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Exploring the potential of *Ulva lactuca* from the Gulf of Aqaba for Micro-scale Biodiesel Production: Designing a Small-scale Photo-bioreactor

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Abstract

Algae are photosynthetic organisms which can be cultivated and produced in photo-bioreactors; therefore, choosing the design parameters and conditions are critical in order to achieve high efficiency and maximum productivity. Algae are of interest to biochemical industries because of their diverse species. This project seeks to build a photo-bioreactor tank to maximize *Ulva lactuca* non-seasonal survival to produce biodiesel from its biomass. The chosen design was because of the ease of monitoring, and the tank design effectively kept *Ulva lactuca* alive and fresh. Biodiesel was accomplished using an alkaline esterification for 50 g of dry mass of *Ulva lactuca* giving a relatively limited yield of approximate 13% of *Ulva lactuca* oil (4 ml) that was converted to (0.5 ml) biodiesel, compared with other oils such as soybean (42%), corn oil (60%) and cooked corn oil (40%). The yield of *Ulva lactuca* oil was statistically significantly lower than the other three types of oil (*p-value* = 0.0032). However, this project still aids in the economics of Jordan, as *Ulva lactuca* is grown locally in the Gulf of Aqaba. *Ulva lactuca* investment is worth considering because of its natural exposure, high yield, low cost, reduced algae contamination risk, and space requirements.

Keywords: Aqaba; Biodiesel; Bioreactor; Energy Sustainability; *Ulva lactuca*.

1. Introduction

A Photo-Bioreactor (PBR) is commonly used to culture phototrophic organisms (Richmond, 2004). Photo-Bioreactors are used to cultivate microalgae because of many characteristics such as sterility, efficiency, and convenience. These characteristics vary based on the type of photo-bioreactor if it laboratory or industrial scale reactors (Spier et al., 2011). The body of a PBR is described as a closed tank, which is subjected to automatic or manually controlled conditions (Singh & Sharma, 2012). Photo-bioreactors come in different sizes and shapes and can operate in batch, fed-batch, or continuous modes to convert raw material into products. Using PBRs for the cultivation of algae means fewer contaminants entering the system because of the closed system structure. Maintaining the levels of CO_2 and H_2O during the operation with minimum losses is also easier. Controlling the system is more accurate by using the features related to the PBR such as pH, light, and temperature meters (Spier et al., 2011). High productivity is important, but low operation and maintenance costs are crucial when choosing a photo-bioreactor. Therefore, choosing the design of the PBR depends on the species, location, costs, the final product, and profit. PBRs come in numerous designs, such as a stirred tank, a bubble column, an airlift, a membrane, a tube, a flat plate, a helix, and a pyramid. These designs reach a high surface-to-volume ratio, which improves the photosynthetic efficiency (Carvalho et al., 2006). Many limitations and problems are faced when processing in a photo-bioreactor; therefore, a study in 2013 included simple solutions for commonly occurring problems, such as biofouling which can be avoided by providing perfect mixing, and ensuring a light–dark cycle, while loss of algae density is prevented when the algae is continuously filtered and harvested (Borowitzka, 2013). All parameters should be controlled to prevent complications from happening during the operation; therefore, sensors for CO_2, pH, and temperature should be installed and monitored regularly. In addition, O_2 build up can be avoided if high mass-transfer capacity is provided. This work focuses on making biofuel from a type of green seaweed called *Ulva lactuca* (Guiry & Guiry, 2013). *Ulva lactuca* reproduces asexually through accidental fragmentation, where fragments become mature clones. This type of reproduction explains the rapid growth rate of the species. *Ulva lactuca* is ideal for biodiesel because of its rapid growth and high lipid content. The attraction to biodiesel is because of the high availability of the different biomasses needed to produce it, the non-toxicity of the fuel emissions as well as the biodegradability of the fuel. It has also been reported that the consumption of biodiesel resulted in a 78% reduction of CO_2 emission compared to

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conventional diesel fuel, thus further increasing the fuel's demand (El Maghraby & Fakhry, 2015). Biodiesel is made from a mixture of different sources like vegetable oils, animal fats, and other living things such as algae and bacteria. The most commonly used oils to produce biodiesel are soybean, sunflower, palm and rapeseed. Waste vegetable oils and non-edible vegetable oils are cheaper than edible, fresh vegetable oil. This is because of the differences in their natural properties. In regards to animal fats, they are highly viscous and solid (Singh & Singh, 2010). Vegetable oils can be used as diesel fuels in traditional diesel engines, but depending on the kind and grade of oil, some drawbacks may arise. Vegetable oil in diesel engines has unique qualities in atomization and combustion compared to regular diesel. Poorly combined oil and air leads to incomplete combustion, causing smoke and lower volatility (Barnwal & Sharma, 2005). Biodiesel can be produced through the transesterification of a variety of oils. In transesterification, an ester is transformed into a different one by exchanging the alkoxy group. Transesterification is an equilibrium process; therefore, mixing the two components will cause a reaction. However, in order to accelerate the process, acid or base catalysts are usually utilized (Otera, 1993).

2. Methods

2.1. The design of bubble-tank photo-bioreactor

The *Ulva lactuca* species was kept alive in artificial seawater using a bubble-tank photo-bioreactor. The photo-bioreactor was built locally with the assistance of Ja'afar Aquatics, a company based in Amman, Jordan (Figure 1). The photo-bioreactor tank was built using Plexiglas® with specifications as shown in Table 1. The water, along with the added sea salt, was changed weekly because the bioreactor was treated as a fish aquarium which needs a water change every week. Ja'afar Aquatics experts recommended the weekly water change and adding a CO_2 pump to maintain the level of CO_2 for algae survival, which was one bubble per second. Sea salt was used to maintain alkalinity and the water's salinity. Another important parameter that was considered was the light intensity; a light-dark cycle was provided by using a light-emitting diode (LED) lamp operating at 8 hours of light and 16 hours of darkness to mimic nature.

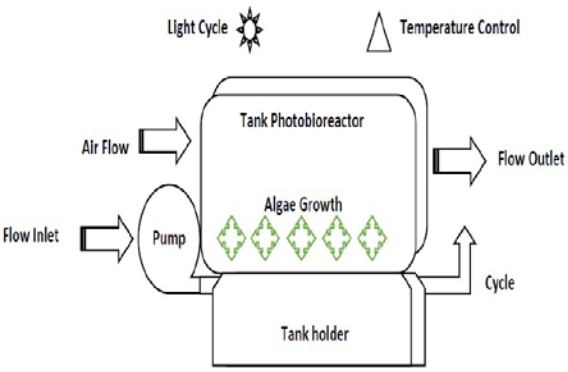

Figure 1. The designed tank photo-bioreactor.

Table 1. Dimensions of the designed photo-bioreactor tank.

Dimensions	
Length(cm)	60
Width(cm)	60
Height(cm)	55
Volume (L)	198
Water Level (cm³)	24
Water Volume(L)	86.4

2.2. Providing the algae species

Fresh and pure *Ulva lactuca* were verified and handpicked by the scientists of the Marine Science Station (MMS) from the beach near the Station in Aqaba, Jordan (GPS: 29.458344881113, 34.97665168083726) (Figure 2). The fresh algae (Figure 3) with seawater were transferred by car to Amman using water gallons and portable aeration pumps. The algae were later transferred into the photo-bioreactor tank along with the seawater (Figure 4).

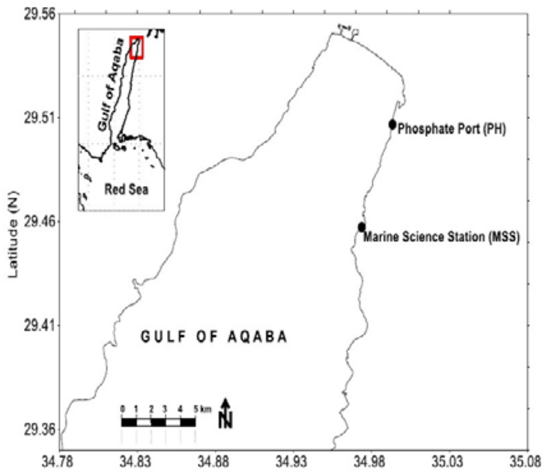

Figure 2. The map location of *Ulva lactuca* collection site on the beach of Aqaba (MSS).

Figure 3. *Ulva Lactuca* found near the beach of Aqaba.

Figure 4. The designed photo-bioreactor

2.3. Optimal conditions for Ulva lactuca

For optimal growth and survival of algae, the pH level should be between 6.5 and 7.5, and the temperature should be between 23˚C and 27˚C (Kusumaraga et al., 2021). A certain amount of CO_2 is needed for the algae to grow, and the recommended amount is between 500 and 1400 µatm (Bockmon et al., 2013). The photo-bioreactor was set to these parameters: pH 7.5, 1500 watts of light, 500 µatm of CO_2, temperature of 24.5 ˚C, and salinity around 30 ppt to get the best conditions. Algae were incubated for 7 weeks. Two sensors for CO_2 and pH were inserted in the water to make sure that the values of CO_2 and pH are properly controlled.

2.4. Oil extraction for biodiesel production

Oil extraction from the *Ulva lactuca* is necessary since the fundamental ingredient for the production of biodiesel is the oil. The oil extraction process was conducted through the use of a Soxhlet extractor (Luque de Castro & Priego-Capote, 2010). 50 g of the algae collected from the aquarium was placed into the thimble and fitted into the Soxhlet extractor. 200 ml of the solvent extractor *n*-hexane was added into the round bottom flask and placed onto the heating mantle (Suganya et al., 2013). Tubes were connected to the inlet and outlet of the condenser. The heat was adjusted so that the boiling point of the hexane is reached since leaching occurs at the boiling point of the solvent, which is 69°C. The oil could leach continuously from the algae for several days until complete extraction was achieved. Extraction was executed inside a fume hood to allow for proper ventilation and to prevent harmful fumes from escaping to the surroundings. Prior to the transesterification of the oil, oil purification was required to remove any impurities.

2.5. Purification using a rotary evaporator

The rotary evaporator, also known as the rotarvap, was used to separate the hexane from the extracted oil (Suganya & Renganathan, 2012). To separate the *n*-hexane from the extracted oil, the following steps were conducted: First, water was added to the water bath, whose temperature was set to 40°C. Then the vacuum pump was turned on with the pressure reaching around 370 mbar. Finally, the flask was lowered into the water bath with a rotation of 45 rpm. The hexane could evaporate for around 20 min to ensure full separation. The oil was collected, measured, and processed after removing it from the rotating flask. To purify the oil, a syringe filter with a 0.2 µm disc filter was used after removing the hexane solvent with rotarvap.

2.6. Transesterification of oils

Following the extraction and purification of the oil, the transesterification reaction was performed. A solution of potassium methoxide with KOH and methanol was prepared before commencing the procedure. The potassium methoxide solution was prepared through the following steps: 4 g of KOH was added to 50 ml of methanol and stirred a stir plate; 4 ml of extracted algae oil was placed into a 100 ml glass beaker and onto the hot plate, and a thermometer was used to ensure that the oil temperature remained around 55°C. 1.25 ml of the potassium methoxide solution was added to the heated algae oil. An additional three types of ready oils (soya bean oil, cooked corn oil, and corn oil) were transesterificated for comparison. The beaker covered with aluminum foil was placed onto the stir plate for 30 min. The mixture was removed from the stir plate and placed into a burette because of its very small volume and left for 24 hr to allow the layers to settle. The lower glycerol layer was slowly removed from the biodiesel top layer, and the biodiesel was collected and measured. The transesterification reaction is shown in Equation (1) (Mata et al., 2010):

$$\text{Triglycerides} + \text{Methanol} \rightarrow \text{Biodiesel} + \text{Glycerol} \qquad \textit{Equation 1}$$

3. Results

3.1. Photo-bioreactor tank

The first aim of this project was achieved, as shown in Figure 1 and Table 1, from the first stage of building a photo-bioreactor, applying some modifications on the system to meet the requirements needed, until the last step of producing biodiesel. The photo bioreactor was constructed for simulating artificial seawater that was needed for the survival of algae. Many modifications were made to the system for an appropriate algae environment. Cultivating the algae was insignificant in terms of the growth of the biomass. However, the non-seasonal survival was achieved, and oil extraction could still be performed. From 2500 g inoculum, the algae retrieved for oil extraction was around 50 g in wet mass and included sludge and excess material from the tank that was too difficult to remove from the algae to avoid further damaging the organisms.

3.2. Oil extraction for biodiesel production

As previously mentioned, the oil extraction was conducted using the Soxhlet extractor. The oil was separated from hexane using the rotarvap after extraction. Considering the discolored and somewhat stable size of the algae used, even a tiny volume of extracted oil was considered a success (Table 2) since it was predicted beforehand that the algae would yield no oil. In addition, from the difference in volume of the hexane before and after extraction, about 50 ml of hexane is lost in each extraction cycle.

Table 2. Extraction and separation results

Material	Volume
Inoculum	2500 g
Wet mass utilized for oil extraction	50 g
Extracted Algal Oil	4 ml
Recycled hexane	150 ml
Algal biodiesel yield	13%

3.3. Transesterification of oils

Table 3 shows the results of the transesterification of the different oils used. From the results, it can be deduced that the larger the volume of oil used in the transesterification reaction, the better the biodiesel yield. Larger volumes were used because they were easier to manage during the experiment and the procedure was not suitable for small-scale production. Using a general formula in the Equation 2, the % *yield* can be calculated, where *Vf* is the final biodiesel volume and *Vi* is the initial oil volume. The statistical analysis was calculated by using *t*-distribution table.

% Yield = (Vf/Vi)* 100% Equation 2

Table 3. Oil transesterification results

Oil Type	Oil Volume	Biodiesel Volume	Yield	*p-value*
Soya Bean oil	10 ml	4.2 ml	42%	
Cooked corn oil	5 ml	2 ml	40%	0.0032
Corn oil	5 ml	3 ml	60%	
Ulva lactuca algae oil	4 ml	0.5 ml	~13%*	

* *Ulva lactuca* algae oil was with the lowest yield compared with other oils.

4. Discussion

This study investigated the potential of *Ulva lactuca*, a readily available algae species in Jordan, as a sustainable feedstock for biodiesel production. We aimed to establish a non-seasonal survival system within a designed bubble-tank photo-bioreactor, while simultaneously highlighting the economic probability of biodiesel production, even with potentially low oil yields. Despite the careful design of the photo-bioreactor to mimic *Ulva lactuca's* natural habitat, successful cultivation proved challenging. This highlights the crucial role of in-depth understanding and consideration of the native environment for effective cultivation of any biomass resource. Despite the relatively low oil yield, *Ulva lactuca*-derived biodiesel could be a cost-effective fuel source compared to corn oil-based biodiesel because of that fact that corn production includes expensive activities linked with its agriculture. This paves the way for further optimization and scaling up of the process for large-scale biodiesel production in Jordan. Our findings align with prior research regarding *Ulva lactuca's* suitability for biodiesel production in Egypt (Abomohra et al., 2018). However, deeper investigation of the fatty acid methyl ester (FAME) profile and optimization of growth conditions are crucial for yield improvement. This study shows the potential of *Ulva lactuca* as a sustainable biodiesel source in Jordan. Future research should address challenges related to yield optimization, biomass availability, and energy balance, while exploring avenues for by-product valorization. These efforts will be key to maximizing the economic and environmental benefits of this renewable fuel resource. This might be the first of its kind study in Jordan; however, a larger scale study, similar to the one by (Bruhn et al., 2011) at Seden Beach (Odense Fjord) in Denmark is needed to determine the realistic economic feasibly of biodiesel production in Jordan and even in the countries that have beaches on the Red Sea such as Yemen, Egypt and Saudi Arabia. The above-mentioned study of Denmark suggested the adoption of bio-refinery concept and revealed that *Ulva lactuca* has a relatively high biomass production potential of 45 T (TS) ha^{-1} y^{-1} where (TS) is the total solid; however, the study does not address the scalability and feasibility of large-scale cultivation and harvesting of *Ulva lactuca* for bio-energy production.

5. Conclusion

This study well designed and implemented a non-seasonal bubble-tank photo-bioreactor, demonstrating its potential as a closed-system approach. This system offers the advantage of maintaining algal viability during storage, facilitating further research and potential commercialization. However, further optimization is crucial for large-scale biodiesel production. The study identified two key limitations; Low oil yield: *Ulva lactuca* exhibited a relatively low oil yield in this study. Future research should focus on optimizing cultivation parameters and exploring alternative extraction methods to enhance oil production. Small sample size: The limited sample size employed in this study restricts the generalizability of our findings. Future studies should utilize larger sample sizes to validate the observed trends and refine the economic feasibility analysis. Despite these limitations, this research provides a valuable foundation for future studies investigating *Ulva lactuca* as a sustainable biodiesel feedstock in Jordan. Further research addressing yield optimization, biomass scalability, and economic viability assessment will be crucial for maximizing the potential of this renewable fuel source.

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