HIGH SCHOOL EDITION
Journal of Student Research

Impact of Environmental Variations on Biodiesel Harvesting from Chlorophyceae in Raceway-Style Ponds

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ABSTRACT

In a world that is increasingly reliant on energy, it is unsustainable to be dependent on fossil fuels such as coal, petroleum, and natural gas. Finding renewable sources of energy that can efficiently support the needs of the modern world is crucial. The following scientific review closely examines microalgae cultured in wastewater as a source of biodiesel, specifically, the well-studied Chlorophyceae class. Chlorophyceae primarily live in freshwater bodies and contain high levels of lipids, making them an ideal type of microalgae for biodiesel extraction. One of the most commonly used methods of mass-producing biodiesel from microalgae is open paddlewheel raceway-style ponds. In these open pond systems, various environmental factors can fluctuate which could reduce biodiesel production in microalgae. Several such limitations exist when considering biodiesel obtained from microalgae as an alternative to fossil fuels. Any light intensity over 600 μE resulted in an optimal lipid level by maximizing photosynthetic activity. Temperatures need to be maintained between 28 and 43 ℃ to avoid thermal stratification. Furthermore, lower pH values yield greater quantities of lipids which are used for biodiesel production. This investigation serves to highlight the drawbacks which may help determine optimal microalgae growth conditions for biodiesel production.

Introduction

The gradual changes to Earth caused by fossil fuels are leaving lasting impacts. Emissions from burning fossil fuels accounts for 65% of the excess mortality rate attributed to air pollution (Lelieveld et al., 2019). Furthermore, the world's population is set to exceed 8.5 billion people in 2030, 9.7 billion people in 2050, and 11.1 billion people in 2100 (Sadigov, 2022). This rise in population along with the rapidly growing technological world means that there will be a greater need for energy and consequently, a greater need for fuel. Hence, the value of finding alternative fuels that can substitute fossil fuels is greater than ever.

Biodiesel is an alternative fuel derived from plants or animals consisting of long-chain fatty acid esters (Schmidt, 2007). One method of obtaining biodiesel is extracting the lipids present in green microalgae. Microalgae converts solar energy into chemical energy through the process of photosynthesis, from which the necessary sugars, starch, and lipids for the formation of biodiesel are produced. These lipids are extracted and processed into biodiesel that can be used as fuel.

Of the many possible means of cultivating and harvesting microalgae, the most common are photobioreactors and open pond systems (Benedetti et al., 2018). However, the price of constructing and maintaining photobioreactors often exceeds that of open pond systems (Costa & de Morais, 2014). Hence, the latter is often the favored method. There remain certain drawbacks to using open pond systems such as the possibility of fluctuations in temperature, pH, salinity, light intensity, as well as carbon dioxide concentration (Chavan et al., 2014). These drawbacks will be further examined in order to identify as to whether biodiesel derived from microalgae cultivated in open pond systems can be efficiently produced.

Biodiesel from Algae

Of the numerous types of microalgae, Chlorophyceae, commonly known as green microalgae, is the most efficient class of microalgae to cultivate for biodiesel due to their high lipid content at roughly 50% of the dry biomass (Udayan et al., 2022). Furthermore, under the appropriate conditions, Chlorophyceae can be enhanced to provide even greater quantities of lipids (Udayan et al., 2022).

The process of extracting the lipid content of microalgal cells can be done through various means such as electroporation, solvent extraction, or supercritical fluid extraction (Ranjith Kumar et al., 2015). All of these methods extract at least 70% of the lipids present in the microalgal cells (Ranjith Kumar et al., 2015). The next step is trans-esterification, a process in which triglycerides extracted as lipids are mixed with methanol and an acid to produce glycerol and methyl esters (Wen, 2019). These methyl esters, when separated from the glycerol, make up the biodiesel.

Paddlewheel-raceway style ponds are the most commonly used design to cultivate and grow microalgae. The most cost-effective way to grow microalgae is in Paddlewheel-raceway ponds as they require less capital investment than other growth methods such as photobioreactors (Costa & de Morais, 2014). These ponds are typically structured in a rectangular loop of shallow water. A motorized paddle is then placed to circulate the water as well as distribute the necessary nutrients. The constant mixing of the medium prevents flocculation (Costa & de Morais, 2014). Flocculation is a process wherein solid particles separate within a liquid to form a sediment (Muruganandam et al., 2017). By flocculating, the microalgae would sediment and not circulate evenly throughout the medium. Furthermore, the formation of a sediment would result in certain areas of the open pond where microalgae are dense to create an imbalance in the spread of necessary resources for microalgal growth (Hannon et al., 2010). Moreover, the constant mixing also prevents thermal stratification. Thermal stratification is a phenomenon where a body of water organizes into layers determined by temperature (Jin et al., 2018). For instance, warmer water would rise up while milder and colder temperatures would be found lower in the pond. This causes an imbalance in the temperature in the open pond system. Therefore, the optimal temperature for microalgal growth would not be maintained. Through the constant mixing from the paddlewheels, any thermal stratification is disrupted and a uniform temperature level is maintained (Hannon et al., 2010). A single paddlewheel can sufficiently maintain a large microalgae culture.

Wastewater is used as a medium for microalgal growth, providing necessary nutrients inexpensively. The food and drink industry produces substantial amounts of wastewater each day. By using microalgae, the wastewater can be treated as the microalgae absorb the unwanted nutrients present in wastewater whilst eliminating the need of supplying the culture with a nutrient solution (Vu et al., 2022). The Camporosso Microalgae Farm in northern Italy is an organization that uses wastewater to cultivate microalgae (Fourneris, 2019). Here, a local dairy factory's wastewater is redirected into raceway style ponds and is treated using microalgae. Furthermore, the microalgae grown is separated from the medium to produce biodiesel and biomass (Fourneris, 2019). This specific farm sells the remaining biomass as various consumer products such as foods or as a source of electricity as it is used for combustion. Furthermore, the wastewater is purified and available for further use (Fourneris, 2019). Many microalgae farms utilize wastewater as a cost-effective and environmentally friendly solution.

Several environmental factors such as temperature, pH, light intensity, carbon dioxide levels, etc. can affect the efficiency of these ponds. Of the many factors taken into account for microalgal growth, those that are most susceptible to outside influence as well as those that could negatively impact the cultivation of microalgae in raceway-style ponds include temperature, pH levels, and light intensity at the site. At times, fluctuations in these variables can greatly impact the yield of each harvesting plant. Understanding the optimal conditions for high-lipid microalgae cultivation is imperative to developing an efficient and sustainable microalgae plant.

Volume 11 Issue 3 (2022)

Examining the Variables

Light Intensity

Light intensity fluctuations may be caused by varying weather patterns or changes to the surroundings of a microalgae farm. Due to the increased cost of developing indoor, artificially controlled and regulated light sources, most microalgae raceway-ponds are built in the open. This leads to more room for fluctuations of light intensity.

Green microalgae are light-dependent organisms. They undergo photosynthesis in order to supply their cells with the energy required to grow and function. Hence, it is important for light to be present when microalgae are cultivated. However, various types of microalgae demonstrate a varied response to light in terms of the impact it has on growth (Zhao & Huang, 2021). Therefore, it is imperative that studies be done to provide a clearer understanding of the most suitable conditions for a specific microalga to be cultivated.

When analyzing the impact of light intensity, it should be noted that many aspects of microalgal growth are impacted by the intensity of light such as biomass production and fatty acid content. For *Chlorella vulgaris*, a type of Chlorophyceae, increasing light intensity from 50 μE to 300 μE showed a trend of increasing amounts of biomass after a 10-day growth period (Chinnasamy et al., 2009). However, it appeared that the rate at which the amount of biomass increased with respect to light intensity began to decrease beyond 150 μE (Chinnasamy et al., 2009). This represents that a threshold is present in the light-absorbing pigment of microalgae.

While biomass content may be important for the manufacturing of the resources derived from the by-products of microalgae cultivation for biodiesel, being able to extract the maximum amount of fatty acids from microalgae blooms is key to obtaining the sustainable fuel source. Since the only types of lipids present in microalgae that can be used for biodiesel production are lipids, any manipulation of external variables such as light intensity can prove to be beneficial. Research has shown that Chlorophyceae are able to maximize lipid production around 600 μE (Hogan et al., 2019). This specific intensity may be beneficial to Chlorophyceae as the higher light intensity may be countering the photooxidation process, a process in which light and oxygen begin to degrade the surface of an object, through conversion of the excess products of light-dependent reactions into fatty acids (Hogan et al., 2019). Therefore, more fatty acids are accumulated within the algae.

Temperature

Temperature changes are the most common fluctuations to monitor when harvesting microalgae in an open pond system. An array of factors including the rate at which the paddlewheel mixes the pond, outdoor temperature, wind patterns, as well as cloud cover can all impact the temperature of the growth medium. Managing and monitoring temperature changes is important in maintaining an efficient microalgae farm.

Both algal viability for biodiesel production and photosynthetic activity were reduced for the *Chlorella M082* strain when outdoor temperatures were above 43 ℃ (Béchet et al., 2017). However, outdoor temperatures between 28 and 43 ℃ did not show any difference in photosynthetic activity or algal viability as the impact of temperature on fatty acid production was relatively unchanged (Béchet et al., 2017).

In most open pond systems, two types of thermometers are used to record temperature data that allows for appropriate manipulation of the system (*Pond Algae Solutions* 2020). Floating thermometers are buoyant units that provide a reading of the surface temperature of the pond while submersible thermometers show readings of temperatures deeper in the pond (*Pond Algae Solutions* 2020). Ensuring a near uniform temperature throughout the pond is integral to maintaining the optimal growth rate of the microalgae (Ras et al., 2013). While constant mixing from a paddlewheel plays a major role in this, surface temperatures often vary based on weather and cloud patterns (Eltanahy et al., 2018). Oftentimes, algal farms are developed in hotter regions due to the need for relatively higher temperatures when compared to the surrounding climate (Ras et al., 2013). Hence, overheating of the open pond system is a common challenge. In such instances, colorants may be used (*Pond Algae Solutions* 2020). Colorants are essentially dyes that can be used to transform the surface of the medium into a reflective layer which allows for cooler temperatures at the surface and throughout the medium as well (*Pond Algae Solutions* 2020).

pH Levels

The influence of pH levels on cell growth and lipid production of *Chlorella sorokiniana* was evident when tested in a laboratory (Qiu et al., 2017). The pH levels had been manipulated by increasing and decreasing CO_2 concentration in the growth medium. It has been observed that in a laboratory environment, a pH of about 7.0 is optimal for maximizing biomass yield (Qiu et al., 2017). However, to maximize biodiesel rather than biomass, a low pH value of 5.8 allowed for the highest yield of biodiesel (Qiu et al., 2017). This is not a cost-effective value of pH as a large amount of CO_2 gas is pumped into the growth medium in order to bring down the pH value to 5.8. This concentration would require 2.5 times the CO_2 pumped into the pond at a pH value of 7.0.

The main correlation observed is that pH levels slightly lower than that of freshwater, which ranges from a pH of 6 to 8, yields a greater lipid content in the algal cells while a pH slightly higher than freshwater yields a greater amount of biomass when separated from the lipid content of the cell (Suter et al., 2022). The pH values of (7 to 8.5) provided results that demonstrated a decreased rate of algal growth but an increased yield in the biomass obtained from the culture. Therefore, the higher pH values not only produced less lipids and more biomass but also took a longer growth period to bloom. Finding a compromise between the more efficient, most cost effective, and greatest lipid-producing pH values at which the microalgae can be harvested is the next phase of optimizing the potential of microalgae.

When applying these results to an outdoor open-pond setting, there are additional variables at play. Since the acidity of the medium in which the algae grows will affect the pH of the system, CO_2 levels play a large role in the manipulation of pH levels. Fluctuations in CO_2 levels in the pond would be able to shift pH values greatly. Furthermore, during night time, the lack of light-dependent reactions causes an increase in the CO_2 content of the medium and hence an increase in pH. Therefore, not only does the yield of biomass for the bloom reduce but also the potential yield of biodiesel reduces. In order to combat this, certain decarbonation methods such as aeration can be used to manage CO_2 levels in the medium (Anthony DeLoach, 2019).

Conclusion

Finding renewable sources of fuel that can be widely used is important to sustain the ever-growing human population. Microalgae provides the means of harnessing a biologically produced fuel source to substitute the unsustainable practice of burning fossil fuels. While there are certain hindrances to maximizing fuel production such that it is an economically and environmentally favorable practice, researching these factors can pave the way to more efficient practices. The variability of environmental factors such as temperature, pH, and carbon dioxide concentration are some of the largest causes of inefficient microalgae harvesting. Keeping these variables within the optimal limits for biofuel production is integral to the continued use of microalgae as a fuel source. Due to the urgent need for finding an alternative and sustainable fuel source to replace fossil fuels, there is a significant amount of research and development focused towards combating these drawbacks. The outlook for biodiesel derived from microalgae is bright due to the continued efforts of researchers in the field.

Acknowledgements

I would like to thank Alina Thokkadam, a PhD candidate at Princeton University, for her guidance throughout the exploration, research, as well as the writing process. Her support allowed for a streamlined research experience while enabling me to pursue a review of a topic that suited my interests.

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