Biodiesel production and Environmental CO₂ cleanup using Oleaginous Microorganisms from Al-Hassa area in Saudi Arabia

Research Article

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Abstract: Biodiesel production is rapidly moving towards the mainstream as an alternative source of energy. Algae oil is one of the viable feed stocks among others to produce Biodiesel. However the difficulties in efficient biodiesel production from algae lie not in the extraction of the oil, but in finding an algal strain with a high lipid content and fast growth rate. This paper presents an experimental work performed to study the production of biodiesel from local algae strains in Al-Hassa territory of the eastern province in Saudi Arabia which was found to contain high lipid contents and show rapid growth. The collected results predict that those types of desert algae are promising and are considered to be a potential feedstock for biofuels.

Keywords: Algae • biodiesel • esterification • Carbon dioxide • Global warming renewable energy

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1. Introduction

With the increased atmospheric carbon dioxide (CO₂) levels, global warming has become a major focus in the environmental agenda. The world's CO₂ production from fossil fuel burning is on a rise and exceeded thirty three billion metric tons of CO₂ from top emitting countries during the year 2011 [1, 2]. Nowadays CO₂ capture and storage technology are being heavily used in order to mitigate the climate change and reduce CO₂ in the atmosphere, however this technology is expensive and

costly [3]. It occurs in a four-step process. First, CO₂ is separated from other flue gases of power generation plants and concentrated into a nearly pure form. It is then liquefied by compression to roughly 100 bars. Next, it is pumped into a pipeline and transported to the storage location. The last step is a geological sequestration step where CO₂ is injected into a deep geological formation for long term or permanent storage [3]. However, the process modeling and feasibility assessment of this technology seems to be very complicated due to the unclear and complex transport phenomena process appearing in the disposal procedure [4, 5]. Moreover, the safety concerns of CO₂ leakage present challenges to researchers and necessitate more technical attention to deal with this

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uncertain area by nature [6].

Among many attempts to reduce the quantity of CO₂ in the atmosphere, Algae cultivation is found to be one of the cleanest, safest and cost effective processes. Algae use CO₂ as a carbon source to grow. Hence 1.8 tons of CO₂ are needed to grow 1 ton of biomass. An insufficient supply of CO₂ is often a limiting factor in algae productivity [7]. Thus using a waste source of CO₂ like flue gas from power plants and boilers can contribute to resolve part of the major problem of air pollution resulting from CO₂ evolution [8, 9] and increase productivity of Algae cultivation process. Flue gas typically contains 4 to 15 percent CO₂ and it is free, where the only cost is the supply from the source to the cultivation system [7].

With the biological approach, CO₂ is converted into algal biomass and then into value-added products such as proteins, vitamins, food, and feeds [3, 8]. It can be inferred that algae grown in CO₂ enriched air can mitigate the CO₂ level by consuming and converting it to oily substances. Such an approach may resolve part of the future crisis of diminishing energy sources [8, 9].

Algae has emerged as one of the most promising source of biodiesel product through the development of technologies that efficiently produce biomass, and convert it to more convenient forms of energy [9, 10]. Consequently; research and development of algal biofuels are currently receiving much interest and funding, but they are still not commercially and economically viable at today's fossil fuel prices range. However, a niche opportunity may exist where algae is grown as a by-product from high rate algal ponds (HRAPs) operated for wastewater treatment.

Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass biodiesel derived from micro algal oil and photobiologically produced biohydrogen. The use of algae as energy crops has potential, due to their adequate adaptability to different conditions, the possibility of growing either in fresh- or saline waters and due to unnecessary arable land [10–12]. Furthermore, as two thirds of earth's surface is covered with water, algae would truly be a proper option to meet the global energy needs [12, 13].

Algae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure [11, 14, 15]. More attention has been drawn to various oleaginous zygomycetes species, such as *Mortierella isabelina* and *Cunninghamella echinulata*, which may accumulate up to 86% and 57% lipids in dry biomass basis [17]. *Oleaginous Algae* are economical choice for biodiesel production, because of their wide availability and low cost.

Biodiesel is made entirely from vegetable oil or animal fats and is renewable and biodegradable. It is produced by transesterification of oils with short-chain alcohols or by the esterification of fatty acids [16, 17]. Triglycerides can then be transformed into fatty acid alkyl esters, in the presence of an alcohol, such as methanol or ethanol, and a catalyst, such as an alkali or acid, with glycerol as a byproduct. However, the lack of oil feedstock limits the large-scale development of biodiesel to some extent.

High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters was studied by Han *et al.* [16]. The aim of their study was to obtain high quality biodiesel production from a microalga *Chlorella protothecoides* through the technology of transesterification. The technique of metabolic controlling through heterotrophic growth of *C. protothecoides* was applied, and the heterotrophic *C. protothecoides* contained the crude lipid content of 55.2% [18]. Large amount of microalgal oil was efficiently extracted from the heterotrophic cells by using n-hexane, and then transmuted into biodiesel by acidic transesterification. The biodiesel was characterized by a high heating value of 41 MJ/kg, a density of 0.864 kg/L, and a viscosity of 5.2×10^{-4} Pa s (at 40°C) [18].

The current study aimed to:

1. Explore and compare the yield of biodiesel product through the esterification process of the most common species of Algae components such as *Oedogonium* and *Spirogyra* in Al-Hassa territory in Saudi Arabia.
2. Use Dimethyl ether as an extraction solvent and determine the residual amount of sediments available in the extracted Bio oil.

2. Experimental procedure and materials

2.1. Sampling

The water samples were collected from different aquatic habitats in the Al-Hassa area (such as, Al-Asfar Lake, Al-Akdoud Spring). Surface water samples were collected in 2 liters' containers which were taken directly to the laboratory for testing.

2.2. Isolation and cultivation

Oedogonium species were isolated from the drainage canal in Al-Hassa area in Saudi Arabia *Spirogyra* species were also isolated from Al-Asfar Lake, and from the Al-

Figure 1. Photo of the cultivation system of isolated algal species.

Hassa area as well. Isolation and purification were made by dilution and plating technique. The isolated algae was placed in an incubator at $27 \pm 1.0^\circ\text{C}$ and exposed to a 16 hour light cycle and 8 hour dark cycle with light intensity $70 \text{ mol m}^{-2} \text{ s}^{-1}$. Seven days cultures (optimal growth period) were diluted under aseptic conditions to provide stock cultures. All solutions were prepared using Millipore membrane filter. All glass wares were soaked for 24 hours in 10–15% nitric acid, rinsed in distilled water, and air-dried before use.

2.3. Cultivation Medium Acidity

10% by weight Monosodium glutamate wastewater solution was used to grow slant seeds of *Oedogonium* & *Spirogyra*. Sample growth was tested at different solution's acidity within a pH range of (2.0) to (8.0) and the culturing temperature of the samples was kept at 30°C . The results of the biomass and lipid contents of both types of Algae species (i.e. *Oedogonium* & *Spirogyra*) were found to be changing with the cultivation medium acidity as shown in Figure 2 and Figure 3. It was found that the cell growth ratio was less at lower pH values (2.0); however, the productivity was relatively stable between pH (4.0) and (7.0). And the two target parameters, biomass (dry cell weight/culture broth volume) and lipid content for both species peaked at pH (5.7). As a result pH (5.7) was chosen as the optimal value.

2.4. Oil extraction

Algae were ground with motor and pestle to certain extent then dried for 20 min at 85°C in an incubator to remove water. Dimethyl ether (DME) was mixed with the dried ground algae and then the contents were refluxed for 4

Figure 2. % of Total Lipid content of *Spirogyra* and *Oedogonium* Algae samples grown in the monosodium glutamate wastewater solution at different pH values.

Figure 3. Biomass content (g/L) of *Spirogyra* and *Oedogonium* Algae samples grown in the monosodium glutamate wastewater solution at different pH values.

hours to extract oil. After the extraction, the contents were cooled and filtered to separate the oil from biomass then the residue was washed with 15 ml of DME twice to extract the residual oil from biomass. The extracts were collected in a separating funnel and washed with 50 ml of 1% aqueous sodium chloride solution twice. The extracted oil was evaporated in a vacuum medium to release DME. The collected amount of algal oil was taken to determine the oil content in biomass.

2.5. Biodiesel production through transesterification

A catalyst mixture consists of 0.25 g NaOH and 24 ml of methanol is mixed with the extracted algal oil in a conical flask and shaken for 3 hours by electric shaker at 300 rpm

Figure 4. Trans esterification reaction to produce Biodiesel.

then kept for 16 hours to be settled and separated to two layers one of them biodiesel product and the other is impure coproduct such as glycerol see Figure 4.

2.6. Separation of biodiesel

The biodiesel product was separated from sediments by using a flask separator. Sediments quantity, which consisted of (glycerin, pigments, etc.), was measured and the extracted biodiesel was washed using 5% distilled water until it became clean. It was then dried with a dryer under a running fan for 12 hours. The final quantity of the produced Biodiesel was measured by a measuring cylinder. The pH was found and stored for further analysis.

3. Results and discussion

Different solvents could be used to extract oil from Algae; yet in this study the extraction with Dimethyl ether is the preferred solvent to be used in this process. Dimethyl ether is found to be an effective solvent in extracting and producing high conversion of oil and it can be easily evaporated with minimum energy.

Figure 5 shows the fresh weight and dry weight of tested algae in milligrams per 100 ml sample. The data predicts that the percent of wet and dry weights of algae (before oil extraction) were higher in *Spirogyra* than in *Oedogonium sp.*

In Table 1 the chemical compositions of the tested algae species of the samples collected directly from the source at pH (7.0) show that the total protein, carbohydrates and total lipids of *Spirogyra* are higher than *Oedogonium sp.*, However the total cellulose content of both species are relatively close to each other and no nucleic acid was detected in either species.

Figure 4 shows the biodiesel content of the tested algae species which indicates that the percent of extracted oil from *Spirogyra* algae was higher than that of

Figure 5. The wet weight and dry weight of *Spirogyra* and *Oedogonium sp.*

Table 1. The chemical composition of tested algae expressed on a dry weight (%).

Components	<i>Spirogyra</i>	<i>Oedogonium</i>
Protein	30	12
Carbohydrates	52	43
Lipids	18	16.6
Cellulose	32	30
Nucleic Acid	-	-

Oedogonium sp. The remaining biomass quantity of both species (after oil extraction) were relatively comparable to each other which is truly consistent with the composition of both species shown in Table 1 hence the cellulose amount of both species are therefore comparable as well. On the other hand, sediments such as glycerin, pigments and other impurities were lower in *Oedogonium sp.* than that in *Spirogyra* (see Figure 5).

Figure 6. The Biodiesel production (% of dry weight) in *Spirogyra* and *Oedogonium sp.*

Figure 7. Sediments after algal biodiesel extraction from *Oedogonium sp.* and *Spirogyra*.

4. Conclusion

Continuous use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. As a result algae is expected to be an economical choice for biodiesel production and green algae species appear to be a good source of renewable biodiesel capable of meeting the global demand for transport fuels. Our results indicate that biodiesel can be produced from both species (i.e. *Oedogonium sp.* and *Spirogyra*) in Al-Hassa.

Algal oil and biodiesel (ester) production was higher in *Spirogyra* than *Oedogonium sp.* However, the remaining biomass and sediments such as glycerin, water and pigments (after oil extraction) were higher in *Spirogyra* than *Oedogonium sp.* There was no difference of pH between *Spirogyra* and *Oedogonium sp.* Using Dimethyl ether as an extraction solvent was effective hence the determined residual amount of sediments available in the extracted Bio oil was very low in both species and no solvent traces remained in the extract.

It is clear that Al-Hassa territory is a promising area for Algae cultivation, mainly the *Spirogyra* and *Oedogonium* type of Algae species as it is an Oasis with plentiful water resources and suitable weather.

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