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Extraction and Characterization of Oil from Algae

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Abstract: ***The extraction of oil from wet algae culture, samples of algal species Chlamydomonas algae were collected from waste water at the management science walk path, University of Abuja Permanent site. Oil from algae was extracted using n-Hexane as a solvent. In the Experiment there were three processes involved, the isolation and identification of the algae, the extraction process and the separation process. The algae samples were collected and Oil from the algae was extracted using the soxhlet apparatus by repeated washing with hexane. Results show that at increasing contact time the extracted oil efficiency increases. The physiochemical properties of the oil were compared with the physiochemical properties of the extracted oil. In conclusion, from the results of the composition obtained, the oil was found to have high values of saponification, which makes it good for the production of soap and creams.***

Keywords: Algae, waste water, oil, physiochemical properties, n-Hexane, isolation and identification

1. Introduction

Microalgae also known as microphytes commonly found in freshwater and marine systems, they exist individually or in chain groups both in water columns and sediments. Microphytes specifically adapt to environments preeminent by viscous forces such as thick, viscid, sewage waste water. They are consequential for life on earth, capable of achieving photosynthesis in which approximately half of the atmospheric oxygen is produced (Thurman, 1997). Algae are primary producers that are predominantly found in aquatic environments. They have been found to be beneficial to human beings due to their application in drug development and environmental remediation. Using wastewater as a medium for microalgae culturing provides an economical medium and leads to efficient removal of nutrients and metal from wastewater (Madhurya, 2018).

Although Corolus Linnaeus (1754) included Algae along with Lichens in his 25th class Cryptogamia, he did not elaborate further on the classification of Algae.

Vaucher (1803) was perhaps the first to propose a system of classification of Algae and he recognized three groups, Conferves, Ulves and Tremelles. While Link (1820) classified Algae on the basis of the colour of the pigment and structure, Harvey (1836) proposed a system of classification on the basis of the habitat and the pigment. J. G. Agardh (1849 -1898) divided Algae into six orders: Diatomaceous, Nostochineae, Confervoideae, Ulvaceae, Floriadeae and Fucoideae. Around 1880, Algae along with Fungi were grouped under Thallophyta, a division created by Eichler (1836). Encouraged by this, Engler and Prantle (1912) proposed a revised scheme of classification of algae and included fungi in algae as they were of opinion that fungi have been derived from algae.

The presence of a variety of chemical constituents, such as saponins, phenols, glycosides, flavonoids and alkaloids. Their antioxidant activities were studied, Phytochemical screening showed the presence of active molecules having antioxidant potential (Kannan, 2014).

Algae fuel, algal biofuel, or algal oil is an alternative to liquid fossil fuels that uses algae as its source of energy-rich oils. Also, algae fuels are an alternative to commonly known biofuel sources, such as corn and sugarcane. When made from seaweed (macro algae) it can be known as seaweed fuel or seaweed oil. In a 2007 report, a formula was derived estimating the cost of algal oil in order for it to be a viable substitute to petroleum diesel:

$$C_{(\text{algal oil})} = 25.9 \times 10^{-3}\, C_{(\text{petroleum})} \qquad (1)$$

where: $C_{(\text{algal oil})}$ is the price of microalgae oil in dollars per gallon and $C_{(\text{petroleum})}$ is the price of crude oil in dollars per barrel.

This equation assumes that algal oil has roughly 80% of the caloric energy value of crude petroleum (Organization of the Petroleum Exporting Countries, basket prices, 2013).

Based on the cost and extraction capability, hexane has been found to be the most efficient solvent in lipid extraction (Elsayed, 2014). In one case about 80 % of the total lipids were extracted by the two-step method, using methanol to extract the lipid, followed by hexane to purify them (Elsayed, 2014). This method has several drawbacks including the large volumes of solvent that are required and the fact that most organic solvents are toxic and highly flammable.

Thermochemical liquefaction is a method used to convert the wet algal biomass into liquid fuel by heating the biomass at high temperatures (200 to 500 ◦C) and pressures (greater than 20 bar) in the presence of a catalyst to yield bio-oil.

The general extraction techniques of algae are Mechanical extraction/ cell disruption methods and Solvent extraction coupled with mechanical cell disruption methods. Other novel methods are Supercritical CO_2 extraction and direct conversion of algal biomass to biodiesel (Harvind, 2012).

Solvent extraction is a common practice used to extract oils from the algal biomass and other biomasses. The solvent should be selected based on efficiency, selectivity towards

the different classes of lipids and ability of solvent to prevent any possible degradation of lipids. In order to achieve maximum extraction, the linkages between the lipids and other organelles of the algae cells which are connected with van der Waals interactions, hydrogen bonding and covalent bonding should be broken (Harvind, 2012). The most common solvents used for extraction are n-hexane, chloroform, petroleum ether, methanol, ethanol, isopropanol, dichloromethane and mixture of any of these solvents depending upon method and desired class selection of lipids. The conventional solvent extraction methods are Bligh and dyer, folch, soxhlet extraction. The steps involved in the solvent extraction at micro level were explained by Halim et al. When the algal cells interacted with the organic solvents, these solvents penetrate through the cell wall and interact with the selective class of lipids depending upon its dielectric constant to form a solvent lipid complex. This complex diffuses in to the bulk solvent due to the concentration gradient continues until this process reaches equilibrium (Harvind, 2012).

Raw materials used

Chlamydomonas, microalgae were obtained from University of Abuja Permanent site, waste water at the management science walk path. The table below shows the manufacturer, the purity as well as the source of the materials used to carry out the investigation.

Table 1: List of Chemicals and Materials

S/ No	Materials	% Purity	Manufacturer	Source
1	*Chlamydomonas*, Microalgae	93	Photosynthesis. Naturally occur from water, sunlight, nutrients and carbon dioxide,	Vet. Medicine walk path, Market and Management science walk path
2	Distilled water	100	-	AUST Lab
3	NaCL	100	Acros Organics	Mbakwe Chemicals
4	Glacial acetic acid	100	-	Mbakwe Chemicals
5	Chloroform	100	Acros Organics	Mbakwe Chemicals
6	Ethanol	100	-	AUST Lab
7	Sulphuric Acid	100	-	Mbakwe Chemicals
8	Hexane	100	Acros Organics	Mbakwe Chemicals

Instruments and Equipments

Below are the list of instruments and equipment that was used in carrying out the experiment

Table 2: List of Instruments and Apparatus

S/No	Equipments/ Apparatus	Model/ Year	Manufacturer	Availability/ Source
1	Soxhlet Extraction apparatus	B34/35Q, 1999	Quick Fit, England	AUST Lab
2	Digital weighing Balance	AR2130	Citizen (Mp 360)	AUST Lab
3	Rotary Viscometer	NDJ-5S	-	AUST Lab
4	Measuring Cylinder	3D Model	Pyrex, England	AUST Lab
5	Pipette	E1020, 1999	Pyrex, England	AUST Lab
6	Beakers	3D Model	Pyrex, England	AUST Lab
7	Filter paper	1001110	Whatman	AUST Lab
8	Thiele Tube	PAC	Seta	AUST Lab
9	Compound Microscope	E200	HHT, America	AUST Lab

2. Methodology

Isolation and Identification of Algae

Water samples for algae isolation were collected aseptically from pond of waste water at the management science walk path. The chemicals used n-Hexane, Methanol and NaOH were of analytical grade and purchased from Mbakwe Chemicals at Gwagwalada. The chemicals were used without any further purification. The complete experiment was carried out in the African University of Science and Technology (AUST). Collected Algae sample was examined under Eclipse E200 Compound Microscope. 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 to fine powder. 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.

Oil Extraction from Algae

The algae samples collected were dried (100%) and powdered. Oil from the algae was extracted using the soxhlet apparatus by repeated washing with hexane (boiling point of 50 to 70^{o}C). After 5 hours, the soxhlet extraction flask containing oil and the solvent mixture were removed from the soxhlet apparatus and the oil and hexane were separated using a rotary evaporator.

The remaining solvent traces were evaporated in a water bath to release hexane. The oil obtained was stored in a closed bottle and kept for further analysis. All extraction was performed in triplicates for the different parameters of solvent extraction process. The oil yield (wt. %) was then calculated by utilizing the equation.

$$\text{Extracted Oil Efficiency (wt.\%)} = \frac{\text{Mass of Oil Extracted (grams)} \times 100}{\text{The Total Mass of Dried Algae}} \quad (2)$$

Physiochemical Properties Determination

Determination of Acid Value

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty

acids present in one gram of fat. It is a relative measure of rancidity as free acids are normally formed during decomposition of oil glycerides. The value is also expressed as percent of free fatty acids calculated as oleic acid. The acid value is determined by directly titrating the oil in an alcoholic medium against standard potassium hydroxide/ sodium hydroxide solution (Ejim, 2013).

Procedure

The oil sample (1.0g) was weighed and dissolved with 50ml of ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated to pink end point (which persisted for 15 minutes) with 0.1N potassium hydroxide solution (KOH). Acid value was calculated as in equation below (Ejim, 2013).

$$\text{Acid value} = \frac{56.1\ V \times C}{m} \quad (3)$$

where;
56.1 = the equivalent weight of KOH
V = Volume in mol of standard volumetric potassium hydroxide of sodium hydroxide used.
C = Exact concentration in potassium hydroxide solution used (0.1N); and
m = the mass in gram of the test portion (1g)
Also, the pH value was 6.8 and was determined using a pH meter.

Determination of Saponification Value

The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil/fat (Ejim, 2013). The oil sample saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid (Ejim, 2013).

Procedure

Saponification value was determined according to titrimetric method discussed by Pearson (1981). Two grams of oil sample were weighed into a conical flask and 25ml of ethanoic potassium hydroxide was added. The solution was refluxed for 2 hours with time to time shaking. 1ml phenolphthalein was added and titrated with 0.5N hydrochloric acid (HCL). The same process was conducted for blank determination. The value was calculated (Ejim, 2013) as;

$$\text{Saponification Value} = \frac{(V_0 - V_1) \times C \times 56.1}{m} \quad (4)$$

Where;
56.1 = equivalent weight of potassium hydroxide
V_0 = Volume in mol of standard hydrochloric acid used for the blank test
V_1 = Volume in mol of standard hydrochloric acid solution for the sample
C = the exact concentration of the standard hydrochloric acid (0.5N) solution; and
m = mass in gram of the oil/ fat taken from the test portion (2g)

Determination of peroxide value

This is an indication of the extent of oxidation suffered by oil

Procedure

Peroxide value was evaluated according to AOCS, 2003. Five grams of oil sample were weighed into a conical flask and 30ml of solvent mixture of glacial acetic acid – chloroform in the ratio of 3:2 was added to the oil sample. $^1/_2$ ml saturated potassium iodide (KI) solution and allowed to stand for 1 minute thereafter, 30ml of distilled water were added and titrated with 0.01N sodium thiosulfate solution using starch indicator until the yellow colour was discharged. A blank test was prepared alongside the oil sample. Peroxide value was calculated as;

$$\text{Peroxide value} = \frac{10 \times (V_1 - V_2)}{m} \quad (5)$$

Where;
V_1 = Volume of Na_2SO_3 for determination of test sample in ml
V_2 = Volume of Na_2SO_3 for determination of blank solution in ml; and
m = mass of test portion in gram (5.0g)

Determination of Viscosity Value

In determining the viscosity of the sample oil, a rotary viscometer is used. In this test, the oil is placed in a glass tube, housed in an insulated block at a fixed temperature.

Procedure

A metal spindle is then rotated in the oil at a fixed rpm, and the torque required to rotate the spindle is measured. Based on the internal resistance to rotation provided by the shear stress of the oil, the oils absolute viscosity is reported in centipoise (cP), equivalent to mPa.s in SI units.

Determination of Color

By observation the color of the obtained oil was determined; it was seen to be greenish yellow in color.

Determination of Boiling point

In determining the boiling point of the obtained oil, a distillation method called Thiele tube method was used. The boiling point is the temperature when the oil just begins to enter the capillary tube.

Procedure

A small tube was half-full with sample and a capillary tube was inserted closed end up. The tube was attached to a thermometer with a small rubber band, the sample was inserted into a Thiele tube, so that the sample is near the middle of the oil. The arm of the Thiele tube was heated with a burner, gently and continuously until a vigorous stream of bubbles emerges from the capillary tube, such that individual drops can be barely distinguished. The temperature was recorded and heat removed to allow oil to cool (Ejim, 2013).

3. Results

Effect of Contact time to the Extracted Oil Efficiency

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. The contact time was varied from 3 to 6 hours. It was observed that extracted oil efficiency increases as the contact time increases.

Table 3: Oil extracted at different contact time.

Sample	Algal Biomass (g)	n-Hexane (ml)	Times (Hours)	Extracted Oil (g)	Extracted Efficiency %
1	30	50	3	1.02	3.4
2	30	50	5	1.24	4.13
3	30	50	6	1.57	5.23

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 [Ejim Ikechukwu, 2013]. Hence oil is extracted from all portions of the algae species by increasing contact time.

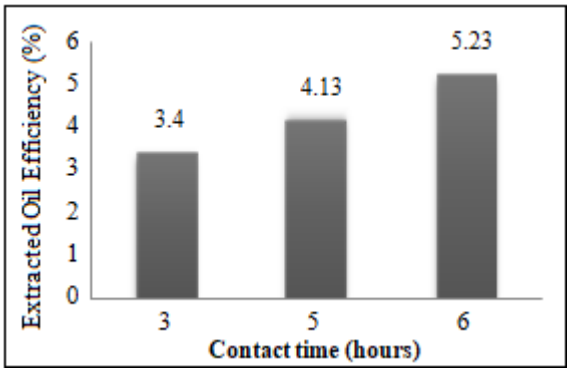

Figure 1: Effect of contact time on extracted efficiency

Table 4: Physiochemical properties according to Ejim Ikechukwu in 2013

Parameters	Units	Values
Acid value	MgKOH/gOil	1.9
Saponification Value	MgKOH/gOil	175
Peroxide Value	meq/kg	1.8
Viscosity	mPa.s	23.53

Table 5: Physiochemical properties of the obtained oil

Parameters	Units	Values
Acid value	MgKOH/gOil	0.5
Saponification Value	MgKOH/gOil	245.44
Peroxide Value	meq/kg	0.59
Viscosity	mPa.s	25.58
Colour	-	Greenish yellow
Boiling point	^{0}C	84
pH value	-	6.8

4. Conclusion

The focus of this research work was to characterize obtained oil from algae collected from a pond at the management science walk path, University of Abuja. The properties of the algae oil obtained were determined and compared with Ejim Ikechukwu's thesis (2013).

- From the results of the composition obtained, the oil was found to have high values of saponification, which makes it good for the production of soap and creams
- The extracted oil efficiency increases as the contact time increases.
- As the temperature increased to the boiling point of the solvent (hexane), the extraction rate was rapid. But as the boiling point was exceeded, the extraction rate reduced with time which is as a result of the solvent and oil back to the still pot of the soxhlet apparatus.

5. Recommendations

This present work did not explore the suitability of other solvent for extraction purposes as such it could not come to a better conclusion, I hereby recommend;

- That work should be done exploring the suitability of other solvent (such as petroleum ether, absolute ethanol etc.) on the extraction of the algae oil to know or determine which is more effective.
- 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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Appendix

1) Extracted Oil Efficiency (wt.%) = $\frac{\text{Mass of Oil Extracted (grams)}}{\text{The Total Mass of Dried Algae}} \times 100$

For Sample 01 = $\frac{1.02}{30} \times 100 = 3.4\%$

For Sample 02 = $\frac{1.24}{30} \times 100 = 4.13\%$

For Sample 03 = $\frac{1.57}{30} \times 100 = 5.23\%$

2) Calculation of Acid value

Acid value = $\frac{56.1\ V \times C}{m}$

Where;
56.1 = the equivalent weight of KOH
V = Volume in mol of standard volumetric potassium hydroxide of sodium hydroxide used (0.892mol).
C = Exact concentration in potassium hydroxide solution used (0.1N); and
m = the mass in gram of the test portion (1g)

$= \frac{56.1 \times 0.0892 \times 0.1}{1}$ = 0.50 MgKOH/gOil

3) Calculation of Saponification Value

Saponification Value = $\frac{(V_0 - V_1) \times C \times 56.1}{m}$

Where;
56.1 = equivalent weight of potassium hydroxide
V_0 = Volume in mol of standard hydrochloric acid used for the blank test
V_1 = Volume in mol of standard hydrochloric acid solution for the sample
C = the exact concentration of the standard hydrochloric acid (0.5N) solution; and
m = mass in gram of the oil/ fat taken from the test portion (2g)

$= \frac{(23.7 - 6.2) \times 0.5 \times 56.1}{2} = \frac{17.5 \times 0.5 \times 56.1}{2}$

= 245.44 MgKOH/gOil

4) Calculation of Peroxide value

Peroxide value = $\frac{10 \times (V_1 - V_2)}{m}$

Where;
V1 = Volume of Na2SO3 for determination of test sample in ml
V2 = Volume of Na2SO3 for determination of blank solution in ml; and
m = mass of test portion in gram (5.0g)
$= \frac{10 \times (0.841 - 0.545)}{5} = \frac{10 \times 0.295}{5}$
= 0.59 meq/kg