Extraction of oil from algae for biodiesel production, from Quetta, Pakistan

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Abstract: The world is facing the energy crises and environmental issues in this century due to increased industrialization and overuse of natural resources for energy such as fossil fuels. The burning of fossil fuels generates greenhouse gases which aggravate the global warming. Researchers across the world are focusing on renewable, less CO₂ and NOx emissions fuels. These fuels are important for sustainability and green economy. Biodiesel production from Algae is emerged as the promising alternative fuel, technically and environmentally acceptable and easily available. In this study, different parameters of the oil extraction process from algae biomass were studied. Samples of algal species Spirogyra were collected from Chashma Achuzai, Quetta, Pakistan and employed as a feedstock for biodiesel production. Oil from macroalgae Spirogyra was extracted using n-Hexane as a solvent. Effects of n-Hexane to oil ratio, size of algal biomass and contact time on the percentage yield of extracted oil was studied and analyzed. It was concluded that maximum amount of oil was extracted from Spirogyra by using a greater ratio of solvent to algal biomass, maximum contact time, smaller algal biomass size.

Keywords: Spirogyra, solvent extraction, biodiesel, macroalgae, Quetta

1. Introduction
Presently, global warming effect, depletion in fossil fuel reserves, and higher petroleum prices are the main issues driving worldwide interest on the development of alternative renewable, biodegradable and sustainable biofuels [1]. Biofuels produced from algae considered to be a potential candidate to replace conventional fossil fuels [2]. Furthermore, it is the fastest developing alternative to petrodiesel fuel in many developed and developing countries worldwide [3]. Besides, its decreases particulate emissions, unburned hydrocarbons, and sulfur dioxide generated through its combustion process [4]. The use of Macroalgae for biodiesel production is the most promising alternative to fossil fuels depletion and pollution caused by these fuels. The certain species of algae contain more than 50% of oil content, which can be extracted and refined into transportation fuel easily using currently available technologies [5]. Microalgae have some benefits than other feedstocks for biodiesel as they have fast growth rate, permit the use of non-arable land, can be grown in wastewater, do not replace the edible traditional crops, they can be harvested daily, and it is not a seasonal production. Its waste can be used as a feed for animals or other purposes [6].

Algae can be used to generate energy in several ways. One of the most efficient ways is to produce biodiesel from algal oil by transesterification. One of the main obstacles to fully taking advantage of lipid-producing microalgae is the ability to successfully and efficiently extract oil from the biomass cells. A number of methods are used for extracting the algal oil i.e., mechanical extraction, chemical extraction [7]. Algal oil can be efficiently extracted using chemicals by solvent
extraction method [8]. Solvent Extraction technique is favored than mechanical pressing method since it recovers all the oil leaving just 0.5–0.7% leftover oil in the algae biomass [9]. Benzene and di-ethyl ether can also use but the popular chemical for solvent extraction is hexane, which is less expensive and hazardous than another solvent [6]. Spirogyra is the green algae, available abundantly in springs, ponds and brackish water. It has a structure like a cellular hair and includes unbranched cylindrical fibers, which is about 1/10 mm in diameter and few centimeters long. Spirogyra produces lipids, carbohydrate, and proteins that can be utilized for the production of biodiesel, or bioethanol [10].

2. Materials and methods
The purpose of the study is to chemically extract oil from Macroalgae species collected from an open pond at Chashma Achozai site in Quetta, using n-Hexane as a solvent. Effects of n-Hexane to oil ratio, mesh size of algal biomass and contact time on the percentage yield of oil extracted were studied. The chemicals utilized, and the analysis procedure of study are discussed in this section.

2.1 Chemicals
The chemicals used n-Hexane, Methanol and NaOH were of analytical grade and purchased from Merck. The chemicals were used without any further purification. The complete experiment was carried out in the laboratory of Biotechnology, Department of Biotechnology, Balochistan University of Information Technology Engineering and Management Science Quetta, Pakistan.

2.2 Algae sample collection
The algae samples were collected from Chashma Achuzai Quetta, Pakistan. The samples collected from an open pond, illustrating in figure 1.

![Figure 1. Collection of algae from the open pond](image1)

![Figure 2. Algae identification and drying associated with the algal biomass.](image2)
2.3 Algae Identification and Preparation for Oil extraction

Collected Algae sample was examined under Eclipse E200 Compound Microscope. The photograph in figure 2 was taken by enlarging it 40 times and 100 times. The samples were spread under the sun in the roof of the hostel for 2 days (48 hours) to evaporate the amount of water. The dried samples were ground with the help of pestle and grinder and the fine powder was passed through different micron sieves, to get different mesh size algal biomass, figure 2. The ground algae were dried for 30 min at 80°C in an incubator for releasing leftover water. Then the algae powder was stored in different jars for extraction experiment in a sealed container.

2.4 Oil extraction from Algae

The algae samples collected was dried (100%) and powdered. Hexane was mixed with the dried ground algae to extract oil in separating funnel of 250 ml. Then the mixture was kept for 24 h for settling and for separation of the two layers in the funnel. The organic phase containing the algae oil was emptied in the pre-weighted 50 ml beaker. The Algal oil was separated from Algae biomass by filtration and weighted it by using electronic weight balance. The extracted oil was evaporated in a water bath to release hexane. All extraction was performed in triplicates for the different parameters solvent extraction process. The oil yield (wt. %) was then calculated by utilizing the equation-1 [4, 8].

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\text{Extracted oil efficiency (wt. %) } = \frac{\text{Mass of oil extracted (grams)}}{\text{The total mass of dried algae}} \times 100
\]  

(1)

![Figure 3. Solvent extraction in laboratory](image)

3. Results and Discussion

Various Parameters of oil extraction from open pond algae by using solvent extraction method were studied. The study focused on to find out which parameter causes the increase in oil yield and improved the extracted efficiency.
3.1 Varying n-Hexane to Algae ratio
Experiments have been performed using the constant mass of dried Algae (Spirogyra) and by changing the volume of solvent. Derived results are then tabled in table 1. As it can be seen that increasing the solvent amount also improved the extracted oil efficiency.

| Sample | Algal Biomass (g) | n-Hexane (ml) | Algae to n-Hexane ratio | Oil Extracted (g) | Extracted Efficiency % |
|--------|------------------|----------------|-------------------------|------------------|------------------------|
| 01     | 30               | 30             | 1                       | 0.79             | 2.63                   |
| 02     | 30               | 40             | 1.33                    | 0.92             | 3.07                   |
| 03     | 30               | 50             | 1.66                    | 1.57             | 5.23                   |

Experiments have been performed using the constant volume of solvent and by changing the mass of dried Algae. Derived results are then tabulated in Table 2. It was observed that the percent yield of oil increased as the solvent to algae ratio increased. However, from the tabulated data, it is noted that extraction efficiency is good when the algae biomass to n-Hexane ratio is kept to 1:2. A very slight Increase is observed when the ratio is kept to 1:3 than the efficiency of 1:2 in extraction. It is concluded that the best optimum value for the biomass to n-Hexane ratio is 1:2.

| Sample | Algal Biomass (g) | n-Hexane (ml) | Algae to solvent ratio | Extracted Oil (g) | Extracted Efficiency % |
|--------|------------------|----------------|------------------------|------------------|------------------------|
| 01     | 20               | 30             | 1.5                    | 0.7              | 4.1                    |
| 02     | 15               | 30             | 2                      | 0.8              | 5.33                   |
| 03     | 10               | 30             | 3                      | 0.82             | 7                      |

The effect of solvent to algae ratio on percent yield of extracted oil is shown in figure-4. It was observed that the percent yield of oil increased as the n-Hexane to algae ratio increased. The higher yield at a solvent to algae ratio is attributed to the excess solvent available to extract oil from the algal biomass, so it is concluded greater will be the ratio between solvent to Algae biomass, greater will be the extraction efficiency [10, 11]. Increasing the algae to solvent ratio from 1:1 to 1:3 the extracted oil yield is 2.5 times more than the equal ratio.
3.2 Varying the algal biomass size

Experiments have been performed using different Algal biomass size by keeping the constant mass of Algal biomass and volume of solvent. The algae to solvent ratio were kept constant to 1: 1.66. Derived results are then tabulated in table 3.

| Sample | Algal Biomass (g) | Mesh number | Algae biomass size (mm) | n-Hexane (ml) | Extracted Oil (g) | Extracted Oil Efficiency % |
|--------|------------------|-------------|-------------------------|--------------|------------------|--------------------------|
| 01     | 0.841            | 30          | 20                      | 50           | 1.55             | 5.16                     |
| 02     | 0.595            | 30          | 30                      | 50           | 2.02             | 6.73                     |
| 03     | 0.297            | 30          | 50                      | 50           | 2.3              | 7.66                     |

It was observed that (Figure-5) when the size of biomass decreased to 50 mesh from 20 mesh the oil extracted efficiency increased from 5.16% to 7.66%. This can be defensible by the improved contact area between the algae biomass and solvent. The smaller sized particles have maximum interaction with solvent as compared to large particles of Algal biomass and thus increased the yield [10, 12].
3.3 Varying contact time between n-Hexane and algae biomass

Experiments have been performed at different contact time while the other parameters i.e. solvent to algae ratio and size of algae remains constant. Derived results are then tabulated in Table 4.

| Sample | Algal Biomass (g) | n-Hexane (ml) | Times (Hours) | Extracted Oil (g) | Extracted Efficiency % |
|--------|-------------------|---------------|---------------|-------------------|------------------------|
| 01     | 30                | 50            | 10            | 1.02              | 3.4                    |
| 02     | 30                | 50            | 15            | 1.04              | 3.46                   |
| 03     | 30                | 50            | 25            | 1.57              | 5.23                   |

The contact time was varied from 10 to 25 hours. It was observed (Figure-6) that extracted oil efficiency increases as the contact time increases. Maximum yield at maximum contact time can due to enhanced interaction between the solvent and algal biomass, which lead to homogenous mixing and increased in solubility of oil by solvent [10, 13]. Hence oil is extracted from all portions of the algae species by increasing contact time.

![Figure 6. Effect of contact time on extracted efficiency](image)

4. Conclusion

In this study, oil was extracted from Algae (Spirogyra) by using solvent extraction process. Also studied different parameters of the solvent extraction process. It was noted that the maximum amount of oil was extracted from algal biomass by using 3:1 ratio of n-Hexane to algal biomass, maximum contact time, smaller algal biomass size. Increasing the algae to solvent ratio from 1:1 to 1:3, the extracted oil yield is 2.5 times more than the equal ratio.

Biodiesel produced from Algae biomass can be considered an alternative choice as of easy availability and eco-friendly nature. A significant amount of biodiesel can be produced from macroalgae (Spirogyra). Spirogyra can be successfully used as feedstock for the producing biodiesel. The extracted oil can be converted to biodiesel by the trans-esterification reaction. Further study related to trans-esterification reaction and biofuel characterization will be needed.
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