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The effect of light on oil production from green algae: *Spirogyra varians (Hass.)*., and *Oedogonium grande (Kuetz.)*

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Abstract
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. 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 spp* and *Spirogyra spp.*, 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 spp.* and *Spirogyra spp.*,

Keywords: biodiesel, transesterification, *Spirogyra varians (Hass.).*, *Oedogonium grande (Kuetz.).*, glycerin, biomass

Introduction

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).

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). 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).

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) [13].

Materials and Methods

Study Area

Shivamogga District is situated in the Western Ghats of Karnataka state in India. A significant piece of Shimoga locale lies in the Malnad district or the Sahyadri. Shimoga city is its managerial focus. Run Falls is a significant vacation spot. There are seven taluks: Soraba, Sagara, Hosanagar, Shimoga, Shikaripura, Thirthahalli, and Bhadravathi.

Geology

Shivamogga locale is an aspect of the Malnad area of Karnataka and is otherwise called the Door to Malnad' or Malenaada Hebbagilu' in Kannada. The locale is landlocked and limited by Haveri, Davanagere, Chikmagalur, Udupi and Uttara Kannada areas. It is spread over a region of 8465 km2. (Public Informatics Center, 2007)

Shivamogga lies between the scopes 13027' and 14039' N and between the longitudes 74038' and 76004'E at a mean height of 640 meters above ocean level. The pinnacle Kodachadri slope at an elevation of 1343 meters above ocean level is the most noteworthy point in this area. Streams Kali, Gangavati, Sharavati and Tadadi start in this area. The two significant streams that course through this locale is Tunga and Bhadra which meet at Koodli close to Shimoga city to pick up the name of Tungabhadra, which later joins River Krishna and it joins ocean at sangameswara, Andrapradesh.

As the area lies in the tropical district, blustery season happens from June to October. In the years 1901–1970, Shimoga got a normal yearly precipitation of 1813.9 mm with a normal of l86 days in the year being blustery days. The normal yearly temperature of Shimoga locale is around 260C. The normal temperature has expanded generously throughout the long term. In certain areas of the region, the day temperature can arrive at l400C during summer.

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.

Light

Algae can grow under various sources including natural light and artificial light (Red light and blue light). The T-shaped 15W (B-22D) LED lamp 22 cm in length was used as a light source and it was fixed 20 inches (55 cm) above the containers, the dimensions of each container were 20 cm (length) x 15 cm (width) x 5 cm (depth). For Red and Blue light the colour papers were used.

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, The density, Viscicity 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 spp.,* 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 spp.,* used for the biodiesel production. Biodiesel is produced with a process known as transesterification. Biodiesel produced using *Oedogonium spp.,* 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 spp.,* 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 growth rate analysis of algae strain was assessed in different light *viz.,* Red, Blue and Visible light. The growth rate of the *Spirogyra varians (Hass.).,* and *Oedogonium grande (Kuetz.).,* algae strain in culture media was determined by weighing the dry weight of algal biomass. Biomass productivity and Lipid productivity of algae for different light (mg/l) was tabulated in Table 1.

The *Spirogyra varians (Hass.).,* showed maximum dry biomass weight for Red light (16.25±0.14), followed by Blue light (14.01±0.03) and minimum in visible light (13.33±0.03). In Red light, the lag phase on first six days, the exponential growth phase was seen on 18th day and stationary phase was observed on 21st day. In Blue light the lag phase on first nine days, the exponential growth phase was seen on 20th day and stationary phase was observed on 21st day. And in visible light the lag phase on first nine days, the exponential growth phase was seen on 20th day and stationary phase was observed on 21st day. So the remarkable growth rate was observed in Red light followed by Blue light. The biomass productivity of *Spirogyra varians (Hass.).,* in Red light was observed 0.77 gl^{-1} d^{-1} and lipid extracted from biomass was 0.5614 gl^{-1} d^{-1}, the 16.72% lipid content was obtained. In Blue light biomass productivity was observed 0.67 gl^{-1} d^{-1} and lipid extracted from biomass was 0.5214 gl^{-1} d^{-1}, the 16.23% lipid content was obtained. In visible light biomass productivity was observed 0.63 gl^{-1} d^{-1} and lipid extracted from biomass was 0.4944 gl^{-1} d^{-1}, the 16.11% lipid content was obtained.

The *Oedogonium grande (Kuetz.).,* showed maximum dry biomass weight for Red light (17.03±0.02), followed by Blue light (14.33±0.05) and minimum in visible light (12.67±0.04). In Red light, the lag phase on first nine days, the exponential growth phase was seen on 19th day and stationary phase was observed on 21st day. In Blue light the lag phase on first nine days, the exponential growth phase was seen on 20th day and stationary phase was observed on 21st day. And in visible light the lag phase on first twelve days, the exponential growth phase was seen on 20th day and stationary phase was observed on 21st day. So the remarkable growth rate was observed in Red light followed by Blue light. The biomass productivity of *Oedogonium grande (Kuetz.).,* in Red light was observed 0.81 gl^{-1} d^{-1} and lipid extracted from biomass was 1.2421 gl^{-1} d^{-1}, the 33.15% lipid content was obtained. In Blue light biomass productivity was observed 0.68 gl^{-1} d^{-1} and lipid extracted from biomass was 1.1420 $gl^{-1}d^{-1}$, the 31.23% lipid content was obtained. In visible light biomass productivity was observed 0.60 gl^{-1} d^{-1} and lipid extracted from biomass was 1.0001 gl^{-1} d^{-1}, the 31.01% lipid content was obtained.

Light is an essential energy source for photosynthetic activity of algae. Algae are mostly autotrophic which required light for their growth. The effect of light depends on the quality and intensity of light. The green algae shows better growth in red and blue light because they contain chlorophyll a and b which are sensitive to red and blue light wavelength.

Steman Nielsen (2011) was observed that high light intensity is harmful to algae. Using artificial light will provide algal growth during the day, as opposed to sunlight which is only available for a certain number of hours each day. Light emitting diodes or fluorescent light may be used for algal growth. The LEDs are more energy efficient than fluorescent lights but cost more. LEDs are capable of converting 80% of electrical energy into radiation energy thus making them more efficient light sources (Chen *et al*., 2009).

The red LED is the most attractive for photosynthesis because its emission spectrum corresponds with the energy required for a photon to reach the first excited state of chlorophyll a and b. In the conversion of photons to biomass, it was concluded that the red LED resulted in the highest growth rate for the species *Spirogyra spp.,* (Chen *et al*., 2009). Round (1973) reported that the red light has the ability to increase carbohydrate formation in algae.

Chen *et al*. (2009) and Rai and Gaur (2001) noted that light consisting of red wavelength was found to be the most efficient in biomass production. Algae are in competition for the red photons because they are strongly absorbed by water molecules and as such are not available for algal photosynthesis in most habitats (Rai and Gaur, 2001). Light of shorter wavelength may also not reach algae because the photons may be absorbed by the solute and other organic materials that are capable of retaining the light. It may also be scattered from the surface by suspended materials (Rai and Guar, 2001). Only 10-30% of the light intensity reaches the molecules under the water surface forming a gradient in the quality and quantity of light going from the surface of the water to deeper layers (Rai and Guar, 2001).

The blue light photons consist of 40% more energy than those of the red light, making them suitable for chlorophyll absorption. However, in the conversion of photons to biomass, it was concluded that the blue LED showed the least efficiency (Chen *et al*., 2009).

A study done on the growth of *Oedogonium spp.,* under artificial fluorescent light illustrated no difference in the overall total biomass, but are comparable to LED (Chen *et al*., 2009). Lee and Palsson (1996) noted that the *Oedogonium spp.,* species grown resulted in double the cell volume of that cultivated under red light.

Table 1: Productivity of algal biomass under different Wave length

Name of algae	**Wave length**	**Dry weight of algal biomass (gms)**						
		Day 3	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**
Spirogyra varians	Red	1.66 ±0.07	3.01 ±0.04	5.21 ±0.09	7.65 ±0.04	10.24 ±0.04	13.33 ±0.06	16.25 ±0.14
	Blue	1.23 ±0.24	2.44 ±0.03	4.66 ±0.04	6.65 ±0.05	8.66 ±0.02	11.24 ±0.06	14.01 ±0.03
	Visible	1.10 ±0.02	2.15 ±0.02	4.19 ±0.02	6.25 ±0.02	9.33 ±0.02	10.11 ±0.03	13.33 ±0.03
Oedogonium grande	Red	1.67 ±0.02	2.66 ±0.02	4.68 ±0.03	7.23 ±0.32	11.33 ±0.03	14.45 ±0.02	17.03 ±0.02
	Blue	1.40 ±0.22	2.33 ±0.03	4.45 ±0.02	6.68 ±0.03	9.43 ±0.03	11.55 ±0.05	14.33 ±0.05
	Visible	1.33 ±0.02	2.11 ±0.02	3.68 ±0.04	6.01 ±0.02	8.33 ±0.03	10.10 ±0.04	12.67 ±0.04

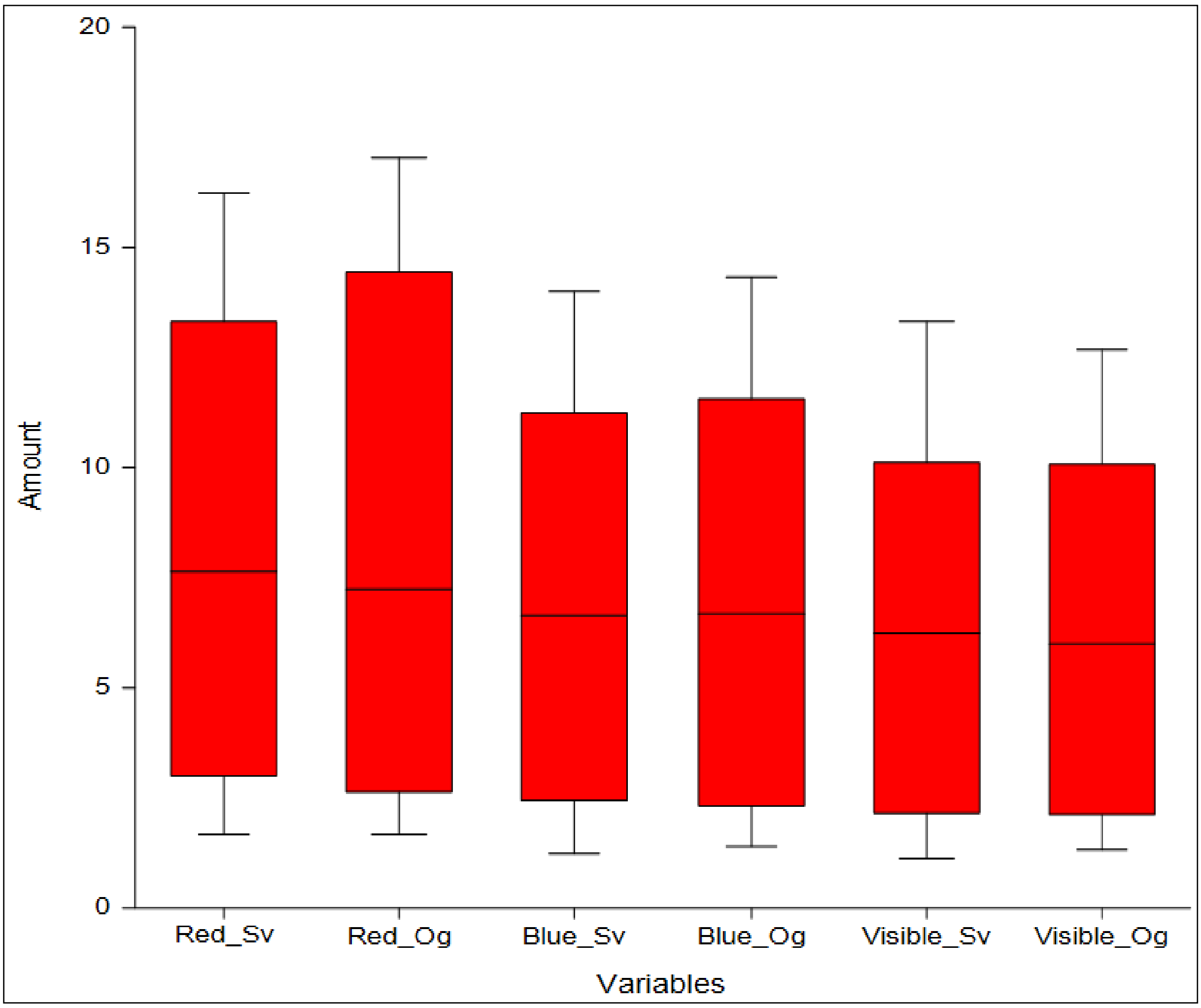

Fig 1: Amount vs Variables

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