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Environmental, Economical and Financial Aspects of Production of Biodiesel from Algal Biomass

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Abstract:
Biodiesel is an environment friendly fuel. It has a substantially better energy balance than ethanol, causes a dramatic reduction in carbon emission per mile driven relative to petroleum based diesel, is 100% renewable, and it can go into existing diesel engines without modifications. Production of biodiesel using conventional methods, make social and economic sense. Jatropha is being planted in India, China and other parts of the world. Since Jatropha grows in sub-prime non-food crop lands, it is potentially useful because land becomes less of an issue. Biodiesel made from algae is another potential source, however, large tracts of water are only available in open oceans and that creates the related issue around using genetic engineering techniques to optimize algae which would be an environmental risk. Biofuels are expected to reduce dependence on imported petroleum with associated political and economic vulnerability, reduce greenhouse gas emissions and other pollutants, and revitalize the economy by increasing demand and prices for agricultural products. Microalgae feedstocks are gaining interest in the present day energy scenario due to their fast growth potential coupled with relatively high lipid, carbohydrate and nutrients contents. All of these properties render them an excellent source for biofuels such as biodiesel, bio ethanol and bio methane; as well as a number of other valuable products.
In the present study the production of Biodiesel from kinds of algae was investigated. The important parameters like reaction temperature, pH, medium and reaction time was studied. Biodiesel is produced by trans esterifications process. Base catalysed transesterification process is more efficient and is less corrosive than an acid catalysis process which makes it more applicable to industrial use. In the other way Oedogonium, Spirogyra and Chlorella were taken for the biodiesel production. After the experimental analysis, it was found that, the percentage of the dry weight of algae (before oil extraction) was higher in Oedogonium torn in Spirogyra, It was 11.3 g than 8.09 g of Spirogyra and 9.20 g of Chlorella. Hence it is encouraging going into the business of biofuels production to empower rural dwellers thereby alleviating poverty and reducing environmental destruction and enjoining sustainable energy usage. The fuels, which showed the greatest reductions in greenhouse gases (over 50 %) when compared with fossil fuels were biodiesel made from waste and algae. The numerous benefits of biofuels include sustainability, mitigation of greenhouse gas emissions, regional developments, employments, energy security, conflict resolutions, agriculture and socioeconomic benefits will make a lot of changes for the benefit of man when their production and usage are fully practicalised globally.

Key words: *Algal Biomass, Biodiesel, Ethyl alcohol, Fermentation, Hydrolysis*

1. Introduction

Algal biofuels are generating considerable interest around the world. They may represent a sustainable pathway for biofuel production. Microalgae are single-cell, photosynthetic organisms known for their rapid growth and high energy content. Some algal strains are capable of doubling their mass several times per day. In some cases, more than half of that mass consists of lipids or triacylglycerides mostly found in vegetable oils, which can be easily converted in to biofuels. These bio-oils can be used to produce such advanced biofuels as biodiesel, green diesel, green gasoline, and green jet fuel. Biofuels production represents a major opportunity for the country's economy. Developing innovative technologies can secure new jobs in rural areas, but also within industrial companies. In addition, new job opportunities could also arise from technology export. Microalgae have been suggested as very good candidates for fuel production because of their advantages of higher photosynthetic efficiency; higher biomass production and faster growth compared to other energy crops [1]. Algal biomass can be produced on lands not suitable for higher plants; therefore resulting in a more effective use of global land surface [2] Therefore microalgae production does not compete with the production of food for a growing population and is the only viable alternative for a large scale biodiesel production seen today. In India most of the places villagers use the biomass in terms of burning fuel for different purposes. The burning of an enormous amount of fossil fuel has increased the CO_2 level in the atmosphere, causing global warming. Biomass have been focused on as an alternative energy source, since it is a renewable resource and it fixes CO_2 in the atmosphere through photosynthesis. If biomass are grown in a sustained way, its combustion has no impact on the CO_2 balance in the atmosphere, because the CO_2 emitted by the burning of biomass is offset by the CO_2 fixed by photosynthesis [3, 4]. Among biomass, algae

(macro and microalgae) usually has a higher photosynthetic efficiency than other biomass [5]. Biomass is one of the better sources of energy [6]. Large-scale introduction of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economic [7]. Now in these days algal biomass is the rich source of production of biofuel. Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass [8] biodiesel derived from micro algal oil [9, 10, 11] and photo biologically produced bio hydrogen [12, 13]. The idea of using microalgae as a source of fuel is not new[14,15] but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels[16]. Algal biomass is one of the emerging sources of sustainable energy. The large-scale introduction of biomass could contribute to sustainable development on several fronts, environmentally, socially and economically [17].

1.1. Advantages of Biodiesel from Algae oil

Producing biodiesel from algae has been touted as the most efficient way to make biodiesel fuel. The main advantages of deriving biodiesel from algae oil include: **(a)** rapid growth rates **(b)** a high per-acre yield (7 to 31 times greater than the next best crop – palm oil) **(c)** certain species of algae can be harvested daily [18,19] (**d)** algae biofuel contains no sulphur **(e)** algae biofuel is non-toxic (**f)** algae biofuel is highly biodegradable, and algae consume carbon dioxide as they grow, so they could be used to capture CO_2 from power stations and other industrial plant that would otherwise go into the atmosphere. Research into algae for the mass-production of oil is mainly focused on micro-algae. The preference towards micro-algae is due largely to its less complex structure, fast growth rate, and high oil content. Some species of algae are ideally suited to biodiesel production due to their high oil content – sometimes topping out near 50%. Some commercial interests into large scale algal-cultivation systems are looking to tie into existing infrastructures, such as coal-fired power plants or sewage treatment facilities. This approach not only provides the raw materials for the system, such as CO_2 and nutrients; but it changes those wastes into resources.

The most extensive research into the development of biofuels from algae was performed by the National Renewable Energy Laboratory (NREL) from 1978 to 1996. The main focus of the program, known as the Aquatic Species Program (ASP) was the production of biodiesel from high lipid content algae grown in ponds and utilizing waste CO_2 from coal fired power plants. In this program they were reported more than 3000 species of algae and the best candidates were some green algae and diatoms [20].

Microalgae	Oil content	Crop	Oil yield (gallons/acre)
Botryococcus braunii	25–75	Corn	18
Chlorella sp.	28–32	Soybeans	48
Crypthecodinium cohnii	20	Canola	127
Cylindrotheca sp.	16–37	Jatropha	202
Nitzschia sp.	45–47	Coconut	287
Phaeodactylum tricornutum	20–30	Oil Palm	636
Schizochytrium sp.	50–77	Microalgae	6283–14641

Table: 1 Comparative study of oil extraction from different algae and crop seeds [21]

1.2. Transesterification of Algal Oil

Biodiesel production from microalgae can be done using several well known industrial processes, the most common of which is base catalyzed transesterification with alcohol. The transesterification is the reversible reaction of fat or oil (which is composed of triglyceride) with an alcohol to form fatty acid alkyl ester and glycerol. Stoichio-metrically, the reaction requires a 3:1 molar alcohol to oil ratio, but excess alcohol is (usually methyl alcohol is used) added to drive the equilibrium toward the product side [22].

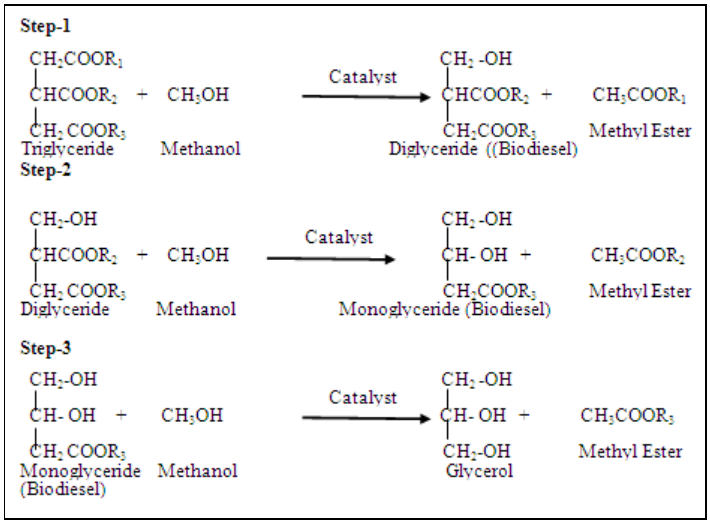

Figure 1: Chemical Reaction Of Esterifications During Production Of Biodiesel

2. Material and Methods

Spirogyra, Oedogonium and Chlorella green algae were taken for the study for both the study of ethanol production and biodiesel production via lipid extraction. In this content Spirogyra and others samples were washed with water to clean the dirt, and then dried for three days in the sun. Spirogyra which had been dried weighed and added aqueduct with compared to aqueduct in the proportion of Spirogyra (5: 1), mashed with a blender, put into 250 ml erlenmeyer [23]. Spirogyra extract sterilized in autoclave 1,5 atm 121°C. Further Spirogyra extracts will be used to process the growth curve of S. cerevisiae and Z. mobilis, and substrate of fermentation. Similarly Oedogonium and Chlorella was collected for the lipid extraction and biodiesel production.

2.1. Pre-treatment (Saccharifiation of Spirogyra biomass by Aspergillus Niger)

A developed mycelial mat of Aspergillus Niger was used for saccharification. Aspergillus Niger is cellulolytic and amylolytic in nature as it produces cellulases and amylases. These enzymes hydrolyze the cellulose and starch present in Spirogyra and releases free sugars. The saccharification was carried out for a period of five to six days at 30^0C and the process was monitored every 24 hrs for sugars released [24] to determine the glucose estimation using standard graph.

2.2.Fermentation By Saccharomyces Cerevisiae

After pre-treatment the mycelial mat of Aspergillus Niger was removed from all flasks under aseptic conditions and 10% of Saccharomyces cerevisiae was added to each flask for fermentative production of bioethanol. The process was carried out for a period of another five-six days at 30^0C during which every 24 hours samples were taken for the estimation of alcohol (bioethanol).

2.3. Measurement of Growth

Z. mobilis was also used for the comparative study of ethanol production. In the first step of activation, Z. mobilis was inoculated into 50 ml erlenmeyer containing 5 ml of sterile Spirogyra extract that has been set pH to 4 by adding 30% HCl solution, then incubated in a rotary shaker with agitation speed of 15 rpm at 30 °C for 24 hours. A total of 1 ml of substrate from first step and inoculated into 50 ml Erlenmeyer containing 9 ml of Spirogyra extract, incubated in a rotary shaker with agitation speed of 15 rpm at a temperature of 30 ° C for 24 hours (Activation II). A total of 5 ml of re-activation II and inoculated into 100 ml Erlenmeyer containing 50 ml of extract of Spirogyra, were incubated in rotary shaker with agitation speed of 15 rpm at a temperature of 30 °C and incubated until the hour in which log phase of Z. mobilis occur (in accordance with the growth curve) (Activation III) [25,26]. Performed dilutions were from 10-1 to 10-9. One ml of culture medium were taken and put into a test tube containing 9 ml of sterile distilled water. Test tube contains the mixture with a vortex mixer, pipet as much as 1 ml and put into a test tube next. The treatment is repeated until retailing to 10-9. The curve of growth was made by measuring the absorbance of cultures of Z. mobilis on Spirogyra extract. Z. mobilis was measured at a wavelength of 600 nm at intervals of once every one hour during 24 hours. Graph the growth curve of absorbance values and the fermentation time [27]. The same step done for S. cerevisiae.

2.4. The Fermentation Process and Measurement

The fermentation process was stopped if ethanol levels have been reduced. Sample measured by picnometer at 20°C. Ethanol Concentration Measured by using specific gravity method. Moisture content of the fresh algae was determined by drying pre-weighed fresh algae on a pre-dried No. 42 What man Filter Paper and drying overnight at 95°C. The dried slurry and paper were weighed until a stable weight was reached. Fresh algae had 9.1% w/w dry algae biomass.

To extract oil from the algae, Algae were ground with motor and pestle as much as possible. The ground algae were dried for 20 min at 80°C in an incubator for releasing water. Hexane and ether solution (20 and 20 mL) were mixed with the dried ground algae to extract oil. Then the mixture was kept for 24 h for settling. The biomass was collected after filtration and weighted. The extracted oil was evaporated in vacuum to release hexane and ether solutions using rotary evaporator. For the effective analysis and product formation 0.25 g NaOH was mixed with 24 mL methanol and stirred properly for 20 min.

2.5. Analytical Treatment

Reducing sugars were estimated by the method of [24]. Ethanol (bioethanol) was estimated by the method of [28, 29].

3. Result and Discussion

Spirogyra extracts as much as 50 ml incorporated into the erlenmeyer. Erlenmeyer was heated on a hot plate for 2 hours in with the temperature 100°C, stirring occasionally open funnel-flops [30]. Cool for two hours until the temperature reaches ± 40 °C. The addition of the α-amylase enzyme with each concentration of 0 grams/50 ml 0.03 grams/50 ml; 0.06 grams/50 ml; and 0.09 grams/50 ml. Incubated at room temperature for 60 minutes [31]. To enhance the production lactose was added. Lactose enhances the production of ethanol and concluded that for the enhancement of bio ethanol production enzyme inducers should be used. On account of 0.9gm of α-amylase enzyme is used with the comparative study of fermentation of Spirogyra biomass by using Zymomonasmobilis and Saccharomyces cerevisiae. The fermentation process using different enzymes also have different production. The comparatives results were obtain in 96 hours of fermentation.

A decline in the number of bioethanol was found on the addition of 0.09 grams of α-amylase enzyme. So the highest yield was obtained from the addition of 0.09 grams of α-amylase enzyme at 96 hours. On addition of α-amylase as the amount of enzyme increases the growth rate of Z. mobilis was found more than S.cerevisiae as shown in Fig: 2. a growth curve gives an overview of the environmental factors that affect the growth of a microorganism such as substrate, ambient temperature, pH, and determine the age of starter [32].

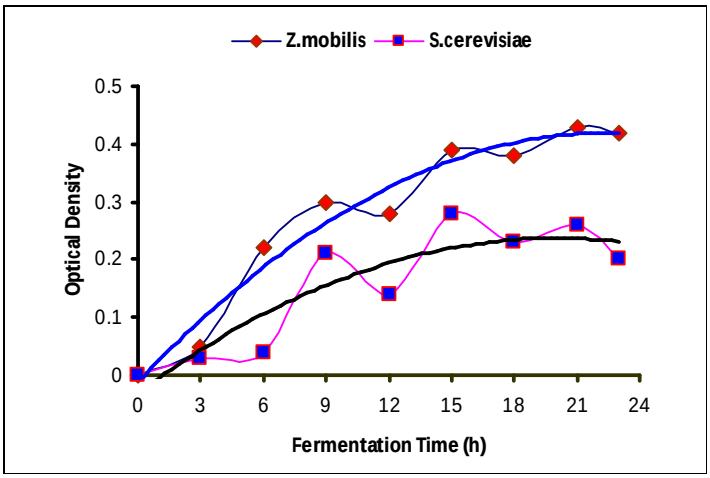

Figure 2: Growth Curves Of Z. Mobilis And S.Cerevisiae

After the experimental analysis it was found that, the percentage of dry weight of algae (before oil extraction) was higher in Oedogonium than in Spirogyra sp. It was 11.3 g than 8.09 g of Spirogyra and 9.20 g of Chlorella (Table 2). The lipid content of Chlorella was found (18%), which was least than others. Extracted oil was higher in Oedogonium than in Spirogyra and Chlorella. It was found 3.0g than 1.8g of Spirogyra. However, biomass (after oil extraction) was lower in Oedogonium than in Spirogyra and chlorella, it was 3.1 g (Table 3). Biodiesel production (methyl ester) was found maximum in Oedogonium 95% than Spirogyra 91% and minimum in Chlorella 87% (Fig. 3).

Microalgae	**Habitat**	**Biomass productivity (g/L/day)**	**Lipid content (% biomass)**	**Lipid productivity (mg/L/day)**
Chlorella	Stationery water	0.314	18.0	34.82
Spirogyra	Freshwater	0.26	21.1	53.9
Oedogonium	Freshwater	0.19	18.4	35.1

Table 2: Biomass Productivity, Lipid Content And Lipid Productivity Of Microalgae Cultivated In 250-Ml Flasks

Microalgae	**Fresh wt (g)**	**Dry Weight (g)**	**Extracted Oil (g)**	**Biomass (g)**
Chlorella	26.4	9.20	1.6	2.2
Spirogyra	24.5	8.09	1.8	3.5
Oedogonium	32.4	11.3	3.0	3.1

Table 3: Measurement Of Fresh And Dry Weight, Extracted Oil And Biomass Of Algae

The results of the following algae are present in Table 3. Moreover, sediment (glycerine, pigments and other elements) were higher in Spirogyra (65%) than in Oedogonium (48%) and Chlorella (42%) Fig.3. There was no significantly difference in pH between both species. Therefore, our results prove that biodiesel can be produced from macro algae though it contains lower lipid content than micro algae. In addition, it seems that Oedogonium is higher biodiesel containing algae than spirogyra .Finally we strongly recommend that biodiesel can be produced from microalgae. By this way algae can be used as renewable source.

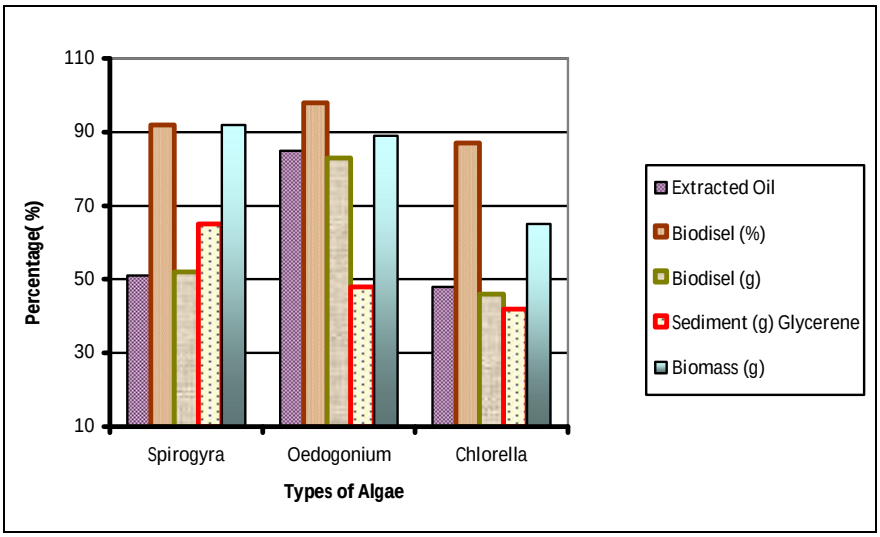

Figure 3: Production of Biodiesel from different kinds of algae

The transesterification of used oil produced biodiesel by, using an alkaline catalyst, KOH [33]. Two types of used oils (partially hydrogenated soybean oil and margarine) were transesterified with methanol, ethanol, 1-propanol, 2-propanol, 1-butanol and 2-ethoxyethanol. Rose and Norris have compared two catalysts such as KOH and a combination of barium and calcium acetate for the preparation of methyl esters from waste cooking oil [34]. Most of the researchers developed four different continuous process flow sheets for biodiesel production from virgin oil or waste vegetable oil using alkaline or acidic conditions [23]. Some researchers, explained about the production of biofuel from microalgae. They had mentioned that biodiesel derived from oil crops is a potential renewable and carbon neutral alternative to petroleum fuels. Like plants, microalgae use sunlight to produce oils but they do so more efficiently than crop plants [35]. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops.

3.1. Effect Of Nutrient Supplementation (KNO_3) On Biodiesel Production

To determine the effect of nitrogen source on production of lipid and biodiesel from chlorella, different concentration were taken in synthetic media. Chlorella is one of the green algae for ethanol and Biodiesel production. The pre-treatment process was done as in previous as prior. The algae were studied for growth and lipid content in different concentrations of nitrate. The original nitrogen source concentration in the medium was 0.2 g L^{-1} KNO_3. The experiment was performed in 0, 0.05, 0.1, 0.2 and 0.4 nitrogen source of concentration. The effect of initial nitrogen concentration on the micro-algal growth and lipid content was investigated. Lipid content was estimated after 24 days (stationary phase) of inoculation. Each measurement was done in triplicate and the mean and standard deviation of the experimental results was calculated.

In the absence of a nitrogen source (0g/l KNO_3), no growth was observed and the cells appeared bleached. At day 24, maximum biomass concentration of 0.315 g/l was recorded in the culture with double the concentration of nitrate (0.4 g/l KNO_3). Dry matter of 0.296, 0.246 and 0.127 g/l was observed in cultures with 0.2g L-1 KNO_3 (control), 0.1g L KNO_3 and 0.05 g/l KNO_3 respectively. (Fig. 4.57) and Table 4.29, shows the lipid content of Chlorella Vulgaris in different concentrations of nitrate in stationary phase. An increasing trend is observed in lipid content as the concentration of nitrate was decreased. Also, at the same concentrations of nitrate, stationary phase cultures showed higher lipid accumulation in comparison to that of exponential phase. When the concentration of nitrate was doubled (0.4 g/l KNO_3), the lipid content in the exponential phase was 11% dry cell weight as opposed to control (15%). However, in stationary phase, 18% was recorded in both the cultures. Moreover, when the nitrogen source was decreased from 0.2 g/l KNO_3 to 0.1g L-1 KNO_3, the lipid content in exponential phase increased to 18% from 15%. Stationary phase cultures did not show much difference (19%) in lipid content when grown in 0.1 g/l KNO_3.

Concentration of KNO_3 (g/L)	**Max. Biomass Production (g/l)**	**Lipid Content (%) Stationary Phase**
0.00	0.075	19
0.05	0.127	26
0.1	0.246	19
0.2	0.296	18
0.4	0.315	18

Table 4: Effect Of Nitrogen Concentration On Biomass Production And Lipid Content Of Chlorella Vulgaris

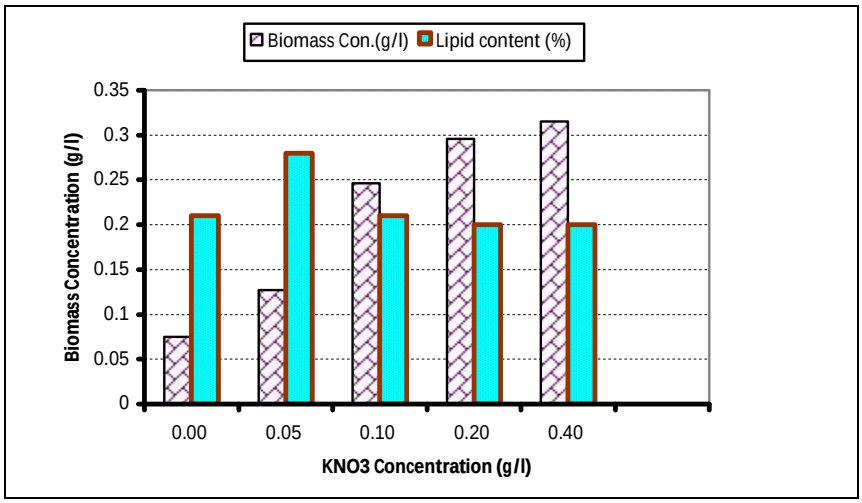

Figure 4: Comparison of lipid content of Chlorella and Biomass Concentration

For economical production of biofuel from microalgae, biomass as well as lipid content plays an important role. In the present study, the results mentioned above indicate that high concentration of nitrogen source supported the biomass concentration in contrast to the lipid content. The alga, Chlorella, cannot grow without a nitrogen source and its growth is directly proportional to the concentration of nitrate in the medium. As nitrate source is increased in the medium, enhancement in biomass concentration was recorded. This result is in accordance with that of [36]. Fig. 4 shows that when cells are transferred to nitrogen rich medium, lipid content was decreased by 4% as compared to control. However, in stationary phase, lipid content was found (18%) as control. This might be because as the algae reaches stationary phase, it has used up its nitrogen reserves for growth and now, it starts accumulating lipid for its survival. This is in parity with [37].

4. Conclusion

Effective parameters of fermentation are also the important features that justified the conditions and maximum production of Alcohol. In presence of higher sugar concentration the ethanol concentration decreases. In case of different pH ethanol fermentation is more favourable at pH 5-6. Similarly higher temperature for fermentation decreases the concentration of ethanol. At different time of fermentation for hydrolysed biomass 24 hours was found optimum. The fermentation media YEPX or YEPD was found optimum than YEP for the growth of enzymes for fermentation. Addition of nutrient supplementation as KNO_3, phosphates and sulphates increases the growth of enzymes as well as alcohol concentration.

Stationary fermentation was found the optimum process for ethanol production from algal biomass in comparison to shaking fermentation. Fermentation of scarified biomass was not sufficient for maximum production of ethanol; to enhance the ethanol concentration addition of lactose is necessary, which increases the ethanol concentration. Addition of α-amylase also increases the production ethanol %, it was found maximum for Zymomonasmobilis than Saccharomyces cerevisiae. On addition of α-amylase as the amount of enzyme increases the growth rate of Z. Mobilis. Hence addition of α-amylase with Zymomonasmobilis enhances the ethanol production from algae. Addition of 0.04(g/100ml) of NH_4NO_3 nutrient enhances the production of lipid as well as biodiesel from algal biomass.

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