturing facility designs, and the potential for low-cost production. Three thousand strains of algae were narrowed down to three hundred which were determined to be viable feedstocks for biofuel production (Sheehan et al., 1998). These early studies led to a massive amount of research and development effort to bring microalgae-based biofuels to the forefront of the renewable energy revolution.

Current Research

    Strain selection.

Current algae-based biofuel technologies focus on the cultivation of individual strains in land-based systems. In an effort to produce large quantities of economically viable biofuel, considerable research has been devoted to

determining individual species of algae that meet modern day energy demands.
Over 50,000 species of microalgae are estimated to exist -- 30,000 of which have
been identified -- with only a small fraction studied (Henry, 2004). For the
purposes of this study, microalgae are characterized as both prokaryotic and
eukaryotic photosynthetic microorganisms. The prokaryotic -- or blue-green
photosynthetic bacteria -- include the *Cyanophyceae* (cyanobacteria) and
Prochlorophyceae. The eukaryotic algae include *Bacillariophyceae* (diatoms),
Chlorophyceae (green algae) and *Chrysophyceae* (golden algae) which have been
the focus of second-generation biofuel research. Studies show that diatoms are
the dominant form of marine microalgae and probably have the largest influence
on biomass production in our world's oceans (Demirbas, 2010).

Microalgae fix carbon dioxide via photosynthesis into biomass which
includes lipids. These lipids come in many different forms including
triglycerides, diglycerides and phospholipids (Singh, Nigam, & Murphy, 2011).
A large percentage of polyunsaturated fatty acids making up as much as 50% to
65% of the total microalgae lipid composition is common (Demirbas & Fatih
Demirbas, 2011; Gouveia & Oliveira, 2008; Packer, 2009). Many lipids
contained in marine diatoms are in the form of phospholipids (Singh et al., 2011).
Oil percentages are equally important in the production of biofuel. Some of the
most common microalgae can have oil levels between 20% and 50% of their total
cell weight or volume. A few strains have been discovered to contain levels as
high as 80% (Amaro, Guedes, & Malcata, 2011; Metting, 1996; Spolaore,

Joannis-Cassan, Duran, & Isambert, 2006). A detailed analysis of microalgae lipid compositions and oil content will be discussed later in this review.

Academic institutions have begun to conduct massive culturing and research efforts on microalgae due to their potential as a source of biodiesel, bioethanol, bio-oil, bio-syngas and bio-hydrogen. Several prominent laboratories exist in the United States at Arizona State University, Colorado State University and the University of California, San Diego (Batan, Quinn, Willson, & Bradley, 2010; Chen, Sommerfeld, & Hu, 2011; Franklin and Mayfield, 2004; Li, Han, Sommerfeld, & Hu, 2011; Rousch, Bingham, & Sommerfeld, 2003). The University of Coimbra (Portugal), Gottingen University (Germany), University of Texas (USA), National Institute for Environmental Studies (Japan), and CSIRO Collection of Living Microalgae (Australia) all maintain extensive collections of algae (Mata et al., 2010). Genetic modification has also been introduced as a potential source of lipid and oil-rich microalgae strains (Amaro et al., 2011). As previously stated, current research efforts primarily focus on land-based cultivation of microalgal resources. For this reason it is essential to have an absolute understanding of the growth parameters necessary to produce large quantities of microalgae in an enclosed non-natural environment.

Growth parameters.

A great deal of scientific study has been done to determine the ideal growth conditions for individual strains of microalgae. Microalgae absorb carbon dioxide and assimilate nutrients via photoautotrophic production, heterotrophic production or mixotrophic production (Brennan & Owende, 2010).

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Photoautotrophic production has been the primary focus in previous biofuel studies. All photosynthetic microalgae need light, water and nutrients for growth. Access to light is necessary to the photosynthetic cycle and allows for assimilation of nutrients and expansion of populations. Studies indicate that only light radiation between 400nm and 700nm wavelengths is captured in the photosynthetic process and these wavelengths make up 42.3% of the total energy from the light spectrum (Bolton & Hall, 1991). Studies also demonstrate that both light limitation and photoinhibition from excess light can lead to decreased productivity of microalgae cultures (Myers & Burr, 1940; Wen & Chen, 2003). For these reasons land-based systems must have appropriate culture densities, quantities of light and flow to encourage optimal growth.

Photosynthesis is a two-step process with photosynthetic activity occurring during the day and respiration occurring at night. Chisti (2007) determined that as much as 25% of algal biomass can be lost at night due to respiration. He also found that the frequency of light/dark cycling had an impact on the productivity of land-based microalgae cultures. Along with adequate amounts of light, sufficient supplies of carbon dioxide must be present for microalgae to grow at appropriate rates (Demirbas & Fatih Demirbas, 2011). Supplementing growth media with high amounts of carbon dioxide can lead to increased concentrations of dissolved oxygen from photosynthetic activity. High oxygen levels in conjunction with intense sunlight have the potential to result in photo-oxidative damage to microalgal cells. This damage can be prevented by not exceeding 400% of the air saturation value in the growth medium (Chisti,

2007). Nutrient limitations within the growth media have the potential to cause the greatest change in chemical composition of microalgal cultures (Amaro et al., 2011; Liu, Wang, & Zhou, 2008; Miron et al., 2003; Mutanda et al., 2011; Packer, 2009; Widjaja, Chien, & Ju, 2009). Several studies corroborate the effects of nitrogen limitation on algal lipid composition. Nitrogen (silica for diatoms) limitation prevents the expansion and proliferation of algal cultures but boosts the assimilation of carbon within each individual in the forms of fatty acids and triglycerides (Brennan & Owende, 2010; Chisti, 2007; Li et al., 2011; Meng et al., 2009; Packer, 2009). Field studies conducted in the Antarctic Ocean along with bench-scale laboratory experiments demonstrated that iron concentrations have the ability to induce considerable lipid production response in the marine microalgae *C. vulgaris* (Boyd et al., 2000; Liu et al., 2008; Uduman, Qi, Danquah, Forde, & Hoadley, 2010). The results of these studies have led to commercial growers supplementing their growth media with nitrate and phosphate fertilizers in an attempt to boost population sizes and increase yield quality. Supplementation adds to cultivation costs, has the potential to cause environmental damage, and decreases the sustainability of the final product. The need for water resources also makes land-based cultivation of microalgae challenging and potentially adds to production costs. Along with light, nutrient fluctuations and temperature (Rousch et al., 2003); shear stress between cells can also have a negative impact on algal production (Chisti, 2007; Miron et al., 2003). All of these growth parameters have led to the design of different growth systems for the commercial cultivation of algae.

Growth systems.

The focus of this study is on natural production of microalgae and subsequent conversion for use as biodiesel. Within this review photoautotrophic production systems will be the primary focus. By definition photoautotrophic cultivation systems require either naturally occurring or artificial light. Land-based cultivation systems exist in two forms: open pond systems and closed photobioreactors. Many scientific works have focused on economically feasible designs of these two forms (Batan et al., 2010; Brennan & Owende, 2010; Chisti, 2007; Demirbas, 2010; Mata et al., 2010). No peer-reviewed scientific studies examining the potential of natural marine filtration as a production source of biofuel feedstock currently exist.

Land-based outdoor open pond systems are the oldest, cheapest and simplest mass cultivation designs for microalgae (Demirbas, 2010). Open pond designs include "raceway" style ponds, circular ponds with rotating arms, and inclined systems with gravity flow. In the "raceway" system, algae and nutrients are circulated around an oval pond via paddle wheel or pump. Several studies have determined that these open "raceway" systems are the only economically feasible large-scale production design (Molina Grima, 1999; Terry & Raymond, 1985). Raceway systems vary between 15 cm to 35 cm deep and between 0.2 ha and 0.5 ha in total size (Demirbas, 2010). Though cheap to construct and operate, open pond systems are susceptible to contamination from the outside environment and do not provide the high production yields of photobioreactors. Also, only

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hardy algal strains can be cultivated to cope with the variety of external stimulus present.

Thirty times the algal biomass can be cultivated in a closed photobioreactor facility as compared to an open "raceway" style pond (Chisti, 2007). Higher biomass concentrations, shorter harvest times, and increased surface to volume ratios are also possible in these closed photobioreactor systems (Demirbas, 2010). Photobioreactors come in many different forms including column, tubular and flat plate designs. Yields of 9.2 g/L per day dry weight biomass are possible for these systems (Hu, Kurano, Kawachi, Iwsaki, & Miyachi, 1998). The higher yields of closed photobioreactors are generally offset by the higher production costs of pumps to maintain circulation, carbon dioxide supplementation to the liquid media and artificial illumination (if necessary). Closed systems also have the potential of accidental biomass sedimentation and photo-oxidative stress if internal conditions are not monitored continuously (Chisti, 2007). To prevent these occurrences, design studies conducted by Miron et al. (2003) determined that aeration velocities could directly impact sedimentation, shear stress and cell viability, with bubble columns outperforming airlift vessel designs.

Opinions on the practicality and functionality of open pond systems versus closed photobioreactor systems are mixed and hotly debated. Benemann (2008) and Walker (2009) concluded that photobioreactors were worse than open pond systems in design and production aspects. Other scientists believe any system that cannot produce a high quality and quantity of microalgae for biofuel is

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uneconomical. Since the feedstock grows in a liquid medium, harvesting, dewatering and drying microalgae before conversion into biofuel is an essential step in the process chain and can account for 20% to 30% of the total end cost (Gudin & Therpenier, 1986).

Harvesting.

Algal harvesting processes include filtration, flotation, flocculation, ultrasonic separation, sedimentation and centrifugation (Bosma, van Spronsen, Tramper, & Wijffels, 2003; Brennan & Owende, 2010; Demirbas, 2010; Mata et al., 2010; Uduman et al., 2010). Harvesting methods depend greatly on the concentration of cells, the type of microalgae feedstock and the desired flow rate. Choosing an appropriate harvesting method is essential to maintaining the viability of microalgal cells for chemical conversion. Biomass filtration is generally suited for microalgae greater than 70 μm (Brennan & Owende, 2010). Filtration yields are low for cells less than 30 μm, with the exception of ultrafiltration membranes which are effective down to a cell size of 3 μm (Zhang, Hu, Sommerfeld, Puruhito, & Chen, 2010). Froth flotation can be used as a solitary technique or in combination with other extraction methods. Several studies determined the addition of chemical flocculants aids in the aggregation of microalgal cells (Danquah, Ang, Uduman, Moheimani, & Forde, 2009; Edzwald, 1993; Molina Grima, Belarbi, Acien, Robles, & Chisti, 2003; Sukenik, Bilanovic, & Shelef, 1988; Tenney, Echelberger, Schuessler, & Pavoni, 1969; Uduman, Qi, Danquah, & Hoadley, 2010). Since flocculation processes depend greatly on charge neutralization they are affected by both pH and temperature. Indeed, pH

values greater than 10.5 negate the need for chemical flocculants completely as magnesium and calcium ions precipitate at this level and naturally floc microalgae (Ayoub, Lee, & Koopman, 1986). Ultrasound may also be used to increase cell aggregation which promotes sedimentation (Bosma et al., 2003).

Density-dependent processes like sedimentation and centrifugation are commonly used to remove microalgae from its natural environment. Sedimentation is one of the most common techniques employed for harvesting large-sized (>70 micron) microalgae and can be used in conjunction with flocculation and ultrasonic separation. The principles of sedimentation are described by Stokes' law which determines the maximum sinking velocities of perfectly round microalgae in non-turbulent fresh water (Oliver, Kinnear, & Ganf, 1981). Since not all microalgae are perfectly round nor are bodies of water perfectly calm or fresh, a modification of Stokes' law by McNown and Malaika (1950) takes into account the varying sizes, densities and shapes of microalgae and directly relates these variables to their terminal sinking velocities. An alternative to sedimentation is forced settling by centrifugation. Centrifugation is an energy intensive process that is only economically viable for high-value end products such as pharmaceuticals and nutraceuticals (Brennan & Owende, 2010). Heasman, Diemar, O'Connor, Sushames, and Foulkes (2000) found that harvesting efficiencies in excess of 95% are possible with centrifugation. More recent studies have focused on the potential of electrolytic harvesting methods but temperature increases and cathode fouling issues have made them economically non-viable (Amaro et al., 2011).

Dewatering and drying.

Traditional processing of microalgae has called for the dewatering and drying of the cells before chemical conversion. Processing techniques are slowly changing with the advent of new conversion technologies that allow for larger percentages of water in the transesterification reaction. Several methods of drying microalgae are currently used -- including sun drying, spray drying, drum drying and freeze-drying -- which use less energy than inefficient thermal drying processes (Henry, 2004). Much like cultivation and harvesting, drying and dewatering processes greatly increase the overall cost of production. While cost effective, solar (sun) systems are prohibitively slow emphasizing the need to develop new drying processes that will decrease cost and improve the economic viability of the final product. Alternatively, spray, drum and freeze-drying all require energy which contributes immensely to overall production costs and decreases the sustainability of the final product. A successful microalgal biofuel must be more sustainable than first-generation biofuels and remain cost competitive with fossil fuels.

Comparison to first-generation biofuels.

Second-generation biofuels have several advantages over their first-generation predecessors. Studies estimate that several million species of algae exist in the world as compared to 250,000 species of terrestrial plants (Norton, Melkonian, & Andersen, 1996). Though it is widely agreed that the cultivation of algae is more energy-intensive and expensive than growing terrestrial plants, the energy output of microalgae greatly outweighs the input (Batan et al., 2010;

Chisti, 2007; Reijnders, 2009). In general, microalgae produce more oil per unit area, consume less space per unit area, have faster growth rates, and do not compete for arable land that should be used for agriculture (Chisti, 2007; Demirbas, 2010; Demirbas & Fatih Demirbas, 2011; Mutanda et al., 2011).

Microalgal yields as compared to first-generation feedstock such as soy and oil palm can be over 200 times higher. When compared to seeds and vegetable oils an increase of 10 to 20 times is not uncommon (Chisti, 2007). The small size and high lipid percentage of microalgae also contributes positively to its dominance over first-generation biofuels. Studies indicate that microalgae are able to achieve a higher photosynthetic efficiency (PE) as compared to terrestrial plants (Brennan & Owende, 2010; Doucha & Livansky, 2008; Hase, Oikawa, Sasao, Morita, & Watanabe, 2000; Morita, Watanabe, & Saiki, 2002; Xia & Gao, 2003). PE as high as 21.6% have been found in certain microalgae strains (Fernandez, Camacho, Perez, Sevilla, & Grima, 1998). There is evidence that microalgae -- especially diatoms -- utilize C4 fixation, which is a more sophisticated photosynthetic process compared to the C3 fixation used by terrestrial plants (Reinfelder, Kraepiel, & Morel, 2000; Reinfelder, Milligan, & Morel, 2004). Increased efficiencies have also been explained by microalgae's varying use of the enzyme glucose-6-phosphatase and ATP transesterification as compared to terrestrial plants (Woodward et al., 1996), though some researchers are skeptical of these findings.

Walker (2009) reported a maximum potential photosynthetic efficiency of 4.5% for microalgae and concluded that there was no credible evidence

supporting the claims that microalgae produce more biomass per unit area than terrestrial plants. His findings corroborated work by Radmer and Kok (1977) who determined that microalgae often yield less production than terrestrial plants. Using transportation sector fuel data from the United States, Chisti (2007) determined that 24% of the total cropland would need to be devoted to oil palm biomass production in order to replace 50% of the nation's transportation fuel. By comparison only 3% of the total cropland would need to be devoted to microalgae production in order to satisfy the same percentage. While disagreement exists in the scientific literature over the efficiency of microalgae, scientists do agree there is potential for using microalgae in the reduction of emissions and production of valuable alternative fuels, chemicals and nutritional supplements.

Combining technologies.

Land-based microalgae cultivation systems have increased their overall value by being successfully coupled with other processes. Industrial flue gases have been mixed with microalgae cultivation to reduce overall carbon dioxide emissions while simultaneously producing biomass for biofuel (Wang et al., 2008). Microalgae can also be used in conjunction with wastewater treatment to remove inorganic nutrients such as NH_4^+, NO_3^- and PO_4^{3-} from effluent streams (Aslan & Kapdan, 2006; Wang et al., 2008) or for co-firing in power generation plants (Demirbas & Fatih Demirbas, 2011; Wang et al., 2008).

Various conversion processes can be applied post-harvest to microalgae. Depending on the process a variety of products can be produced including oil,

gas, ethanol, biodiesel, bio-hydrogen and bio-methane (Amaro et al., 2011; Amin, 2009; Girard & Fallot, 2006). In addition to the inherent energy value of microalgae, biomass left over after oil extraction has been used for chemical production, fertilizers, animal feeds, cosmetics, pharmaceuticals and food additives (Chisti, 2007; Chisti, 2008; Mata et al., 2010; Miron et al., 2003; Raja, Hemaiswarya, Kumar, Sridhar, & Rengasamy, 2008; Rosenberg, Oyler, Wilkinson, & Betenbaugh, 2008; Singh & Gu, 2010). The concept of bio-refineries for the production of these byproducts has recently emerged. This system -- commonly used in the petroleum industry -- has the ability to reduce costs by increasing efficiency. Following the laws of nature and natural food chains, microalgae have also been used in the cultivation of several different types of zooplankton including rotifers, cladocerans, brine shrimp and copepods (Borowitzka, 1997; Brown, Jeffrey, Volkman, & Dunstan, 1997; Pauw, Morales, & Persoone, 1984). The resulting zooplankton are in turn used as feed in fish and mollusk aquaculture. Cultivation of microalgae has yet to achieve economic viability despite the vast number of potential end products achieved from the process.

Economics

Feedstock growth and processing.

The inability of the biofuel industry to make its product economically viable has been the main reason for failure to compete with petroleum-based fuels. Many companies have begun to explore this potential energy source in an attempt to streamline production costs and create viable returns for investors

(Torrey, 2008). Several studies have been published that explore, outline and quantify the costs associated with biofuel production (Amaro et al., 2011; Chang, Kim, Kang, & Jeong, 2010; Chisti, 2007; Demirbas, 2010; Demirbas & Fatih Demirbas, 2011; Girard & Fallot, 2006; Lardon et al., 2009; Mata et al., 2010; Uduman et al., 2010). Feedstock growth, harvesting/drying and oil extraction/transesterification are the primary costs of microalgal fuel production.

A review of the available scientific research reveals that the economic viability of this product is linked closely with the energy utilization in the process chain. A study conducted by Lardon et al. (2009) found that the energy used in the production of biodiesel frequently eclipses that which is contained in the fuel itself. Demirbas and Fatih Demirbas (2011) determined that in order for biofuels to compete with fossil fuels, they would require large quantities of cheap biomass. Much (60% - 75%) of the final cost of biodiesel can be attributed to the cost of feedstock growth (Canakci & Sanli, 2008). As previously stated the primary feedstock acquisition strategies currently employed involve growing microalgae in land-based systems. The cost of production in open ponds is drastically lower than similar closed systems (Chisti, 2007; Demirbas, 2010; Demirbas & Fatih Demirbas, 2011; Mata et al., 2010). The current cost of biodiesel production is dependent on the type of growth system and the oil percentage of the strain. Thurmond (2009) estimates the cost of production at $9/gal - $25/gal in open ponds systems and $15/gal - $40/gal in closed systems. Conversely, Chisti (2007) estimates that closed systems have the potential to produce biomass cheaper than

open ponds. He estimates that in order to make production competitive with fossil fuels a final cost of $0.48/L ($1.85/gal) is necessary for biodiesel.

Land-based systems require immense amounts of electricity, water, fertilizers and artificial light (depending on design), which significantly contribute to the overall cost of production. Harvesting and drying also contribute a great deal to the final value of biofuels. Demirbas and Fatih Demirbas (2011) and Molina Grima et al. (2003) estimate that harvesting alone accounts for nearly 20% - 30% of the total production costs while Chisti (2007) estimates values as high as 50%. Filtration, floatation, sedimentation, flocculation and centrifugation all consume energy and require infrastructure (Uduman et al., 2010). Singh and Gu (2010) report that centrifugation alone can add over $1/gal to production costs. In an attempt to increase economic efficiency several companies have developed unique harvesting strategies to reduce cost. Algae Venture Systems created a combination separation, dewatering and drying system that contributes less than $0.30/gal to the final biofuel cost. Oil extraction and transesterification have also been developed to increase efficiency and decrease costs. An extraction strategy that decreases process costs by several dollars per gallon has been patented by Missing Link Technologies and is currently being used by Algae to Energy. Chemical conversion technologies have also become more cost effective. Transesterification methods continue to be improved in an attempt to eliminate the expensive drying step in the process chain. Even with current developments there is still a great deal of progress that must be made before second-generation biofuels become economically viable.

Comparison to other sources.

A brief comparison of microalgae to first-generation feedstocks elucidates some reasons why they haven't reached financial prominence. For example, the cost of producing biodiesel from palm oil is approximately $0.66/L (Demirbas & Fatih Demirbas, 2011). Rapeseed and soy production costs range between $0.35/L and $0.55/L, respectively, depending on the price of oil (Girard & Fallot, 2006). The production cost of microalgae is greater than fuel produced from terrestrial crops which is greater than petroleum fuel. The economic viability of biodiesel from microalgae depends on the production costs as well as the concurrent price of petroleum diesel. As crude oil supplies decrease, petroleum production costs will climb making biofuels more viable. Chisti (2007) predicted a price point of $0.55/L for microalgae oil if crude petroleum reached $80/barrel. In 2011 we have seen petroleum prices eclipse $100/barrel. Using the estimate that 47,000 - 308,000 l/hectare/year of oil can be produced by microalgae, the cost per barrel would be a mere $20 (Demirbas, 2010). Unfortunately, these estimates have not come to fruition due to lower than expected microalgal production/yields. With land-based cultivation consuming a substantial portion of fuel production costs, the acquisition of a large quantity of cheap biomass such as that found in our world's oceans may be economically beneficial to the biofuel industry.

Future implications.

Much research needs to be done regarding the potential use of marine microalgae and zooplankton as a feedstock for biofuels. Marine sources may

possibly decrease overall production costs by eliminating land-based production systems and focusing more energy on the harvesting and downstream processing of these sources into potential fuel and subsequent commercial products. Researchers have assumed that there is temporal variability in marine microalgae and zooplankton populations resulting in the majority of current scientific research focused on individual strains of algae and their inherent lipid compositions. Current scientific thought has neglected the potential viability of naturally occurring marine biomass. Many believe the cultivation cost of microalgae is a limiting factor in its competitiveness with fossil fuels; therefore, a baseline study of marine microalgae's and zooplankton's potential as biofuel is necessary. Lipid quantity and quality is of the utmost importance to a potential biofuel feedstock. For these reasons an understanding of the lipid and fatty acid compositions of marine microalgae and zooplankton is important for successful exploration of this biomass source.

Chemical Composition

Types of fatty acids.

The lipid type present in a biofuel feedstock has one of the greatest influences on the economic viability of that feedstock. Fats and oils are generally water insoluble and hydrophobic. They come in many forms including triglycerides, diglycerides, free fatty acids, wax esters, phospholipids, etc. Triglycerides are composed of 3 moles of fatty acid linked to one mole of glycerol and are the ideal molecules for transesterification into biodiesel. By comparison, free fatty acids can complicate the transesterification process due to their

hygroscopic nature (Karmakar, Karmakar, & Mukherjee, 2010). For these reasons it is beneficial to find feedstocks that are high in triglycerides and low in free fatty acid content. Studies completed by Van Mooy and Fredricks (2010) demonstrated that intact polar diglycerides were likely the most abundant group of fatty acids found in the marine environment. Diglycerides, triglycerides and other components play important roles in energy storage for microalgae and zooplankton as well as regulate hormone production, cell signaling and chemical defenses (Irigoien, 2004; Miralto et al., 1999; Vardi et al., 2008).

Several scientific studies have explored the chemical composition of the ocean's surface. Research highlights the importance of the sea surface as a convergence layer for organic matter as well as organic and inorganic pollutants, which contribute to microalgae and zooplankton chemical compositions (Ewing, 1950; Garrett, 1966; Morris, 1974). The rate of uptake of these chemical components by microalgae is determined by a variety of factors including availability, temperature, salinity, population size, turnover rate and physiological state (Martin, 1970; Martin & Knauer, 1973). Redfield (1934) determined the ratio of carbon, nitrogen and phosphorus in the biomass of a mixed population of marine plankton to be 106:16:1, respectively. This study revealed the enormous abundance of oceanic carbon and highlighted the potential for nitrogen and phosphorus limitations which is now known to induce high lipid percentages in microalgae.

With the invention of gas chromatographic techniques, the individual fatty acid components of marine organisms can be identified. A variety of saturated

and unsaturated fatty acids of both long and short chain forms occur in all the world's oceans (Goutx & Saliot, 1980; Keenicutt & Jeffrey, 1981; Parrish & Wangersky, 1987). Studies demonstrate that polyunsaturated fatty acids ($C_{20:5}$ and $C_{22:6}$) are the predominant forms in marine microalgae and macroalgae along with saturated palmitic acid ($C_{16:0}$) (Gunstone, 1967; Orhan, Sener, & Atici, 2003). Palmitic acid is also a main component of marine zooplankton, bacteria and cyanobacteria (Falk-Petersen, Sargent, Gatten, & Hopkins, 1981; Goodloe & Light, 1982; Morris, 1984; Parker, Van Baalen, & Maurer, 1967; Ratledge, 1993). Discovery of an immense variety of potential chemical compositions in marine plankton necessitated detailed studies of the make-up of specific microalgae and zooplankton in order to investigate trophic interactions and potential anthropogenic contributions. Marine plankton varies geographically, temporally and bathymetrically; therefore, a detailed understanding of the chemical composition is needed to support its use as a feedstock for biofuel.

Microalgae.

Microalgae oils differ from those of terrestrial plants because they contain a higher percentage of polyunsaturated fatty acids (Belarbi, Molina Grima, & Chisti, 2000). Several scientific studies have determined the fatty acid composition within microalgae cells to be anywhere from 5% to 25% with some ranging even higher due to nutrient limitation (Falk-Petersen et al., 1981; Lee, Nevenzel, & Paffenhofer, 1971; Parrish & Wangerksy, 1987). Liu et al. (2011) found that photoautotrophic cells contain 25.8% of their dry weight as lipids. This study also reported that glycolipids and phospholipids comprised 70.6% of

the lipid fraction. Volkman, Jeffrey, Nichols, Rogers, and Garland (1989) conducted research on microalgae chemical composition in their examination of 10 species commonly used in mariculture. Their findings corroborated those of Liu et al. (2011) showing that glycolipids, phospholipids and chlorophylls made up over 65% of cellular fatty acids. The only outlier in this study was the diatom *Chaetoceros gracilis* -- a marine form -- which reported high levels of triglycerides and free fatty acids. Larger amounts of free fatty acids were noted to occur in cells that had been lysed or broken down due to stress. Within the diatoms examined, isolated individual fatty acids included chain lengths of 16:1, 16:0, 14:0 and 20:5. These four acids made up 62% to 70% of the total lipid fraction (Volkman et al., 1989). Lee, Richard et al. (1971), Parrish (1987) and Parrish and Wangersky (1987) all concluded that triglycerides are one of the primary fatty acid classes found in marine diatoms. Green microalgae are comprised of a variety of fatty acids with 16 carbon chain lengths including 16:2, 16:3 and 16:4. They also contain a few 18 carbon chains including 18:2 and 18:3. For the most part, green algae contain small amounts of fatty acid chains greater than 20 carbons excepting the common marine microalgae *Chlorella* which can have as much as 30% in the form of 20:5 (Watanabe, Kitajima, & Fujita, 1983). Two marine microalgae of the genera *Isochrysis* and *Pavlova* were the focus of a study by Volkman et al. (1989) and were found to contain a variety of polyunsaturated fatty acids including 20:5 and 22:6 in addition to the usual forms 16:0 and 16:1. However, microalgae in general lack a large number of saturated fatty acids longer than 18 carbons.

James et al. (2011) examined the chemical response of algae grown under nitrogen deprivation and found the essential fatty acids linolenic (18:3), linoleic (18:2), oleic (18:1) and eicosenoic acid (20:1) were the predominant forms. Studies have also demonstrated that microalgae in the open ocean substitute non-phosphorus lipids for phospholipids during periods of phosphorus limitation (Van Mooy & Fredricks; 2010; Van Mooy, Rocap, Fredricks, Evans, & Devol, 2006). Tonon et al. (2002) determined that polyunsaturated fatty acid production also depended on the growth phase within a species. Their study -- which focused on four microalgae -- found that the percentage of fatty acid accumulation usually increased when cells shifted from an exponential growth phase to a stationary phase. They discovered that the transition from the growth to stationary phase coincided with a depletion in nitrate levels in the fluid medium leading to a rapid increase in the accumulation of fatty acids within the cells (Tonon et al., 2002). Of the fatty acids present in the stationary phase, 75% were triglyceride neutral fatty acids.

The temporal variability of fatty acid composition in microalgae is also of interest in this study. Fraser, Sargent, Gamble, and Seaton (1989) found that polyunsaturated fatty acid accumulation in microalgae increased and peaked at a maximum of 58% on day 12 of their nutrient study then declined to a minimal 22% by day 54. These findings are similar to those of Lee (1975) who reported temporal variation in microalgae polyunsaturated fatty acid percentages within an Arctic food web. Geographic location also plays a role in chemical composition. For instance, microalgae found in polar waters vary greatly from those that are

encountered near the equator (Garrett, 1967; Lee, 1975). The primary

composition of fatty acids in plankton from the eastern subtropical south Pacific

are composed of intact polar diglycerides (Viso & Marty, 1993; Volkman et al.,

1989; Wakeham, Hedges, Lee, Peterson, & Hernes, 1997), further supporting the

fact that chemical composition is not only species and nutrient-specific, but

geographically specific as well. The strains of microalgae within a species,

interactions between species and the nature of their growing environments all play

a role in determining their fatty acid composition. These conditions also have a

major effect on the chemical composition of the zooplankton that reside one step

higher on the trophic ladder.

Zooplankton.

Determining the chemical composition of various zooplankton is a

difficult endeavor. Studies show that many zooplankton contain over 95% water

and low lipid percentages (Clarke, Holmes, & Gore, 1992). A study by Clarke et

al. (1992) of the chemical composition of zooplankton in the southern ocean

determined the largest fractions to be proteins, lipids and carbohydrates. From

species present in net tows, lipid percentages between 1.79% and 4.56% were

found comprising between 61% and 85% of the samples dried weight. In a study

of the eastern Mediterranean waters, Morris (1974) determined lipid percentages

to be between 2% - 3% of the samples' wet weights with 17% - 33% of this lipid

fraction composed of hydrocarbons. Lee (1975) showed that some Arctic

amphipods have the ability to sequester 12% to 56% of their organic matter in the

form of lipids. These findings match earlier studies where he noted geographic

differences in lipid composition (Lee, Richard et al., 1971). In this particular study he discovered a greater percentage of lipids existed in zooplankton collected from higher latitudes as compared to those collected closer to the equator. Much like microalgae, chemical composition of zooplankton has significant geographic variability.

Marine zooplankton exist in many different sizes, shapes and life stages. Adult copepods incorporate fatty acids directly from microalgae food sources. Lee, Nevenzel et al. (1971) found little trophic alteration of fatty acids between microalgae and zooplankton. This can be both a good and bad occurrence. A potential positive correlation between biofuel feedstocks and zooplankton exists in the findings that triglycerides comprise the major component of eggs, euphausiids and copepods (Falk-Petersen et al., 1981; Gatten, Sargent, Foresberg, O'Hara, & Corner, 1980; Kattner, Krause, & Trahms, 1981). Large amounts of dissolved fatty acids are created during zooplankton degradation (Parrish, 1988). In fact, large die-offs can lead to a milky white marine layer primarily consisting of wax esters (Volkman, Gatten, & Sargent, 1980), a potential precursor to biodiesel. Alternatively, a study conducted by Saba, Steinberg, Bronk, and Place (2011) revealed that the predatory relationship between zooplankton and harmful algal blooms can lead to decreased growth rates, increased mortality and decreased zooplankton egg production. Decreased production densities due to toxic microalgae (Sunda, Graneli, & Gobler, 2006) could have a deleterious effect on biofuel feedstocks.

Several studies have focused on the effects of food availability on zooplankton composition and abundance, highlighting the importance of the effects of microalgae on the chemical composition of zooplankton that feed on them. A 50% decrease in fatty acid percentage was witnessed in zooplankton that had been food-starved for four days (Hoeger, 1983). These findings echoed those of Conover and Corner (1968) who showed that lipid storage decreased temporally between summer and winter presumably due to food availability. Lee (1975) collected zooplankton samples between fall and winter in the Arctic and reported that in a two-month span between November and February, lipid concentrations decreased by 1.2 mg per individual. He also found that during summer months the quantity of polyunsaturated neutral lipids increased, presumably due to populations feeding on blooms of microalgae. Fraser et al. (1989) noted that changes in zooplankton polyunsaturated fatty acid percentage mirrored those of microalgae within an enclosed marine food chain. The most prevalent polyunsaturated fatty acids in the zooplankton studied were 20:5 and 22:6, both of which peaked several days after microalgae populations containing the same fatty acid chains peaked. The vertical distribution of zooplankton within the water column is an important contributing factor to lipid densities, as are the geographic distributions, temporal variations and the link between microalgae and zooplankton chemical compositions.

A keystone study conducted by Lee, Hirota et al. (1971) demonstrated a large difference in zooplankton caught at varying depths. However, a sample bias exists in this study. Samples were collected between 1m - 250m of depth yet only

individuals larger than 2 mm were retained for chemical analysis. Lipid percentages between 8% and 42% of sample dry weight were found with fractions as high as 37% of those lipids being in the form of triglycerides. On average, neutral lipids accounted for nearly 70% of the total lipid fraction and structural lipids including phospholipids, sterols and wax esters were less than 25%. These findings seem to contradict other studies that report higher percentages of structural lipids versus triglycerides. These contradictions are most likely explained by the sample bias resulting from the size limitation of the study specimens. Results also indicated that zooplankton in the middle water column retained the highest percentage of lipids in the form of triglycerides and some of these zooplankton conducted daily vertical migrations into shallower water. The vast majority of previous zooplankton studies have been conducted in open water with very few inorganic contaminants to taint analysis. In order to harness microalgae and zooplankton as potential feedstocks for biofuel, some form of filtration will be necessary to remove the organisms from the liquid media. By utilizing a sand filtration system like the one in this study, a large percentage of inorganic material is introduced into the samples. These inorganics must be separated before transesterification and analysis can occur.

Separation of Organics

   Background.

       In the current study significant amounts of inorganic contaminants were found mixed with the targeted organic material in the filter beds making separation of these two components necessary before transesterification could

occur. The separation and dewatering technologies discussed previously have been designed for systems with negligible quantities of inorganic contaminants. The separation processes discussed below have not been a typical part of modern algal biofuel research. Several bench-scale strategies for the separation of organic and inorganic components exist with many having been developed by marine geologists attempting to recover organics from benthic core samples.

A majority of the organic fraction in marine sediments is in the density range of 1.6 g/cm^3 to 2.2 g/cm^3 (Arnarson, Thorarinn, & Keil, 2001; Bock & Mayer, 2000; Dickens et al., 2006; Keil, Tsamakis, Fuh, Giddings, & Hedges, 1994; Wakeham et al., 2009). Organic marine plankton fall within the density range of 1.0 - 1.6 g/cm^3 (Price, St. Onge-Burns, Colton, & Joyce, 1977). By safely assuming a density of >2.2 g/cm^3 for silica sand (SiO$_2$) a noticeable separation becomes evident. For this reason density gradient centrifugation has become a common strategy for sample component separation.

Density gradient centrifugation.

Density gradient centrifugation acts on the principle of density differences by using a heavy liquid to band sample components into definable layers. Heavy liquid colloidal silica sols were developed over 40 years ago and used successfully to separate both dead and living marine plankton and meiofauna from sediments (Burgess, 2001; Price, Reardon, & Guillard, 1978; Schwinghamer, Anderson, & Kulis, 1991). The majority of early research focused on the utilization of artificial solutions such as Percoll and Ludox AM/TM as the primary heavy liquids. Several problems existed with these

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original liquids including low density gradient potentials (1.2 - 1.4 g/cm^3 max), high toxicity to marine organisms, gelling on contact with seawater, increased animal respiration rates and potential catalysis of oxidation reactions (Price, Mendiola-Morgenthaler, Goldstein, Breden, & Guillard, 1974; Price et al., 1978; Schwinghamer, 1981; Slawson, Adamson, & Mead 1973). For these reasons it became necessary to develop better chemicals for this task. Aluminate-stabilized sols were developed to resist gelling but their cost was too high for use on an everyday basis or in large-scale processes. Nalco 1060 -- a silica sol mixed with sucrose and buffered to a moderate pH -- has also been used for density gradient centrifugation. This solution was found to be isosmotic with seawater but was extremely viscous making recovery of samples difficult (Schwinghamer et al., 1991). Ficoll -- a mixture of sucrose and seawater -- was also tested but only a small fraction of marine microalgae formed bands in the available gradients (Price et al., 1978). The heavy liquids, bromoform and zinc chloride, were commonly used for separation purposes until the mid 1990s when their toxicity and viscosity became known risks (Munsterman & Kerstholt, 1996). In the late 1980s the new heavy liquid sodium metatungstate was developed and is currently used in the fields of geology, paleontology and palynology.

Sodium metatungstate.

Sodium metatungstate has a variety of benefits over other heavy liquid mediums and is perfectly suited for use in marine plankton research. Density gradients as low as 3 g/ml can be achieved and comparative studies show a larger variety of organic material isolated with sodium metatungstate as opposed to

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similar treatments of bromoform (Munsterman & Kerstholt, 1996). Bolch (1997) successfully used sodium metatungstate in a density gradient centrifugation experiment to separate living dinoflagellate cysts from marine sediments. The recovery efficiency has been noted to correlate directly with the type of material being separated and the density gradient chosen. Along with increased gradient potentials, sodium metatungstate is relatively neutral, non-toxic, less viscous than its silica sol counterparts, and can be recycled after each treatment to prolong chemical life. In recent studies it has been used to separate a variety of living marine microalgae all of which showed excellent viability after treatment and required little or no post-treatment before chemical analysis (Swann & Leng, 2009; Touzet, Franco, & Raine, 2008; Wakeham et al., 2009).

The high cost of sodium metatungstate makes it suitable for bench-scale studies only, and the availability and ease of use were major determining factors in choosing it for this experiment. With a mixed sample of organic and inorganic material the separation step of the process is by far the most critical and time consuming. Though labor intensive, it is imperative to have a purified sample free of inorganic contaminants before samples can be chemically converted into biofuel.

Chemical Conversion

> **Background.**

Several methods exist to convert raw biomass and oils into biofuel, including pyrolysis, micro-emulsification and transesterification. Transesterification has been the primary means of modern day biodiesel synthesis

and is therefore the focus of this current study. The transesterification process was first developed by E. Duffy and J. Patrick in 1853 (Demirbas, 2005). Several patents were issued in the 1940s to DuPont and Colgate-Palmolive-Peet, who used the process to extract glycerol during soap processing (Allen & Kline, 1945; Arrowsmith & Ross, 1945; Bradshaw & Meuly, 1942; Dreger, 1945; Keim, 1945; Percy, 1945; Trent, 1945). The byproduct of these chemical reactions was the creation of biodiesel. Triglycerides -- the ideal building blocks of biodiesel -- consist of three fatty acids ester bonded to a glycerol molecule. In order to utilize these fats as fuel they must first be extracted and then converted into fatty acid methyl esters (FAME) via transesterification with methanol in the presence of a catalyst.

Extraction.

Chemists utilize two primary strategies to extract bound fatty acids from the cellular matrix. A simple strategy is the expeller/press. This mechanical solution squeezes the oils directly from the feedstock and can achieve 75% extraction efficiency (Demirbas & Fatih Demirbas, 2011). Solvent extraction creates a better yield; in this method, polar organic solvents are combined with an alcohol to remove bound fatty acids in cell membranes and organelles. Two mainstream processes for solvent extraction are commonly used in science and industry today. The Folch method -- developed in 1957 -- utilizes a chloroform/methanol/water mixture to extract high percentages of fatty acids (Folch, Lees, & Sloane-Stanley, 1957). This method requires large quantities of solvent to achieve a ratio of 20:1 solvent to sample. The quantities of solvent

used in this extraction method are often costly and unnecessary, leading to the development of a second method by Bligh and Dyer (1959). Their method uses a chloroform/methanol mixture for extraction. The main difference between their method and Folch's is the solvent-to-sample ratio of 3+1:1. Iverson, Lang, and Cooper (2001) compared the two methods demonstrating similar extraction efficiencies >95% when samples of low fatty acid percentage were used. When samples contained more than 2% fatty acid these scientists reaffirmed the suggestion of Christie (1973) that a pre-extraction step utilizing a non-polar solvent was necessary in order for the Bligh and Dyer method to yield similar results to the Folch method. Hundreds of studies to date have used variations of these two methods. The inherent toxicity of chloroform has led to an increase in the use of hexane for extraction in recent years.

Other strategies have also been used with varying success. A pressurized hot solvent extraction method was tested and found to yield similar results to that of Bligh and Dyer (Macnaughton, Jenkins, Wimpee, Cormier, & White, 1997). Both supercritical carbon dioxide extraction and microextraction were also tested with results varying depending upon initial fatty acid compositions (Halim, Gladman, Danquah, & Webley, 2011; Reisenbichler & Bailey, 1991). Microalgae contain a wide variety of cellular fatty acids in varying percentages. For this reason several specific techniques have been developed for the extraction of their lipids (Kita et al., 2010; Kuchkina, Gladyshev, Sushchik, Kravchuk, & Kalachova, 2011; Lewis, Nichols, & McMeekin, 2000; Sheng et al., 2011). Post-

extraction fatty acids from the desired feedstock must be chemically converted via transesterification into useable fuel.

Transesterification.

Transesterification is the breakage of an ester bond by an alcohol in the presence of a catalyst and is a step reaction whereby triglycerides are converted to their subsequent di- and monoglycerides and eventually reduced to alkyl esters and glycerol. When methanol is used as the alcohol the reaction is termed methanolysis and the products are fatty acid methyl esters (FAMEs). Several factors affect the reaction including molar ratio, type of alcohol used, free fatty acid quantity, type/amount of catalyst, temperature, presence of water and extent of mixing (Canakci & Van Gerpen, 2003; Carvalho & Malcata, 2005; Freedman, Butterfield, & Pryde, 1986; Freedman, Pryde, & Mounts, 1984; Lewis et al., 2000; Ma & Hanna, 1999; Meher, Vidya Sagar, & Naik, 2006; Ozgul-Yucel & Turkay, 2002; Wright et al., 1944). Methanol is commonly used due to its low cost and ability to concurrently separate glycerol. Ethanol, propanol, isopropanol and butanol can also be used.

No matter which alcohol chosen, the molar ratio has a large effect on the efficiency of the reaction. The stoichiometric ratio for the alcohol transesterification of fatty acids is 3:1, respectively. A ratio of 6:1 alcohol to fatty acids is used in modern-day science to drive the reaction towards the products. Some studies have reported ratios as high as 40:1 for the conversion of vegetable oil to FAMEs (Demirbas, 2002). The quality of the feedstock and its inherent chemical composition can have a large influence on the eventual products. High

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free fatty acid and water percentages (>3%) cause deactivation of the catalyst and excessive soap production (Helwani, Othman, Aziz, Fernando, & Kim, 2009; Meher et al., 2006; Rived, Canals, Bosch, & Roses, 2001).

Both alkali and acid catalysts are used to boost reaction speeds and efficiencies (Fukuda et al., 2001; Gerpen, 2005; Nye et al., 1983; Peterson & Scarrah, 1984; Schuchardt, Sercheli, & Vargas, 1998). Alkali catalysts provide the fastest reaction time and best reaction efficiency (Freedman et al., 1986); of these, sodium hydroxide and potassium hydroxide are most commonly used today. Some studies report reaction speeds 4000 times faster for alkali catalysts over comparable acid catalyst (Formo, 1954). However, alkali catalysts also have several drawbacks including high energy input, difficult glycerol recovery, necessary catalyst removal/treatment, and interference by free fatty acids and water. To avoid these complications acid catalysts have been explored and are often used. Acid catalysts such as sulfuric and sulfonic acid aren't as susceptible to feedstock free fatty acids and can accommodate higher percentages of water (Freedman et al., 1984). They also have the ability to convert low-quality feedstocks to FAMEs as demonstrated by Haas, Michalski, Runyon, Nunez, and Scott (2003). To avoid complications from chemical catalysts, enzymatic catalysts such as lipases have recently been developed. While these allow for easier recovery of glycerol they increase production costs significantly. Supercritical methanol is an environmentally friendly solution that allows catalysts to be completely avoided (Patil et al., 2011; Saka & Kusdiana, 2001). While this process decreases reaction times and produces high yield, it also

increases production costs due to the high temperatures and pressures needed (Demirbas, 2002).

Direct transesterification.

Some modern day methods eliminate the lipid extraction step all together and focus solely on a one step extraction-transesterification reaction. Direct transesterification is an example of a one-step reaction that avoids the extraction phase of traditional FAME production and the potential complications associated with multi-step processes (Garces & Mancha, 1993; Lepage & Roy, 1984). Several studies report increased yields from direct transesterification when compared to separate extraction-transesterification processes (Rodriguez-Ruiz, Belarbi, Sanchez, & Alonso, 1998; Tran et al., 2009; Wen & Johnson, 2009). Lewis et al. (2000) tested the direct transesterification reaction on microheterotrophs and found that all classes of fatty acids (saturated, unsaturated and polyunsaturated) were extracted in higher quantities as compared to separate extraction-transesterification procedures. In addition to higher yields there is less loss from oxidation of pre-extracted fatty acids, and either dried or wet feedstocks can be used (Lepage & Roy, 1984). Though large percentages of water can potentially decrease reaction efficiency, Wahlen, Willis, and Seefeldt (2011) found that a small increase in methanol could potentially offset the detrimental effects of water on the reaction and increase the yield of FAME. Direct transesterification provides a simple, low cost means of producing FAMEs for use in biodiesel and can achieve higher yields for greater accuracy in chemical analysis.

Analysis

Analytical techniques.

Both quantitative and qualitative analysis of FAMEs is necessary to determine their potential as biofuel. Depending on the quantity and nature of the components, FAMEs may be suitable for biodiesel, bio-jet fuel or bio-gasoline. Thin layer chromatography, high performance liquid chromatography, gravimetric analysis and gas chromatography are several of the analytical techniques available to the modern-day chemist who wants to analyze FAMEs. Many of these systems can be coupled with flame ionization detectors or mass spectrometers for secondary analysis and determination of specific compounds within samples. Gas chromatograph-mass spectrometers (GC-MS) are commonly used for the analysis of FAME samples in modern laboratories (Halim et al., 2011; Kuchkina et al., 2011; Patil et al., 2011; Wahlen et al., 2011). With the addition of an internal standard, analyzers are able to quantify components, cross-reference individual compounds with a known database, and provide the investigator with a detailed description of the samples' chemical composition.

Kennicutt and Jeffrey (1981) conducted an extensive study of marine dissolved lipids utilizing GC-MS as their analysis method. Using this system they were able to determine fatty acid quantities at varying depths in the water column, however, certain problems arose with such a sensitive analysis. Rodriguiz-Ruiz et al. (1998) reported that the FAMEs of directly transesterified dried biomass contained higher impurities than those from separate extraction-transesterification procedures and had the potential to interfere with GC-MS analysis. GC samples

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also take a considerable amount of time to process. For this reason, alternative

rapid techniques have been developed to speed analytical procedures.

Fourier transform infrared micro-spectroscopy, Nile red fluorescence and

colorimetric quantification are all used as quick means of determining lipid

quantities and qualities (Chen et al., 2011; Dean, Sigee, Estrada, & Pittman, 2010;

Huang, Chen, & Chen, 2009; Wawrik & Harriman, 2010). No matter what

analytical technique used with biodiesel, researchers must determine if the

FAMEs meet the industry standards.

Biodiesel quality standards.

Biodiesel feedstocks are converted to suitable FAMEs to ensure that

modern-day engines are not unnecessarily compromised and that emissions are at

suitable levels. Used vegetable oils are viscous, cause difficulties with fuel

injection and leave harmful engine deposits (Demirbas, 2003). In order to avoid

mechanical issues, standards were developed to ensure appropriate testing of

biofuels. Austria led the way with the development of the first standards for

FAME diesel fuel. They were quickly followed by Germany, Italy, France and

the United States (Meher et al., 2006). In the U.S., quality standards are set by the

ASTM D6751-02. These regulations are defined by several parameters applicable

to biodiesel produced in this country. Viscosity, flash point, cetane number, cold

filter plugging point, iodine value, water content, cloud point and pour point all

define the quality of biodiesel. These attributes are determined by the quality of

the feedstock and the effectiveness of the conversion. An increase in cetane

number is correlated with the presence of straight-chain, saturated hydrocarbons

(Demirbas, 2005). As the number of double bonds increases, the cetane number

drops. The American Society for Testing Materials determined that biodiesel

derived from microalgae biomass is similar in properties to that of standard

biodiesel while maintaining a more stable flash point. Without a sustainable and

quality feedstock, biodiesel fuel cannot achieve viability. For this reason it is

necessary to have a global understanding of the availability of potential feedstock

sources.

Spatial Distribution

  Factors affecting distribution.

   Difficulty finding reliable quantities of microalgae and zooplankton in our

world's oceans is presumably one of the main reasons why they have not been

explored as a viable second-generation biofuel feedstock. A lack of investigation

from a bio-prospecting perspective is evident in the current scientific literature.

The interest of this study is partially derived from the lack of scientific evidence

supporting bio-prospecting. Oceanic feedstocks are controlled by a variety of

interrelated factors that influence population dynamics beginning with microalgae

primary production. This production in turn influences zooplankton biomass and

distribution and so on up the food chain to the highest trophic predators: humans.

   The Pacific Decadal Oscillation, wind-driven forcing and dominant

current systems all play a major role in the vertical and horizontal circulation of

water which determines the availability of nutrients to microalgae feedstocks

(Eppley, Renger, & Harrison, 1979; Keister et al., 2011; Mantyla, Bogard, &

Venrick, 2008; Miller et al., 1991). Surface water chlorophyll concentrations -- a

microalgae indicator -- show strong temporal changes that coincide with seasonal variations in wind patterns and sea surface temperature (Barnett & Jahn, 1987; Eppley, 1992; Hebbeln, Marchant, & Wefer, 2000; Kim, Miller, McGowan, & Carter, 2009; Lynn et al., 1998; Otero & Siegel, 2004). Both physical and chemical processes directly influence microalgae populations. Nitrate concentrations (silicic acid for diatoms) brought to the surface from deep ocean upwelling have been linked to high chlorophyll concentrations in near-shore habitats (Barnett & Jahn, 1987; Dugdale & Wilkerson, 1989; Eppley, 1992; Mantyla et al., 2008; Moberg, 1928; Shipe & Brzezinski, 2003). As available nutrients are exhausted by microalgae populations their concentrations gradually decrease in surface waters and populations are re-established at deeper depths near the nitracline (a level of increased nitrate availability) (Eppley, 1992; Mantyla et al., 2008). Diatom populations especially follow this trend with increased concentrations in areas of coastal upwelling, smaller individuals inhabiting near-shore environments, and larger constituents residing in offshore populations (Anderson et al., 2008; Putt & Prezelin, 1985; Riebesell et al., 2007). These population differences are most likely due to the increased availability of nutrients and relatively constant mixing of the near-shore environment (Barnett & Jahn, 1987; Eppley, 1992; Kim et al., 2009).

Biological, physical and chemical processes in the ocean are intimately connected to each other and are sporadically influenced by land-based inputs from anthropogenic sources and coastal watersheds. Boyd et al. (2000) demonstrated a six-fold increase in microalgae concentrations in response to artificial "seeding"

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of oceanic waters with iron. Rivers have a large effect on coastal microalgae populations by input of essential nutrients such as nitrogen, phosphorus and silicon (Corcoran, Reifel, Jones, & Shipe, 2010). These organic and inorganic inputs have been shown to have both positive and negative effects on microalgae populations. Sunda et al. (2006) correlated the positive effects of coastal nutrient inputs on harmful algal blooms such as those that cause paralytic shellfish poisoning and domoic acid. Their findings were replicated by other studies (Cloern, 2001; Laroche et al., 1997), and by researchers who focused on non-harmful microalgae (Dagg & Breed, 2003; LaPointe & Matzie, 1996). Along with increasing nutrient concentrations, rivers add a large quantity of suspended solids to coastal waters, thus decreasing available light levels and negatively effecting microalgae growth (Hu, Muller-Kargen, Vargo, Neely, & Johns, 2004). Zooplankton populations are also effected by these inputs.

Zooplankton abundances, locations and species assemblages are also influenced by oceanic processes. Barnett and Jahn (1987) showed that smaller individuals of the subphylum *Crustacea* were found in near-shore environments when compared to offshore samples. Kimmerer (1993) observed population density differences of an order of magnitude across relatively short spatial scales of 2km - 4km. These near-shore populations can be transported large distances offshore by currents and wind-driven circulation (Mackas, Washburn, & Smith, 1991). Other scientific works have focused on the temporal variations in zooplankton taxa revealing distinct links to seasonal forcing and climate change (Keister et al., 2011; Lavaniegos & Ohlman, 2007). Without a thorough

understanding of ocean systems and the relationship between their chemical and physical processes, we would be unable to determine the biological relationships between microalgae and zooplankton that form the basis of marine biofuel studies.

Zooplankton densities often mimic microalgae populations by having a high prevalence in near-shore, cold, nutrient-rich waters (Barnett & Jahn, 1987; Kimmerer, 1993; Mackas et al., 1991). This is best seen in the poleward zooplankton populations that are larger in size and have increased lipid stores as compared to warm water species (Lee, Hagen, & Kattner, 2006). Climate change has recently forced some southern species to higher latitudes causing detrimental effects on local microalgae and zooplankton populations (Keister et al., 2011). Copepods, for example, currently dominate the southern and central California ocean but a strong temporal shift has occurred in predator-prey interactions since 1984 (Lavaniegos & Ohman, 2007) and will presumably continue into the future. These environmental shifts leave unanswered questions as to the location and abundance of naturally occurring biofuel feedstocks in the near and distant future. The study site for this investigation provides a unique set of circumstances that directly affect the local marine assemblage and is linked to a larger network of trophic interactions which have been studied for many years.

Study site description.

The Santa Barbara Channel, where the California Current and California Counter-Current converge, is located at the northernmost edge of the Southern California Bight, an area which encompasses the oceanic waters from Point

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Conception to the Mexico border. A strong upwelling center at Point Conception provides cold, nutrient-rich waters to the west end of the channel (Barnett & Jahn, 1987; Eppley, 1992). This point also serves to deflect wind pressures in the channel causing spatially variable water circulation (Harms & Winant, 1998). All of these factors contribute to some of the highest primary productivities seen in the entire Bight (Mantyla, Venrick, & Hayward, 1995). Much like other areas of the Pacific, nutrient concentrations and species assemblages in the Bight show significant seasonal variability (Anderson et al., 2008; Otero & Siegel, 2004; Shipe & Brzezinski, 2003; Shipe et al., 2002). Terrestrial runoff is a major contributor to nutrient levels in the Santa Barbara Channel and northern Bight. The Santa Clara River in Ventura County has the ability to produce runoff plumes in excess of 200 km^2 and severely impacts local plankton populations by introducing excess nutrient and inorganic suspended solids (Nezlin, DiGiacomo, Stein, & Ackerman, 2005). Depending on the characteristics of the watershed, quantity of rainfall and period between concurrent rains, these plumes can be transported alongshore and offshore for miles with far-reaching influences in southern and central California (Nezlin et al., 2005; Warrick et al., 2007). Local microalgae bloom studies show the highest concentrations around river mouths (Corcoran et al., 2010) as well as north of Point Arguello (Otero & Siegel, 2004). In the east Santa Barbara Channel where the study site is located, microalgae blooms are less frequent and intense as those seen in other areas. The sporadic and somewhat unpredictable nature of microalgae in the east channel makes it a challenging location to conduct feasibility studies for biofuel feedstock harvest.

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Biofuel perspective.

Data on the oceanographic conditions necessary to produce reliable feedstocks for biofuel must be applied in a creative way in future exploration. Our knowledge from land-based cultivation systems gives us a solid background about the conditions necessary in the open ocean to produce good quality/high-yield feedstocks for biofuel. A review of the available oceanographic literature on plankton provides an understanding of the potential geographic locations where these conditions will exist and how often they may be upset. Historical data from the Southern California Bight reflects annual variations in microalgae and zooplankton population abundances and relates these numbers directly to nutrient availability and weather conditions (Barnett & Jahn, 1987; Eppley, 1992). Satellite monitoring conducted by Kim et al. (2009) demonstrated that near-shore blooms were quite different from those offshore in terms of spatial distribution and concentration. Future investigations cannot overlook the influence of anthropogenic sources of nutrients, pollutants and suspended solids. All of these factors contribute to the quality and quantity of microalgae and zooplankton in coastal systems which have the potential to provide a solution to our future global energy needs.

<center>**Methods**</center>

Quantitative Analysis

    Collection and filtration.

    Sample collections occurred between April and August, 2011. Each collection day consisted of one 2L subsample of backwash water collected in a plastic screw cap container at three intervals during the backwash cycle: 0 minutes, 2.5 minutes, and 5 minutes, for a total of three 2L samples per backwash event. The date, sample time, filter bed number, flow-rate (L/minute), and duration of backwash cycle (minutes) were recorded for each collection. Each subsample was gravity-filtered through stacked partitioning sieves made of 4 inch schedule 40 PVC pipe and Nitex nylon mesh, sizes: 500µm, 210µm, 100µm, and 20µm. Solids retained in the 210µm, 100µm, and 20µm sieves were consolidated in a glass beaker by backwashing the mesh with filtered seawater (20µm). The consolidated solids were then transferred into three 50mL disposable polypropylene centrifuge tubes (Dow Corning) marked: 0 min, 2.5 min, and 5 min and centrifuged (Thermo Electron Corp, IEC Centra CL3, swinging bucket rotor # 243) at 4000 rpm for 11 minutes. After centrifugation, the supernate was discarded via a vacuum sipper (AIR-VAC, AVR093H) and the tubes left to dry at 60°C for 72 hours (Precision Economy Oven).

    Ash-free dry weights.

    Ash-free dry weight determinations were done following the established protocols of Clarke et al. (1992), Hedges et al. (2002), Larson (1986), and Martin and Knauer (1973).

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After drying, each sample tube was allowed to cool to room temperature (~24°C) and then transferred into aluminum weigh boats (Fisher brand, 1 3/8 fl. oz.). Dry weights (DW) were measured on a Mettler PM100 balance. After recording DW, each aluminum weigh boat was covered in aluminum foil and combusted at 450°C for 5 hours (Thermolyne 47900 muffle furnace). Samples were again allowed to cool to room temperature (~24°C) after which post-combustion ash-free dry weights (AFDW) were measured on the same balance (Mettler PM100). Organic quantification was calculated by subtracting the AFDW from the DW and dividing by 2L to determine grams per liter (g/L) of organics.

$$\frac{DW\ (g) - AFDW\ (g)}{2L} = g/L\ Organics \tag{1}$$

Sample averages were calculated and extrapolated upwards using recorded meta-data in order to determine the potential weekly organic yield of the system.

$$Organics\ (g/week) = organics\ (g/L)\ x\ flow\text{-}rate\ (L/minute)\ x\ cycle$$
$$duration(minutes/backwash)\ x\ 3\ backwashes/day\ x\ 3\ days/week \tag{2}$$

The quantity of seawater filtered each week was 24,417,792 L/week which was calculated by multiplying the supply pump flow-rate (3,028 L/min) by the filtration time (10,080 min/week) and used a correction factor of 80% to account for the quantity of water filtered versus that which was kept unfiltered.

Qualitative Analysis

Collection and filtration.

The five qualitative sample collections occurred during the month of August, 2011. On each collection date two 5 gallon plastic buckets (37.85L total)

of backwash water were collected at time 0 minutes of the backwash cycle. The date, time, filter bed number, flow-rate (L/minute), and duration of the backwash cycle (minutes) were recorded for each collection. Each bucket was gravity filtered through 500µm, 100µm, 28µm, and 20µm Nitex nylon mesh and the solids from the 100µm, 28µm, and 20µm filters were collected by backwashing the mesh with filtered seawater (20µm) into a glass beaker. The solids were transferred into 200mL polypropylene centrifuge tubes (Falcon) and centrifuged (Thermo Electron Corp, IEC Centra CL3, swinging bucket rotor # 243) at 4000 rpm for 10 minutes. The supernate was discarded with a vacuum sipper (AIR-VAC, AVR093H) and the solids transferred into 50mL disposable polypropylene centrifuge tubes (Dow Corning). Each tube was mixed well by hand in order to break up any small chunks of solids before separation.

Density gradient separation.

Separation of organics was done using protocols established by Bolch (1997) and Price et al. (1978) modified to effectively separate the mixed samples in this study.

In a new 50mL polypropylene tube, 20mL of homogenized solids were delicately layered on top of 20mL of a sodium metatungstate solution (ACROS Organics, CAS# 7732-18-5) adjusted to a density of 1.8g/cm^3 via addition of nanopure water. The mixture was centrifuged (Thermo Electron Corp, IEC Centra CL3, swinging bucket rotor # 243) at 2500 rpm for 10 minutes. After centrifugation, the top organic layer was pipetted off and transferred into a new 50mL polypropylene centrifuge tube containing a fresh 20mL of 1.8 g/cm^3

sodium metatungstate. Centrifugation and separation were repeated until inorganics were no longer visually present in the lower sodium metatungstate layer. After the inorganics had been removed, the remaining organics were transferred into a clean 50mL polypropylene tube, washed with 40mL of filtered seawater (20µm), and centrifuged at 4000 rpm for 5 minutes.
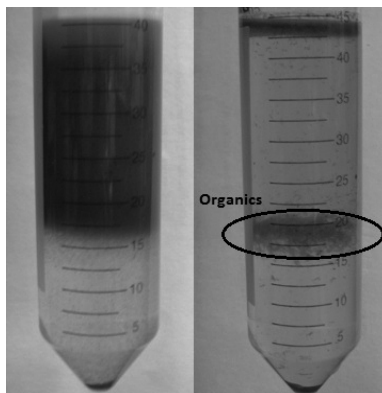
Figure 1. Sodium metatungstate separation of organics from inorganics.

The supernate was discarded via vacuum sipper and the organics re-washed, centrifuged, and the supernate discarded three more times to ensure all sodium metatungstate had been removed. After the final wash, the remaining organics were flash frozen in liquid nitrogen and lyophilized for 48 hours (Labconco, Lyph-Lock 6L bench top freeze dry system, model #77520). The lyophilized organics were weighed, transferred to glass vials with screw cap lids, and immediately frozen under nitrogen at -80°C in an ultra-cold freezer (Thermo Scientific, Revco Ultima Plus) for no longer than 1 month before transesterification.

Direct transesterification.

Direct transesterification was done following the protocol established by Lepage and Roy (1984) with modifications by Wen and Johnson (2009) and Whalen et al. (2011).

Frozen lyophilized biomass was allowed to come to room temperature (~24°C) and then ground to a fine powder with a porcelain mortar and pestle. 50mg of lyophilized powder was added to a 5mL glass ampoule along with 2.0mL of methanol (Fisher Chemical, A412-4), 50µL of sulfuric acid (Fisher Chemical, Cat #: A300-500), and 2.0mL of hexane (Fisher Chemical, Optima grade) and the top hermetically sealed. The mixture was heated to 80°C in a block heater (Denville Scientific Inc., IncuBlock™) for 45 minutes while being mixed on an orbital shaker (Gene Mate, OS350, Setting #4). After cooling to room temperature, the mixture was transferred to a 10mL glass centrifuge tube with Teflon lined screw cap lid (Kimble Kimax®) and 4mL of de-ionized water was added. The tube was mixed thoroughly by hand for 45 seconds and then centrifuged at 3100 rpm (IEC Clinical centrifuge, swinging bucket rotor) for 10 minutes to accelerate phase separation. Once phase separation occurred, the upper hexane phase was recovered and filtered through a 0.2µm syringe filter (Whatman, Anotop 10) into two pre-weighed 4mL glass vials with Teflon lined screw cap lids.

Gravimetric quantification.

Each pre-weighed vial containing the FAME/hexane mixture was evaporated to dryness under a stream of nitrogen. After drying, the samples were

re-weighed (Fisher Scientific A-160 Balance) and the mass of the FAMEs determined by subtracting the original vial weight from the final FAME/vial weight. The potential quantity of FAME/Biodiesel produced by the system per week was calculated using a FAME density of 0.864 g/L (Xu, Miao, & Wu, 2006).

$$FAME \text{ (ml/week)} = \frac{[Organics \text{ (g/week)} \times 0.00004]}{0.864 \text{ g/L}} \qquad (3)$$

FAMEs were re-suspended in 750µL of hexane, sealed, and immediately placed in the freezer until analysis via gas chromatograph with mass spectrometer.

Gas chromatography and mass spectrometry.

FAMEs were analyzed at the ASU Polytechnic Laboratory for Algae Research and Biotechnology by an experienced technician. Analysis was done on an Agilent 7890 series gas chromatograph with 5975C series mass spectrometer operated in electron ionization full scan mode (EI = 70 eV). The instrument was equipped with an Agilent HP-88 capillary column (60m x 0.25mm I.D., 0.20mm film thickness) and a split-less capillary injector heated to 250°C. The oven temperature program began at 100°C and remained so for 5 minutes after which it was increased by 3.5°C per minute to 240°C and remained again for 5 minutes. Helium carrier gas was used at a flow rate of 1.5mL per minute. Heptadecanoic acid (C17:0) was added to each sample as an internal standard. FAME composition was calculated from the chromatograms as a percentage of the total fatty acids present in each sample.

Results

Quantitative

Organics.

Subtracting the ash-free dry weight (AFDW) from the dry weight (DW) measurements provided baseline quantification of organic material being produced by the system as shown in Table 1. The greatest quantity of organic material was expelled during the first sample (0 min) of each backwash cycle.

Table 1.

Quantity of Organics Produced Over Time (g/L)

Date	Bed #	Sample Time			Average
		0 min	2.5 min	5 min	
4/8/11	4	0.083	0.015	0.018	0.039
4/18/11	4	0.040	0.010	0.007	0.019
4/22/11	4	0.046	0.016	0.009	0.024
4/25/11	4	0.035	0.010	0.006	0.017
5/6/11	4	0.043	0.012	0.006	0.020
5/20/11	4	0.028	0.010	0.007	0.015
6/3/11	3	0.032	0.006	0.007	0.015
6/10/11	4	0.045	0.011	0.009	0.022
6/17/11	3	0.038	0.010	0.006	0.018
7/1/11	4	0.024	0.009	0.011	0.014
7/8/11	3	0.058	0.015	0.006	0.026
7/15/11	4	0.030	0.005	0.008	0.014
7/22/11	4	0.024	0.007	0.003	0.011
7/29/11	4	0.020	0.010	0.006	0.012
8/5/11	4	0.024	0.008	0.004	0.012
8/12/11	4	0.031	0.012	0.005	0.016
8/19/11	4	0.017	0.007	0.003	0.009
8/31/11	4	0.031	0.010	0.006	0.016
	Average	0.036	0.010	0.007	0.018

A maximum of 0.083g/L was recorded on April 8th, a minimum of 0.017g/L on August 19th, with an average organic quantity of 0.036g/L produced over the

duration of the sampling period. Sample two (2.5 min) showed significantly

reduced values of organics with a maximum of 0.016g/L observed on April 22nd,

a minimum of 0.005g/L on July 15th, and an average of 0.010g/L during the

sampling period. Sample three (5 min) showed even lower values as compared to

samples one and two with a maximum of 0.018g/L on April 8th, a minimum of

0.003g/L on July 22nd and August 19th, and an average of 0.007g/L during the

sampling period. Total averages of the organic production from each sample date

ranged from 0.039g/L on April 8th to 0.009g/L on August 19th with an overall

average of 0.018g/L of organic material produced during the study period. The

quantity of organics varied over the five-month sample period. Overall a

temporal decline in organics was observed between April and August, 2011 as
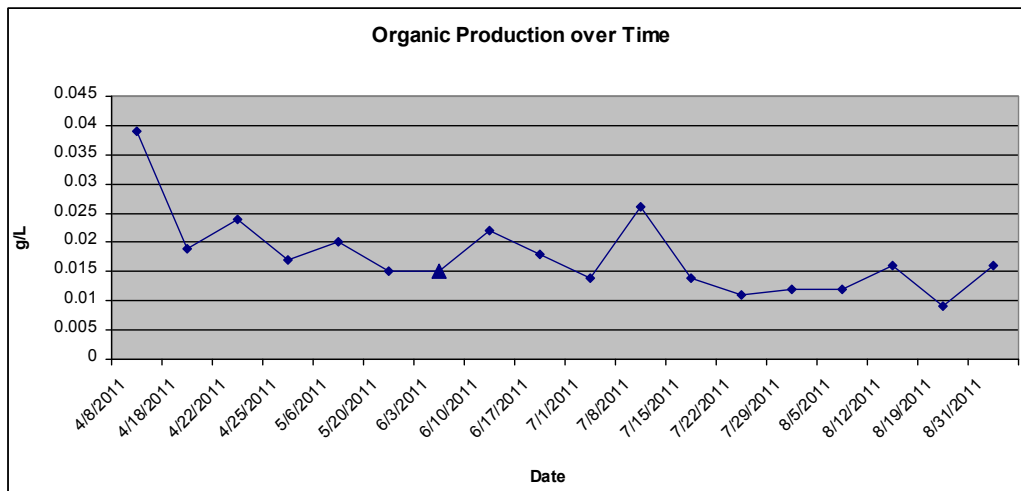
seen in Figure 2.

Figure 2. Quantity of organic material produced by the UCSB seawater system
over the course of the study from April to August, 2011.

Two months into the sampling period, between May and June 2011, the

seawater supply line was switched from one containing large quantities of bio-

fouling in the form of mussel (*Mytilus californianus*) growth to a clean, un-fouled

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supply line. Quantities of organic material were not intuitively affected by this switch with all quantities showing higher values in the bio-fouled supply line as compared to the un-fouled line (Table 2).

Table 2.

Comparison Between Bio-fouled and Un-fouled Seawater Supply Line (g/L)

	Sample Time		
Line Condition	0 min	2.5 min	5 min
Bio-Fouled	0.046	0.012	0.009
Un-Fouled	0.031	0.009	0.006

Note. Average values shown

Organics from sample one (0 min) were 0.015g/L higher in the bio-fouled line and samples two (2.5 min) and three (5 min) both showed 0.003g/L increases when compared to the un-fouled line. The supply line switch is denoted in Figure 2 by the large triangular data point on June 3rd.

Weekly backwash production quantities of organic material (Table 3) were calculated from recorded sample measurements. Maximum/minimum weekly quantities used the highest/lowest average value from the sample dates and extrapolated upwards using Equation 2.

Table 3.

Weekly Dry Organic Production (g/week)

	Organics
Maximum	16,628.63
Minimum	3,837.38
Average	7,674.75

During the sample period the maximum weekly dried organic production value, occurring during the first week of April, was calculated to be 16,628.63g.

Alternately, a minimum of 3,837.38g of dried organics per week was produced

during the third week of August. An average of 7,674.75g of dried organics were

produced by the system per week over the course of the study.

FAMEs.

Results of the FAME production via the direct transesterification of

freeze-dried organic biomass as determined by gravimetric quantification are

shown in Table 4.

Table 4.

Quantity of Fatty Acid Methyl Esters (FAMEs) Produced

Date	Dry Organic Mass (mg)	FAMEs (µg)	FAME (%)
8/5/11	35	1.6	0.005
8/12/11	55	2.2	0.004
8/17/11	54	1.4	0.003
8/19/11	52	2.4	0.005
8/31/11	52	2.2	0.004

Values between 1.6µg and 2.4µg of FAMEs were produced from the direct

transesterification reaction. FAME quantities expressed as a percentage of the

initial organic biomass ranged from 0.003% to 0.005% and averaged 0.004%.

These FAME yields (g) were converted to milliliters per week utilizing Equation

3 and the calculated weekly organic values from Table 3 with results shown in

Table 5. During the sample period the maximum weekly production of FAMEs

was calculated as 0.77ml per week during the first week of April. A minimum

value of 0.18ml per week was observed during the third week of August. An

average of 0.36ml of FAMEs were produced by the system per week over the

course of the study.

Table 5.

Fatty Acid Methyl Ester (FAME) Production per Week

	Organics (g/week)	FAME (ml/week)
Maximum	16,628.63	0.77
Minimum	3,837.38	0.18
Average	7,674.75	0.36

Qualitative

GC-MS analysis of the FAMEs produced during the month of August 2011 yielded consistent results (Table 6). Replicates (A and B) from each sample date were analyzed separately to verify accuracy. Both saturated and unsaturated fatty acid compositions ranging from C11:0 to C22:6n3 carbon chains were detected. The largest fatty acid percentages were in the form C22:6n3. C22:6n3 comprised a maximum value of 37.02% of the FAME total on August 17th and a minimum of 26.57% of the sample on August 19th. The fatty acid C16:0 was also highly prevalent with a maximum of 27.89% of the FAME sample on August 17th and a minimum of 18.49% on August 19th. The fatty acids C18:0, C18:1n9C, and C20:5n3 were relatively common as well with maximum values of 7.81%, 10.23%, and 8.35%, respectively. C16:1 and C20:4n6 were found to be minor sample components with maximum values between 5% - 6%. Total sample composition indicated a dominance of unsaturated fatty acids as opposed to saturated forms (Table 7). Saturated fatty acids ranged between 30.71% and 44.09% of the total composition while unsaturated fatty acids made up 55.90% to 66.32% of the total composition.

Table 6.

Fatty Acid Methyl Ester Composition

						Date				
	8/5/11		8/12/11	8/17/11		8/19/11		8/31/11	
Compound	Sample A	Sample B	Sample A	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
C11:0	n.d.	n.d.	n.d.	n.d.	n.d.	0.23	n.d.	n.d.	n.d.
C12:0	n.d.	n.d.	0.59	0.47	0.48	0.28	0.34	0.30	0.33
C13:0	0.14	n.d.	0.24	0.12	0.15	0.37	0.26	0.13	0.12
C14:0	2.31	2.55	3.17	3.42	3.45	1.99	2.01	4.05	4.03
C14:1	0.57	0.60	0.66	0.45	0.46	0.49	0.51	0.36	0.40
C15:0	2.07	2.20	2.11	1.95	2.08	2.36	2.51	1.94	1.94
C15:1	n.d.	n.d.	0.31	0.62	0.65	n.d.	n.d.	0.37	0.40
C16:0	23.90	24.93	24.93	25.79	27.89	18.49	20.91	24.64	25.69
C16:1	5.45	3.59	2.19	1.68	1.82	1.80	2.05	1.68	1.74
C17:1	0.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:0	6.66	6.94	7.81	5.29	5.79	4.83	5.50	4.91	5.16
C18:1n9	3.70	3.91	3.11	2.75	3.04	10.23	7.00	2.81	2.85
C16:4	3.85	4.08	n.d.	n.d.	n.d.	9.71	6.82	2.85	2.93
C18:2n6T	0.26	0.49	0.40	0.28	0.31	0.18	0.28	0.18	0.20
C18:2n6C	0.66	0.61	0.66	0.68	0.78	0.14	0.08	0.74	0.77
C18:3n6	n.d.	n.d.	0.63	0.66	0.60	n.d.	n.d.	0.37	0.37
C20:0	0.84	0.74	0.82	0.56	0.53	n.d.	n.d.	0.95	0.57
C18:3n3	0.58	0.52	0.80	0.29	0.37	6.02	3.44	0.32	0.27
C20:1	0.27	0.57	0.74	0.40	0.35	0.29	0.39	0.27	0.34
C21:0	0.28	0.58	1.06	1.11	1.14	1.02	1.05	0.60	0.63
C20:2	0.49	0.56	0.53	n.d.	n.d.	4.23	2.98	n.d.	n.d.
C22:0	n.d.	n.d.	1.48	n.d.	n.d.	n.d.	n.d.	1.06	1.00
C20:3n6	n.d.	n.d.	0.16	0.10	0.06	n.d.	n.d.	0.29	n.d.
C20:4n6	5.00	4.84	4.91	4.82	4.95	2.51	5.98	5.20	4.83
C22:1n9	n.d.	0.01	1.20	0.02	0.07	0.96	0.77	0.02	n.d.
C23:0	0.64	0.68	1.88	0.54	0.49	1.14	1.10	0.40	0.45
C22:2	n.d.	n.d.	0.31	3.27	1.11	0.24	0.23	1.40	1.44
C20:5n3	6.44	6.56	8.35	6.27	6.42	5.94	6.46	8.07	8.30
C24:0	0.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:6n3	35.10	33.15	30.94	35.76	37.02	26.57	29.33	36.09	35.24

Note. Values are a % of total fatty acids. FAMEs produced were separated into 2 vials (Sample A and B) and analyzed separately to verify accuracy. n.d. = non-detect.

Table 7.

Saturated vs. Unsaturated FAMEs

	Date								
	8/5/11		8/12/11	8/17/11		8/19/11		8/31/11	
	Sample A	Sample B	Sample A	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
Saturated	37.38	38.62	44.09	39.25	42.00	30.71	33.68	38.98	39.92
Unsaturated	62.65	59.49	55.90	58.05	58.01	65.08	66.32	61.02	60.08

Note. Values are a % of total fatty acids. FAMEs from each sample date were separated into 2 vials (Sample A and B) and analyzed separately to verify accuracy.

Minimal temporal variation was observed between samples with all samples displaying similar trends in FAME composition. An exception was the August 19th sample which showed elevated quantities of C18:3n3 and C20:2 fatty acids as high as 5.75% and 4.23%, respectively, over the other sample dates (Table 6). C16:4 was observed as high as 4.08% and 9.71% in early and late month samples but was non-detect in samples from August 12th and 17th. Alternately, C22:0 was non-detect in the majority of sample dates with the exception of August 12th and 31st where it was observed to be as high as 1.48% of the total fatty acid composition. Minus these exceptions, fatty acid compositions mirrored each other throughout the limited study period.

Quantitative

> **Organics.**

Determining the quantity of organic material produced by natural marine filtration and relating it to land-based system production was one focus of this study. It should be noted that the size of the organisms captured for analysis was between 20µm and 500µm. This range inevitably excluded many marine microalgae whose size was significantly smaller than the 20µm minimum, consequently creating a sample bias towards larger microalgae and smaller zooplankton. This bias complicates empirical comparisons to many land-based production quantities and much of the currently published data.
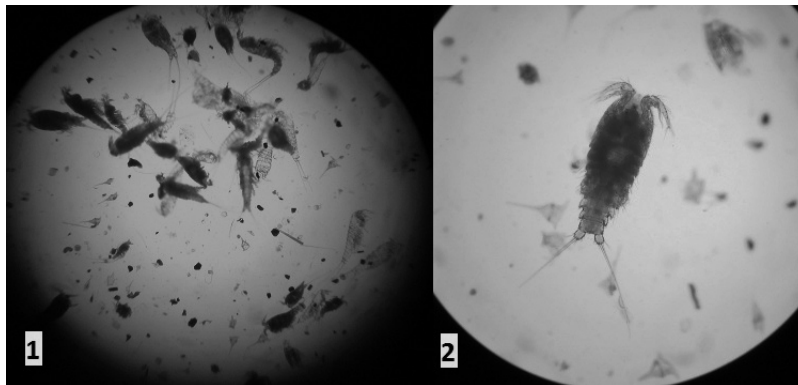

Figure 3. Dissecting microscope image of mixed plankton sample.

A maximum average value of 0.039g/L of dried biomass was produced by the system during the study. This value was the result of 3 days of seawater filtration. If we assume a backwash recovery percentage of 100% this equates to a maximum average concentration of 0.013g/L per day of dried biomass, sizes 20µm to 500µm. Land-based microalgae cultivation studies on *Nannochloropsis* sp. (~2µm) have reported concentrations of 0.48g/L per day (Chiu et al., 2009).

However, due to the variation in target organism size between this study and previous works, it is difficult to say whether a larger daily quantity of microalgae can be produced on land than can be filtered by the UCSB seawater system. Approximately 24,417,792L/week of seawater are filtered by the UCSB system depending on pumping rates. Calculated weekly organic quantities ranged from 16,628.63g/week of dried organic material to 3,837.38g/week which equates to concentrations of 0.68mg/L and 0.16mg/L of incoming organics, respectively. Even if the maximum quantity is assumed to occur throughout the year the system would produce less than 1 metric ton of dried organic material per year. This figure is over 100 times less than yields reported in other studies (Sheehan et al., 1998). We can definitively conclude that more organic biomass can be produced in land-based systems when compared to the quantity of material between 20µm - 500µm that was naturally filtered from the seawater in this study which suggests an advantage to the production of biomass in land-based systems.

Making exact statements about the temporal variation of organics is difficult due to the relatively short sample period (April - August). Overall there was a slight decline in the quantity of organic material produced by the system which seems to mirror the temporal trends found in published studies. Many studies have identified spring and summer as times of increased primary productivity within the California Bight, with microalgae concentrations generally peaking in April and then declining into late November and December (Anderson et al., 2008; Barnett & Jahn, 1987; Mantyla et al., 2008; Mutanda et al., 2011; Otero & Siegel, 2004; Shipe & Brzezinski, 2003). These population increases are

correlated directly with nutrient availability from upwelling, which was not tested for in this study. Broader seasonal and yearly trends have also been identified, especially those which coincide with El Nino events (Lavaniegos & Ohman, 2007). This type of event could directly affect both population densities as well as size distributions in the near-shore community (Barnett & Jahn, 1987). Given that the majority of these studies have focused on the seasonal variation of microalgae, and that many microalgae were excluded from this study's samples, we cannot decisively conclude that organic quantities followed any previously identified temporal trend.

Though not the original intent of this study, logistical errors by facilities management allowed for a comparison between bio-fouled and un-fouled seawater supply lines. Results from this comparison showed counterintuitive results with decreased quantities of organics being produced by the un-fouled line as compared to the fouled supply line. These results contradict the theory that bio-filtration reduces the quantity of organic material in the incoming seawater. One potential explanation for this result is the size selection of the filtering organisms. California mussels (*Mytilus californianus*) filter microalgae from the water. Since this study's size specification neglected the majority of microalgae this could explain why a bio-fouling effect was not seen in the resulting data.

Effective backwashing was identified as a potential source of error in the experimental data and was accounted for by taking multiple samples at varying times throughout the cycle (0 min, 2.5 min, 5 min). Results indicated decreasing organic quantities from samples taken at the various sample times which revealed

that the majority of organic material contained within the filters was expelled during the flushing process. Furthermore, the data shows that most of the organic material was expelled during the first few minutes of the backwash process. Though backwashing was as complete as could be expected some material could have inevitably remained trapped after the 5 minute flush time (Zhang et al., 2010). Seawater system design could also have skewed results. As shown in Figure 4, the seawater supply coming from the ocean dumps into four filter beds. During the course of the study, filter bed 1 was inoperable forcing bypassed seawater into beds 2, 3, and 4. Organic subsamples were only collected from beds 3 and 4. Very little variation was seen between organic quantities in these two filter beds; however no empirical comparison was made between their yields and those of bed 2.

Bed Configuration

Figure 4. Seawater filter bed configuration.
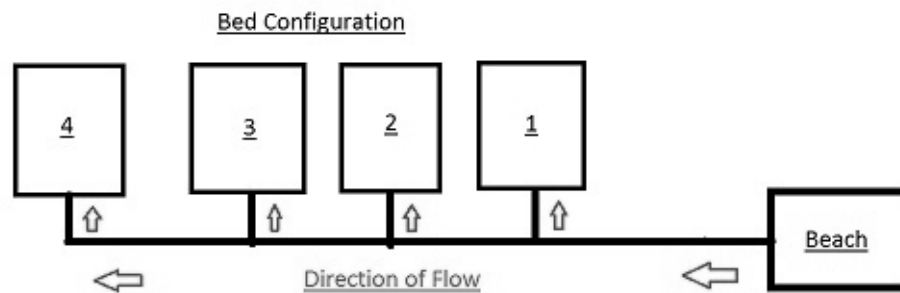
Therefore, it should be noted that the weekly calculations were done under the assumption that filter bed 2 produced similar quantities of organics as beds 3 and 4. It should also be noted that the backwash design could have impacted quantification results. The filtration beds are designed with two troughs that receive the backwash effluent, as seen in Figure 5. As the backwash process

proceeds the effluent is split into each of these troughs. Preliminary tests

determined that the quantity of organics was equal in the subsamples from these

two troughs, therefore only one trough was sampled. This data was then

extrapolated upwards to quantify the total g/L being produced in each backwash

event as well as the potential weekly production.
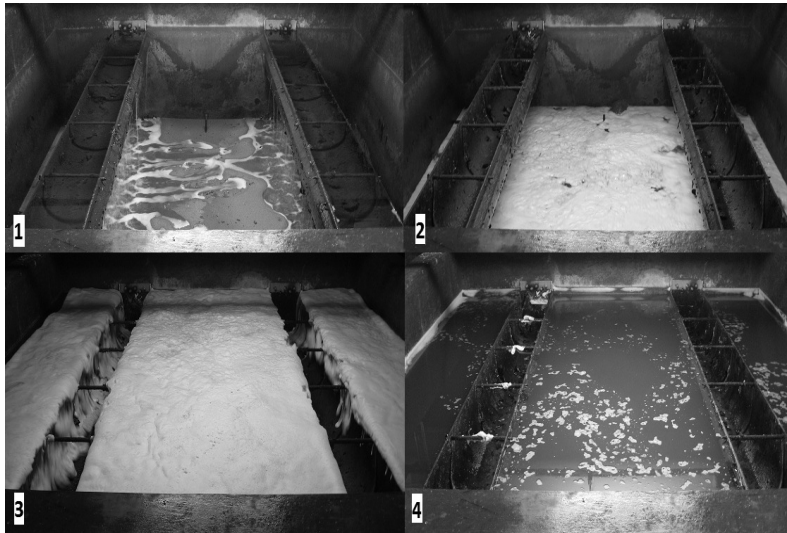
Figure 5. Seawater filtration system backwash from start (1) to finish (4).

Given the system design, organisms were exposed to periods of

desiccation, increased shear stress, and light limitation while trapped in the filter

beds, which may have inhibited the overall yield or changed the chemical

composition of the trapped organics (Miron et al., 2003). Undoubtedly, spatial,

temporal, oceanographic and geographic factors all played critical roles in

determining the quantity of organic material that ended up within the filter media.

The seawater intake structure is located at a depth of 18m. It could be argued that

this depth does not fully capture microalgae from the photic zone which also

reduces the capture of zooplankton that feed on them. The physical density of

marine-based organic material has previously been identified as a major factor in

the depth distribution of these organisms (Steele & Yentsch, 1960). Marine

plankton densities range between $1g/cm^3$ and $1.6g/cm^3$ (Price et al., 1977). This

variable could cause the capture efficiency of the system to vary with mortality,

sinking velocities and weather-related events, all of which were not quantified in

this study. Since microalgae come in motile and non-motile forms, this too

directly impacts their depth distribution and geographic location.

Several published studies have documented difficulty in determining

accurate dry weight measurements (Clarke, Holmes, & Gore, 1992; Hedges et al.,

2002; Larson, 1986; Martin & Knauer, 1973). They show that even after drying at

60°C a small percentage of bound water could remain in the sample causing

elevated dry weight measurements and an overestimation of organics post

combustion. These studies primarily focused on gelatinous marine species such

as ctenophores and jellyfish. The filters sampled in this study would most likely

have destroyed these animals and effectively negated their impacts on dry weight

measurements. Regardless, currently adopted procedures of pre-drying followed

by combustion must suffice as more suitable organic quantification methods were

unavailable.

Greater quantities of material can be grown on land for biofuel production.

Increased yields from land-based systems can potentially be explained by a

variety of factors including the ability to precisely control growth conditions and

isolate robust microalgae species. This study's sample size limitation cannot be

overlooked when making comparisons. Unfortunately, due to availability of

resources and time, the majority of microalgae were excluded from final dry

weight quantification. Even after considering this limitation it is evident that the availability of oceanic feedstocks is patchy, temporally variable, and affected by a variety of seawater system based factors.

FAMEs.

Along with developing a basic understanding of the amount of organic material the UCSB seawater system can generate for biofuel production this study also sought to quantify the amount of fuel that could be produced from this source. Fatty acids comprised less than 0.01% of the dried mixed organic matter. These results are drastically lower than published data from microalgae pure cultures (Bruton, Lyons, Lerat, Stanley, & BoRasmussen, 2009). The fatty acid values observed in this study are also quite low when compared to wild-caught zooplankton species, whose percentages range from 3% to 42% (Lee et al., 1971). It should be noted that the zooplankton in Lee's study were caught in offshore waters between the depths of 0 - 250m versus the 18m depth of this study, making accurate comparisons difficult. Limited sample time and processing requirements made temporal variability difficult to determine as only one calendar month (5 total samples) could be analyzed. For this reason there can be no definitive determination of year-round biofuel potential. Weekly production results showed an average of 0.36ml of FAMEs produced per week or 18.8ml of biodiesel per year. This quantity of biodiesel is not only unremarkable but is also extremely uneconomical from a production standpoint. In order to scale up production, facilities would have to be located in areas of constant feedstock availability, filter extremely large quantities of seawater, and target appropriately sized organisms.

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Near-shore microalgae and zooplankton undergo more frequent disturbance and cycling causing population sizes to be smaller and less robust (Barnett & Jahn, 1987). Since populations are inevitably dependent on nutrient influxes as well as trophic interactions, local weather and temporal variability can directly affect sizes, distributions, and chemical composition. In general, species from higher latitudes have greater quantities of fatty acids as compared to those in lower latitudes (Lee et al., 1971). Lee (1975) found that copepods from the arctic have fatty acids in excess of 60% of their body weight. The UCSB seawater system location is typically regarded as temperate. One could argue that the study site is geographically challenged; however, it may be more pertinent to claim that the seawater system design poses more real challenges to recovering fatty acids in optimal quantities than its location.

Shear stress and auto-oxidation which possibly occurred in the filter beds may have contributed to lower than expected lipid percentages, especially in feedstocks with high polyunsaturated fatty acids (Lepage & Roy, 1984). Natural lipase enzyme activity may have increased free fatty acid quantities in post-mortem cells potentially reducing their biodiesel potential (Bruton et al., 2009). Light limitation has also been found to limit the quantity of fatty acids in recovered organics (Miron et al., 2003). Along with the organics, the backwashing process flushed large quantities of inorganic material from the filter beds which had to be removed via density gradient centrifugation with the heavy liquid sodium metatungstate ($1.8g/cm^3$). If any type of chemical alteration occurred during this step, separation could be identified as a potential source of

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error. Several studies have proven the viability of living marine microalgae post-separation, showing that little if any chemical alteration occurs to the organisms (Bolch, 1997; Price et al., 1974). For this reason it was assumed that negligible chemical change occurred to the samples during the separation step of the process.

The direct transesterification reaction is a relatively new process that allows for a continuous reaction in a single tube. The presence of excess water in the sample has the potential to hydrolyze triglycerides effectively negating methanolysis. Samples in this study were lyophilized for 48 hours to minimize this possibility. It is essential to select a transesterification method suitable to the samples being converted. Many studies have found direct transesterification yields to be higher than those from separate extraction-transesterification steps, especially when large quantities of polyunsaturated fatty acids are present (Fukuda et al., 2001; Lewis et al., 2000; Sheng et al., 2011; Tran et al., 2009; Wen & Johnson, 2009). Higher yields from direct transesterification combined with the convenience of conducting this type of reaction were the primary reasons for choosing this method.

This study is one of the first to document the quantity of organic material being produced by natural marine filtration. These results indicate there is a small amount of organic material (20µm to 500µm) that can be recovered for use as biofuel. The majority of limiting factors seen in this study are related directly to the seawater system location and construction as well as the study's organism size restriction. Due to these limitations it is impossible to make definitive statements

as to the total potential feedstock production of the UCSB seawater system. However, this study and others help to clarify some uncertainties of this type of bio-prospecting. Any system that can avoid the complication of inorganic contaminants is much better suited for biofuel feedstock production. Filtering seawater from higher latitudes at varying depths could potentially increase the overall yield of organic material. By targeting smaller sized microalgae there is also potential to increase fatty acid yields.

Qualitative

A large quantity of biofuel feedstock is powerless unless it is composed of appropriate components for fuel production. Chemical analysis of study samples sought to determine their suitability for use as biodiesel. Two fatty acids dominated the sample chemical composition. C16:0 and C22:6n3 comprised 18.49% to 27.89% of the total sample and 26.57% to 37.02% of the total sample, respectively. Overall saturated fatty acids comprised 30.71% to 44.79% of the total and unsaturated fatty acids comprised 55.90% to 66.32% of the total fatty acid composition. These results correlate well with published data from land-based microalgae systems which show a prevalence of C16:0, C16:1, C20:5, and C22:6n3 fatty acids (Gunstone, 1967; Tonon et al., 2002; Tran et al., 2009; Van Mooy, 2010). Other studies show a great deal of species variability (Meireles, Guedes, & Malcata, 2003; Tonon et al., 2002) which is also evident in this study in the variety of fatty acids present within the samples. A strong correlation exists between the results of this study and those of other zooplankton studies where

both C16:0 and C22:6n3 were common (Fraser et al., 1989; Lee, 1975; Lee et al., 1971).

Bias was evident in the density gradient centrifugation step of sample processing which used the heavy liquid sodium metatungstate to separate out inorganic contaminants. Any organic material that was denser than this liquid was also lost during this step. Published data by Price et al. (1977) on plankton densities along with the knowledge that inorganic silica sand (SiO_2) with a density of $2.6g/cm^3$ made up the primary components of the sample helped to determine the separation solution density. For these reasons, a density of $1.8g/cm^3$ was used to isolate the majority of organic material from the inorganic contaminants.

As evidenced in the quantification data, a temporal bias could affect the chemical composition of the microalgae and zooplankton being filtered by the system along with potential desiccation and auto-oxidation. Stressed and lysed cells caused by pumps, piping, and turbulent flow could all generate increased quantities of free fatty acids which react negatively in the transesterification reaction thereby decreasing the total end product. To counteract this potential error, excess methanol and sulfuric acid were used in an attempt to force the reaction towards the products before sacrificing all of the catalyst. In general, sample analysis yielded repeatable results with minor variability regardless of sample date or composition.

The samples were presumably mixed cultures of microalgae and zooplankton which increased the potential for error during the chemical conversion of their respective fatty acids into FAMEs. Studies have indicated that

phospholipids and other cellular components of marine species do not behave well during transesterification (Packer, 2009). This concern was offset by running the reaction at a high temperature with an acid catalyst (sulfuric acid) which has been shown to extract cellular fatty acids most effectively (Ozgul-Yucel & Turkay, 2002). Some studies have found decreased yields of specific fatty acids (ex. C22:6n3) when samples are extracted with hexane (Carvalho & Malcata, 2005); however, this was not evident in this study's analytical results as shown in Table 6. Analysis of FAMEs was conducted at Arizona State University by a trained technician. Reports of fatty alcohol interference with the fatty acids C14:0 and C16:0 during GC analysis have been published (Cripps & Tarling, 1997) though none were reported in the analysis conducted for this study. During shipping one sample replicate from August 12th 2011 spilled; this is reflected in Table 6 and 7 by the lack of duplicate data for this sample date.

Large quantities of polyunsaturated fatty acids are evident in the mixed sample chemical compositions. These types are generally minimal or non-detect in first-generation feedstocks. Land-based crops such as soy and rapeseed produce large quantities of C18:1 and C18:2 fatty acids (Gerpen, Shanks, Pruszko, Clements, & Knothe, 2004); which is most likely due to their role as terrestrial primary producers. Conversely, oceanic primary production is often bundled into polyunsaturated forms. This is the most likely reason for the prevalence of C22:6n3, which is generated by microalgae in large quantities and then consumed by zooplankton whereby it is directly transferred up the trophic ladder. Fatty acid composition is also affected by the types of cellular

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components that are extracted in the chemical reaction. Phospholipids and wax esters yield different fatty acids from those of the traditional tri- and diglycerides found in many microalgae.

FAMEs from this study would not be suitable as biodiesel in Europe without some chemical alteration to reduce the number of polyunsaturated fatty acids to below 1% (molar) of the total composition (Chisti, 2007; Mutanda et al., 2011). This alteration is common in the health sciences and could be achieved by partial catalytic hydrogenation as described by Jang, Jung, and Min (2005). American Society for Testing and Materials (ASTM) standards are specified by alternate properties that were not tested for in this study. Therefore, further testing would be required to determine if the biodiesel produced was suitable for use in the United States. Biodiesel is one of many uses for the fatty acids found in marine-based feedstocks. Alternative uses include: therapeutics, pharmaceuticals, cosmetics, and dietary supplements (Barrow & Shahidi, 2008; Mata et al., 2010). These are just a few of the many reasons it is necessary to continue to study this naturally occurring feedstock.

Further Study

This study raises and answers many questions as to the ability of marine filtration to produce substantial, quality feedstocks for biofuel production. Further exploration into the quantity of microalgae ($\leq 20\mu$m) that can be filtered from oceanic water is definitely necessary. Detailed investigation into the geographic variability of microalgae and zooplankton would also be beneficial in order to pinpoint potential future filtration sites. Along with large quantities,

good quality organisms are necessary to provide high purity biofuels. Many of these answers will come in the form of oceanographic data, nutrient mapping, and seasonal sampling. Knowledge of population assemblages, species composition, and distribution are critical to future exploration.

Systems design for the efficient filtration of marine organisms would help to eliminate the need for inorganic separation and would ideally require limited maintenance. Improved materials science is critical to create filtration media that can handle the pressures and flow volume necessary for scaling up production. Determining optimal backwash cycles for systems that are currently in place to lower the probability that organic material undergoes light limitation, shear stress, and auto-oxidation are also vitally important. Coordinating filtration systems with clean wave energy will help to lower potential emissions and eliminate the need for fossil fuels to run pumps, filters, and processing equipment. Continual improvement in the dewatering and conversion processes and/or the development of new technologies that do not require the energy intensive drying step of the production chain are also essential. The future potential for plankton based biofuels is real and within reach; a little creativity and a lot of hard work will help to successfully move us toward this reality.

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