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“Studies on Potency of Chlorophycean Microalgae for Biodiesel”

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By

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Rational Behind selecting the topic:
Oil provides energy for 95% of transportation and the demand of transport fuel continues to rise. The requirement of Motor Spirit is expected to grow from little over 7 MMT in 2001–02 to over 10 MMT in 2006-07 and 12.848 MMT in 2011-12 and that of diesel (HSD) from 39.815 MMT in 2001-02 to 52.324 MMT in 2006-07 and just over 66 MMT in 2011-12. The domestic supply of crude will satisfy only about 22% of the demand and the rest will have to be met from imported crude. Our dependence on import of oil will continue to increase in the foreseeable future. It has been estimated that the demand for crude oil would go up to 85 MMTPA from about 50 MMTPA in 2001-02 while the domestic production will be around 22% of the demand. The crude prices and availability are subject to great volatility depending upon the international situation and, therefore, attempt needs to be made to reduce dependence on imports. In biofuels the country has a ray of hope. Biofuels are renewable liquid fuels coming from biological raw material and have been proved to be good substitutes for oil in the transportation sector. As such biofuels – ethanol and biodiesel- are gaining worldwide acceptance as a solution to environmental problems, energy security, reducing imports, rural employment and improving agricultural economy. BIS standards for 10% blend need to be drafted after conducting trials and fixing parameters. Biodiesel is made from virgin or used vegetable oils (both edible & non-edible) and animal fats through trans-esterification and is a diesel substitute and requires very little or no engine modifications up to 20% blend and minor modification for higher percentage blends. The use of biodiesel results in substantial reduction of un-burnt hydrocarbons, carbon monoxide and particulate matters. It has almost no sulphur, no aromatics and has about 10 % built in oxygen, which helps it to burn fully. Its higher cetane number improves the combustion.

The current effort is to explore the possibility of an alternative for biodiesel production. The merits of fresh water algal oil over terrestrial plants are easy availability of nutrients to algae through water, throughout availability and Indian environmental condition (Chisti, 2007).

In the light of above mentioned reviews present study was laid out to evaluate the algae as potential feedstock in order to improve the yield, quality and productivity of biodiesel. Such investigations are useful in awaking the farmers, industrialist as well as
nation and achieve maximum oil content that in turn will reduce the green house effect and problem of environmental degradation as well. Further use of genetic engineering have vast scope in it.

The present research endeavor has been divided into six chapters.

Chapter-1: Introduction

This chapter deals with the general introduction on oil crisis and current position of India in oil market. It has highlighted the importance of algae in enhancing biofuel industry. The detailed description given on the green algae, its use as potential biodiesel in the form of feedstock for obtaining oil.

The present situation of environmental problems that has been created due to tremendous use of crude oils. Therefore the need and importance of algae as a feedstock used in the form of biodiesel along with the aims and objectives of the present investigation has been incorporated in this chapter.

Aims and Objectives:

a) To collect and document planktonic chlorophycean microalgal members.
b) To isolate and maintain microalgal cultures.
c) To investigate the potency of chlorophycean members for fuel production.
d) To document the comparison between the various microalgae for oil content and its properties like kinematic viscosity, sulphate ash, total Sulphur, Copper strip corrosion, carbon number, free glycerol etc.
e) To introduce algal lipids in the process of biodiesel production.
f) To check the potentials of algal oils for biodiesel production
g) To check Carotenoid content.

Chapter-2: Review of Literature

A thorough literature survey on previous works of biodiesel from different sources as well as of different algal species was revived critically. For the detailed account of the studied algal taxa, monograph and different relevant literature was also reviewed. The reference work on biodiesel production, involvement of multinational companies, various sectors, government policies, current research emphasis by other countries, involvement of researchers in this sector were reviewed in detail and compiled in this chapter.

Chapter-3: Material and Methods

This chapter comprises detailed methodology for collection of algae, isolation procedures, biochemical studies of selected chlorophycean algae. Different types of
isolation media were tested and finally Bold Basal Media and Chu 13 media were selected for further studies.

In biochemical studies isolation and quantification of pigments had been carried out by the method suggested by Arnon (1949). Total carotenoids were determined by the method of Jensen (1978), Total oil/lipids were extracted by soxhlet apparatus (AOAC, 1984 method) using petroleum ether (boiling point 60-80°C).

Different methods were used for mass multiplication of algae, viz. raceway pond method, indigenously prepared photobioreactor method and bottled culture method etc. for mass multiplication purpose indigenously prepared photobioreactor was used. For comparative analysis the growth behavior of the strains was assessed in Bold Basal Media (BBM) under laboratory conditions (Nitumani, 2011)

Biodiesel was prepared by using the oil extracted from algal source. Procedures followed by transesterification settling and separation.

Properties of biodiesel obtained from microalgal lipids were checked by the standards given by BIS (Bureau of Indian Standards) viz. Kinematic viscosity at 40 °C, sulphated ash, % wt, Sulphur % mass, Copper strip corrosion at 100 3 hrs, TAN, mg KOH/gm, Carbon residues, % wt. and Free glycerin, %wt. were checked and presented in tables of result and discussion..

Chapter 4- Results and Discussion

This chapter deals with the experimental results obtained during the investigation and the collected data regarding the studied parameter for selected chlorophycean algae is depicted in the table and graphically represented in figure separately for each algal species.

During study period many samples of water were collected as per the procedure given by Singh (1961). Total 38 species of 17 different genera of Chlorophycean microalgae and one genus of submicroscopic algae were collected from the study area.

The following genus Ankistrodesmus (2) Botryococcus (1), Chlamydomonas (1), Chlorella (1), Chlorococcum (1), Closterium (2), Coelastrem (2), Cosmarium (3), Ererelle (1), Euastrum (1), Hydrodictyon (1), Kirchneriella (1), Pediastrum (2), Scenedesmus (5), Selanastrum (1), Stauroastrum (5) and Tetradron (1) with species were identified from the study area. The collected microalgae were identified by using monograph Chlorophyta by Philipose (1967), Anand (1998), Prescott (1951, 1954) Randhwa (1959), Krishnamurthy (2000) and arranged according to the classification
system of G. M. Smith. The green algal species were identified and then confirmed under the supervision of Dr. S. D. Pingle at P. G. Department of Botany, P. V. P. College, Pravaranagar (Loni) and K.J. Somaiya College, Kopargaon.

Green algae from the water samples were isolated by serial dilution method. Green algal growth appeared on the petriplates were isolated and purified by repeated subculturing in liquid medium followed by dilution and streak plate method on solid media like Bold Basal Media Chu-10 medium, Beijerinck Medium, Cramer and Meyers Medium, CHU13 Medium, CHU 13 (Modified) were made for studying growth of green algal forms from the water samples collected from Ahmednagar district of Maharashtra state. The most suitable media observed for isolation of algae was found as Bold Basal Media followed by Chu 13 (modified). Most of the isolated species were microphotographed by using SONY Cybershot camera by attaching it to microscope.

Unialgal isolates were grown and replicated in conical flasks containing 50 ml liquid BBM and Chu 13 media and incubated for 28 days at 28±2 °C under 16/8 hr. light/dark cycles with 2-5 K Lux light intensity from white fluorescent tubes.

From the isolated species only six species were selected and analysed for their lipid content for biodiesel production efficiency and biochemical content- crude proteins, growth behavior, carotenoids, biodiesel production capacity, etc.

The results obtained during the present investigation are compiled as follows:

- **Selection of Chlorophycean microalgae for different biochemical studies:**

  On the basis of previous literature and growth and culture characteristics total six algae were selected for different biochemical studies viz *Ankistrodesmus spiralis, Botyococcus braunii, Chlorella vulgaris, Chlorococcum humicola, Scenedesmus quadricauda, Chlamydomonas pertusa*. The pure cultures were maintained in the laboratory and used periodically as per requirement.

  Biochemical studies were carried out after 28 days of inoculation. These are fully grown culture. The variation in growth characters were checked.

1. **Carettenoid content:**

  Total carotenoids were determined by the method of Jensen (1978). Results obtained for carotenoid content were: maximum carotenoid content was observed in *Botyococcus* (0.2947 mg/L) whereas minimum was observed in *Chlorococcum* (0.1653 mg/L).
2. Total Lipid content:

Maximum lipid content was observed in *Botyococcus* (58.87%) and minimum in *Chlamydomonas* (23.13%). From this study it is clear that *Botryococcus* seems to be most compatible algal from as row material for biodiesel production. It is followed by *Ankistrodesmus* and *Chlorococcum*. Further it is also observed that *Chlorella*, *Scenedesmus* and *Chlamydomonas* are also found good source of lipid as compared with terrestrial plants.

3. Effect of cultivation methods on mass yield:

During present study it had been observed that photobiorectors are more convenient as compared to raceway pond for mass culture of algae. There were almost nil contamination in photobioreactor where as air borne algae were found in racewaypond while mass culturing.

4. Growth characteristics of algae

It had been observed that log phase of the chlorophycean algae was up to 6 days of inoculation, exponential phase was observed to be 8 - 20 days whereas stationery phase was observed after 22nd days. However minor variation had been observed in exponential phase according to species variation.

5. Preparation of biodiesel:

The total lipid extracted from algae by soxhlet apparatus was introduced in the process of biodiesel production. Traditional method of transesterification was used for biodiesel production. The mixture of catalyst and methanol was poured in to the algal oil in conical flask. The following reaction and steps were followed

a) Transesterification:

The extracted oil from different green algal cultures was introduced in the process of transesterification by using NaOH as catalyst (Hossain and Salleh, 2008). Before introducing the lipids in the process it was heated constantly for about 60°C for 20 minute and cooled. Mixing of methanol and lipid in 3:1 concentration was done and NaOH was added as catalyst. The mixture was shaken for 15 minutes and kept for settling.

\[
\text{RCOOR'} + R'' \rightarrow \text{RCOOR}'' + R'O\text{H}
\]

Triglycerides are rapidly trans-esterified in the presence of alkaline catalysts at atmospheric pressure and at temperature of approximately 60-70 °C. with an excess of methanol.

b) Settling:
After shaking the solution was kept for 16 hrs to settle biodiesel and sediments layer clearly.

c) Separation of Biodiesel:

After settling for about 12 hours two layers were clearly observed which were separated by using separating funnel. Upper layer was collected and named as biodiesel whereas lower layers was glycerin. For each type of algal oil same process was repeated and different concentrations were preserved. Though the concentration of oil and methanol was same for each case concentration of biodiesel obtained was vary. Maximum concentration of biodiesel was obtained from *Botryococcus* followed by *Chlorococcum, Chlorella* and *Chlamydomonas*. lowest amount of biodiesel was obtained from oil obtained from *Scenedesmus*. It is observed that the oil obtained from *Botryococcus* have biodiesel savy fatty acids.

6. Properties of biodiesel obtained from microalgal lipids.

a) **Kinematic viscosity at 40 °C (mm²):**

Kinematic viscosity was checked by using KV-4 low temperature viscosity bath. The range of viscosity observed during this study was 2.8 to 6.2 mm²/s

b) **Sulphated ash, % wt.**

This is measure of the residue left after the sample has been carbonized at 775 ± 25 °C and subsequent residue treated with sulphuric acid. Determination of sulphated ash is according to ISO 3987 specifications. This test method determines the amount of mineral ash remaining after a fuel is burned. For biodiesel, this test is an important indicator of the quantity of residual metals in the fuel that could come from the catalyst used in the esterification process. Maximum value for sulphated ash was recorded as 0.02%

c) **Copper strip corrosion at 50 °C 3 hrs (Thermal stability)**

Copper strip Corrosion test indicates damage to copper alloy components of fuel system. Many of the compounds in diesel fuel can be corrosive. Copper and copper compounds tend to be particularly susceptible to chemical attack. As an indicator of the tendency of a fuel to cause corrosion, ASTM D 130 describes placing polished copper strips in the fuel for 3 hours at 50°C. Then the strips are washed in a solvent and compared to standard descriptions of tarnish and corrosion. Copper strips of Finished to 75 x 12.5 x 2.4mm with 3.0 mm hole for suspension were used for this purpose. It was observed as class 2.

d) **TAN, mg KOH/gm**
Acid Number determines FFA in fuel. This is essentially the amount of Potassium Hydroxide (KOH) required to neutralize the free fatty acids present in 1 g of the oil sample. The acid number is given by the expression:

\[ \text{Acid Number} = \frac{56.1M (A-B)}{W} \]

Where,
- M= molar concentration of KOH, A= Volume of KOH needed to reach end point, corresponding to blank buffer (ml).
- B= volume corresponding to A for blank titration (ml)
- W= mass of sample (g)

The acid number found was 0.5 as maximum.

e) Carbon residues, % wt.
Carbon residue tester was used for this purpose. Carbon residue indicaes unburnt carbon to be left after combustion. It was observed in the range of 0.68 to 8.0

f) Free glycerin, % (m/m)
Total glycerin obtained after filtration of biodiesel was measure and calculated in the form of % glycerine. It was observed in the range of 18-40% according to species varied.

5. Summary and Conclusions

This chapter of the thesis summarizes the main finding of the entire research work, conclusions drawn and its future application and utility were as follows:

- There is great diversity of Chlorophycean microalgae in Ahemadnagar district of Maharashtra state.
- Great variations were observed in different algal members for biochemical characters, such as chlorophyll content lipid content and proteins.
- In comparison with angiospermic plants lipid content of algae were more. However mass production of algae may be limit initially.
- Algae grown in photobioreactor produce more mass compared with raceway pond.
- While studying characteristics of biodiesel produced from algal sources it had been noticed that all standards for biodiesel decided by BIS prove satisfactory except sulphur content. However it can be overcome by adding some additives.
Use of algae as feedstock for biodiesel is cheaper, ecofriendly and cost effective than other sources of oil since algae species to be used are abundantly available in fresh water bodies of Maharashtra. Emphasis need to be given for mass multiplication methods.

Growth characteristic studied of different algae shows maximum growth rate for *Chlamydomonas* as well as *Botryococcus*

The use of algae as source of oil would certainly help in reducing environmental pollution and green house gases concentration from environment.

To conclude algae as source of biodiesel production may prove efficient tool for boosting green fuels and to lead scenario of fuel crisis in India and world as well.

6. References

The investigations and methods used in the thesis are supported by relevant references. A collage of materials, text and figures from different sources used to produce this work are also cited.

I wish to express a deep sense of gratitude towards my research guide Prin. Dr. S. D. Pingle, for his able guidance and constant encouragement and inspiration throughout the period of investigation.