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Prospectus for Future Research: Temperature Effects on Green Macroalgae

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Prospectus for future research: Temperature effects on green macroalgae

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Abstract

Research on temperature change has received attention from scientists. Not only has temperature change attracted scientists who study the Earth's weather and climate, but also those who study plants and animals on land and in oceans. The research being conducted looks at how these temperature changes affect the anatomy and physiology of organisms, but other research also studies how terrestrial and aquatic ecosystems have changed and the types of responses that have occurred as a result. For many years, research in terrestrial ecosystems has dominated research in aquatic ecosystems. Limited research has been conducted in aquatic ecosystems involving green macroalgae, which are primary producers and an essential part of these ecosystems. Within the last fifteen years, research regarding the effects of temperature change on green macroalgae has shown that temperature affects photosynthesis and biochemistry. Overall, photosynthesis is almost completely inhibited after sufficient exposure due to damage in Photosystem II. There is also a substantial change in the production of chemical molecules known as reactive oxygen species that caused physical damage to the algal cells as well as a change in structure for proteins that is essential for function. I reviewed the literature within the past fifteen years, regarding the effects of temperature on a variety of green macroalgae species. The results indicated that after sufficient exposure, the photosynthetic rate of macroalgae is reduced significantly and that the change in chemistry lead to alterations in the physiology of green macroalgae. In this thesis, I propose two possible directions for algae research that involves genetic sequencing of a model species to identify a possible temperature tolerance gene, as well as the potential use of green macroalgae in photobioreactors as a source of renewable energy.

Introduction

Climate Change and Marine Environments

Climate change has been occurring over the last century due to changes in atmospheric composition from increased emissions of greenhouse gases such as carbon dioxide (Thomas, et. al. 2003). Anthropogenic increases in greenhouse gas emissions have led to global increases in temperatures. As greenhouse gas emissions have increased, global warming in the last century has occurred at an increased rate (Mann et al. 1998; Ruedy et al. 2010). Over the past 100 years, global climate temperature has increased by 0.6°C, and the rate at which it has been increasing has now doubled and is now greater than it has been in the past 1,000 years (Walther, et. al. 2002). Research on climate change has revealed that over the years, this increase in climate temperature has affected organisms that are known to live in a broad range of geographical locations and has affected species diversity (Parmesan, 2006). The effects that anthropogenic changes have had on organisms are not limited to a certain part of the globe; the effects have been traced to every ocean, continent and to the majority of taxonomic groups (Parmesan, 2006).

The ocean can absorb approximately 80% of the heat and 50% of carbon emitted into our atmosphere (Hobday, 2014), resulting in an “air conditioner,” which may lead to terrestrial temperatures not increasing as rapidly as expected with current greenhouse gas emissions. Heat storage relies on the rate at which the ocean can absorb heat and distribute it to be released slowly back into the atmosphere (Thomas, et. al. 2003). However, while the ocean is acting to absorb CO₂ and heat and mitigate terrestrial climate change, large changes in temperature and acidification are occurring in the oceans as a result of the absorption of heat and CO₂ (Hönisch, 2012). This causes several problems, including lowering the pH level of the ocean and causing it to be more acidic (Honish, et. al 2012). While scientists have learned about how temperature

affects terrestrial environments, less is known about how marine environments are impacted by climate change. The effects of climate change in marine systems appear to be greater than in terrestrial systems despite the fact that oceans are warming at a slower rate than terrestrial systems (Burrows, et. al 2011).

The ocean is susceptible to climate change, making it an important factor to consider when looking at the abundance, phenology and life history of marine species. Although temperature is known to have a significant impact in marine systems, according to Hobday (2014), the effect of global warming on the oceans is poorly understood and is neglected when compared to the effects of warming in terrestrial systems. Research into the effects of increased temperature in marine systems can help scientists better understand the redistribution of marine species, as well as to help with sustainable natural resource management, as the oceans warm (Hobday, 2014).

Research has shown that there has been an increase in oceanic temperature (Hobday, Gretta. 2014) particularly in the deep oceans (Cutler, et. al. 2002), but scientists have different views about the extent of the effect on marine life. One thing that can be said, however, is that major changes have been occurring across marine ecosystems due to global warming (Hobday, 2014). In the last few decades, marine temperatures have increased, particularly at the troposphere which is the lowest layer in the atmosphere (Perlwitz, 2014). This increase in tropospheric warming can be attributed to the sea surface temperature increase (Ding, et. al. 2014). This has been in part, caused by the arctic ice loss that has occurred mostly after the year 2000 (Perlwitz, 2014) and is expected to increase the temperature by 4 degrees Celsius by the end of this century (Hobday, 2014). Aside from a change in global temperature, within the last 112 years, the ocean temperature has also increased by 1 °C (Hader, 2014). An increase in air

temperature can occur quickly and can raise the surface water temperature up to two times and lead to thermal stratification. Typically nutrients are found in deeper waters but with thermal stratification this then leads to the formation of strong barriers that make it more difficult for organisms to use nutrients, especially plants that need nutrients for photosynthesis (Hader, 2014).

Xianyao (2014), however, has a different interpretation for the recent temperature changes. In his research, he proposes that there is currently a slowdown in global warming, which he concludes is due to heat moving into deeper oceans. Not only does he propose that global warming is now slowing down, but that this is a pattern that has occurred before. This pattern is referred to as “multi-decadal variation,” and it consists of two 65 year cycles of accelerated warming, followed by a period in which temperature increases at a slower pace.

The global temperature increases, whether they are going through a recent slowdown period as more heat is transferred into the oceans than into terrestrial systems, are due to anthropogenic production of greenhouse gases due to human activities. This climate change will have significant impacts across ecosystems, particularly in marine systems. Future research should focus on understanding the extent of warming in deep oceans and its effects on marine ecosystems.

Importance of Macroalgae

Green macroalgae are known to live in some of the most extreme environments on earth including deserts, permafrost, and hypersaline environments (Rothschild, et. al, 2001). Algae can be found across the planet, whether that is on land or in aquatic environments and in both hot and cold environments (Rothschild, et. al, 2001). Despite being able to thrive under different

condition, algae are still susceptible to changes in the environment, and it is important to understand how the environment plays a role on their regulation processes and survival.

Green macroalgae, are important components of ecosystems. Green macroalgae are large photosynthetic algae such as seaweed. They are primary producers that are able to convert carbon dioxide into sugars, which can then be consumed by other organisms higher up the trophic structure. Not only are they able to absorb CO₂, but they also provide somewhere between 40%-50% of atmospheric oxygen that is essential for many organisms (Andersen, 2005). Seaweed are one of the most dominant autotrophic by biomass in many ocean environments and may include up to 1550 species (Mineur et. al, 2014). Macroalgae act in both structural and functional roles within the ecosystem. For instance, they create additional diversity by providing habitat for other species (Mineur et. al, 2014). They are functionally important in marine communities not only as primary producers of energy but also as partners in mutualistic interactions (Andersen, 2005). Additionally, macroalgae are able to increase photosynthetic rates, biomass accumulation, nutrient uptake, and invasion resistance (Mineur et. al, 2014). According to Mineur (2014), within the last two decades researchers have been troubled by the loss of diversity of macroalgae at a global level.

Another great advantage that algae provide is that they are “carbon sinks” (Mineur et. al, 2014). In other words, they are able to absorb CO₂ from the environment that could otherwise cause harmful effects, such as increasing temperatures. Green macroalgae is able to absorb anywhere from 0.46 - 2.55Pg of carbon per year, which is not as effective as phytoplankton, but efficient nonetheless (Mineur et. al, 2014). Not only can macroalgae absorb CO₂, but they can also provide carbon back to the ecosystem within a year, which is faster than phytoplankton. Mineur (2014) claims that approximately 40% of the carbon is exported out of the system, 30%

is decomposed, and the remaining 30% is consumed by grazers. This exported material is what gets deposited on the shores where it provides benefits to decomposers and herbivores or it goes into deeper parts of the ocean where it will become a part of the detrital process (Mineur et. al, 2014).

Climate Change Effects on Macroalgae

Photosynthesis is the process that allows plants, including algae, to convert CO₂ into sugars, using solar energy, and this process is vital for plant growth. As temperature increases, the rate of photosynthesis generally increases (Davison, 1991). However, this does not always hold true. There is an optimum temperature at which a plant's photosynthetic rate is greatest, and, once the temperature gets past this optimum, the rate of photosynthesis begins to decline rapidly (Davison, 1991). For macroalgae, rather than a fixed temperature, this optimum temperature can vary by several degrees. The effects that temperature will have on photosynthetic rate vary based on whether algae are in their species-specific optimum temperature range or not (Rautenberger, 2015). The rate at which photosynthesis occurs is limited by the photosynthetic electron transport chain, and the carbon fixation rate of RUBISCO (Davison, 1991).

Reactive oxygen species (ROS) are chemicals that are released by organisms during metabolic processes such as photosynthesis, and are produced by chloroplasts and mitochondria in the electron transport flow (Lu, 2006). ROS are able to rapidly react with cellular components and water, but also contain a short life time. Oxidative stress occurs when a biological system cannot keep up with the ROS that are being formed and cannot repair the damage being done. Because of that, the presence of ROS can be a good indicator of oxidative stress that result from

a disruption of cellular homeostasis, including UV radiation. This is due to the fact that UV light affects the biomolecules that block enzymatic reactions that ultimately induce the formation of ROS. Due to the stress, ROS can cause damage to membrane lipids, proteins and nucleic acids which can lead to cell death (Colleen, 1996). To counteract the effects of ROS, plants have developed non-enzymatic, as well as enzymatic systems in which superoxide (O_2^-) is converted to hydrogen peroxide (H_2O_2) via enzymes (Lu, 2006). Some of these enzymes include ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT), and for the non-enzymatic system phenolic compounds are present (Dummermuth, 2003; Lu, 2006). Although plants have this defense system, an increase in CO_2 concentration and ocean acidification due to an increase in temperature can change the chemistry and alter the UV protection that algae have, leaving them at higher risk of UV damage (Hader, 2015). In the end, the side effects that occur to algae as temperatures increase, only add more stress to the plant caused by the direct effects of increasing temperatures (Rautenberger, 2015).

Temperature has additional lasting effects on marine plants. An increase in ocean temperature means that habitat will change as well as species composition, especially for sensitive organisms. The ecological effects would then alter the evolutionary adaptation for many of these organisms (Reusch, 2014). Algae are known to be geographically widespread which could mean that local adaptation is possible.

According to Reusch (2014), ocean warming can result in immediate effects which will help macroalgae survive. Macroalgae tend to grow in warmer temperatures due to an increased CO_2 availability. This means that an increase in temperature will result in longer growth periods for algae, allowing them to enhance their reproduction and growth rate. One study done by Navarro (2016), showed that one of the green macroalgae species *Monostroma hariatii* had a

very high temperature tolerance of 30°C for its reproductive cells. The study also revealed high photosynthetic capacities at high temperatures than those compared to brown macroalgae. For those with greater phenotypic plasticity, it allows acclimatization to the changing environment. Even though increased temperature does provide some benefits, it adds stress to algae which can result in detrimental effects.

Objective

In this paper, I seek to 1) review the literature on the effects of increased temperatures on macroalgae physiology and 2) provide future directions for research in this area. Below I will summarize the important research conducted on macroalgae physiology as it relates to global warming from 2000 to 2016. I will then use this review as a starting point to focus researchers' efforts on macroalgae physiology. Global warming is greatly impacting ocean temperatures and we need research more about how this affects primary producers such as macroalgae. This could then perhaps give future direction to other research that could break many cycles. If scientists were to better understand how temperature affects macroalgae, they can better counteract the effects and prevent death. With many different species of macroalgae, it would be beneficial to find a model species which can be used to represent a vast majority, if not all. This type of information could be used to enhance research that has been conducted regarding biofuels.

Review of Literature

Temperature Effects on Photosynthesis in Macroalgae

Based on research conducted within the last fifteen years, scientists have some understanding of how temperature affects the photosynthesis process of macroalgae. Research has shown that the effects of temperature are species-specific (Rautenberger, Bischof, 2006). Not only are the effects of temperature species-specific, but the degree of damage is also dependent on the temperature and length of exposure. Green algae especially tend to have high net photosynthetic respiration, meaning that they break down organic molecules into ATP at a fast rate (Dummermouth, 2003). Species such as *Ulva bulbosa* are able to keep high respiration rates over a wide range of temperatures, and for some green algae they are also able to maintain photosynthesis at high temperatures. Unfortunately, higher temperatures tend to lead to detrimental conditions because production decreases (Nechi, 2004). This is due to the algae's short term response that allows them to acclimate to their environment. Macroalgae are able to acclimate easier by altering chlorophyll concentration to match concentration and activity levels of enzymes such as Rubisco (Nechi, 2009). This ability to acclimate is a common trait to have for many primary producers like algae in lotic ecosystems that include streams, rivers, channels and other bodies of flowing water. Due to adaptations of different species, algae are able to thrive at either lower or higher temperatures, ultimately leading to variations in survival strategies among species (Dummermouth, 2003).

Even though green macroalgae live in different environments and experience different types of temperature changes, they all experience detrimental effects. Overall, the results indicate that algae are sensitive to temperature which leads to inhibition of photosynthetic activity due to damage to pigments and proteins (Rautenberger, Bischof, 2006). Both too high and too low of

temperatures result in photosynthetic inhibition, but the effects are not immediate. Even if there is no direct damage being done to photosynthetic proteins, temperature still has a great influence over photosynthetic rates. After long enough exposure to high temperatures, dependent on the species, photosynthetic abilities of the algae are almost completely inhibited and results in irreversible damage. A great example of this is from Alstynne's (2014) study in Washington which involved intertidal environments. Due to their environment, the algae *Ulva lactuca* experienced rapid changes in temperature especially during the tides which made them more vulnerable to temperature changes. This resulted in the death of many algae during the summer months unlike many of the other algae that lived in other environments where the temperature is more stable.

Heat has been shown to cause some physical changes in algae that affect its photosynthesis rate. Turgidity, vacuoles and plasmolysis all help algae keep their cell structure, and in the presence of heat stress these structures decrease. Thermal stress has been shown to lead to slower growth (Kakinuma, 2006). Not only does heat alter the shape of algal cells, but it damages organelles that are essential for photosynthesis such as chloroplasts, mitochondria, nuclei (Lee, 2009). However, the altering of cell shape is not the main issue for algae caused by temperature. Damage to Photosystem II appears to be the primary site of heat stress in photosynthetic transport systems (Lee, 2009), leading scientists to believe this to be the main cause for photosynthetic inhibition. This results in a reduced amount of energy flux passing through photochemical reactions. In the freshwater macroalgae, significant effects in the electron transport rate (ETR) were due to changes in the photosynthetic ratio respiration (Nechi, 2004). Some macroalgae, however, is still able to have high photosynthetic rates despite higher temperatures. The study conducted by Dummermuth et. al (2003) indicated that the species *Ulva*

bulbosa had a high stress tolerance and still showed signs of activity even after it was exposed to high temperatures for eight days. Unlike Alstyne's research that indicated that indicated *Ulva lactuca* did not have the same type of tolerance and did not survive in environments when temperature fluctuates.

Temperature Effects on Biochemistry in Macroalgae

Similar to photosynthetic changes, the alteration of molecular production in macroalgae is also species-specific (Abrahamsson, 2003). An increase in temperature leads to an increase in the production rates of many ROS, but this rate varies among algal species. This is due to the fact that temperature affects algae differently. The formation of ROS can be used to measure the photosynthetic efficiency of photosystems by measuring at the amount of proteins present. Some of the more common ROS proteins are superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Dummermouth, et. al., 2003).

UV absorption has shown to also affect the physical properties of algae resulting in a higher exposure to thermal stress and damage. Proteins within the cells are capable of absorbing short wavelengths of UV, which are able to break the disulfide bridges that help give a protein its shape. The protein's shape is essential for structure as well as the function, so an alteration in its shape means that it could drastically change how it interacts with its environment. Not only does heat break disulfide bonds, but also free amino acids increase, meaning that the proteins themselves are being broken down (Kakinuma, 2005). The effects that algae suffer damage its own DNA (Rautenberger, Bischof, 2006).

The way in which temperature affects photosynthesis also has to do with how it affects the molecules present. As other researchers have found, heat causes damage mainly to

photosystem II. In a study by Dummermouth (2003), photoinhibition was due to H_2O_2 stress which also happens to be due to temperature as among other potential factors. There was high photosynthetic activity as well as anti-oxidative potential like high enzyme activities of CAT and SOD. In all the studies conducted, a point was reached where production can no longer be balanced. This type of protection typically comes from the chloroplast compartments, which include photosystem II. An increase in temperature can lead to oxidative stress because the algae cannot maintain a certain level of chemicals such as H_2O_2 as mentioned earlier. Marine algae especially, produce halocarbons while they are under oxidative stress as chemical degradation occurs (Abrahamsson, et.al. 2003). In studies mentioned by Abrahamsson (2003), halocarbons have shown to have the potential to contribute to ozone depletion which takes part by changing the oxidizing capacity of the atmosphere through a series of reactions. Algae are known to produce up to 70% of global bromoform, meaning that they only feed the cycle and allowing longer lifetime for harmful chemicals such as methane and other greenhouse gases. Algae in warmer temperatures tend to release higher concentrations of these chemicals into the atmosphere than those in lower temperatures. Algae functions through a series of cycles. These all come together in some way, as some seem to feed and influence each other, although there have been different types of studies being done in the past fifteen years little is still known about how these systems all work together.

Future Research

Research that has been conducted in the past fifteen years has revealed the species-specific consequences that temperature has on both the biochemistry and photosynthetic rates of green macroalgae. Both the degree, as well as the duration of the temperature change are species-specific (Rautenberger and Bischof. 2006). Once there has been sufficient stress to cause damage, there is a drastic change in the photosynthesis rate due to photoinhibition which is caused by damage to organelles (Lee, et. al. 2009), as well as changes in chemical molecules (Kakinuma, 2006). While research can answer, current questions that we have, it also raises new ones. Can we find common ground between the different types of macroalgae, and perhaps even find a model species? What is it that makes the results that we see species-dependent? These questions can expand to other fields. How can we take the information that we already know about algae and temperature and apply it to other research?

Macroalgal Physiological Research

The research that has been done in past years has ranged from studies in oceans, reefs, lotic and brackish environments and even in ponds. Despite a wide range of environments being studied, the studies do not go into sufficient depth to gain insightful information into the species. There are approximately 1,500 different species of green macroalgae worldwide (Waaland, et. al. 2004). Based on studies done on a few species; such as *Cladophora glomerata*, *Ulva chlorophyta*, *U. bulbosa*, and *U. lactuca*, we know that the results are species-specific, certain species have been shown to be better adapted to temperature change than others. Scientists, however, still do not fully understand why some species have a higher tolerance than others to temperature change. Studying all of the different types of algae would not only be expensive, but

it would also be time consuming. Conducting research requires money for equipment, as well as time and energy from researchers. In order to measure temperature, devices need to be purchased. If the algae is being cultivated in the lab, as most experiments do, then money needs to be spent in order to provide transportation from the sample area to the lab and to be able to grow it as well. To fully understand the effects of temperature several trials need to be conducted, and these trials need to be conducted at a range of temperatures. While studying multiple species would be ideal, some may reveal similar. With so many species, can we find one which represents the full range of them and can be used as a standard model? There are species such as the microalgae *Chlamydomonas reinhardtii* and yeast *Saccharomyces cerevisiae* that are already being used as models for their own macroalgal fungi respectively and have been used to study the effects of genetic engineering (Diaz-Santos et. al, 2015). These species have allowed researchers to analyze the way in which gene transformation will affect similar species, without having to test each species individually. Finding a model to represent all 1,500 macroalgae species would be too much, but it would be beneficial if scientists could identify a species that represents a similar tolerance to temperature. Finding a model species could take time because there are still many questions to which we do not have the answers. Some of the questions include: What gives some algae advantage of tolerance to high temperatures? Are species tolerant to high temperatures due to specific genes? Or is it simply their environment? For several years now, macroalgae have been viewed as unique species and have caught the attention of scientists mainly for three reasons; its taxonomy, physiology, and biochemistry (Chan, et. al. 2006).

Since the 2000s, scientists have been interested in finding a model plant based on genomic studies, in order to apply the species to multidisciplinary research (Chan, et. al. 2006).

Despite macroalgae being important providers for their environment, there has been little effort to sequence the genome of any of the species, and studies have been lagging behind those involving microalgae and terrestrial plants (Waaland, et. al. 2004). By sequencing an organism's genome, or part of it, scientists can learn information about the organism including evolution, molecular biology, biochemistry, physiology, and developmental biology. Recently, the red algal species *Cyanidioschyzon merolae* had its full genome sequenced (Walker et. al, 2005). Some benefits that have emerged from this are studies involving recombinant techniques which can be used to identify promoter sites for genetic engineering. A promoter site is a section of DNA in which transcription begins (Freeman, et. al. 2008). This is important because having different promoter sites means variability in gene expression (Stormo, 2000). In the case of the red algae, this information can be used to enhance the metabolism of other microalgae via transgene expression in which a gene is transferred from one organism to another. Microalgal species involved in these studies include species in the *Volvox* and *Chlorella* genera. Transgene expression is beneficial because it reduces the likelihood for gene silencing to occur, and can lead to higher metabolisms for microalgae (Walker et. al, 2005). For green macroalgae, if we are able to identify sequences for different phenotypes, then we can see if they influence temperature tolerance or not. The answer to the difference in species tolerance could potentially lie in the genome. This is where identifying a model species would be beneficial. Rather than sequencing the genome of a range of species, scientists can focus on those that have been established as a model species.

If scientists were able to sequence part of a species' genome, or even perhaps all of it, then we could potentially determine whether there is a gene for temperature tolerance. This has already been done in species like wheat where research has shown that there has been an

enhanced temperature tolerance due to gene expression (Wang, 2016). These genes have been linked to the production of ABA, cytokinin and auxin signaling transduction pathways. If this approach could be applied in green macroalgae then scientists could identify what allows certain algae to have a high temperature tolerance as well. This type of research could further build our understanding of temperature tolerance in algae and perhaps prevent some of the damage that is caused by high temperature. One potential direction could be to transfer this knowledge to non-model species with a lower temperature tolerance to identify that gene and study whether it affects that species in the same way or not. Perhaps scientists can work on genetic engineering for green macroalgae, similar to how they have with red algae and possibly expand towards more than just green macroalgae but other applications as well.

Biofuels

The world's main source of energy comes from the burning of fossil fuels that include petroleum, coal, and natural gases (Hubere, 1949). Fossil fuels are formed when organic matter decays to 98-99% completion resulting in a fossil (Schobert, 2013). These are typically found in the earth's crust. The burning of fossil fuels result in the release of CO₂ into the atmosphere, which can accumulate and ultimately result in climate change (Schobert, 2013). This has led many scientists to find more sustainable and economic energy sources (Jung, et. al. 2015). Some of the "clean and alternative" sources include wind, solar, geothermal and tidal. All of these are currently being put into practice, but biomass energy is actually the form that is more common around the globe.

Biomass energy produces combustion fuels, also known as biofuels. These biofuels are exclusive to biomass energy because they are derived from plants via a fermentation process

(Jung, et. al, 2015). There are two main reasons why biomass and more specifically, biofuel energy has become a great alternative to fossil fuels. As mentioned before, fossil fuels take millions of years to form. Plants on the other hand do not take nearly as much time to grow and can easily be grown and replaced. This makes biofuels a renewable resource for energy. Not only is biofuel easy to replace, but it is a material which can be grown in greater mass quantities and is also readily available in nature. Another advantage to using biofuel is that it helps to eliminate the amount of CO₂ that is released into the atmosphere (Schobert, 2013). It is able to do this through a cycle system Schobert (2013) identifies as a “short circuit”.

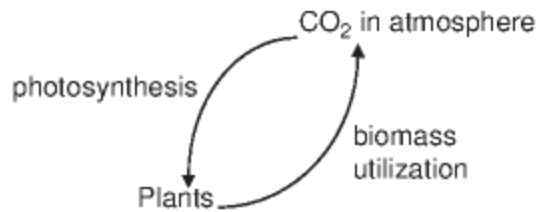


Figure 1. This figure demonstrates the cycle that occurs as a result of plants being used to create biomass taken from Schobert (2013).

The cycle can be seen in Figure 1, and begins with a plant being used for biofuels. Naturally, this results in a release of CO₂ into the atmosphere, similar to fossil fuels. Although the burning of fossil fuels and biofuels both result in CO₂ release, the total amount of CO₂ released by the first set of plants will then be absorbed by the next generation of plants. Because every cycle of plants is the same species as the previous one used, they should in theory absorb

the same amount of CO₂ that was initially released (Schobert, 2013). Ultimately, this results in no net change in the concentration of atmospheric CO₂. There are several great advantages to using biomass energy, and now scientists have been trying to find ways in which they could improve the efficiency which I will mention in the next few paragraphs.

Photobioreactors are one of the applications of biomass that scientists have been working on in recent years which are designed to help decrease the amount of CO₂ found in the atmosphere. A photobioreactor is a good alternative because it uses some type of light source in order to grow different organisms such as terrestrial biomass and microalgae. These organisms are able to convert light and carbon dioxide into biomass via photosynthesis, which will be used for energy in the form of biofuels. Having a larger optical cross sectional area, allows the culture of organisms to receive more energy and therefore have a more efficient light distribution. There are many factors to consider when designing a photobioreactor, as it is still something scientists have been trying to explore and better understand. The two types of systems that have been incorporated into these bioreactors are open and close. An open system is hard to control due to a fluctuation of both temperature and light availability (Kumar, et. al 2011). This is due to not just seasonal changes, but even changes that occur within 24 hours. This type of system is also more susceptible to light contamination which would result in a decrease in biomass productivity. With a closed system however, it is easier to control variables and maintain a more suitable environment for the organisms and improve their productivity levels. Based off of that, scientists have designed multiple types of bioreactors. The different kinds of bioreactors are also able to provide different nutrients that are important for growth, allowing for better results based on the design. These include airlift, the bubble column, tubular reactors, flat panels, and a hybrid combination. Aside from different possible systems, one major factor that seems to affect the

CO₂ absorption by these organisms is temperature. There seems to be a higher ability to absorb CO₂ when there is some sort of heat exchanger system or a species which thrives in higher temperatures. There are some plant and algal species that can tolerate higher temperatures, which leads to the question of: Which organisms are better fit and can provide higher biomass?

Some of the more common biofuels today what are known as “first-generation biofuels”. These fuels are derived from, but not limited to plants such as the sugarcane, corn and soybeans (Jung, et. al 2015). The problem that occurs with these sources of energy is that there is a competition with the production of food. This raises alarming questions as to whether or not it is a sustainable and economic alternative. Because of this, a possible solution has emerged known as the “next-generation biofuels” that consist of algal biomass and organic wastes.

The potential of green macroalgae

Green macroalgae are a great candidate for photobioreactors for many reasons. These organisms are known to thrive globally in a range of environments such as freshwater, lotic and oceans and are easy to grow in large population sizes. Occurring in a wide range of environments is a great advantage because it means they are readily availability for research. Green macroalgae occur at a great abundance, but only 1% of it is currently being used (Chen, et. al. 2015). Not only is it easy to find and grow, but they are also unique when compared to other organisms (Kumar, et. al 2011). A great example is seaweed, one of the most common species of green macroalgae, which is already considered a next-generation biomass. Macroalgae contain small amounts of lignin making them good for the fermentation processes of microbial conversion (Jung, et. al 2015). Lignin is a biopolymer that is essential for structure and strength of the cell wall (Boerjan et. al, 2003). This means that the biogas energy that is produced will be more

feasible than other fuels, the small amount of lignin, also allows the macroalgae to biodegrade faster than microalgae (Chen, et. al. 2015). Growing seaweed is more convenient and economical than growing a terrestrial plant because you do not need land mass to grow it or fertilizers to ensure that it grows. The use of algae as a form of biofuel is still something that needs further research in order to find its true potential. With all of the potential species to use, having a model species would make it easier to find a more effective specimen to use.

Not only are macroalgae able to help decrease atmospheric CO₂, but it has also shown to have other environmental impacts (Jung, et. al. 2016). “Green tide” is a special phenomenon which many coastal areas experience due to a substantial accumulation of green algae. As the algae continue to pile up on the beach, it can cause hygiene and environmental problems. If green algae were to be used for biofuels, then the severity of green tide would decrease. What once would have been waste piled up along the beaches can now be converted into energy.

The fluctuation of temperature plays an important role in the effectiveness of the photobioreactor so understanding how temperature affects the photosynthetic organism, in this case macroalgae, is an important component. Temperature plays an important role for the algae on not just photosynthesis rate, but even something as essential as growth. In order for photobioreactors to work, the algae have to be able to be cultivated. Being able to cultivate the algae will ensure better results and a higher productivity of biofuel production (Jung, et. al 2015). As we have seen from the research conducted within the last fifteen years, the way in which temperature affects green macroalgae is species specific. Some of the algae are better suited for higher temperature ranges and can withstand rapid changes in temperature (Dummermouth, 2003), making them a better candidates for photobioreactors. If we can understand how temperature affects the different species of green macroalgae, then scientists can use that

information in order to design better photobioreactors that produce more biofuel. With so many different species possible there is already an infinite number of possible combinations that change from the type of system, the design of the photobioreactor, the photosynthetic organism, all the way down to which species of that organism would yield the best results.

Conclusion

Green macroalgae have been shown to be an important part of ecosystems. They not only provide food and structure to other organisms, but they also help regulate and absorb CO₂ from their environment (Hobday, 2014). Understanding how factors such as temperature affects green macroalgae could help prevent damage and death of these organisms. This would help maintain more suitable conditions for algae so that ecosystems could stay balanced and thrive. The algae would not be the only organisms benefiting, however, since there are many other organisms that depend on algae for a variety of reasons. If green macroalgae, one of the primary producers were removed or damaged, the results can be extremely negative to the whole ecosystem. Furthering research to such a key organism has many benefits. One of them is that we can conserve and help maintain an adequate environment in which it can thrive along with the other species that depend on it. Unfortunately, studying a number of macroalgae species would take a long time which is why I propose that a few model species would be used to represent different temperature tolerance levels. Once those model species are identified, whether it was because of abundance, ability to cultivate in labs, or for convenience, we can then begin to compare them and learn more about why some have a greater tolerance for temperature. These differences can be studied, not just by comparing the physiology of the species, but by looking into their genomes. There are many different species within green macroalgae that have been underrepresented in the studies

within the last few years that could hold new information. Within the past several years, there has been an interest in genetic sequencing, but there have been little efforts to sequence the genome for green macroalgae. The amount of information that is gathered would allow for many applications and perhaps reveal what the differences among these types of algae are. Promoter sites like those in the red microalgae *Cyanidioschyzon merolae* have been used in transgene expression as an effort to enhance metabolisms in other microalgae. Species like wheat have also shown to possess the gene which provides temperature tolerance. These two species are examples of potential applications that have been found as a result of genetic sequencing. Green macroalgae could perhaps also hold new applications.

Once we have a better idea of how macroalgae functions, and how temperature affects macroalgae, we can begin to apply that information towards new research and perhaps find solutions to problems. An increase of CO₂ in the atmosphere and resulting temperature increases have led to a push for new energy sources and conservation efforts. One new energy source is biomass energy which has shown to mitigate the increase of CO₂ with the use of photobioreactors. Although green macroalgae are not currently being used as biomass energy, there has been research to suggest that they may provide some benefits that others organisms like microalgae cannot. There is potential for green macroalgae to be used as a biofuel. However, research needs to occur in this area particularly once genetic tools are available to increase production in thermotolerant macroalgae.

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