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Review of Production of Biofuels

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Abstract: *Biofuels being derived from biomass, they are one of the most renewable and biodegradable sources of energy present. It is very important to harvest this technology due to the increasing fear of the impact that the conventional petro fuels have on the environment. This study reviews some of the methods for the production of biofuel. Some of the existing and emerging technologies have been discussed. It can be seen in the course of the paper that the production processes are extremely environment friendly as compared with the production processes of conventional fuels. In the process of biofuel production, the choice of the feedstock plays a major role. The efficiency of the process is also decided to a large extent by the downstream processes. Advantages of using certain materials as feedstock over others have been discussed. The process of biomass catalytic pyrolysis has been reviewed in brief. This study focuses on the manufacture of biofuel surrogates using genetic engineering to modify different metabolic pathways as well as to enhance the resistance of the organisms to harsher conditions. The paper talks about the feasibility of large scale production of biofuels. Some examples of successful running of such plants have been discussed. Particularly, large scale production of biodiesel from microalgae has been analyzed. India is an emerging nation that can be harnessed totally by biofuel run machines to tackle our ever increasing need for fuels.*
Keywords: *Biofuels, Biodiesel, Microalgae, Genetic engineering, Bioethanol.*

I. Introduction

With an increased demand for fuel oils, it is imperative to look for alternative fuel sources. Petroleum derived fuel reserves are dwindling fast and their detrimental effect on the environment is a cause for grave concern. Development of alternative fuels which are dependable, sustainable and cost effective is the need of the hour. Biofuels have been considered as viable alternatives for petro-fuels. They have the advantage of being renewable sources of energy in addition to being non-toxic and biodegradable. Further, their combustion doesn't show any increase in the carbon dioxide emissions. Also these fuels have very low sulphur content and have excellent lubricity. First generation biofuels include biodiesel from plant oils and ethanol obtained from the fermentation of starch from cornflour or sugarcane molasses. Use of food crops for the production of biofuels will adversely affect the cost of food. There is a possibility of increasing the biofuel yields using the non-food based feedstock. This study reviews all the different methods for the production of biofuel. Some of the existing and emerging technologies have been discussed. The process of biomass catalytic pyrolysis has been reviewed in brief.

Microalgae are a promising source of biofuels. Bio hydrogen, diesel fuel substitutes, alcohols obtained from starch etc. are the biofuels which can be produced by genetically engineering microalgae. A lot of the current research aims at altering the content of energy containing compounds like lipids, polysaccharides and other hydrocarbons stored in organisms like algae by manipulating their genetics and thus modifying their metabolic pathways. Genetically engineering algae can produce strains which not only give high yields of the desired biofuels, but they can also give the prerequisite quality of fuels in a cost effective manner.

Since biofuels are derived from biomass, they are one of the most renewable and the most biodegradable sources of energy present. It is very important to harvest this technology due to the increasing fear of the impact that the conventional petro fuels have on the environment. Short chain alcohols and alkanes, which are potential advanced biofuels, can be used as potential surrogates for gasoline [1]. To replace diesel, we can use fatty acid methyl esters (FAMEs, biodiesel), fatty alcohols, alkanes, and linear or cyclic isoprenoids [1]. Fatty acid and isoprenoid based biofuels can act as supplements for or replacements of jet fuels. But when using biofuels, the engines had to be modified to a large extent which added greatly to the cost. Making a biofuel engine takes a lot of investment and so the existing technology makes it very difficult to use biofuels directly in engines.

Background

Biofuels obtained from vegetable oils are very highly viscous and cause a lot of maintenance problems. Research enabled blending of biofuels with the petro fuels without modifying the existing gasoline and diesel engines. As a result of this work, a lot of carbon dioxide and other greenhouse gases emission was arrested to a large extent. A lot of the current research aims at altering the content of energy containing compounds like lipids, polysaccharides and other hydrocarbons stored in organisms like algae by manipulating their genetics and thus modifying their metabolic pathways. Genetically engineering algae can produce strains which not only give high yields of the desired biofuels, but they can also give the prerequisite quality of fuels in a cost effective manner.

But there are many limitations to the use of biofuels. The first and the most important one is the high cost of biofuel production. In addition, biomass required for biofuel production has to compete with the resources required for food leading to the fear of decreasing food security. There are a lot of other parameters that decide the feasibility of the production and the use of biodiesel in a particular region. India, in particular is a country which flourishes with the type of feedstock required for this purpose. Taking certain steps and the development of certain technologies can enable the large scale production of biofuels.

II. Existing and emerging technologies

2.1. Conventional agricultural products

Sugar rich crops constitute 60% of the world's ethanol production [2]. Sugarcane and beet root are used as sources of ethanol production worldwide. The process of production of ethanol from sugarcane is a well-established process [3]. Sweet sorghum when used in particular led to the development of a cost- effective process. Starch from starch rich crops can be easily converted to smaller sugars and thus can be used for biofuel production. Fuels produced successfully used crops like sweet potato, cassava, potato, wheat and maize. Maize is the largest worldwide producer of ethanol. Oil seeds, particularly industrially used rapeseed oil [4], palm oil [5], sunflower oil [6] also are used for producing biodiesel. The basic criterion for selection of seeds is the number of free fatty acid (FFA) groups present. These FFA ease the process of transesterification. Many by-products are obtained from agricultural products which generate competition and so it is preferred not to divert the huge profit making products for the production of biofuels.

2.2. Lignocellulosic products and residues

The most important advantage of using cellulose for producing biofuels is that its feedstock does not compete with food products. The major source is tree barks and branches, energy plantations and agro- based residues. The efficiency of biofuels is expressed in terms of toe/ha (tonnes of oil equivalent biofuel per hectare). In the developing countries, wood gives 60% of the total primary energy demand. Very less input in terms of fertilizers and pesticides is required for the cultivation of energy plantation. But energy plantation decreases the nutrients in the soil and so it is not very widely accepted as a feedstock for biofuel production. Rice- husk, bagasse and cane trash are also used as feedstock. Because it is an agricultural based economy and leading producer of many crops in the world, India can harness these agro- based residues feedstock to a large extent.

2.3. Inedible feedstock for biodiesel

Per unit price of biodiesel is almost 1.5- 3 times more than the conventional petroleum derived fuels. One way to tackle this problem is to use feedstock that does not compete with food demand. Inedible vegetable oils were used for production of biodiesel [7]. *Jatropha Curcas* L oil is 40- 60% w/w after extraction and contains more than 75% unsaturated FFA [7]. Mahua oil is also rich in saturated fats and yields high FFA. Production process was revolutionized by the two step process. In the first step, the extracted oil is pre- treated with acid and then a basic catalyst is used for transesterification to produce biodiesel. Optimum output could be obtained by using this method and led to the improvement in reaction time, temperature requirement, methanol to oil ratio.

2.4. Use of brown grease

Brown grease is composed of triglycerides (TG), diglycerides (DG), monoglycerides (MG) and more than 35% by weight of FFA. Though FFA boosts the rate of transesterification, its presence is detrimental base catalyzed transesterification due to formation of foam. So Canacki et. al suggested a two-step process very similar to the one discussed earlier. The oil is catalysed with sulphuric acid to esterify the FFA to biodiesel and then the base catalysed transesterification to produce more amount of biodiesel. A pilot plant was set up to test the efficiency of this process [7]. It was found that the two step process was very effective but large amount of base was required for catalysis. So the production costs increased.

2.5. Microalgae for production of biodiesel

Biodiesel from biodiesel hold the potential for large scale production of biodiesel because of its non-competing nature with food security and it yields more biodiesel per hectare of land utilized. The growth rate and the lipid content of algae is very high. Some common classes of algae used for biodiesel production are *Bacillariophyceae, Chlorophyceae, Chrysophyceae* [7]. Most commonly NaOH, CH_3OH and KOH are used as catalysts. It is observed that base catalyzed reactions are 4000 times faster than acid catalyzed reactions.

Enzymatic catalysis was found to be the most promising because it allows easy product separation, there are no side reactions, it increases the purity of glycerol and enzymes can be reused for a long time as catalysts.

III. Bio fuel conversion

3.1. Bioethanol

Bioethanol is obtained from any feedstock that contains sugar, starch or cellulose. The sugar in the form of glucose is broken down and then fermented. The obtained ethanol mixture is then distilled. The anhydrous ethanol thus obtained can be blended with petrol. If starch is used, then it is first hydrolyzed. The mixture is then fermented and then distilled. In this process, glucose breaks down into simpler sugars and eventually starchy material is formed which is converted into short chain carbohydrates. Cellulose is made to undergo saccharification, then fermentation and finally it is distilled. It is a two-step process in the first step, cellulose and hemicellulose is broken down into smaller carbohydrates. This carbohydrate mixture is fermented to obtain alcohols [Fig. 1]. This process leads to large scale release of CO2 gas. The major cost component is due to feed stock (58%- 65%) specific investment (40%) and operating costs (15%- 20%) [2]. Almost 50% of this cost can be cut down by the sale of by-products obtained out of the process.

3.2. Vegetable oil as straight vegetable oil

The properties that decide if a material is fit to be used as a fuel are its freezing point, cetane number, viscosity. All of these depend on the number of FFA and fatty acid composition as also the presence of other minor compounds. Presence of smaller molecules of triglycerides avoids the need for engine modification and reduces environmental impacts. Conversion of vegetable oil into biofuels can be mechanical or using solvents like hexane which give higher yields. For example, palm oil obtained from palm leaves is one of the most productive feedstock sources of vegetable oil. Special engines are available that can be installed that use SVO directly as fuel but they are very costly [2].

The first large scale experimental facility of transesterification was set up at New Mexico, USA [8] where different tests on strain selection and seed culture preparation were carried out. Other tests involved biomass and lipid yield optimization, bioreactor configuration, physico- chemical parameters and harvesting and extraction of lipid from biomass. The yield of pretreated oils and fat added with alcohol and catalyst is about 99% [2]. Methanol is better than ethanol because ethanol is more hydrophobic and so it does not easily get separated from water. Also, kinetics with methanol is faster and specific costs are lower. Nowadays, direct and indirect gassification is used to convert biomass into fuel. To do this, biomass feedstock is converted to CO, H2 rich gases after undergoing pretreatment. The obtained gas is cleaned, conditioned and sent for synthesis [27]. The most common harvesting methods are flocculation, microscreening, centrifugation [9].

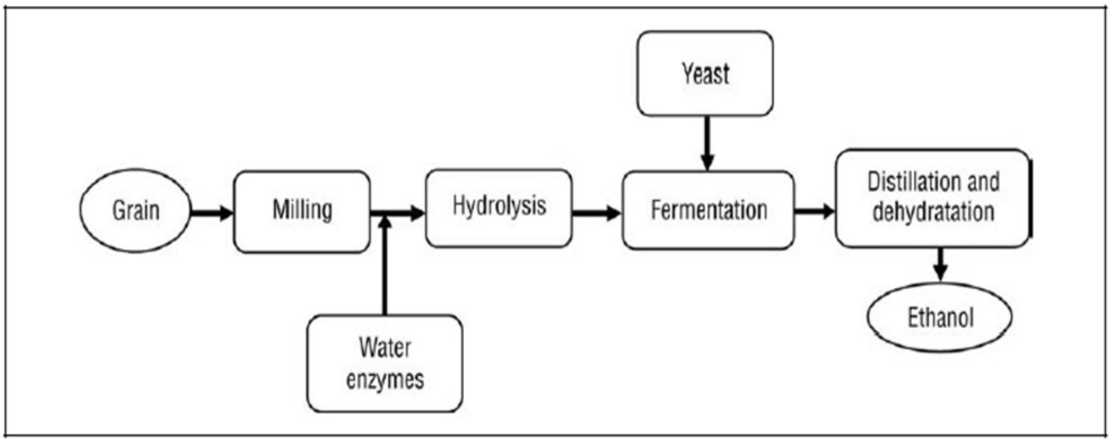

Fig 1. Ethanol production from grain dry milling (Reith et. al, 2001)

Transesterification is a very widely used process of utilizing vegetable oil for making biodiesel. Biodiesel basically is methyl ester. In transesterification, oil is catalytically transesterified with methanol. Thus it is broken into esters and glycerol, separated and then purified.

IV. Co processing in conventional refining process

Biomass can also be produced by catalytic pyrolysis of biomass over FCC zeolitic catalysts, by co-processing gas oil mixed with hydro-treated biomass pyrolysis liquids or by co hydro processing vacuum gas oil (VGO) with sunflower oil [10]. To maximize liquid yields bubble fluidized beds, circulating fluid beds (CFB), transported beds and rotating cone beds are used. Biooil cannot be directly used as transportation fuel due to high oxygen (40- 50%) and water (15- 30%) content, less stability, high acidity and high viscosity. So, two downstreaming processes can be performed- hydrooxygenation (HDO) using neutral COMO- NIMO catalysts, under high pressure and moderate temperature (330- 450^oC) and also by catalytic cracking (FCC) using zeolite catalyst.

The catalytic pyrolysis unit consists of a biomass and solid feed section, reactor, and a product recovery line. The regenerator regenerates and supplies catalyst with the required heat for reactions. It is connected to the reactor with a slide valve. Reactor consists of an injector that enables direct mixing of hot catalyst with the biomass particles. The stripper is used for solid removal. Isothermal operation is ensured by temperature controller over various regions. Steam inlet is provided at the bottom of the stripper vessel for stripping. Gas and liquid flows are separated in the stabiliser.

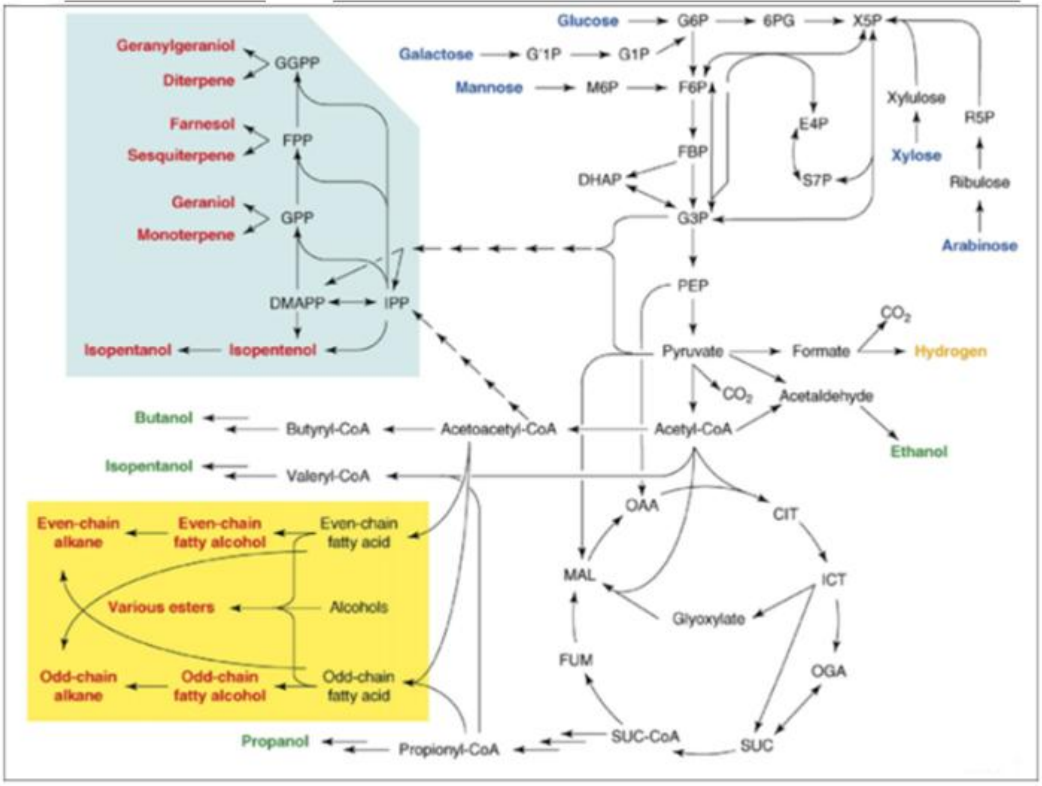

Fig. 2 Central metabolic pathways and the potential fuel molecules derived from them. [28]

V. Production of bio fuels using microalgae by genetic engineering

Biodiesels are obtained using the transesterification reaction between the triacylglycerols (TAGs) (plant or animal origin oils) with an alcohol (generally methanol) [11]. Thus, the biodiesel precursors, TAGs are the ones obtained from the plant or animal sources. Triglycerides are thus the main materials in the production of biodiesel. Large proportions of fatty acid triglycerides are preferred in the biodiesel raw materials. In order to study the production of biodiesel, it is imperative to study the lipid metabolism taking place within these organisms. Further, in order to optimize the production of the biodiesel precursors, genetic modifications need to be performed to enhance specific aspects of the lipid synthesis pathways. Biodiesel precursors should be available in sufficient quantity. Also, the quality of these precursors will ultimately decide the quality of the biodiesel and should also be analysed. The genes that are responsible for lipid metabolism have been studied in great details for terrestrial plants but not for algae. However, these genes present in the terrestrial plants have homologs in algal genomes and hence, it is possible to work with the algal genomes too.

Microorganisms have a simpler genome and can be manipulated efficiently using biotechnological means. Further, their ability to store lipids is high. Also, their growth cycles are short. Microalgae have a higher photosynthetic efficiency and growth rate as compared to higher plants [12].

5.1. Lipid biosynthesis

Microalgae can consume both organic as well as inorganic carbon sources for the production of lipids. Many transgenic overexpression strategies have helped increase the TAG levels in oil seeds. It has been proposed that fatty acid supply is responsible for the regulation of the synthesis of oils [13]. Naturally, initial steps involved trying to increase the expression of enzymes responsible for the synthesis of fatty acids. The first step in the fatty acid production pathway for most organisms is the conversion of acetyl-coenzyme A (CoA) to malonyl-CoA, catalyzed by acetyl-CoA carboxylase (ACCase). The attempts made to make use of an overexpression of ACCase to help increase the lipid contents however were not very satisfactory [14]; [15]; [16]; [17]**.** Continuing along similar modifications, the increased expression of 3-ketoacyl-acylcarrier protein synthase III (KASIII) was tried. The aforementioned protein is involved in fatty acid synthesis. This method too could not increase lipid production satisfactorily [18].

Another approach, thee overexpression of genes involved in the TAG assembly has provided significant results. Various studies conducted have indicated that genes involved in TAG assembly are important for the synthesis of oils in seeds. Further, metabolic pathways which lead to the accumulation of starch and other energy storage compounds if blocked can help increase the cellular lipid content. [19] Organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* have metabolic pathways which have been studied extensively. Making genetic modifications in their pathways is relatively easier. Many genes and enzymes and genes can be knocked off or overexpressed simultaneously and their effects can be studied [20].

5.2. Lipid catabolism

In order to increase the accumulation of lipids in the cell, another approach can be to reduce lipid catabolism**.** If genes responsible for the activation of TAG and fatty acids are inactivated, there can be an increase in the cellular lipid content. However, reducing lipid catabolism may have adverse effects on cell growth and proliferation. Microalgae depend upon catabolism for the production of energy essential for cell division. Also, many of the precursors for cell division are attained from catabolism processes. Further, enzymes which participate in the β-oxidation of fatty acids have overlapping functions. So, those functions cannot be completely nullified. Also, some studies show that lipid oxidation if inhibited can lead to the manifestation of unexpected phenotypes [21].

5.3. Lipid Characteristics

For any fuel to be suitable for use, it should not only have good availability, but it should also have suitable quality. With regards to the quality, the carbon chain length and the degree of unsaturation of the fatty acids are the important factors. The chain length of the fatty acids changes depending upon the species of the microalgae**.** Most microalgal fatty acids have a chain length between 14 and 20. Acyl-ACP thioesterases help set the fatty acid chain length. These thioesterases are specific for fatty acid chain lengths. The chain lengths dictate the fuel applications. Short chains can be used for gasoline and jet fuel production.

5.4. Direct synthesis of biofuels

In order to reduce the requirement for processing of the fuel product, possible means for the direct synthesis of fuel products in microalgal cells are being looked into. However, introducing modified pathways into a cell to produce a compound not directly used by the cell may not be economically favourable. Also, many of the fuel molecules maybe toxic for the cell and tolerant species will have to be engineered. For every production step which can be inculcate in the cellular pathway, there is a potential for improvising the overall economics. With advances in genetic transformation methods, some biochemical pathways maybe altered for the direct production of fuels.

5.4.1. Straight chain alkanes

Fatty acids can be converted into alkanes. Hence, microalgae which can synthesize an appreciable quantity of lipids can be used to manufacture alkanes. However, in order for the conversion of fatty acids into alkanes to be successful, the system should contain a functional decarbonylase enzyme. So far, no viable decabonylase enzyme has been cloned. In order for the direct manufacture of short chain alkanes to be successful, suitable enzymes need to be engineered. A few instances have been reported where long chain alkanes have been obtained. However, these long chain alkanes need to be processed further to yield usable products.

5.4.2. Alcohols

Algal starches are known to be fermentable by yeast. In order to reduce the overall steps of the process, it is preferred to have an in situ production of the ethanol. Attempts have been made to couple ethanol production with photosynthetic carbon fixation. Ethanol has lower energy density than gasoline. Higher alcohols however have energy densities similar to that of gasoline. Isopropanol and butanol are naturally produced by bacteria of the genus *Clostridium*, and production has been industrialized using *Clostridium acetobutylicum*. Recent attempts aim to include isopropanol and butanol producing pathways in *E. Coli.* The use of transgenic overexpression of entire production pathways may cause trouble in the host cell. There is a possibility that the foreign enzymes may affect the normal metabolic pathways of the organism.

5.4.3 Terpenoids

Terpenoids if processed appropriately can act as substitutes for gasoline. Diesel or jet fuel surrogates can also be obtained using terpenoids. Further, these compounds can be produced using the isoprenoid pathways. Branched chain alcohols, alkanes, alkenes and cyclic hydrocarbons can be produced through the isoprenoid biosynthetic pathway. Isoprenoid pathways produce complex mixtures or hydrocarbons. If extracted, the extract can be regarded as biocrude. It needs to undergo further purification and processing to give the desired products. Isoprenoid pathways can also produce gasoline additives. Also, monoterpenes and sesquiterpenes may also be used as diesel and jet fuel surrogates.

5.5. Secretion of fuel precursors

To reduce the cost of downstream processing of the biofuels, one alternative is to engineer the organisms so that they can secrete the fuels or the feedstock directly into the growth medium. Any genetic modification which leads to a large accumulation of intracellular free fatty acids will in theory lead to a secretion of free fatty acids. There are known pathways for the intracellular transport of fatty acids between organelles. As of now, knowledge pertaining to secretion pathways is limited. It is not easy to transfer the manipulations in secretion pathways to microalgal cells. If there are a number of other microorganisms coexisting in the cultivation system, the secretion of the fuel intermediates into the growth medium will provide energy rich nutrients for their growth. Thus, there will be a reduction in the product yields.

5.6. Carbohydrate metabolism

Carbohydrates can be metabolized into ethanol, butanol, H2, lipids and or methane. Microalgae store glucans in a variety of ways. The rate limiting step of starch synthesis is the ADP-glucose pyrophosphorylase (AGPase)-catalyzed reaction of glucose 1-phosphate with ATP, resulting in ADP-glucose and pyrophosphate. In order to increase the starch content in the algal cells, starch synthesizing enzymes can be introduced into the cytosol. Also, the catabolism of starch can be reduced to increase the starch accumulated in the cells. Phosphorylation steps are critical for starch catabolism. Thus, to ensure starch accumulation, these genes can be probable targets for knockout. The production of soluble sugars is preferred over the production of polysaccharides. Soluble sugars are easier to process and their secretion can be engineered easily. Both, the secretion and accumulation of soluble sugars is preferred.

5.7. Hydrogen production

Few species of algae have the ability to produce H2 as a fermentation metabolite. Enzymes called hydrogenases are capable of a reversible reduction of protons to H2. However, H2 production is only observed transiently before accumulation. *C. reinhardtii* is an attractive candidate for hydrogen production due to its relatively high hydrogenase activity. Bio hydrogen presents a viable fuel alternative since it does not give carbon dioxide by-products. Further, it can be used as a means to generate electricity using fuel cells.

5.8. Stress tolerance

The metabolic pathways leading to the manufacture of biofuels or their precursors should be efficient. The accumulation of the storage compounds such as lipids and starch should be in significant quantities. Further, in order for the processes to be economically viable, the microorganisms should be able to rapidly produce large amounts of biomass. In order to attain adequate overall rates of oil production, several factors need to be considered. Adequate supply of nutrients and sunlight are a basic requirement. Nutrient deficiency will cause a lower rate of biomass production and hence the amount of lipids produced will also be lesser. A prerequisite Carbon to Nitrogen ratio is essential for a sufficient quantity of lipids to be synthesized in the microalgae. Environmental factors also affect lipid synthesis. Modes of cultivation also significantly affect the levels of lipid accumulation [22]. Thus, manipulating the growth conditions can also help change the lipid accumulation in microalgae.

Several factors such as salt concentration, temperature, pH, and light intensity have the potential to limit the synthesis steps. These stress factors need to be controlled. The tolerance levels of the microalgae to these extreme conditions can be increased with the use of genetic modifications. Also, if the tolerance levels of the microalgae can be increased with genetic engineering techniques, these select organisms can be grown under extreme conditions and the growth of other organisms can be suppressed. Thus, we can achieve a sort of selective growth of the desired species over that of other invasive species. Genetic engineering approaches have helped increase the production of a target molecule. However, if a cell-wide approach is adopted, the rate of biosynthesis as well as the yield of the desired molecule can be altered.

The metabolic engineering strategies should ensure a number of factors. There should be a production of targeted and efficient transport systems, also, the biofuel producers should be made resistant to the toxic nature of the accumulated biomolecules. In order to have maximum quantities of the required molecules, the carbon flux should be optimized to the desired products. Further, since the microorganisms will be exposed to processing, they should be robust under the industrial process conditions [23]**;** [24]; [25]; [26]. Apart from microalgae and yeast, fungi can also accumulate oils under special conditions. They are currently being explored for the production of special lipids. Bacterial performances with regards to oil accumulation can be enhanced using genetic engineering. Different microbes have different oil yield, lipid coefficient, and lipid volumetric productivity.

VI. Feasibility of large scale production and the position of India

Not many efforts have been made towards the scale-up of biofuel production facilities. It is difficult to predict the behaviour of large scale plants due to nonlinear changes. Besides, there are many other socio-economic factors that make scaling up very difficult. The land area required for biofuel plants is very large and many times it is not sufficiently available. Because of different priorities of developing nations, directing resources to the production of biofuel is not advised. Also, biomass is used for many other purposes that earn more fiscal profits. In India, around 70% petroleum is consumed by automobiles [9]. The need for using petroleum fuels increases by 5- 6% every year (2005- 2006). The demand for cereal is first for food and then for biomass. So, the government decided to focus on Jatropha oil seeds through which large scale production is possible. The solution for India’s go-ahead is the use of algal farming because it does not compete with food demand.

VII. Conclusion

Biofuels are an effective means to help reduce the energy crisis and to battle the ever growing concerns regarding climate change. In order to meet the growing demand for fuels, the manufacture of biofuels should be scaled up considerably. Biofuels can thus reduce the dependence on petroleum derived fuels. There are many socio- economic parameters that decide the feasibility of using biofuels for large scale production. Given the present technology, biofuels cannot be used to replace existing conventional fuels. The best way to meet the economic viability of the same is to use genetic engineering methods to enhance the production. Microalgae can be considered as extremely malleable sources of biofuels. Other alternative routes can also be studied in depths. Various critical challenges still exist in the use of this technology. However, this technique still holds a lot of promise.

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