

INTRODUCTION TO BIO-FUEL AND ITS PRODUCTION FROM ALGAE: AN OVERVIEW

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ABSTRACT

In today's scenario where there is too much dependence on fuel for meeting the requirements of the common masses and industries, and due to failure in the replenishment of non-renewable natural sources, arises an urge to supplement the requirements from renewable natural resources. Biofuel or in simple language fuel obtained from biological sources like crop wastes, exhausted mineral oils or fats etc. is a cogent alternative but none has proven to be a complete surrogate for petroleum and coal due to various reasons. In this context, a path breaking development of mass production of biofuel from algae comes into picture. In this following article the production of biofuel and methods of obtaining the same has been discussed.

KEY WORDS

Bio-fuel, Algae.

INTRODUCTION

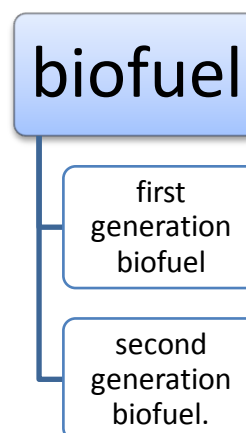
Now-a-days the common notion around the world is to reduce the consumption of fossil fuels and bring down effects of global warming. It will be an ideal state to live in a pollution free world without cutting on power requirements, however for this kind of environment we urgently need to reduce our dependency on fossil fuels. Thinking tanks find it difficult to come up with such an alternative fuels which could replace conventional fuels. No energy source has still been proven perfect. Like some fuels are environment friendly but not effective for mass production due to high initial prices etc. environmental friendly fuel derived from natural source is termed as biofuel.[1]. Bio-fuel has become attention-grabbing topic; this is attributed to the higher demands of fuel, power, depletion of fossil fuels, green house effect, rise in crude oil prices. Thus this is being worked out

as an alternate source of energy to the present day liquid fuels.

So what is a biofuel?

A biofuel is a type of fuel whose energy is derived from biological carbon fixation. Biofuels include fuels derived from biomass conversion, as well as solid biomass, liquid fuels and various biogases.

Classification of biofuel: biofuel can be classified as:



FIRST GENERATION BIOFUEL

Bioalcohol

Alcohol is produced by fermentation, generally from carbohydrates from starch rich plants like sugarcane, molasses to name a few and Bioethanol is a type of alcohol. Ethanol is also being produced from cellulose biomass which is being derived from non-food sources such as trees and grasses. Use of ethanol as a fuel is not new to the industry as earlier also it has been used as an additive up to 15% with gasoline fuel to increase the octane number of it, in turn increasing efficiency. Biobutanol (also called biogasoline) is often claimed to provide a direct replacement for gasoline, because it can be used directly in a gasoline engine. Methanol is currently produced from natural gas, a non-renewable fossil fuel. It can also be produced from biomass as biomethanol. Methanol could be an alternative to the hydrogen economy, compared to today's hydrogen production from natural gas. Butanol is considered of higher value as compared to other bio-fuels as it can be directly used with the current gasoline engines (without modification to the engine or car), [2] and they would produce more energy. ABE

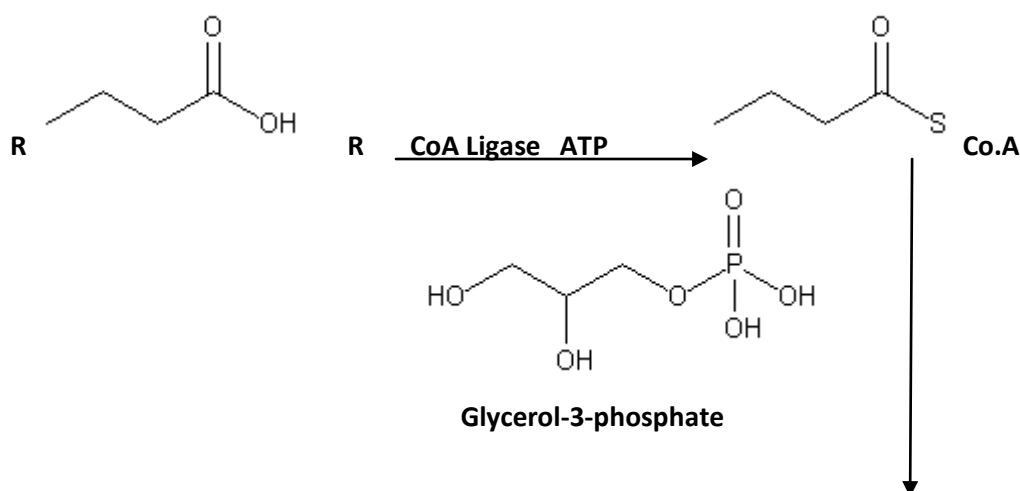
fermentation [acetone, ethanol] yields butanol. (Without modification to the engine or car), [2] and is less corrosive and less water soluble than ethanol.

Biodiesel

The most common biofuel in Europe is Biodiesel. Trans-esterification from oils or fats yields a liquid similar in composition to the crude oil by-product diesel (see chemical reaction for the synthesis of biofuel from transesterification of oils below). Chemically, it consists mostly of fatty acid methyl (or ethyl) esters. Animal fats, vegetable oils, jatropha, mahua, mustard, flax, sunflower, palm oil, hemp, field pennycress, pongamia pinnata and algae are amentionable few of biodiesel feedstocks. Pure biodiesel (B100) is the lowest emission diesel fuel.

In a number of countries, 5% biodiesel blend is widely used and is available at thousands of gas stations.[3][4] Biodiesel is an oxygenated fuel, meaning that it contains a reduced carbon content and higher hydrogen and oxygen content than conventional diesel. This improves the combustion of biodiesel and reduces the particulate emissions from un-burnt carbon.

Production of biodiesel



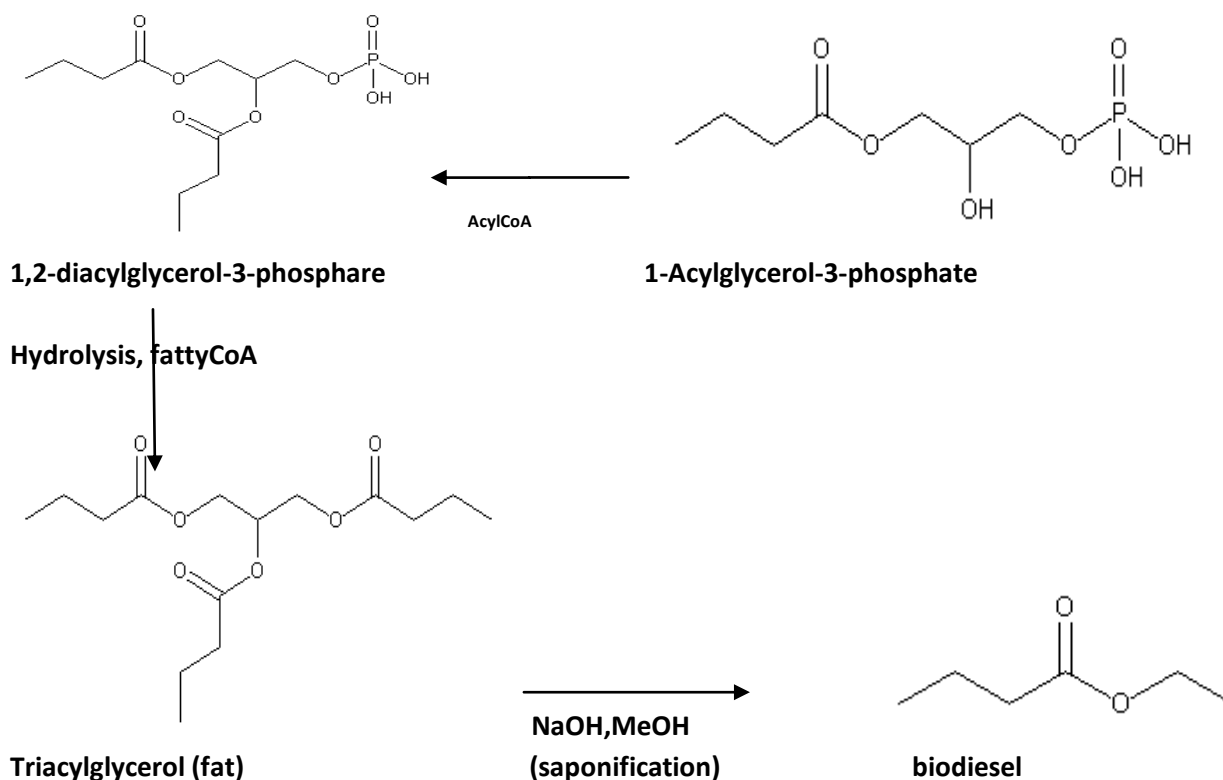
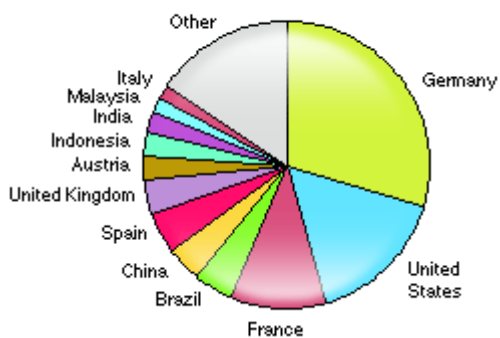


Fig:2 Chemical reactions involving production of biodiesel from exhausted fats/oils

The estimated world consumption of biodiesel is as follows:[15]

World Consumption of Biodiesel—2007



Green diesel

Green diesel result from hydrocracking of biological oil feedstocks which include vegetable oils and animal fats.[5][6] Long chain hydrocarbons are broken down into smaller chains in hydrocracking which is a method that

uses elevated temperatures and pressure in the presence of a catalyst to break down the long chains.[7]

Vegetable oil

Lower quality oil can and has been used for this purpose. Used vegetable oil is increasingly being processed into biodiesel, or (more rarely) cleaned of water and particulates and used as a fuel. Vegetable oil fuel must be heated to reduce its viscosity to that of diesel, either by electric coils or heat exchangers. This is easier in warm or temperate climates. Oils and fats can be hydrogenated to give a diesel substitute. The resulting product is a straight chain hydrocarbon with a high cetane number, low in aromatics and sulfur and does not contain oxygen. Hydrogenated oils can be blended with diesel in all proportions.[8]

Biogas

Biogas is methane(CH₄) produced by anaerobic digestion of organic material by anaerobic microbes.[9] It can be produced either from biodegradable waste materials, and the use of energy crops fed into anaerobic digesters to supplement gas yields. Farmers produce biogas from manure from their cows and other farm wastes by using an anaerobic digester (AD).[10]

Syngas

Syngas is a mixture of hydrogen, carbon monoxide and other hydrocarbons. Partial combustion of biomass, the process proceeds with combustion with an amount of oxygen that is not sufficient to convert the biomass completely to carbon dioxide and water.[8] Before partial combustion the biomass is dried, and sometimes pyrolysed.

- Can be used directly in internal combustion engines, turbines.[11]
- Can be used to produce methanol, hydrogen, to produce a diesel substitute, or a mixture of alcohols that can be blended into gasoline. Gasification normally relies on temperatures >700°C.

Solid biofuels

Solid bio-fuels include wood, sawdust, grass trimmings, domestic refuse, charcoal, agricultural waste, non-food energy crops, and dried manure. One of the disadvantages of using the oldest solid bio-fuel wood is the amount smoke it creates. Advantage of solid biomass fuel is that it is often a by-product, a residue or waste-product of other processes, such as farming, animal husbandry and forestry, this way we do not invest separately to get the fuel.[12] In theory this means there is no competition between fuel and food production, although this is not always the case.[30]

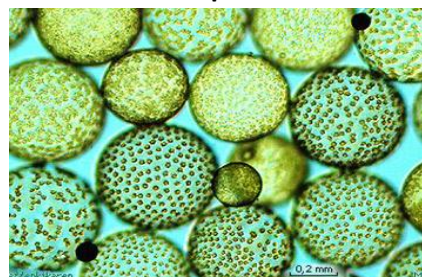
The other disadvantages of raw biomass are that it emits considerable amounts of pollutants such as particulates and PAHs (polycyclic aromatic

hydrocarbons). A derivative of solid biofuel is biochar, which is produced by biomass pyrolysis. Bio-char made from agricultural waste can substitute for wood charcoal. As wood stock becomes inadequate this alternative is gaining ground. For example, in eastern Democratic Republic of Congo, biomass briquettes are marketed as alternatives to charcoal to protect Virunga National Park from deforestation related with charcoal production.[13]

Second generation biofuels (advanced biofuels)

These are also known as advanced biofuels. Many of the biofuel in this class has been derived from algae and more recently from microalgae which are genetically modified version of algae of common use. Co-cultures of *Shewanella* and *Synechococcus* which have been genetically modified has is now commercially utilized for biofuel production but many of such biofuels are under development, examples of these include Cellulosic ethanol, Algae fuel [14]. BioDME, Fischer-Tropsch diesel, DMF, bio-hydrogen, biohydrogen diesel.

Why biofuel from algae is more preferred to biofuel from plant sources?



Even though many crops like corn can be utilized for the production of biofuel, but none of the methods can be utilized effectively when yield is a major concern. Biofuel from plant source gives very low yield per hectare of land. Fuel obtained from Algae yields considerably more energy than other bio-fuels (per unit area). Algae can be grown on land that has become unsuitable for agriculture, and arable lands as well. This advantage is also commercially exploited for

mass production of biofuels. Companies include Solazyme, Sapphire Energy and OPXBIO. Further the yield can be increased by using photo-bio reactors which can drastically change the yield of the biofuels from few kgs. to gallons. [16]

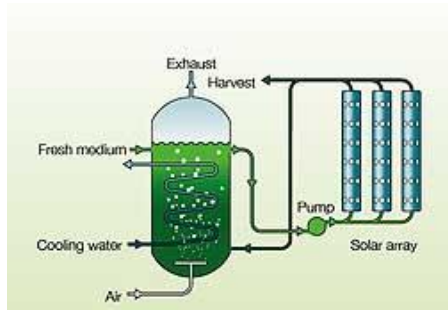


Fig: a photo bio-reactor

Algae-based biofuels has both its pros and cons which includes:

Pros

- Reduction in the use of Crude Oil based fuel
- Reduction in carbon dioxide (CO₂) levels
- Increased energy output as compared to other bio-fuel substituent
- Reduction in degradation of land due to oil mining
- Renewable source of energy can be grown anywhere provided temperature conditions are met

Cons

- High initial cost as in case of every other renewable source of energy
- Needs some rare elements in extraction of fuel
- Requirement of proper temperature conditions and sizeable amount of land for growth

Methods of extraction of biofuel from algae:

Lipid Extraction from algae

Lipid extraction from algae is one of the methods for the production of biofuel. Lipid extraction includes the subsequent approaches:

- Solvent-based extraction depending on microwaves

- sonication for cell interruption for example using solvents to extract algal cells without disrupting cellular functions
- Extraction bypass schemes that try to obtain algal systems that exude products directly into the growth medium.

Mechanical Disruption (i.e., Cell Rupture)

For the biofuels dependent on the accretion of intra-cell lipids to be successful,

- extracting solvent should infiltrate through the lipid enclosing matrix
- Physically contact the lipid material
- Solvate the lipid

Use elevated temperatures and pressures which force the solvent into the required biopolymer contact and be compensated for by effective mechanical disruption. Mechanical disruption can include bead mills (or bead-beating), cell homogenizers, autoclaving [17], ultrasounds. Non-mechanical techniques include processes such as freezing, acid, base, and enzyme reactions [17], use of organic solvents, and osmotic shock to name a few. The use of microwaves to disturb cells and augment efficiency of vegetable lipid and oil extraction is a noteworthy advancement [18], even if applications outside methodical labs are blurred. Treatment of sewage sludge with pulsed sonication has enhanced methane gas production and reduction in biosolids in sludge digestion [19].

Organic Co-solvent Mixtures

In the co-solvent extraction procedure [20], after the reaction is accomplished, water is added to the co-solvent (chloroform) mixture until a two-phase system develops where water and chloroform break up into two immiscible layers. Lipids can be recovered for analysis as they separate to the chloroform layer. Subsequently, other combinations of co-solvents projected for the extraction of lipids: hexane/isopropanol for tissue [21]; hexane/ethanol for microalgae [22];

and hexane/isopropanol for microalgae [23]. The hexane system is supported because hexane and alcohol will readily separate into two separate phases when water is added, thereby improving downstream separations. Likewise, less volatile and hazardous alcohols have been suggested in place of methanol. One example is the hexane/ethanol extraction co-solvent system [24]. In other cases, single alcohol (e.g., 1-butanol and ethanol) solvents have been tested [23]. In these applications, the alcohol is first added as the extracting solvent. Separation is then achieved by adding both hexane and water in proportions that create a two phase system (hexane and an aqueous hydroalcoholic) that partition the extracted lipids into the nonpolar hexane [25].

The results suggest that the most vital condition when selecting a co-solvent system to extract lipids is:

- The capability of a more polar co-solvent to disturb the cell membrane and thus make it adequately porous and
- The ability of a second less polar co-solvent to better match the polarity of the lipids being extracted.

Application of Organic Two-Solvent Systems for Lipid Extraction from Microalgae:

The sequence of solvent addition also affects extraction [26]. Starting from freeze dried biomass, it has been verified that, the extraction of lipids was notably more efficient when solvents were added in order of increasing polarity (i.e. chloroform, methanol, and then water)[26]. The results can be justified as, in terms of initial contact of the biomass with nonpolar solvents deteriorating the connection between the lipids and cell structure, preceding their dissolution in the monophasic system of water, chloroform, and methanol. These results have a key influence on liquid phase extraction systems applied to moist biomass as, it is

suggested that the water will form a solvent covering around the lipids, making it difficult for less polar solvents such as chloroform to connect, solubilise, and extract the lipids.

Direct Transesterification of Lipids into Fatty Acid Methyl Esters (FAMES)

Lepage and Roy (1984) offered the direct transesterification of human milk without prior extraction or refining for improved recovery of fatty acids. In general, the approach proposed a one-step reaction that added the alcohol and acid catalyst directly to the biomass sample and followed with heating at 100°C for an hour under sealed conditions would increase fatty acid concentrations measured, and provide relatively high recoveries of volatile medium chain triglycerides, and eliminate the need to use antioxidants to protect unsaturated lipids. Efforts in this direction have shown that when employing direct transesterification with an acid catalyst (i.e., acetyl chloride), the efficiency of the reaction improved when a second “less polar” solvent for example diethyl ether or toluene were mixed with the methanol to alter the polarity of the reaction medium [27]. These results suggest that the usefulness of the second co-solvent depends upon its ability to solubilise the target lipids along with its miscibility with methanol. However the earlier co-solvent systems stay largely bench-scale methods which are difficult to scale up to industrial processes due to solvent toxicity and the low carrying capacity of the solvents. As a result, single solvent systems at elevated temperature and pressure have the following advantages:

- High temperature and pressure increase the rate of mass transfer and degree of solvent access
- Increased pressures reduce the dielectric constant of otherwise immiscible solvent [28].

Accelerated Solvent Extraction

Accelerated solvent extraction was proposed in the mid 1990s [29], using the technique on 1 - 30 g samples of dried biomass. It uses organic solvents at high pressure and temperatures usually above their boiling point. The process involves, enclosing a solid sample in a sample cartridge which is prior filled with an extraction fluid and used to statically extract the sample under elevated temperature (50 - 200°C) and pressure (500 - 3000 psi) conditions for short time periods (5 - 10 min). Compressed gas is used to remove the sample extract from the cell into a collection vessel. This method is applicable to solid and semi-solid samples that can be retained in the cell during the extraction phase. Apart from improving yields and reducing extraction time, Accelerated Solvent Extraction can also be applied to efface co-extractable material from various processes, to selectively extract polar compounds from lipid-rich samples. Accelerated Solvent Extraction process is more effective if extracting solvent, sample-solvent ratio, extraction temperature, and time have been optimized [30]. The performance of Accelerated Solvent Extraction was compared to that of traditional Folch method for microalgae grown on dairy manure effluent [31]. The Accelerated Solvent Extraction method, depending on the solvent, extracted 85 - 95% of the fatty acid substance in the reaped microalgae compared to 44 - 55% of the fatty acids by the Folch method in the first solvent extraction cycle.

Subcritical Water Extraction

This method is based on the use of water, at temperatures below the critical temperature, and pressure high enough to keep it in the liquid state [32]. The technique, was initially applied to biomass hemicellulose as a pre-treatment to its use as a fermentation substrate [33]. However, now it has been tested for the selective extraction of essential oils from plant matter

[34], functional ingredients from microalgae [35], and saponins from oil-seeds [36]. In technique the water, under condition consideration, becomes less polar and organic compounds tend to be more soluble than at room temperature. In addition, products can easily be separated as the water is cooled back down to room temperature, and products miscible at the high temperature and pressure become immiscible at lower temperatures. A major constraint, however, is the difficulty with proposing a large scale system and the high-energy load required to heat the system up to subcritical temperatures. Additional energy challenges are created as a large-scale design would require a considerable cooling system to cool down the product to room temperature to avoid product degradation.

Heterotrophic Production

One of the many other methods for extraction and fractionation is the production of oils using heterotrophic algae. In this approach non-photosynthetic algae are developed using sugars as energy source and using traditional industrial fermentation equipment [37]. Several companies have contrived algae that secrete oil into the fermentation media which can be recovered and later refined into a biofuel; this methodology drastically reduces capital and operating cost for the extraction procedure. The benefits of this approach range from the use of conventional fermentation systems ease of scale-up, averting expensive extraction schemes, ability to maintain the integrity of the fermentation catalyst to the use of sugar-based feedstock.

Direct Production of Biofuels from Algae

Heterotrophic growth has certain benefits in terms of process cost as it can eliminate many process steps and also permits for maintaining controlled conditions. Such a system can engender very high biomass and a high proportion of that biomass as lipid. There are several biofuels that can be produced directly

from algae, including alcohols, alkanes, and hydrogen.
Alcohols [38]

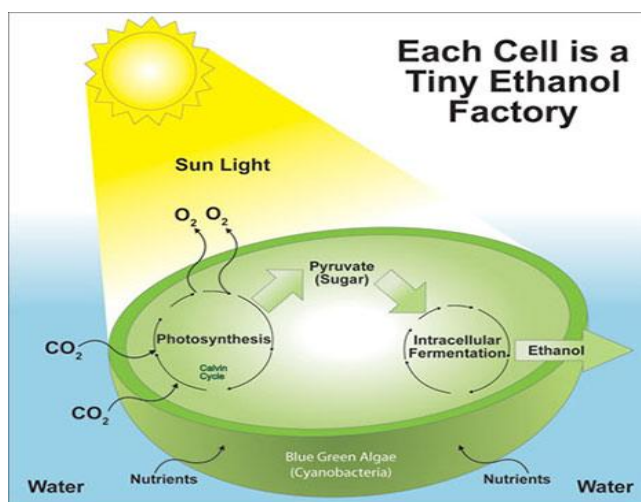


Fig: scheme for production of ethanol in algae

Production of alcohols like ethanols is accomplished through the creation and storage of starch during photosynthesis inside the algae, or by feeding sugar to the algae directly, and subsequent anaerobic fermentation of these carbon sources to produce ethanol under dark conditions. Algae, such as *Chlorella vulgaris* and *Chlamydomonas perigranulata*, are competent of yielding ethanol and other alcohols during heterotrophic fermentation of starch [39]. If alcohols can be mined directly from the algal culture media, the process can be considerably less energy demanding than competitive algal biofuel processes. This process usually consists of closed photo-bioreactors employing sea-water with metabolically superior cyanobacteria [40]. (One key aspect of the system is that a source of cheap carbon, such as a power plant, is typically used to supply CO₂ to the bioreactors to accelerate the algae growth. An example of this process technology links sugar production to algal photosynthesis. There are claims that this process may consume more than 90% of the system's CO₂ through photosynthesis, wherein a portion of the carbon in these sugars is converted into ethanol. The ethanol is secreted into the culture media and is collected in the

headspace of the reactor, purified, and stored.) This expertise is estimated to yield 4,000 - 6,000 gallons per acre per year, with prospective increasing up to 10,000 gallons per acre per year within a few years with noteworthy research. It is theoretically estimated that one ton of CO₂ is converted into approximately 60 - 70 gallons of ethanol with this technology.

Alkanes

In addition to alcohols, alkanes may be produced directly by heterotrophic metabolic pathways using algae. Rather than growing algae in ponds or enclosed in plastic tubes that utilize sunlight and photosynthesis, algae can be grown inside closed reactors without sunlight. The algae are fed sugars, the cheap availability of which is a key consideration for cost-effective production of biofuels; these sugars are themselves available from renewable feedstock such as lignocellulosic biomass, in a pressure and heat-controlled environment.

Hydrogen

Biological production of hydrogen technologies present wide range of methods to generate hydrogen which include indirect biophotolysis, direct biophotolysis, photo-fermentation, and dark-fermentation. Yet several challenges wait to

be addressed before biological hydrogen production can be considered a viable technology, some of which are the constraint of photosynthetic hydrogen production by accretion of a proton gradient, competitive inhibition of photosynthetic hydrogen production by CO₂, necessity for bicarbonate binding at photosystem II (PSII) for efficient photosynthetic action, and competitive drainage of electrons by oxygen in algal hydrogen production.

CONVERSION OF ALGAL EXTRACTS

Chemical Transesterification

This process is engaged to change triacylglycerols extracted from algae to fatty acid methyl esters (FAME), which is simply a process of replacement of an alcohol group from an ester by another alcohol [41]. It can be achieved via catalytic or non-catalytic reaction systems using different heating systems required to initiate the reaction. This technology is relatively mature and has been verified to be a standard in the conversion of vegetable oils into biodiesel [42]. Vaporizing under high vacuum, biodiesel is separated from the ether. Acid catalyzed transesterification is another way of converting algal extracts. This involves soluble bases being replaced liquid acid catalyst some of which are H₂SO₄, HCl [43]. Acid catalysts have advantages, yet they at present are not chosen due to their lower activity than the conventional transesterification alkaline catalysts. Recently, it has been verified that HPA-catalyzed transesterification of vegetable oil accomplishes higher reaction pace than standard mineral acids due to their higher acid strength [44]. The apparent higher activity of certain HPAs with respect to polyoxometallates of higher strength resulted in lower pre-treatment temperatures.

Biochemical Conversion (Enzymatic)

The advantage of chemical processes giving high conversions of triacylglycerols to esters is

dwarfed by the shortcomings such as the process being energy demanding, intricacy in separating the glycerol, and require elimination of alkaline catalyst from the product and treatment of alkaline wastewater. A solution to these limitations is the use of biocatalysts (lipases) in transesterification of triacylglycerols for biodiesel manufacturing [45]. One vital point that needs to be addressed is the solvent and temperature limitations of the enzymes in order to facilitate efficient biocatalytic processing. Although enzymatic approaches have become more attractive, the process stands unproven at a large scale mainly due to the rather high price of lipase and its ephemeral life caused by the effects of excessive methanol and co-product glycerol. The presence of solvents is occasionally necessary to augment the solubility of the triacylglycerols during the extraction process, and the enzymes used in the downstream change must be able to fulfil their purpose in the presence of these solvents to varying degrees to enable cost-effective biofuel production [46]. Solvent engineering method can also be used to enhance the lipase lipase-catalyzed methanolysis of triacylglycerols for biodiesel production [47][48]. Much research is still necessary in discovery, engineering, and optimization of enzymatic processes, which are competent of producing the reactions in a range of environments and on different types of oil feedstocks [49]. Bioprospecting for the enzymes in extreme environments may produce enzymes with convincing characteristics that are more suitable for industrial applications [50]. Enzyme immobilization would play a vital role in developing an economic method of biocatalytic transesterification [51].

Catalytic Cracking

Although catalytic transesterification is a very effective and relatively economical, these catalysts require purification and removal from

the product stream, which increases the overall cost of execution. One likely solution to this is the improvement of immobilized heterogeneous and/or homogeneous catalysts that are very efficient and inexpensive [52]. Acid and basic catalysts are usually classified as Brönsted or Lewis catalysts. Lewis acid catalysts, $AlCl_3$, $ZnCl_2$ to name a few; have been verified as a feasible means of converting triacylglycerols into fatty acid methyl esters. One of the many other methods include, catalysts derived from titanium compounds having the general formula ATi_xMO ; where A is a hydrogen atom/an alkaline metal atom, M a niobium atom or a tantalum atom, and x is number less than 7; is employed in vegetable oil transesterification. The catalysts achieved are steady and give high glycerol yield. A typical FAME yield of 91% and glycerol yield of 91% were obtained in a fixed-bed reactor at $200^\circ C$ and 35 bar, using $HTiNbO_3$ as the catalyst. Vanadate metal compounds are stable, active catalysts during transesterification, with $TiVO_4$ being the most active [53]. This catalyst is also more active than $HTiNbO_3$, producing the same yields with lower residence times. (no language to edit in blue) Double-metal cyanide Fe-Zn proved to be promising catalysts resulting in active transesterification of oil. These catalysts are Lewis acids, hydrophobic (at reaction temperatures of about $170^\circ C$), and insoluble. Moreover, they can be used even with oils containing significant amounts of free fatty acids and water, probably due to the hydrophobicity of their surface. The catalysts are active in the esterification reaction, reducing the concentration of free fatty acids in non-refined oil or in used oil. Other catalyst examples include MgO , CaO , and Al_2O_3 .

At these temperatures about ($220-240^\circ C$), the process pressure is high (40 - 60 bar), which interprets to high construction requirements and high plant design capital. The catalysts used must

most importantly resist poisoning and the leaching of active components. One particular concern is the consistency and the life of the catalysts in the reaction environment.

RECENT APPLICATIONS OF BIO-FUEL

150mpg Algae-Powered Toyota Prius

One of the first successful test was an algae fuel powered vehicle which was a modified Toyota Prius known by the name Algaeus which gave a mileage of about 63 kilometers on a litre of green fuel. This car was launched in San-Francisco. It derived power from green fuel, which was developed by Sapphire Energy. The only drawback was its cost.

Engineers Tap Algae Cells for Electricity

Light energy is converted to chemical energy and stored in bonds of sugar by plants during the photosynthesis process. This is later utilized as their source of energy. The process of conversion takes place inside a chloroplast, which is believed to be the source of power. Chloroplasts are responsible for the green shade of the plants and algae. The actual process that takes place during photosynthesis is that the water is broken down to oxygen, the negative charged electrons and the positive charged protons. The electrons are stored in the chloroplast, this reaches excited levels when leaves are exposed to sunlight. The excited electrons pass through a number of layers of proteins where they are absorbed. After this the energy from the electrons is used to make sugar until the entire energy is utilized.

The New Role of Microbes in Bio-Fuel Production

Plants and microbes both are used to refine and synthesize to produce bio-fuel. This helps conserve fossil fuels and are considered to be conservational and renewable sources of energy. Recent studies indicate that microbes can generate fuel from proteins, as a substitute to

using them for its growth. (The research is being carried out at university of california, LA)

CONCLUSION

Summing up, bio-fuels based on algae are being seen as an alternative source of energy. It is still in the embryonic stages as all results are being analyzed before starting the substitution primary source of energy, Crude Oil. The main factors that have to be looked upon the use of these fuels are their production, the by-products of their use, and the cost of production. The source of energy from algae seems to be promising, provided the above mentioned factors are taken care of.

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