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Biodiesel from fresh water algae: Effect of pH on oil production

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Abstract
In this investigation algal oil was used as a raw material for biodiesel production. The decreasing fossil fuel resources cause both insufficiency in providing demand and increase in prices and it triggers the structural change in energy production and resources. In this context, the innovations in encouraging the use of renewable energy sources will make it possible to manage the passage from an unsustainable structure to a more sustainable structure. The necessary conditions for the world oil supply can be said to enter into a new era with the increasing demand pressure. In this study naturally occurring fresh water algal samples were collected from different sites of Shivamogga. Algae was identified as *Oedogonium grande* and *Spirogyra varians.*, inoculated into the selective media, which favor the growth of algae oil was extracted from dried algal samples and pH were analyzed. These results indicate that biodiesel can be produced from *Oedogonium grande.* and *Spirogyra varians.*,

Keywords: biodiesel, transesterification, *Oedogonium grande. Spirogyra varians*, glycerin, biomass

Introduction

An innovative and attractive life style of human is fulfilled by primary energy source fossil fuel. The energy demand flies higher due to increasing population and industrialization. The world may face the challenges like rising prices of petroleum fuel, energy security, deforestation and growing global warming. Hence researcher seriously focused on the renewable energy sources as key solution for replacement of fossil fuel (Dayananda C. *et al*., 2007). The bioenergy is becoming increasingly relevant as a possible and potential alternative to fossil fuel. Biofuels are liquid or gaseous fuels produced from biomass resources and used in place of, or in addition to diesel and other fossil fuel for transport, stationary, portable and other applications. Biofuels are derived from renewable biomass resources like agriculture, forestry and aquatic environment (Weissman, J.C. and D.M. Tillett 1992). These sources are taken in good consideration as feedstock producer for making the biofuel such as biodiesel, bioethanol, bio-oil and biogas. The utilization of renewable biomass energy in large extent provides sustainable development which link to global stability, economic growth, innovation in local market, reduces Green House gas emission and meeting the energy needs of vast rural population to get quality of life (Sanjaykumar N. D., *et al*., 2013).

Biodiesel is an alternative liquid fuel for diesel engines that is produced by transesterification of vegetable oil or animal fat sources. Biodiesel is made by chemically reacting of oil or animal fat with alcohol in presence of catalyst producing fatty acid alkyl ester along with co-product glycerin. According to National Biodiesel Board, biodiesel is as a mono-alkyl ester (Schneider, D 2008).

Energy is one of the major inputs for the economic development of the country. In the case of the developing countries, the energy sector assumes a critical importance in view of the ever increasing energy needs requiring huge investment to meet them. All energy used by human which originates from the radiant energy emitted by the sun; geothermal energy from the interior of earth; tidal energy originating from the gravitational pull of the moon; and nuclear energy (Meher *et al*., 2006). Solar energy source is the thousands time larger than the other like fossil fuel reserves. The long availability is not the only criterion to judge an energy source. The way it is requiring to convert into other forms, help to meet our needs, environment and health issue at local, regional and global level (Bangboyc A.I. and Hansen A.C. 2008).

Biodiesel is not a new concept, in 1912; Rudolf Diesel used the straight vegetable peanut oil in diesel engine. It is reported that there is no requirement in the modification of diesel engine. The various feedstock used for biodiesel production such as soyabean oil, Pongamia, jatropha oil, coconut oil, and waste vegetable oil, currently algae which use for production of biodiesel (Subha Rao N.S. 1997).

Biofuel has wide applications such as; it is used in railway engine, in aircraft, as heating oil, diesel generator. Biodiesel can be used in pure form or may be blended with petroleum diesel at any concentration in most of injection pump engines. Biodiesel has different solvent properties than petrodiesel hence it require to change fuel filter on engines heaters shortly use of biodiesel blend (Subha Rao N.S. 1997).

Biodiesel has promising lubricating properties and it possess low sulfur content than diesel fuel. The efficiency of biodiesel depends on its blend, quality, and load conditions under which fuel burnt. The quality of biodiesel fuel has set by the American Society for Testing Materials standard. Biodiesel fuel provides economic stability as well as energy security. The National Biodiesel Board reported that, in 2011 biodiesel production supported 39,027 jobs and more than $2.1 billion in household income. Many countries have their own independent policies regarding taxation and rebate of biodiesel use, import and production to reduce oil dependency and to increase use of renewable energies. According to Renewable Fuel Standards Program Regularity Impact Analysis, reported that soy oil biodiesel results 57% reduction in greenhouse gases compared to

petroleum diesel and biodiesel produced from waste grease results in an 86% reduction. Hence current research focused on finding more suitable crop and improving oil yield for biodiesel production (Wang L. *et al*., 2010).

Materials and Methods

Collection of algal Samples

The sample was collected from freshwater bodies of Shivamogga region, and brought to the Department of Botany and Seed Technology, Sahyadri Science College, Kuvempu University, Shivamogga The collected algal sample was observed under electronic microscope and identified with the help of standard literature and monographs: Smith (1950), Fritsch (1935).

Isolation and culture of algal samples

Once the algae were identified, they were inoculated into the selective media, which favor the growth of algae. In case of more than one alga in a sample, serial dilution was performed followed to obtain uni-algal cultures. The samples were cultured in modified BG-11 media at 27-30^{0}C, for 21 days.

pH

The selected algal strain was transferred into each sterile plastic bottle (500ml), and the different pH was adjusted by the addition of small amount of NaOH or HCL. The pH was determined by using hm Digital PH-80 pH meter.

Harvesting

The algal culture was filtered with the help of filter paper then weighed separately. Then the filtrate was dried in Hot Air oven at 80^{0}C for 3hrs.

Oil extraction

The dried algae were ground with motor and pestle as much as possible. The ground algae were dried for 20 min at 80°C in a incubator for releasing water. Hexane and ether solution (1:1 vol) were mixed with the dried ground algae to extract oil. Then the mixture was kept for 24h for settling. Then the biomass was collected after filtration and weighted.

Evaporation

The extracted oil was evaporated in vaccum to release hexane and ether solutions using rotary evaporator, and 0.25g NaOH was mixed with 24ml methanol and stirred properly for 20 min.

Biodiesel production

The mixture of catalyst and methanol was poured into the algal oil in a conical flask. The following reaction and steps were followed.

Transesterification

The conical flask containing solution was shaken for 3h by rotatory shaker at 300rpm. After shaking the solution was kept for 16h to settle the biodiesel and sediment layers clearly. The biodiesel was separated from sedimentation by flask separator carefully. Quantity of sediment was measured. Biodiesel was washed by 5% water until it was become clean. Biodiesel was dried by using dryer and finally kept under the running fan for 12h. And measured by using measuring cylinder; pH was measured by using pH strips and stored for analysis.

FAME analysis and physical properties

LC-MS was used for the analysis of fatty acid, density, Viscosity value of biodiesel were calculated from their percentage.

Results and Discussion

The investigations were carried out to isolate and growth prospecting of fresh water algae for biodiesel production. The result shows that the biodiesel can produced from fresh water algae *Oedogonium grande*, Algae are simple autotrophic organisms and from simple inorganic molecules such as carbon dioxide they produce complex organic compounds using energy from light or inorganic chemical reactions. Lipids extracted from *Oedogonium grande*, used for the biodiesel production. Biodiesel is produced with a process known as transesterification. Biodiesel produced using *Oedogonium grande*, as lipid source. Glycerol is a byproduct of biodiesel production and it can be used in food industries, pharmaceutical industries and cosmetic industries. Amount of glycerol produced using the lipids of respective algal samples was recorded and the comparison between the two algal species showed the lipid extracted from *Oedogonium grande*, was the best feed for glycerol production. Biomass is also a byproduct of algal sample and it can be used as a fertilizer or fodder.

The pH effect on the growth rate and lipid content of selected algal species showed growth at pH 7. Doubling rate of *Spirogyra varians (Hass.)*, has observed as 0.63 g^{-1} at pH 7. *Spirogyra varians (Hass.)*, will show good pattern if pH maintained from pH 7 – 7.5, however decrease in growth rate had been observed at pH 5, 6 and 8. The effect of pH of the growth of the *Spirogyra varians (Hass.)*, was studied by using BG11 media in different pH level *viz*., 5, 6, 7 and 8.

The *Spirogyra varians (Hass.)*, showed maximum dry biomass weight for pH 7 (13.33±0.04), and minimum in pH 5 (3.13±0.03), pH 6 (4.03±0.03), and pH 8 (3.66±0.03). The remarkable growth rate was observed in pH 7, the lag phase on first six days, the exponential growth phase was seen on 20th day and stationary phase was observed on 21stday. The biomass productivity of *Spirogyra varians (Hass.).*, in pH 7 was observed 1.26 gl^{-1} d^{-1} and lipid extracted from biomass was 0.5714 gl^{-1} d^{-1}, the 16.85% lipid content was obtained.

Table 1: Productivity of algal biomass under different pH

Dry weight of algal biomass (gms)				
Spirogyra varians				
Day	pH 5	pH 6	pH 7	pH 8
3	1.03±0.03	1.05±0.02	1.23±0.23	1.03±0.03
6	1.06±0.04	1.33±0.03	2.15±0.03	1.28±0.03
9	1.20±0.28	2.04±0.03	4.19±0.03	2.11±0.45
12	2.01±0.05	2.55±0.03	6.25±0.03	2.45±0.03
15	2.11±0.03	3.10±0.85	9.33±0.02	3.04±0.04
18	2.33±0.04	3.24±0.04	10.11±0.02	3.24±0.03
21	3.13±0.03	4.03±0.03	13.33±0.04	3.66±0.03

The doubling rate of *Oedogonium grande (Kuetz.).*, has observed as 0.71 g^{-1} at pH 7. *Oedogonium grande (Kuetz.).*, will show good pattern if pH maintained to pH 7, however decrease in growth rate had been observed at pH 5, 6 and 8. The effect of pH of the growth of the *Oedogonium grande (Kuetz.)*, was studied by using BG11 media in different pH level *viz*., 5, 6, 7 and 8.

The *Oedogonium grande (Kuetz.)*, showed maximum dry biomass weight for pH 7 (15.03±0.05), and minimum in pH

5 (3.22±0.03), pH 6 (3.44±0.03), and pH 8 (4.06±0.02). The remarkable growth rate was observed in pH 7, the lag phase on first six days, the exponential growth phase was seen on 20th day and stationary phase was observed on 21stday. The biomass productivity of *Oedogonium grande (Kuetz.).*, in pH 7 was observed 1.43 gl^{-1} d^{-1} and lipid extracted from biomass was 1.242 gl^{-1} d^{-1}, the 33.15% lipid content was obtained.

Table 2: Productivity of algal biomass under different pH

Dry weight of algal biomass (gms)				
Oedogonium grande				
Day	pH 5	pH 6	pH 7	pH 8
3	1.03±0.03	1.03±0.02	1.33±0.05	1.02±0.01
6	1.09±0.02	1.11±0.42	2.56±0.25	1.33±0.04
9	1.22±0.04	2.01±0.38	4.44±0.02	2.21±0.30
12	1.32±0.02	2.26±0.04	7.01±0.04	2.45±0.04
15	2.01±0.03	2.55±0.27	10.33±0.03	3.14±0.02
18	2.33±0.04	3.11±0.02	12.11±0.02	3.54±0.03
21	3.22±0.03	3.44±0.03	15.03±0.05	4.06±0.02

Only a few algal organisms are capable of growing in extreme pH environments because the extent of ionization of metabolites is affected by the pH, which in turn affects the reactivity of the organisms and their ability to uptake nutrient. High levels of photosynthesis may result in pH fluctuation, as a result of carbon dioxide removal from an already alkaline culture environment (Rai and Guar, 2001). The pH of a medium affects the toxicity of the surrounding metals present in the algae and in turn the ability of algae to uptake nutrients from the environment is affected by the toxicity of the metal (Peterson *et al*., 1984). This might be attributed to the fact that with increasing pH there is a decrease in competition between the metal ion and the H^+ at the cell surface (Franklin *et al*., 2000; De Schamphelaere *et al*., 2003). Therefore, the pH of the medium must be maintained at the optimum level for sufficient nutrient uptake. Rodolfi *et al*. (2009) reported that the pH of the culture was in the range of 7-7.5 while introducing air/CO_2 into the system at a ratio of 97/3% (v/v).

One-Way Analysis of Variance Report

Analysis of Variance Table and F-Test

Model Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Reject Equal Means? (α=0.05)	Power (α=0.05)
Between	7	257.7468	36.82097	5.6596	0.00008	Yes	0.99695
Within (Error)	48	312.2834	6.505904				
Adjusted Total	55	570.0302					
Total	56						

Welch's Test of Means Allowing for Unequal Variances

Model Term	Numerator DF	Denominator DF	F-Ratio	Prob Level	Reject Equal Means? (α=0.05)
Between Groups	7	20.41	2.3199	0.06532	No

Fig 1

Hsu's Simultaneous Confidence Intervals for Multiple Comparisons with the Best

Response: pH_5_Sv,pH_6_Sv,pH_7_Sv,pH_8_Sv,pH_5_Og,pH_6_Og,pH_7_Og,pH_8_Og
Term A:

Alpha=0.050 Error Term=S(A) DF=48 MSE=6.505904

Group	Count	Mean	Lower 95.0% Simult.C.I.	Minimum Difference	Upper 95.0% Simult.C.I.	Critical Value
pH_5_Sv (Not Best)	7	1.837143	-8.992378	-5.707143	0	2.410
pH_6_Sv (Not Best)	7	2.474286	-8.355235	-5.07	0	2.410
pH_7_Sv	7	6.637143	-4.192378	-0.9071429	2.378092	2.410
pH_8_Sv (Not Best)	7	2.4	-8.429521	-5.144286	0	2.410
pH_5_Og (Not Best)	7	1.75	-9.079521	-5.794286	0	2.410
pH_6_Og (Not Best)	7	2.215714	-8.613807	-5.328571	0	2.410
pH_7_Og	7	7.544286	-2.378092	0.9071429	4.192378	2.410
pH_8_Og (Not Best)	7	2.535714	-8.293807	-5.008572	0	2.410

Fig 2

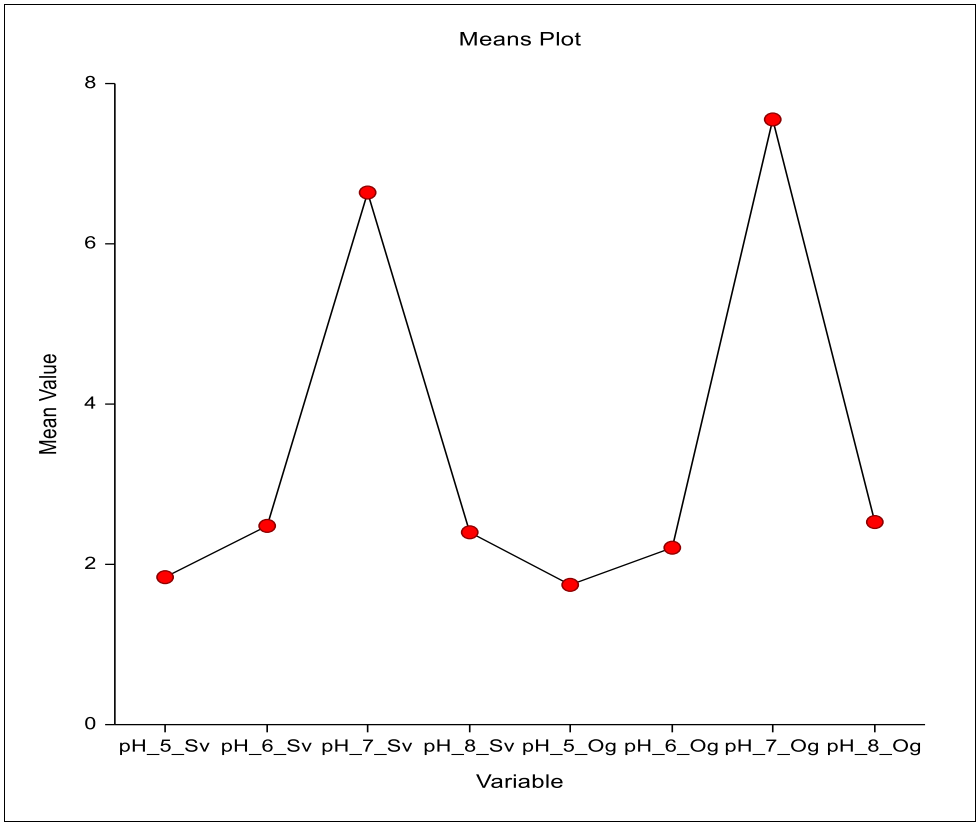

Fig 3

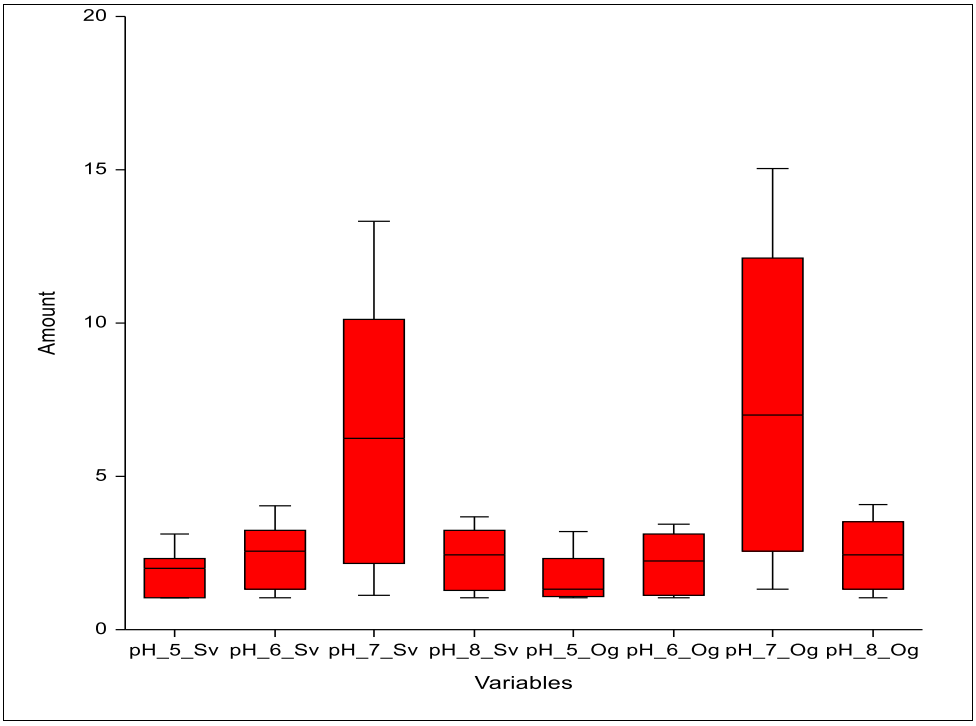

Fig 4

The effects of pH on the specific growth rates of *Spirogyra varians (Hass.)., and Oedogonium grande (Kuetz.).*, were investigated. During the six four days of cultivation, there was a statistically significant difference in the specific growth rate of *Spirogyra varians (Hass.)., and Oedogonium grande (Kuetz.).*, Analysis of variance showed that specific growth rates of the algal biomass were significantly different between culture (F = 5.6596, DF = 48, H=17.1359, P=0.05). Welch's test showed that the positive specific growth rate over the course of the 21st day culture (DF=7, F=2.3199, P=0.0653). Hsu's showed that pH 7 is best for the specific growth rates of selected algal species (DF=48, MSE=6.5059, P=0.05). However, the growth study of *Spirogyra varians (Hass.).*, and *Oedogonium grande (Kuetz.).*, results shows that, the biomass productivity of *Spirogyra varians (Hass.).*, in pH 7 was observed 1.26 gl^{-1} d^{-1} and lipid extracted from biomass was 0.5714 gl^{-1} d^{-1}, the 16.85% lipid content and the biomass productivity of

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