International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064

Production of Biofuel from Algae: An Economic and Eco-Friendly Resource

Devendra Pratap Singh[1], Rakesh Kumar Trivedi[2]

[1]Department of Chemistry, Dr. A. I.T. H., U.P. Awadhpuri, Kanpur, India

[2]Professor & Head, Department of Oil and Paint Technology, H. B.T.l., Kanpur 208002, India

Abstract: ***Biomass is a renewable energy resource derived from the carbonaceous waste of various human and natural activities. Bio-fuels such as ethanol, biodiesel are important because they replace petroleum fuels. Production of bio-ethanol from kinds of biomass is one way to reduce both consumption of crude oil and environmental pollution. Using bio-ethanol blended gasoline fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. Biofuel production from renewable sources is widely considered to be one of the most sustainable alternatives to petroleum sourced fuels and a viable means for environmental and economic sustainability. Microalgae are currently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, CO_2 fixation ability and high production capacity of lipids; they also do not compete with food or feed crops, and can be produced on non-arable land. Lignocellulosic biomass and algae are the rich source of carbohydrates and lipids, which may converted in to biofuels. Microalgae have broad bioenergy potential as they can be used to produce liquid transportation and heating fuels, such as biodiesel and bioethanol. The Spirogyra biomass was selected as a substrate for bioethanol production in the process of work, as it is rich in polysaccharides- starch and cellulose. In this content experiment were done in two ways for comparative study. (a) Pretreatment with acid and scarification of algal biomass by Aspergillus Niger (b) Direct scarification of algal biomass. In the first case ethanol concentration was found 4% (w/w). While in case of direct scarification of algal biomass using Aspergillus Niger, 6% (w/w) of alcohol was produced. Saccharomyces cerevisiae and Zymomonasmobilis was comparatively used to ferment the saccharified algal biomass. Addition of lactose and α-amylase were taken for the effective improvement. (i) On addition of 0.12g of lactose maximum alcohol was found 6.6% for direct scarified biomass, while 4.7% ethanol was found in case of acid treated biomass in 6 days. (ii) Presence of 0.09 grams of α-amylase enzyme ethanol production is recorded maximum by fermentation using Zymomonasmobilis than Saccharomyces cerevisiae.***

Keywords: Pretreatment, Algal biomass, Biofuel, Saccharification, Fermentation.

1. Introduction

Biofuel production from renewable sources is widely considered to be one of the most sustainable alternatives to petroleum sourced fuels and a viable means for environmental and economic sustainability. Microalgae are currently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, CO_2 fixation ability and high production capacity of lipids; they also do not compete with food or feed crops, and can be produced on non-arable land. Microalgae have broad bioenergy potential as they can be used to produce liquid transportation and heating fuels, such as biodiesel and bioethanol.

Microalgal biofuels are a viable alternative for clean, economical and sustainable sources of energy. The research and development of micro-algae needs a massive boost to ease the technical difficulties to overcome the large cost advantage of other biofuel feed stocks. It is a large source of biomass on non-arable lands and capture of CO_2. Lipids produced from algae contain saturated and polar lipids, which are suitable for use as a fuel feedstock and it exceeds the best producing oil crops. Microalgae, recognized as one of the oldest living organisms, are thallophytes (plants lacking roots, stems, and leaves) that have chlorophyll *a* as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cells [1]. While the mechanism of photosynthesis in these microorganisms is similar to that of higher plants, they are generally more efficient converters of solar energy because of their simple cellular structure. In addition, because the cells grow in aqueous suspension, they have more efficient access to water, CO_2, and other nutrients [2].

Mostly algae are found in open ponds, stationery water during raining season and are found in fresh water. Vessels such as tubes, flasks, carboys, bags, etc. or ponds covered with green house or usually a photo bioreactor which allows control over illumination, temperature, nutrient level, contamination with predators and other competing algae can be used. Researchers also took the path of creating heterotrophic strains of algae from obligate photoautotrophs due to inadequate luminance. Algal cultures can be defined (one or more selected strains), or are made up of an undefined mixture of strains [3], [4], [5]. Heterotrophic cultivation of micro algae for lipids production does not involve CO_2 mitigation and wastewater treatment programme along with production of algal biofuel. A photo bioreactor is equipment that is used to harvest algae. Photo bioreactors can be set up to be continually harvested (the majority of the larger cultivation systems), or by harvesting a batch at a time (like polyethylene bag cultivation). Some photo bioreactors types include: tubular photo bioreactors, flat-plated photo bioreactors, an inclined triangular tubular photo bioreactor, rectangular tanks, continuous stirred tank reactors (CSTR).

Algae are far more oil-rich and offer a higher yield of oil per unit of land in a year compared to terrestrial crops (Table 1). Lipids are one of the main components of micro algae (2-60% of total cell dry matter) depending on the species and growth conditions [14]. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. Microalgal strains with high oil or lipid content are of great interest in the search for a sustainable feedstock for biodiesel. A few micro algal

species, including some *Chlorella* species [6], *Dunaliella* species [7], [8], *Nannochloris* sp. [9], [10], *Parietochloris incisa* [11] and *Botryococcus braunii* [12], [13], have been reported to have the capacity of accumulating large quantities of lipids in cells under favourable conditions. Lipid content also varies with season as tested in *Chlorella vulgaris*, *Euglena gracilis* and *Chlamydomonas* sp.

Table 1: Oil output of different biofuel feed stocks Source: [15]

Crop	*Oil yield (gal/acre-yr)*
Corn	18
Cotton	35
Soybean	48
Canola	127
Jatropha	202
Oil palm	635
Microalgae (15% oil)	1,200
Microalgae (50% oil)	10,000

Recent studies have shown that microalgal biomass is one of the most promising sources of renewable biodiesel that is capable of meeting the global demand for transport fuels. Biodiesel production by microalgae will not compromise production of food, fodder and other products derived from crops [1]. Microalgal biomass contains three main components: proteins, carbohydrates, and lipids (oil) [16], [17]. The biomass composition of various microalgae in terms of those main components is shown in Table 2.

Many microalgae strains naturally have high lipid content; it is possible to increase that concentration by optimising growth determining factors such as the control of nitrogen level, light intensity, temperature, salinity, CO_2 concentration and harvesting procedure. However, increasing lipid accumulation will not result in increased lipid productivity as biomass productivity and lipid accumulation are not necessarily correlated. Lipid accumulation refers to increased concentration of lipids within the microalgae cells without consideration of the overall biomass production. Lipid productivity takes into account both the lipid concentration within cells and the biomass produced by these cells and is therefore a more useful indicator of the potential costs of liquid biofuel production [17].

An integrated production of biofuels from microalgae (Fig. 1) includes a microalgal cultivation step, followed by the separation of the cells from the growth medium and subsequent lipid extraction for biodiesel production through trans esterifications.

Table: 2 Biomass compositions of microalgae expressed on a dry matter basis [17]

Strain	Protein	Carbohydrates	Lipid
Anabaena cylindrica	43–56	25–30	4–7
Botryococcus braunii	40	2	33
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	41–58	12–17	10–22
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Dunaliella tertiolecta	29	14	11
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–39
Scenedesmus dimorphus	8–18	21–52	16–40
Scenedesmus obliquus	50–56	10–17	12–14
Scenedesmus quadricauda	47	–	1.9
Spirogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Spirulina platensis	42–63	8–14	4–11
Synechoccus sp.	63	15	11
Tetraselmis maculata	52	15	3

To extract the algae oil, a simple process is to use a press to extract a large percentage (70-75%) of the oils out of algae. The remaining pulp can be mixed with cyclo-hexane to extract the remaining oil content. Algal oil can also be extracted using chemical methods. Benzene and ether have been used, oil can also be separated by hexane extraction, which is widely used in the food industry and is relatively inexpensive. Three chemical methods are hexane solvent method, soxhlet extraction and supercritical fluid extraction methods are widely used to extract oil.

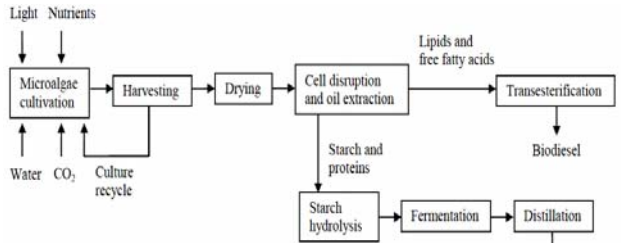

Figure 1: Integrated processes for biodiesel and bio ethanol production from microalgae

Microalgal biomass is a potentially valuable fermentation substrate, and to concentrate on lipids only will exclude the majority of microalgal species. Concerning the fermentation process, similar issues will need to be addressed as for microalgae to determine the inhibitory factors and both optimise species selection and make adjustments to the process for fermentation. To produce the biofuel from algal biomass yeast is best source of fermentation of hydrolyzates. Since the industrially used Saccharomyces cerevisiae is non celluolytic and non amylolytic, the *A.niger* was employed to hydrolyse and produce sugars which can be directly utilized by Saccharomyces cerevisiae for ethanol production.

Algae can be converted into various types of energy for transportation, including biodiesel, jet fuel, electric power, and ethanol. The potential advantages of algae-based biofuel over other biofuel pathways include higher biomass yields per acre of cultivation, little to no competition for arable land, use of a wide variety of water sources, the opportunity to reuse carbon dioxide from stationary sources, and the potential to produce “drop-in” ready-to-use fuels. The need for large amounts of the greenhouse gas CO_2 to grow algae may be a benefit or a concern: algae cultivation could

potentially contribute to emissions neutrality by reusing CO2 from stationary sources.

2. Material and Methods

The alga *Spirogyra* species is multicellular, filamentous and microscopic in nature. The algal mat was collected in sterile containers and transferred to the laboratory. Two fungal cultures *Aspergillus Niger* (for saccharification) and *Saccharomyces cerevisiae* for (fermentation) were procured from (Microbial Type Culture Collection Centre and Gene Bank) Chandigarh, India. The fungi *Aspergillus Niger* was cultured and maintained on potato dextrose agar medium at 30^0C. The yeast *Saccharomyces cerevisiae* was cultured and maintained on YPD (Yeast extract, peptone and dextrose) agar media at 30^0C.

The Spirogyra filaments were sun dried. The dried *Spirogyra* biomass was grinded and filtered through 1mm sieve. Fine powder of *Spirogyra* biomass obtained was used for saccharification and fermentation experiments. Chemical pre-treatment of Spirogyra biomass was done in the following manner, 50% *Spirogyra* biomass was chemically treated with 2% H_2SO_4 or 1% NaOH for a period of 2 hrs [19], [20].

2.1 Fermentation Studies: For comparative studies *Spirogyra* biomass was used for fermentative production of bioethanol in two variations- chemically pre-treated form and untreated form. Fermentation studies were performed in 250 ml Erlenmeyer flasks with three different variations: (a) 5g of the biomass in 100ml of distilled water (b) 5g of the biomass in 100ml distilled water containing 0.5% of lactose and (c) 5g of the biomass in 100ml of synthetic media containing the following components (g/100ml): L-Glutamic acid, 0.04; NH_4NO_3 or 0.04 KNO_3, 0.14; KH_2PO_4, 0.2; $CaCl_2$, 0.03; $MgSO_4$, 0.03; Protease peptone, 0.75; $FeSO_4$, 0.5; $MnSO_4$, 0.16; $ZnSO_4$. The flasks were autoclaved at 120^0C for 15 minutes and inoculated with mycelial mat of *Aspergillus niger*. The same process was followed for both the chemically pre-treated biomass and the untreated biomass.

2.2 Saccharification and fermentation: *Spirogyra* biomass was saccharified by enzymes produced from *Aspergillus Niger* (amylase and cellulase). In the first step the biomass was subjected to saccharification by *Aspergillus Niger* and in the second step *Saccharomyces cerevisiae* was added for fermentative process to produce bioethanol.

Fermentation studies were conducted in 250 ml Erlenmeyer flasks. The flasks were autoclaved at 15 lbs for 15 minutes and inoculated with mycelial mat of Aspergillus Niger. Experiments were carried out in following manner. The following criteria were used for both type of fermentation with Spirogyra and Chemically Treated Spirogyra Biomass.
5g of the biomass + 100ml of distilled water+ Aspergillus Niger + Saccharomyces cerevisiae

Z. mobilis was inoculated into a 50 ml Erlenmeyer containing 5 ml of sterile extract of Spirogyra which had governed the pH to 4 by adding 30% HCl solution [25]. Then it was incubated in a rotary shaker with agitation speed of 15 rpm at a temperature of 30 °C for 24 hours.

2.3 Analytical Treatment: Reducing sugars were estimated by the method of [21]. Ethanol (bioethanol) was estimated by the method of [22]. 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 six days at 30^0C and the process was monitored every 24 hrs for sugars released by *Millers* method of glucose estimation using slandered graph. For the fermentation by *Saccharomyces cerevisiae* 10% of Saccharomyces cerevisiae was added to each flask for fermentative production of bioethanol. The process was carried out for a period of six days at 30^0C during which every 24 hours samples were taken for the estimation of alcohol (bioethanol).

3. Result and Discussion

The sun dried biomass of Spirogyra was grinded, sieved through 1mm sieve and fine powder was obtained. 50% of the powder was used directly for the saccharification and fermentation and remaining 50% of the powder was treated chemically and then used for saccharification and fermentation. *Aspergillus Niger* and *Saccharomyces cerevisiae* were employed in present study. Fermentation experiments were carried out by stationary fermentation process. In general highest yield was recorded for untreated Spirogyra biomass in stationary fermentation. After six days of experiment, it was found that in case of stationery fermentation, the maximum sugar released (consumed), was 19 (g/100g) and the alcohol produced was recorded as 6 (g/100g), as shown in fig.2.

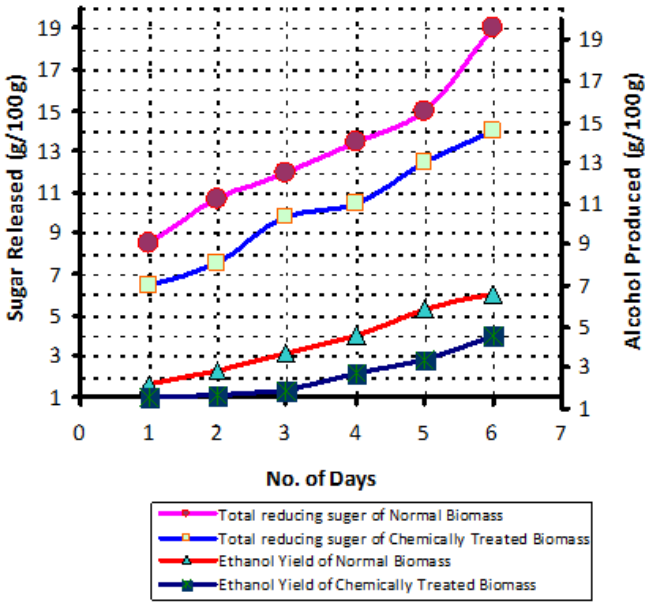

Figure 2: Comparative study of production of ethanol by normally and acid treated algal biomass

The maximum ethanol yield was found 6 (g/100g), and amount of sugar consumed was observed 19(g/100g) in three days of fermentation in case of normally biomass. While in

case of chemically treated biomass the maximum ethanol was produced 4% on 14% of sugar released as shown in Table-3. To check the maximum yield of ethanol different concentration of lactose was used as shown in Fig.3. The maximum ethanol production was found maximum 6.6 % or (g/100g) in comparison to acid treated biomass (4.7%). The optimum addition of lactose was recorded 0.12g for maximum production. The maximum alcohol was found 6.6 (g/100g) in 6 days with the addition of 0.12g of lactose, which was higher than 6 (g/100g) of alcohol concentration without lactose in stationary fermentation, While for acid treated biomass the maximum ethanol concentration was counted as 4.7 on addition of 0.12g of lactose, higher than the ethanol concentration 4 (g/100g) of without lactose in stationary fermentation (Fig: 3). The microbial production of ethanol from cellulosic material is mainly dependent on saccharification enzymes produced by *A. Niger*.

Table 3: Comparative study of production of ethanol by normally and acid treated algal biomass

Biomass Type	*No. Of Days*	*Amount of Sugar Released (g/100g)*	*Alcohol Produced (g/100g)*
Normal Biomass	1	8.5	1.6
	2	10.7	2.3
	3	12	3.2
	4	13.5	4
	5	15	5.3
	6	19	6
Chemically Pretreated Biomass	1	6.5	1
	2	7.5	1.1
	3	9.8	1.3
	4	10.5	2.2
	5	12.5	2.8
	6	14	4

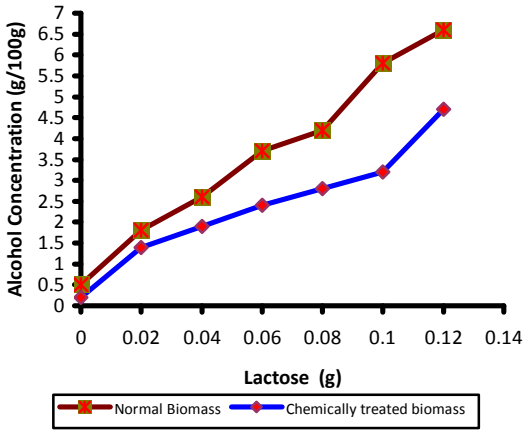

Figure 3: Effect of ethanol production at different concentrations of lactose added

In this content the enzymes converted the biomass into sugars then the sugars released were fermented by Saccharomyces cerevisiae. The production of ethanol is dependent on the availability of sugars and the activity of enzymes (cellulase and amylase) produced by the *A. Niger*. In present study we have noticed that saccharification and fermentation are moving hand in glove with each other. Increased production of ethanol was observed when sugars are more in the suspension and the activity of enzymes decreased gradually when sugars were decreased gradually.

As we discussed above that addition of lactose, enhances the production of ethanol and concluded that for the enhancement of bio ethanol production enzyme inducers should be used. On account of this different concentration 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 results against experiment are given in Table 4. The comparatives results were obtain in between 48-96 hours of fermentation.

Table 4: Comparison of ethanol production between *S. cerevisiae and Z. mobilis*

Amount of α-amylase (g)	*Ethanol produced % in 96 hours*	
	Zymomonasmobilis	*Saccharomyces cerevisiae*
0.00	5.39	2.24
0.04	7.10	3.26
0.06	9.60	4.42
0.08	9.68	4.23
0.09	9.94	4.15

Based on the results, ethanol content increased in accordance with the addition of α-amylase enzyme concentration and long fermentation time. In general, the results tend to increase fermentation. As the amount of α-amylase increase the production ethanol % increases, in case of addition of 0.09 g of α-amylase the ethanol percentage was found maximum 6.34% in 48 hours and 9.94% in 96 hours of fermentation by *Zymomonasmobilis*, while at the same condition ethanol % was found 2.60 % in 48 hours and 4.15 % in 96 hours by *Saccharomyces cerevisiae* (Fig: 4). More and more the addition of α-amylase enzyme concentration, it tends to increase the number of levels of ethanol produced. 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 (Table 4).

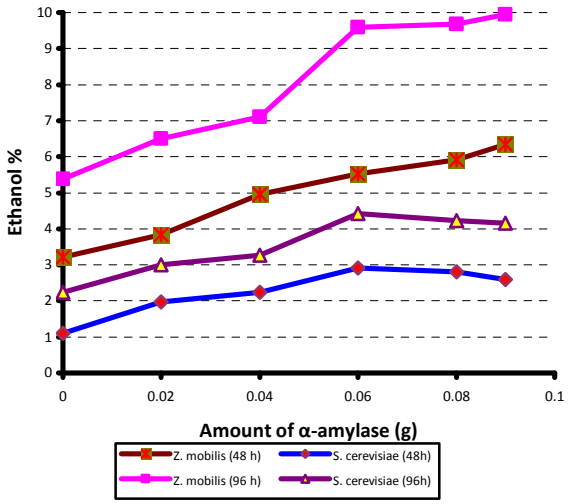

Figure 4: Effect of ethanol production by Z. mobilis and S. Cerevisiae at different concentrations of α-amylase in 48 hours

The addition of α-amylase enhances the metabolism path of *Z. mobilis* than *S.cerevisiae* it was also found by many of the

researcher. *Z. mobilis* can utilize glucose through the glycolysis pathway that converts six carbon atoms becomes redundant three-carbon molecule is pyruvate [23]. Then the molecule of pyruvate is converted into ethanol in anaerobic conditions. Ethanol from the fermentation product may be affected by the addition of α-amylase enzyme. α-amylase enzyme will cut ties α-1, 4 glycoside with the final product dextrin, maltose and glucose. Hence it is clear that, enzyme reaction velocity is directly proportional to enzyme concentration, the greater the amount of enzyme the faster the reaction and the more product produced α-amylase enzyme used for starch hydrolysis process that α-glycosidic bond break into glucose monomers.

Effective parameters such as reaction time, different sugar concentration and Ph also affected the production of biofuel from biomass. The maximum mono sugar production was achieved with low reaction temperature of 120°C and time above 30 min. Results obtained in the condition of higher temperature of 150°C with holding time of 15 min is also similar. However, prolonging the holding time up to 1 h, decreased the mono sugar concentration. There was little effect of reaction time on total sugar production especially after 15 min. Therefore, shorter reaction time is appropriate to optimize total sugar production. Highest glucose production was achieved either at 130°C with holding time of 15 min or at 150°C with holding time of 45 min. Profile of sugar production showed similar trend.

4. Conclusion

From the present study it can be concluded that algal biomass is more beneficial raw material than agro based raw materials for bio ethanol production as it is available abundantly in fresh water as well as marine eco-system and more importantly it is renewable. In case of Pretreatment with acid and scarification of algal biomass by Aspergillus Niger ethanol concentration was found lower (4%) in comparison to 6% by the direct scarification and formation with *Saccharomyces cerevisiae.* Hence these studies also concluded that in general, pre-treatment with Chemicals are not required for the algal material particularly for *Spirogyra*. In practice chemical treatments were employed to remove or denature unwanted materials particularly lignin (biomass) which are present along with cellulose and starch in agriculturally based raw materials which are extensively used in bio ethanol production. As *Spirogyra* cell wall is purely cellulosic and cell contains simple starch. In fact pre-treatment will damage the cellulose leading to less yield of alcohol when compared with untreated *spirogyra* biomass. Also the results obtained from the addition of 0.12g lactose, the maximum alcohol was found 6.6 (g/100g) in 6 days, which was higher than 6 (g/100g) of alcohol concentration without lactose.

Finally the comparative studies revealed that, for alcohol production from *Spirogyra* using *A.niger* chemical pre-treatment is not necessary and stationary method of fermentation is advisable, because it helps in elimination of shaking method and thereby cutting the cost of power consumption. For the enhancement of bio ethanol production enzyme inducers should be used. Addition of α-amylase enhances the production of biofuel from algal biomass. Excess α-amylase enzymes break the bond that is specific to the bond α-1, 4-glucosidic to produce glucose. While the chemical hydrolysis, using sulfuric acid (H_2SO_4) or acid chloride (HCl) will break the starch polymer chains at random, and not necessarily to produce glucose [24]. 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 (9.94%) for *Zymomonasmobilis* than *Saccharomyces cerevisiae* (4.15%). 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. Addition of 10 g/L glycerol and 2 g/L glucose as carbon source increases the growth rate of algal biomass and lipid production. Effect of concentration of sulphuric acid 3% (v/v) was found effective parameter for hydrolysis of algal biomass to produce mono sugars. 120^0C temperature was found optimum for hydrolysis of algal biomass and the hydrolysis time at 120^0C was observed 45 minutes. These results concluded for the maximum production of ethanol as a substitute of gasoline and for environmental friendly product. Domestic production and use of ethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, and reduce global climate change carbon dioxide build up. Ethanol, unlike gasoline, is an oxygenated fuel that contains 35%oxygen, which reduces particulate and NOx emissions from combustion.

In current the worldwide desire to reduce greenhouse gas emission will lead to an increased interest in renewable resources for energy production. Cellulosic and algal biomass materials are among the candidates to be used as a renewable resource. Ethanol has very good characteristics to be used as a fuel either in a neat form or in a mixture with gasoline. Bio ethanol is a domestically produced liquid fuel from cellulosic biomass resources. It is a high-octane fuel that can contribute substantially to the automotive fuel supply; Ethanol is a potentially clean-burning fuel that reduces smog and emissions of carbon monoxide. The use of gasohol (ethanol and gasoline mixture) as an alternative motor fuel has been steadily increasing around the world for a number of reasons.

Ethanol is the original fuel for automobiles and is also extensively used as an octane booster for low grade gasoline. Ethanol is an excellent motor fuel that burns much cleaner and more efficiently then doe's gasoline. Anhydrous, or dried ethanol, blends well with gasoline and ethanol-gasoline blends are increasingly common, even mandated by various government actions. The current standard of biofuel is E10, which are 10% ethanol and 90% gasoline. A change to E15 should be implemented shortly. E10 and E15 will work in any automobile and have been tested to not harm small 2 stroke and marine engines. Brazil has an E25 standard and it is possible that E15 is not the limit for unmodified engines in

the USA in the future. In the meantime, the some countries have adopted an E85 standard (85% ethanol, 15% gasoline) for flex fuel vehicles (FFVs), while Brazil goes all the way to pure ethanol (E100) at present.

References

[1] Brennan L, Owende P. Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews. 2010; 14:557-577.
[2] Chisti Y. Biodiesel from microalgae. Biotechnology Advances. 2007; 25:294-306.
[3] Borowitzka MA (1988). Fats, oils and hydrocarbons. In: Borowitzka MA, Borowitzda LJ, Ed., Microalgal biotechnology, Cambridge University Press, Cambridge, UK, pp. 257-287.
[4] Bosma R, van Spronsen WA, Tramper J, Wijffels RH (2003). Ultrasound a new separation technique to harvest microalgae. Journal of Applied Phycology 15: 143-153.
[5] Boichenko VA, Hoffmann P (1994). Photosynthetic hydrogen production in prokaryotes and eukaryotes-occurrence, mechanism and functions. Photosynthetica 30: 527-552.
[6] Chaumont D (1993). Biotechnology of algal biomass production: a review of systems for outdoor mass culture. Journal of Applied Phycology 5: 593-604.
[7] Fukuda H, Kondo A, Noda H (2001). Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 92: 405-416.
[8] Gaffron H, Rubin J (1942). Fermentative and photochemical production of hydrogen in algae. J Gen Physiol 26: 219-240.
[9] Gerpen JV (2005). Biodiesel processing and production. Fuel Process Technol 86: 1097-1107.
[10] Ghirardi ML, Zhang JP, Lee JW, Flynn T, Seibert M, Greenbaum E, Melis A (2000). Microalgae: a green source of renewable H2. Trends Biotechnol 18: 506-511.
[11] Gordillo FJL, Goutx M, Figueroa FL, Niell FX (1998). Effects of light intensity, CO2 and nitrogen supply on lipid class composition of *Dunaliella viridis*. J Appl Phycol 10: 135-144.
[12] Haesman M, Diemar J, Connor WO, Soushames T, Foulkes L (2000). Development of extended shelf-life microalgae concentrates diets harvested by centrifugation for bivalve molluscs-a summary. Aquaculture Research 31: 637-659.
[13] Harris EH (1989). In: The *Chlamydomonas* sourcebook: a comprehensive guide to biology and laboratory use. Academic Press, San Diego, USA.
[14] Johnson M, Wen Z (2010). Development of an attached microalgal growth system for biofuel production. Applied Microbiology and Biotechnology 85: 525-534.
[15] Dote Y, Sawayama S, Inoue S, Minowa T, Yokoyama SY (1994). Recovery of liquid fuel from hydrocarbon-rich microalgae by thermo chemical liquefaction. Fuel 73: 1855-1857.
[16] Khan S, Rashmi A, Hussain MZ, Prasad S, Banerjee UC (2009). Prospects of biodiesel production from microalgae in India. Renewable and Sustainable Energy Reviews, Elsevier.
[17] Um B H, Karim M N, Henk L L. Effect of sulfuric and phosphoric acid pretreatments on enzymatic hydrolysis of corn stover. Appl Biochem Biotechnol, 2003; 105: 115－125.
[18] Um B-H, Kim Y-S. Review: A chance for Korea to advance algal-biodiesel technology. Journal of Industrial and Engineering Chemistry. 2009; 15:1-7.
[19] Gharphuray, MM. Lee, YH. Fan, LT. (1983). Structural modification of lignocellulosic by treatement to enhance enzymatic hydrolysis. *Biotechnol.* 25, 157-170.
[20] Solomon, BO., Amigun, B., Betikue, TV., Ojumu, T., Layokun, SK. (1999). Optimization of cellulase production by Aspergillus flavus Linn. Isolates NSPR 101 grown on bagasse. JNSCHE. 18: 61-68.
[21] Miller, G.L. 1959. "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar." *Anal. Chem.* 31:426-428.
[22] Caputi A. Veda, M., and Brown, T. (1968). “Spectrophotometric determination of ethanol in wine. *American. J. Enol. Viticul.* 19: 160-165.
[23] Madigan, MT and John M.M. (2006). Biology of Microorganisms. *Pearson Education International*: United Stated of America.
[24] Manoj, S. Pradeep, K. Chandraraj, and N. G. Sathyanarayana (2005). Hydrolysis of starch by amylase from Bacillus sp.KCA102: a statistical approach. *Journal Process Biochemestry*. 40: 2499-2507.
[25] Zhang, Kunn and Feng Hong (2010). Fermentation potentials of Zymomonas mobilis and its application in ethanol production from low-cost raw sweet potato. *African Journal of Biotechnology*. 9 : 37.

Author Profile

Devendra Pratap Singh received the M.Sc. degrees in organic chemistry from Purvanchal University Jaunpur in 2002 and PhD degree in applied chemistry from C.S.J.M. University, Kanpur in 2013. In present he is working in Engineering College (Dr. Ambedker Institute of Technology for Handicapped, U.P., Awadhpuri, Kanpur-208024) as Assistant Professor.