

A Comprehensive Review on Enhancing Algal Biology to Reduce Production Costs

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Abstract- The energy crisis and global warming due to rapid increase in industrialization are the issues which compelled the scientist and the environmentalist to think about the more environment friendly solution which will satisfy the current energy consumption. Biofuel which is a fuel made by contemporary biological process is one such solution which gives energy security and economic development to our motherland. The Microalgae are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms that grow rapidly due to their simple structure. They can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner. The developments in microalgal cultivation and downstream processing (e.g., harvesting, drying, and thermochemical processing) are expected to further enhance the cost effectiveness of the biofuel from microalgae strategy. Our review focuses on the research achievement of metabolic engineering of algae for the biofuel production. It is concluded that the Fourth generation biofuel (FGB) uses genetically modified (GM) algae to enhance biofuel production. This paper reviews on technologies for converting biomass into liquid fuels, Economics of algae biodiesel production and also enhancing algal biology to reduce production costs.

Index Terms- Biodiesel, cost effectiveness, Fourth generation biofuel (FGB), Microalgae, Transesterification

I. INTRODUCTION

Due to the increase in the price of the petroleum and the environmental concerns about pollution coming from the car gases, the biodiesel is becoming a developing area of high concern. There are different ways of production, with different kinds of raw materials: refined, crude or frying oils. Figure 1 represents the first to the fourth generation biodiesel. Also there are different types of catalyst, basic ones such as sodium or potassium hydroxides, acids such as sulfuric acid, ion exchange resins, lipases and supercritical fluids.

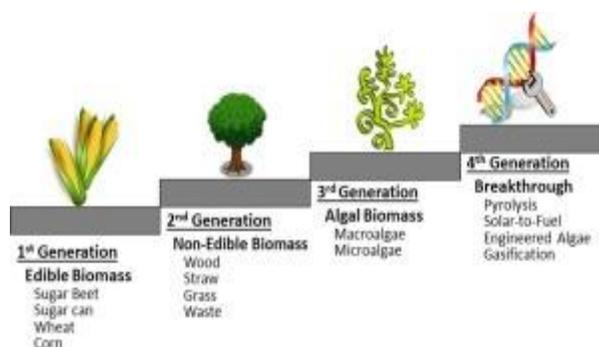


Fig.1. First to fourth generation

One of the advantages of this fuel is that the raw materials used to produce it are natural and renewable. All these types of oils come from vegetables or animal fat, making it biodegradable and nontoxic [1]. The Bio-based alternative fuels such as ethanol, biodiesel have been in focus for the reasons which are by now well understood. Heavy consumption of fossil resources, effect on global warming and concerns of energy security are main drivers for growth of biofuels. Recent studies on life cycle analysis (LCA) of biodiesel have shown a very appreciable reduction of greenhouse gas (GHG) by their use as a blend component of transport fuel. Biodiesel produced by transesterification of vegetable oils and animal fats using homogeneous base catalyst has seen several folds increase in last few years for their commercial production and use as a blending component in transport fuels. Fatty acid methyl esters (FAME) have found favour for use as a blend component of petro-diesel fuel due to lack of aromatics, negligible sulfur content, higher lubricity and very high cetane values. FAME (Biodiesel) mixes freely in all proportions with petro-diesel and its use has been approved by almost all the major automotive manufactures. Biodiesel can be used in conventional compression ignition engines, which need almost no modification. Though biodiesel has been approved for use in automobiles as a blend with normal petro-diesel, there are very stringent quality norms prescribed by several countries. which any biodiesel must meet before it can be used as an auto fuel component. The widely used industrial method for the

commercial production of biodiesel from vegetable oils, fats is a base catalyzed transesterification process using KOH or NaOH as the homogeneous catalyst and MeOH as the lower alcohol.

II. TECHNOLOGIES FOR CONVERTING BIOMASS INTO LIQUID FUELS

It is possible to produce a variety of liquid biofuels from the cellulosic biomass (next generation feedstock), however its cost is not competitive with petro fuels, even with the recent price hikes. The Multiple steps are required for the conversion into a liquid fuel. Recent studies have indicated that 6:10% of the energy in biomass is utilized in feedstock preparation. The two primary conversion pathways are thermo chemical and biochemical process.

2.1. Thermo chemical conversion

These technologies typically use the high temperatures and pressure to depolymerize lignocelluloses into small molecular weight organic and inorganic compounds which can be transformed into hydrocarbons, alcohols, aromatics and other organics.

2.2. Gasification

The two major thermo chemical pathways for the converting biomass to gaseous and liquid fuels are the gasification and pyrolysis, the Gasification is the thermo chemical partial oxidation of hydrocarbons in the biomass at high temperature ((800–1000°C)) to a combustible gas mixture ((typically its containing H₂, CH₄, CO₂ and C₂H₄)). In the gasification procedure, the biomass is thermally decayed at great temperature in O₂ hungry environment to avoid the explosive gas from combustion. This synthesis gas a mixture of ((CO₂, CO, CH₄ and H₂)) is transformed to a liquid fuel such as synthetic diesel using FischerTropsch technology [2].

2.3. Pyrolysis

The Pyrolysis method depends on using high temperature in the non-existence of O₂ to transforms the biomass into bio-oil. Pyrolysis can be classified as fast, conventional or flash according to the heating rate, particle size, operating condition of temperature and solid seat time. For instance, if bio-oil yield is to be maximized fast, this is required that the biomass is heated to (500°C) for around (10 second).The pyrolysis temperatures are approximately (475°C), where gasification is ready at temperatures fluctuating from (600 to 1100°C).

2.4. Biochemical conversion

In Biochemical conversion, the process is described as enzymatic hydrolysis in addition to the microbial digestion. It includes the decomposition of the biomass into hemi cellulose, cellulose and lignin and transforming, the hemi cellulose and cellulose into fermentable sugars, subsequently use of yeast and specific bacteria to transform the sugar to ethanol. This method needs a pre-treatment stage (steam, ammonia and acid) to decomposition of the biomass into fluid slurry. The Use of acid to destroy lingo cellulosic fibers can be used also to destroy much of the hemi cellulose sugar earlier then can be fermented into ethanol, causing low incomes [3].

2.5. Anaerobic digestion

The natural process is called the anaerobic digestion and is the microbiological conversion of organic matter to CH₄ in the deficiency of O₂. The biochemical transformation of biomass is finished throughout alcoholic fermentation to yield liquid fuels, while the fermentation with anaerobic digestion produce biogas ((H₂, NH₄, CO₂ and CH₄)) generally by four stages that includes (hydrolysis, acidogenesis, acetogenesis and methanogenesis). The decomposition is caused by natural bacterial action in different stages and occurs in a variety of natural aerobic environments including water sediment, waterlogged soils, natural hot springs, ocean thermal vents and the stomachs of various animals.

2.6. Fermentation and hydrolysis

There is some methods permit biomass to be converted into gaseous fuel, for instance, (CH₄ or H₂). One genetic-modified procedure uses bacteria and algae to yield (H₂) immediately instead of the usual biotic energy carriers. The second way uses agricultural remains in fermentation for produce biogas. This method is documented and used for waste treatment in a wide range. The high temperature in gasification supplies a crude gas for the production of hydrogen by a second reaction step. in biogas, there is the opportunity of using the compact byproduct as a biofuel. Traditional fermentation plants producing biogas are in routine use, ranging from farms to large municipal plants [4].

2.7. Transesterification

The Transesterification is a chemical combination of bio-oil with an alcohol (the methanol or ethanol) [5]. The resulting of biodiesel is an alkyl ester of the fatty acid, which contains an alcohol group attached to a single hydrocarbon chain comparable in length to that of diesel (C₁₀H₂₂–C₁₅H₃₂). The transesterification method means biodiesel production [5] in which glycerin is extracted from the fat or vegetable oil [6].The Plants late two products are methyl esters and glycerin that is used in the soaps and other products. Transesterification of the triglycerides can be improved by using catalysts which are divided in to alkali, acid and enzyme. Alkali-catalyzed transesterification is the best and faster than acid-catalyzed transesterification, so it used commercially [7, 8].

Chemical composition

The Natural oils and fats are the esters of glycerol and fatty acids.They are called the glycerides or triglycerides. There are two kinds of fatty acids, saturated fatty acids are polarized and contain a single carbon bond, while unsaturated fatty acids include one or more carbon-to-carbon doubled bonds and are polarized. Figure 2 represent the Triglycerides transesterification reaction with methanol. Examples of the common fatty acids are stearic, oleic, linolenic and the palmitic [9].

Transesterification reaction

The Transesterification of vegetable oils with alcohol is the best method for biodiesel production. There are two transesterification methods, which are: (1) with catalyst , (2) without catalyst. The utilization of the different types of catalysts improves the rate and yield of biodiesel. The transesterification reaction is reversible and excess alcohol shifts the equilibrium to

the product side shows the general equation of transesterification reaction [fig.2.] [10]. Many different alcohols can be used in this reaction, including, the methanol, ethanol, propanol, and butanol. The methanol application is more feasible because of its low cost and physical as well as chemical advantages, such as being polar and having the shortest alcohol chain. According to R1, R2, and R3 are the long chains of hydrocarbons and carbon atoms is called fatty acid chains. The reaction is based on one mole of the triglyceride reacting with three moles of methanol to produce three moles methyl ester (biodiesel) and one mole glycerol. Generally the transesterification reaction involves some critical parameters which significantly influence the final conversion and yield. The important variables are: the reaction temperature, free fatty acid content in the oil, water content in the oil, type of catalyst, amount of catalyst, reaction time, molar ratio of alcohol to oil, type or chemical stream of alcohol, use of co-solvent and mixing intensity.

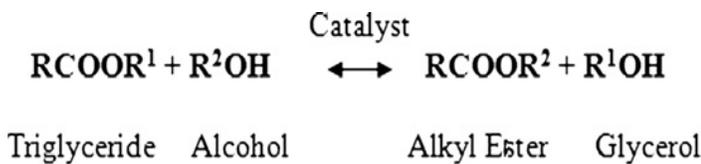


Fig. 2. General transesterification reaction equation.

The Biodiesel is usually obtained by transesterification of the oils or fats by reacting a short chain alcohol, like methanol, in the presence of a homogeneous base-catalyst (typically NaOH). The most common raw materials for production of the biodiesel are vegetable oils from sunflower, soybean, oil palm and others. The transesterification reaction mainly depends on the nature of the feedstock, catalyst concentration, the molar ratio alcohol-oil, temperature, agitation rate, pressure and reaction time as well as moisture content and amount of free fatty acids. The commonly used catalyst for production of biodiesel is the homogeneous catalysis. The homogeneous catalysis used for the biodiesel production can be divided mainly in alkaline and acidic catalysts. The Heterogeneous catalysts (solid phase) is also used.

III. CLASSIFICATION OF BIOFUELS

Biofuels are generally classified into 4 categories

A. First Generation Biofuels

The very first generation of biofuels began in the late 1990's when corn farmers of the United States of America synthesized fuel out of corn to meet the need to run their machinery. The first Generation of Biofuel was derived from sugarcane, feedstocks, corn, vegetable oils.

B. Second Generation Biofuels

The beginning of 21st century marked the entry of second generation of biofuels in the market. The second generation also marked the introduction of advanced biogenesis in this field. In second generation biofuels, the roots of certain sugar producing or sugar rich plants were cultured in a special way such that directly oil is extracted from the roots. This generation was almost Carbon free or Carbon negative in terms of CO₂ released in the whole

process. Common second-generation biofuel sources include lignocellulosic feed stocks, grasses, Jatropha, seed crops, waste vegetable oil, and solid waste and forest residues.

C. Third and fourth generation biofuel feedstock

Recently, the algae have received a significant interest as alternative biofuel feedstock because of their higher photosynthesis and fast growth rate as compared to any terrestrial plant. The Algae may contain up to (70%) of lipid on a dry weight basis [11,12] and can grow in the liquid medium utilizing different wastewater streams ((saline/brackish water/coastal seawater)) resulting in reduced freshwater demand.

Recent research activities have been focused on the search for the ideal combination of algal species with high lipid content and their optimum growth conditions. Several algal species such as *Botryococcus braunii*, *Chaetoceros calcitrans*, several *Chlorella* species, *Isochrysis galbana*, *Nanochloropsis*, *Schizochytrium limacinum* and *Scenedesmus* species have been studied as a potential source of biofuel. Among these, the highest average lipid content and the biomass was obtained in *Chlorella* but has low triglyceride content. Some algal species like *Botryococcus braunii*, *Nannochloropsis* and *Schizochytrium* sp. can produce (25-75%), (31-68%) and (50-77%) of the triglycerides on dry cell weight basis, respectively, though the yield of the biomass is low in each case [13,14]. It remains a common observation that fast growing algae (such as *Spirulina*) have low oil content whereas high lipid containing algae are slow growing organisms. The identification of correct species with high biomass as well as high lipid content is necessary for commercialization of algal biofuel. Type of cultivation (phototrophic and heterotrophic) also affects the biomass and lipid yield in same microalgal strain. The scientists are looking for proper cultivation method for these species which will lead to the maximization of lipid contents to make it more cost effective and sustainable source of biofuel. In this context the genetic modification, metabolic engineering could be promising alternative to increase the lipid content and biomass yield of the algae. The pathways for the lipid anabolism and catabolism are investigated to identify and modify key enzymes of these pathways (TAG), suggesting that the shunting of photosynthetic carbon partitioning from starch to TAG synthesis may represent a more effective strategy than direct manipulation of the lipid synthesis pathway to overproduce TAG, the modification in CoA-dependent 1-butanol production pathway into a cyanobacterium, *Synechococcus elongatus* can produce the butanol from CO₂ directly [15].

D. Fourth generation biofuels—solar biofuels

By synthetic biology technologies the Fourth generation biofuels take advantage of synthetic biology of algae and cyanobacteria [16,17] which is a young but strongly evolving research field. The Synthetic biology comprises the design and construction of new biological parts, devices and systems, and the re-design of the existing, natural biological systems for useful purposes. It is becoming possible to design a photosynthetic, non-photosynthetic chassis, either the natural or the synthetic, to produce high quality biofuels with high PFCE. For the first, second and the third generation biofuels, the raw material is either biomass or a waste, both being results of (yesterday's

photosynthesis), (yet not from fossil resources). While these biofuels often are very useful in a certain region or country, they are always limited by the availability of the corresponding organic raw material, i.e. the biomass, which limits their application on global scales.

The Fourth generation biofuels will be based on raw materials that are essentially inexhaustible, cheap and widely available. The Photosynthetic water splitting into its constituents by the solar energy can become a large contributor to fuel production on global scales, by artificial photosynthesis and by the direct solar biofuel production technologies. Not only the production of the hydrogen but also the production of reduced carbon based biofuels is possible by concomitant enhanced fixation of atmospheric CO₂ and the innovative design of the synthetic metabolic pathways for the fuel production. The generation of (designer bacteria) with new useful properties requires revolutionary scientific breakthroughs in several areas of the fundamental research. The European Union bioeconomy strategy highlights the importance of discovery research for the establishment of functional bioeconomy, and the synthetic biology is a foreseen as a key enabling technology for successful realization of bioeconomy in replacement of the fossil fuels. The Synthetic biology will have the capacity to make bioeconomy much broader by providing means to produce numerous different biological compounds, including an array of various biofuels. There is also a worry about the decrease in (EU's) biocapacity. If there are no actions will be taken, it is forecasted that consumption of the bio resources will exceed their renewal capacity by 2030. It is considered extremely important to produce of the biofuels using the minimal raw material resources in their production.

IV. ENVIRONMENTAL IMPACT THROUGH DIFFERENT BIOFUEL

The Generations greenhouse gas (GHG) emission from the biofuel is not only dependent on the gas coming out from burning the fuel but also from the combinational effect of GHG emission at different supply stages such as production of feedstock biomass (the fuel used in agriculture, N₂O soil emission from N-fertilizer and the residues), transportation to the industrial conversion unit, the

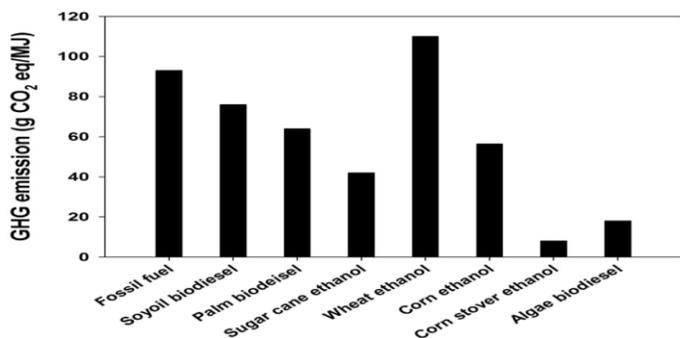


Fig.3. Life cycle of GHG emission from different sources of biofuel.

industrial unit (the crucial issue is the methodology that is used to include co-products from the conversion) and distribution. Three

GHGs mostly studied in the recent past are CO₂, CH₄, and N₂O, which converted to CO₂ equivalent by the global warming potential (GWP) recommended by the Intergovernmental Panel on Climate Change (IPCC). The Life cycle of GHG emissions from different sources of biofuel is shown in [fig.3.]. For the fossil fuel, the net GHG emissions include the emissions from mining, extraction, transport, conversion to primary energy carrier, distribution and end use.

V. OVERALL BIODIESEL PRODUCTION COST

In the world, there are more than (350) oil-bearing crops that identified as potential sources for the biodiesel production. The wide range of available feedstocks for biodiesel production represents one of the most significant factors of producing biodiesel. The feedstock should fulfill two main requirements: (low production costs and a large production scale).

The availability of feedstock for the producing biodiesel depends on the regional climate, geographical locations, local soil conditions and agricultural practices of any country. From the literature, it has been found that feedstock alone represents (75%) of the overall biodiesel production cost as shown in Fig.[4].

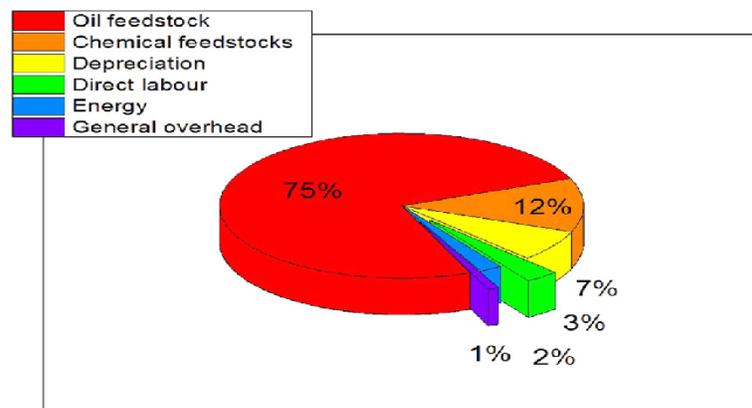


Fig.4. overall biodiesel production cost

VI. ALGAE-BASED BIODIESEL

The Algae-based technologies could provide a key tool for the reducing greenhouse gas emissions from coal-fired power plants and other carbon-intensive industrial processes. The Microalgae are fast-growing organisms with a voracious appetite for carbon dioxide. They have the potential to produce more oil per acre than any other feedstock being used to make biodiesel, and they can be grown on land that's unsuitable for food crops.

The Microalgae are (prokaryotic or eukaryotic) photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. As examples of prokaryotic microorganisms are Cyanobacteria ((Cyanophyceae)) and eukaryotic microalgae are for example green algae ((Chlorophyta)) and diatoms ((Bacillariophyta)) [18]. The Microalgae reproduce themselves using photosynthesis to convert sun energy into chemical energy, completing an entire growth cycle every few days. They can grow

almost anywhere, requiring the sunlight and some simple nutrients, although the growth rates can be accelerated by the addition of specific nutrients and sufficient aeration. The Different microalgae species can be adapted to live in a variety of environmental conditions. It is possible to find species best suited to local environments or specific growth characteristics, which is not possible to do with other current biodiesel feed stocks (e.g. soybean, rapeseed, sunflower and palm oil). They have much higher growth rates and productivity when compared to the conventional forestry, agricultural crops, and other aquatic plants, requiring much less land area than other biodiesel feed stocks of agricultural origin, up to 132 times less when compared to rapeseed or soybean crops, for a 30% (w/w) of the oil content in algae biomass. The competition for arable soil with other crops, in particular for human consumption, is greatly reduced. The Microalgae can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, among others. Algae biodiesel contains no sulfur and performs as well as petroleum diesel, while reducing emissions of the particulate matter, CO, hydrocarbons, and SO_x. However emissions of NO_x may be higher in some engine types.

VII. ECONOMICS OF ALGAE BIODIESEL PRODUCTION

There are small numbers of the economic feasibility studies on microalgae oil. Microalgae biofuel has not been deemed economically feasible compared to the conventional agricultural biomass. Critical and controversial issues are the potential biomass yield that can be obtained by the cultivating macro or microalgae, and the production costs of the biomass and derived products. The basis of the estimates is usually a discussion on three parameters: photosynthetic efficiency, assumptions on scale up, and on long-term cultivation issues. For microalgae the productivity of raceway ponds and photo bioreactors is limited by a range of the interacting issues. Typical productivity for microalgae in open ponds is (30–50) tons/hectare year. Several possible target areas to improve the productivity in large scale installations have been proposed. The Harvesting costs contribute (20–30%) to the total cost of algal cultivation with the majority of the cost contribute to cultivation expenses.

The Genetic engineering, development of the low cost harvesting processes, improvement on photobioreactor, and integration of co-production of high value products, processes are other alternatives in reducing algal oil production cost. The harvested algae next undergo the anaerobic digestion producing methane which could be used to produce electricity. In the commercial photobioreactors, higher productivities may be possible. Typical productivity for a microalgae (*Chlorella vulgaris*) in photobioreactors is (13–150). The Photobioreactors require (10) times capital investment than open pond systems. The estimated algal production cost for open pond systems (\$10/kg) and photobioreactors (\$30–\$70/kg) is two order magnitudes higher and almost three order magnitudes higher than conventional agricultural biomass respectively. Assuming that the biomass contains (30%) oil by weight and carbon dioxide available at no cost (flue gas), estimated production cost for photo bioreactors and raceway ponds to be (\$1.40 and \$1.81) per liter of oil respectively. For the microalgal biodiesel to be competitive with petro diesel,

algal oil price should be less than (\$0.48/L). It is useful to compare the potential of microalgal biodiesel with bioethanol from sugarcane, because on an equal energy basis, sugarcane bioethanol can be produced at a price comparable to that of gasoline. Bioethanol is well established for use as a transport fuel and sugarcane is the most productive source of the bioethanol.

For example, in Brazil, the best bioethanol yield from sugarcane is (7.5 m³) per hectare. Bioethanol has only (64%) of the energy content of biodiesel. Therefore, if all the energy associated with (0.53) billion m³ of biodiesel that the US needs annually was to be provided by bioethanol, nearly 828 million m³ of bioethanol would be needed. This would require planting sugarcane over an area of 111 M hectares or (61%) of the total available cropping area of the United States.

The Recovery of oil from the micro algal biomass and conversion of oil to biodiesel are not affected by whether the biomass is produced in raceways or photo bioreactors. Hence, the cost of producing the biomass is the only relevant factor for a comparative assessment of photo bioreactors and raceways for producing the microalgal biodiesel. If the annual biomass production capacity is increased to (10,000) tons, the cost of production per kilogram reduces to roughly (\$0.47) and (\$0.60) for photo bioreactors and raceways, respectively because of the economy of scale. Assuming that the biomass contains (30%) oil by weight, the cost of the biomass for providing a liter of oil would be something like (\$1.40) and (\$1.81) for photo bioreactors and raceways, respectively. The Biodiesel from palm oil costs roughly (\$0.66/L), or (35%) more than the petro diesel. This suggests that the process of converting palm oil to biodiesel adds about (\$0.14/L) to the price of oil. For palm oil sourced biodiesel to be competitive with the petro diesel, the price of palm oil should not exceed (\$0.48/L), assuming an absence of the tax on biodiesel. Using the same analogy, a reasonable target price for microalgal oil is \$0.48/L for algal diesel to be cost competitive with petro diesel.

The Algae are among the fastest-growing plants in the world, and about (50%) of their weight is oil. That lipid oil can be used to make biodiesel for the cars, trucks, and airplanes. The Algae will someday be competitive as a source for biofuel. The Only renewable biodiesel can potentially completely displace liquid fuels derived from the petroleum. The Economics of producing microalgal biodiesel need to improve substantially to make it competitive with the petro diesel, but the level of improvement necessary appears to be attainable.

VIII. ENHANCING ALGAL BIOLOGY TO REDUCE PRODUCTION COSTS

The biofuels made from the microalgae hold the potential to solve many of the sustainability challenges facing other biofuels today, the production of their biomass using the technology available is not economical and, the existing algal species could not be grown sufficiently cheaply and, at the same time, they produce oil usable as a source of the fatty acids for biodiesel. Based on conventional estimates, algal biofuels produced in large volumes with the current technology would cost more than (\$8) per gallon ((in contrast to (\$4) per gallon for the soybean oil today)) [19,20].

Therefore, to producing low cost microalgal biodiesel requires either improvements of the algal biology through genetic and the metabolic engineering or modifying the culture conditions in some species or combination of both. Concomitant use of tailor-made, rather than wild type, algal strains may help to reduce production costs to a level that could bring algal oil within the reach of economic feasibility [21]. To address these drawbacks, a number of research works have been made for decades. The last few years have witnessed significant progress in the genetic engineering of microalgae. The Transgenesis in the algae is a fastgrowing technology as selectable marker genes, promoters, reporter genes, transformation techniques, and other genetic tools and methods are already available for various species. The commercial application of the algal transgenics is beginning to be realized, and algal biotechnology companies are being established. The Microbial production of natural products has been achieved by transferring productspecific enzymes or entire metabolic pathways. Figure 5, is Schematic that representation of Photanol process.

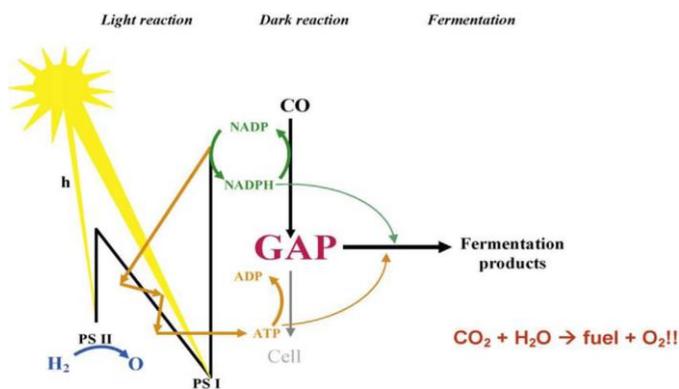


Fig. 5. Schematic representation of Photanol process

From the rare or genetically intractable organisms to those that can be readily engineered. Production of special chemicals, bulk chemicals, and fuels has been made possible by combining enzymes or pathways from different hosts into a single microorganism and by engineering enzymes to have new function. the photanol approach, ((Lightdriven conversion of CO₂ and water into the biofuel)) can be achieved by combining the light reactions of photosynthesis and the Calvin Cycle with a fermentative pathway from a chemoheterotrophic microorganism in one single chimera ((an organism with at least two genetically different components resulting from mutation, the grafting of plants, or the insertion of the foreign cells into an embryo)). This would circumvent the need for converting CO₂ into the complex mixture of biopolymers ((protein, nucleic acids, cell walls, neutral and phospholipids, etc...)) and then applying a series of subsequent processing steps to convert this complex mixture into a specific biofuel with consequent increase in the overall efficiency of the biofuel production process.

The Genetic and the metabolic engineering and transgenics offer the potential to increase the lipid productivity of microalgae and contribute to improving the economics of production of the microalgal diesel.

In this regard, many improvements have been realized, including the increased lipid and carbohydrate production, improved H₂ yields, and diversion of central metabolic intermediates into biofuels. Some of the achievements in genetic and metabolic engineering that involve *Scenedemus obliquus* have been discussed, while some more with other microalgal species are presented here. The transgenic *Chlamydo monas reinhardtii* is now being developed for various biotechnological applications, including the production of biohydrogen. In this species, a mutant created by the genetic blockage of the starch synthesis showed increased accumulation of lipids on a cellular basis during nitrogen deprivation. In an attempt to increase the algal productivity through downregulating expression of the light-harvesting antenna complexes in *C. reinhardtii*, the transgenic alga showed higher resistance to photo-oxidative damage with a concordant (30%) increase in photosynthetic efficiency [22]. Another fascinating finding in this scenario is an engineered blue green alga, the *Synechococcus leopoliensis*, equipped with the cloned bacterial cellulose synthase genes from *Gluconobacter xylinus*. This engineered alga produces extra-cellular deposits of the non-crystalline cellulose, a polymer which is ideal as a feedstock for biofuel production of various alcohols.

The Outstanding achievements reported in enhancing the alcohol production via genetic engineering in two independent cyanobacterial strains ((*Synechococcus* sp.)) through the expression of pyruvate decarboxylase and alcohol dehydrogenase II genes. This was done through cloning the code sequences of pyruvate decarboxylase ((*pdh*)) and the alcohol dehydrogenase II ((*adh*)) from the bacterium *Zymomonas mobilis* into the shuttle vector ((refereed as pCB4)) and then used to transform the cyanobacterium *Synechococcus* sp. (strain PCC 7942). The cloned and transformed cyano bacterium synthesized ethanol, which diffused from the cells into the culture medium. Another recent finding was that of Joule Unlimited, where the engineered, transformed strain was able to secrete ethanol at a rate of (1 mg/L.h), which greatly outpaces rates reported previously ((0.2 mg/ L.day)). Other important finding which involves the iso butanol production, as iso butanol possesses greater energy density. the valine synthesizing enzymes have been used to divert internal pyruvate stores to the precursor 2- ketoisovalerate, which is converted to the iso butyraldehyde a precursor of iso butanol at a rate of (6.23 mg/L.h) by co-expression of keto acid decarboxylase ((*kivD*)) within *Synechococcus elongates* [23].

The genetic approaches to construct the blue green algal strains with a higher and special photosynthetic efficiency could be used to improve product yields from several biochemical pathways. The photosynthetic productivity and light utilization efficiency of the algae, the achievements are also recorded, as these are the important factors in determining the production cost. At high photon flux densities, the rate of photon absorption by the chlorophyll antenna far exceeds the rate at which photons can be utilized for photosynthesis. Thus, the microalgal mass cultures growing under full sunlight have a low per chlorophyll productivity resulting in excess photons ((up to 80%)) dissipated as fluorescence or heat. This reduces of the light conversion efficiencies and cellular productivity to fairly low levels. That this shortcoming could possibly be alleviated by the development of the microalgal strains with a limited number of chlorophyll molecules in the light-harvesting antenna of their photo

systems, (i.e. the strains that have a truncated chlorophyll antenna size). the truncated chlorophyll antenna size minimizes absorption and wasteful dissipation of sunlight by individual cells, the resulting in better light utilization efficiency and greater photosynthetic productivity by the green alga mass culture. In general, metabolic and molecular level engineering can be potentially useful to promote several desirable features of microalgae to increase photosynthetic efficiency to enable increased biomass yield, to enhance biomass growth rate, to increase oil content of biomass, to improve temperature tolerance to reduce the expense of cooling, to eliminate the light saturation phenomenon so that growth continues to increase in response to increasing the light level, to reduce the photo inhibition that actually reduces growth rate at midday light intensities that occur in the temperate and tropical zones, and to reduce susceptibility to photo oxidation that damages cells.

IX. CONCLUSIONS

The Biodiesel is gradually gaining acceptance in the market as an environmentally friendly alternative diesel fuel. However, for biodiesel to establish and continue to mature in the market, various aspects must be examined and overcome. Some of the key issues such as improving efficiency of the production process, using low cost feedstock, developing cost effective catalyst, and managing agricultural land, have been reviewed. As with any new technology or products, the biodiesel will require continuous improvement especially in producing cleaner emissions and having less impact on the environment. Further, the development on the use of the byproduct will enhance the economic viability of the overall biodiesel production process.

It can also be carried out with seawater as the medium, given that marine microalgal species are adopted, providing a feasible alternative for biofuel production to populous and dry coastal regions. Microalgae can produce a large variety of novel bioproducts with wide applications in medicine, food, and cosmetic industries.

Combining the microalgal farming and the production of biofuels using biorefinery strategy is expected to significantly enhance the overall cost effectiveness of the biofuel from microalgae approach. Technological developments, including advances in photobioreactor design, microalgal biomass harvesting, drying, and other downstream processing technologies are important areas that may lead to enhanced cost effectiveness and therefore, effective commercial implementation of the biofuel from microalgae strategy.

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