

International Journal of Research in Biosciences  
Vol. 5 Issue 3, pp. (1-12), July 2016  
Available online at <http://www.ijrbs.in>  
ISSN 2319-2844

## Review Paper

# Biofuel: A Stepping Stone towards Sustainable Development

Mohammad Nadeem Akhtar

School of Bioscience and Technology, VIT University, Vellore-632014 (TN), INDIA

(Received June 07, 2016, Accepted June 24, 2016)

## Abstract

High energy demand coupled with fossil fuel depletion and environmental degradation has compelled the energy sector to search for an alternative means of energy production. Host organisms like *E. coli* and *Saccharomyces cerevisiae* are potent source of biofuel production because of their fast growth rate and their easy genetic manipulation. With the use of modern genetic engineering tools, metabolic pathways of microorganisms can be manipulated to achieve biofuel production. Organic waste materials and even carbon dioxide from the atmosphere (carbon sequestration) can be used as the raw material for biofuel production, thereby making it an environment friendly process. The use of membrane bioreactors has not only reduced product inhibition but has also enhanced separation of products from cell cultures. The activity and stability of immobilized enzymes can be improved by using nanostructures. This has led to the development of highly efficient biofuel cells. There is a close link between biofuel and feedstock prices that needs to be optimized in order to have a stabilized biofuel market. Critical issues such as efficiency of biofuel in comparison to conventional fuel and the use of agricultural land in order to produce raw materials for biofuel industry still need to be addressed.

**Keywords:** Biofuel, Genetic engineering, Host organisms, Membrane bioreactors, Nanostructures.

## Introduction

Biofuel is a non-conventional fuel derived from organic materials (biomass) – plant and animal waste. It is produced through contemporary biological processes, like anaerobic digestion and carbon fixation in contrast to the natural fuel which is produced from buried combustible geological deposits of organic materials. Biofuel is advantageous in the sense that its production rate can be taken care of manually, whereas the conventional fuel generally takes millions of years to form. The production of biofuel does not only reduce the dependence on fossil oil trade, but also reduces the uncertainties caused by the fluctuations in fossil oil prices<sup>[1]</sup>. Biofuel is generally categorized into three types: Bioethanol, Biodiesel and Bio-oil based on the raw material used for its production.

With the advent of twenty first century, the demand of petroleum products has surged exponentially. The modern world is hooked onto fuel for energy production, transportation, industrialization, etc. In order to meet the growing demand there has been tremendous over-exploitation of natural resources, at a much higher rate than at which nature could replenish it<sup>[2]</sup>. Moreover the unequal distribution of natural resources has led to additional spending on the transportation of fuel, particularly to Europe, Australia and Japan. The leakage from oil rigs also causes appreciable damage to aquatic biodiversity. The alarming rate of global warming and air pollution has left the energy sector to look for an alternative source of energy production<sup>[3]</sup>. Compared with other renewable energy forms, liquid biofuels are more compatible with current infrastructure and have a high energy density, which provides a basis for their long term roles in the future energy mix<sup>[4]</sup>.

Host organisms like *E. coli*, *Saccharomyces cerevisiae* and Cyanobacteria can provide an ideal platform for biofuel production. Moreover their entire genome has been sequenced, allowing scientists to engineer their metabolic pathway to produce alcohol from carbohydrate sources<sup>[5]</sup>. With huge quantity of organic waste being produced, it can be used as the raw material for the production of biofuel via microbial fermentation pathway. An alternative technique might be to gasify organic biomass and to use the produced synthesis gas as a feed stock for the synthesis of ethanol and other valuable compounds<sup>[6]</sup>. Efficiently designed photo bioreactors can greatly enhance biofuel production from micro algae. The following parameters has to be optimized to increase the productivity: (a) solar energy and CO<sub>2</sub> utilization, (b) efficient gas transfer and mixing of the culture, and (c) harvesting<sup>[7]</sup>. Biofuel fermentation engineering if integrated with metabolic engineering in order to tune the expression of multiple heterologous genes, improve energy metabolism and construct sensor based regulator system can improve cell productivity in industrial bioreactors.

However, some critical issues like using food crops and cultivable land for biofuel production is posing threat to under developed nations. The scale up of biofuel production from pilot scale remains a big challenge. This review gives a detailed insight about different means of biofuel production and sophisticated techniques required to overcome the hindrance in this field.

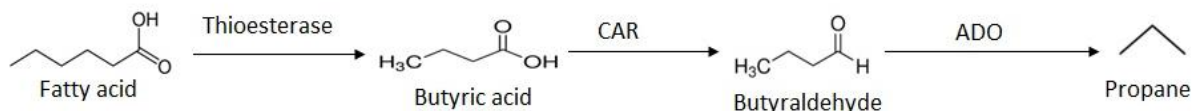
**Table 1: It depicts the classification of biofuel according to generation, the potential feedstock used for producing them and a few examples**

Classification	Example	Feedstock
First Generation	Biodiesel, Biogas, Vegetable oils	Sugar, starch
Second Generation	Biohydrogen, Biomethanol	Cellulosic biofuel, waste biomass
Third Generation	Oilgae (30 times more energy)	Extracting oil of algae

### Host Organisms

#### *E. coli*

*E. coli* has the natural ability to synthesize fatty acids and is amenable to genetic manipulation making it an ideal target for biofuels research. The combination of *E. coli* with new biochemical reactions, realized through synthetic biology, produces structurally tailored fatty esters (biodiesel) from simple sugars. The major obstacle is almost all the fatty acid produced by bacteria is esterified to glycerol or analogous product. The accumulation of glycerol as by product could pose a challenge to bacteria as a host for biodiesel production. There is a three stage biochemical process to produce propane using fatty acid as the starting material. Thioesterase is an enzyme that breaks down fatty acids in a way that interrupts the usual membrane building process, instead producing butyric acid. It is followed by the action of carboxylic acid reductase (CAR) that turns butyric acid into butyraldehyde. Finally, the third enzyme aldehyde-deformylating oxygenase (ADO) converts butyraldehyde into propane<sup>[8]</sup>. The catalytic efficiency of ADO enzyme can be enhanced by stimulating it with electrons. Reducing systems when coupled to ADOs in a specific order can greatly enhance its catalytic efficiency by increasing the rate of electron transfer (reducing power)<sup>[9]</sup>. The resulting complex can be stabilized by covalent bond. The resulting propane gas can be easily liquefied for storage and transport.



**Figure 1: It represents the step-wise conversion of fatty acid to propane with the respective intermediate formation. By stimulating the ADOs with electron, the end product formation can be highly accelerated**

Genetic engineering technique was used to regulate four distinct genotypes of *E. coli* for over production of fatty acids. These are as follows: (a) Fatty acid degradation was blocked by knocking out endogenous *fadD* gene which encodes an acetyl CoA-synthetase, (b) Thioesterase enzyme from

plant is expressed in order to up regulate the production of small chain fatty acids which gives a broad scope for improving fuel quality (low ignition temperature), (c). Acetyl CoA carboxylase (ACC) is over expressed in order to increase the supply of malonyl CoA, (d). Endogenous thioesterase is over expressed in order to overcome the feedback inhibition caused by long-chain fatty acyl-ACPs (Acyl Carrier Proteins)<sup>[10]</sup>.

The length of the fatty acid chain produced can be controlled by expressing alternative thioesterases from plants. Eliminating  $\beta$ -oxidation (knocking out *fadD* and *fadE* gene) and by over expressing thioesterases and acyl-CoA ligases, resulted in an extra three to four fold increase in the yield of fatty acids<sup>[11]</sup>. Acyl-ACP can be hydrolyzed to free fatty acids and ACP by thioesterase. Basically there are two distinct but related thioesterases gene classes found in higher plants, *FatA* and *FatB*. *FatA* encodes for 18:1-ACP and is commonly known as long chain or oleoyl-ACP thioesterases. *FatB* encodes for thioesterases that prefer acyl-ACPs having saturated acyl groups. Moreover *FatB* genes are diverse in functionality. Some groups are specific for saturated ACPs in the C8 to C18 range while the others prefer C14 to C18 acyl ACPs (predominantly 16:0)<sup>[12]</sup>.

Full length clone from *Arabidopsis thaliana* corresponding to the partial cDNA YAP140 can be expressed in *E. coli* which shows highly versatile substrate specificity. It shows high substrate affinity towards 16:0-ACP and 18:1-ACP, while comparatively less preference towards 18:0-ACP and 14:0-ACP. However the gene product might be toxic to the cell due to the free fatty acids released. Notably the toxicity can be eradicated by culturing the *E. coli* cells at 30°C. Over expressing the native *E. coli* thioesterase I or II in *E. coli* resulted in no change in lipid composition or the growth rate in the transformed cells. Henceforth it can be deduced that the high rate of free fatty acid production and increased acyl-ACP hydrolytic activity is due to the action of plant gene product<sup>[13]</sup>.

### ***Saccharomyces cerevisiae***

*Saccharomyces cerevisiae* is an ideal host microorganism for the production of fatty acid ethyl esters (FAEEs) which is the precursor for biofuel production. However a number of constraints have been encountered during the process. To begin with  $\beta$ -oxidation of fatty acids (FAs) is used to generate energy. In addition fatty acids are used to synthesize phospholipids and storage neutral lipids. Phospholipids are integral part of cell membrane while neutral lipids, such as triacylglycerols (TAGs) and steryl esters (SEs) are the main fatty acid reserves and constitute up to 97% of the storage lipids. Phospholipids are important to the cell while the storage lipids are not essential. The synthesis of TAGs and SEs are highly prominent in eukaryotes, but not so in *E. coli*. SEs are synthesized from FAs by the action of an enzyme, acyl-CoA: sterol acyltransferase (ASAT) which is encoded by the gene *ARE1* and *ARE2*. Two different enzymes are responsible for the production of TAGs from FAs, acyl-CoA: diacylglycerol acyltransferase (DGAT) which is encoded by the gene *DGA1* and lecithin: cholesterol acyltransferase (LCAT), encoded by *LRO1*. *POX1* gene encodes for an enzyme, acyl-CoA oxidase which is responsible for  $\beta$ -oxidation. Upon deleting the four genes responsible for lipid storage (*ARE1*, *ARE2*, *DGA1* and *LRO1*) and eliminating the  $\beta$ -oxidation pathway by knocking out *POX1* gene, the production of free fatty acids was found to increase by 2.5 times<sup>[14]</sup>.

An additional enzyme in the form of NADPH dependent 2,4-dienoyl-CoA reductase is required for the  $\beta$ -oxidation of mono- and polyunsaturated fatty acids with the double bond at even position. Mutant cells lacking PTS1 (Peroxisomal targeting signal) receptor shows no  $\beta$ -oxidation of fatty acids by NADPH dependent 2,4-dienoyl-CoA reductase<sup>[15]</sup>. Disruption of *SAT2* gene does not alter the activity of ASAT. However mutant cells lacking *SAT1* gene showed a decrease in 25% activity of ASAT. *SAT1* and *SAT2* correspond to the yeast genes *ARE2* and *ARE1* respectively<sup>[16]</sup>. These finding could prove to be beneficial in the production of biodiesel from free fatty acids.

Environmental conditions like temperature and the impact of end product (ethanol) on biofuel production by *Saccharomyces cerevisiae* play a key role. The optimum temperature for ethanol production is 33°C while 30°C favors the growth of yeast cells. However an interesting phenomenon is that while increasing temperature above 35°C favors glycerol production, which is a byproduct and hinders ethanol production. Ethanol concentration in the medium coupled with temperature inhibits the growth of cells. At the highest temperature, 39°C the growth of cells completely ceased with minimal ethanol production<sup>[17]</sup>.

*Saccharomyces cerevisiae* has an advantage over *E. coli* in terms of fatty acid production. The product of *S. cerevisiae* fatty acid synthase is in the form of fatty acyl-CoA, whereas in *E. coli* it is in

the form of fatty acyl-ACP. This enzyme linked product needs to be hydrolyzed by thioesterase to free fatty acids and subsequently activated to fatty acyl-CoA by a ligase. Thereafter enzymes like acyltransferase and fatty acyl-CoA reductase can act on them to produce free FAEEs and fatty alcohols respectively. *E. coli* are also highly prone to attack by bacteriophage, which could worsen industrial production of biofuel. With the introduction of a strong constitutive promoter, TEF1 overproduction of fatty acids can be achieved<sup>[18]</sup>.

### **Cyanobacteria**

Cyanobacteria are highly advantageous in producing biofuel, considering the fact that they are able to convert atmospheric carbon dioxide to ethanol directly in the presence of sunlight. This inevitably means that they are not totally dependent on any organic substrate. Moreover they can be grown in their natural habitat, thereby eliminating the transportation cost of biofuel so produced<sup>[19]</sup>.

Hydrogen is formed as the byproduct when cyanobacteria are grown under N<sub>2</sub> limiting condition by the action of the enzyme, hydrogenases. The major obstacle is that hydrogenases are highly intolerant to the O<sub>2</sub> produced during photosynthesis. Moreover reducing agents like NADPH and ferredoxin are utilized for respiration process, thereby providing competitive challenge to H<sub>2</sub> production<sup>[20]</sup>.

Hydrogenases are basically categorized into three groups based on the metal composition of the active site: NiFe, Fe and metal free hydrogenases. Cyanobacteria possess two functionally different NiFe hydrogenases, an uptake enzyme and a bidirectional enzyme. An interesting feature is that, both the enzymes have varying thermal stability. The uptake enzyme has a half-life of 12 minutes at 70°C but less sensitive to competitive inhibition by carbon monoxide than is bidirectional enzyme. Cyanobacterial *hupSL* gene encodes for an uptake hydrogenase. The mutant gene, *hupL* exhibits significantly increased rates in hydrogen accumulation and also produces three times more hydrogen than the wild type gene. Cyanobacterial bidirectional enzyme hydrogenases are characterized by their sensitivity to oxygen, thermo tolerance and high affinity towards hydrogen<sup>[21]</sup>. The bidirectional hydrogenases catalyze both, the oxidation of molecular hydrogen and reduction of protons. *hoxFU* genes are responsible for encoding bidirectional enzyme. Mutations caused in the diaphorase genes *hoxU* or *hoxF* are unable to evolve H<sub>2</sub><sup>[22]</sup>.

### **Raw Materials**

#### **Cellulose**

Cellulose is a highly favored raw material for ethanol production due to its low greenhouse gas emissions and more importantly it does not create food versus fuel scenario. The major obstacle for ethanol production is that the fermentable sugars are not easily liberated from cellulose. The current model for cellulose utilization involves saccharification with immobilized enzymes, however the process is extremely slow and expensive. One approach would be to produce ethanol directly from cellulose, bypassing glucose production. Cellulose can be converted into furanic products, mainly 5-(chloro methyl) furfural (CMF) with over 80% yield. CMF itself is not a biofuel candidate, however it can be converted to ethoxymethylfurfural (EMF) by stirring it in ethanol solution at room temperature or it can be converted to 5-methyl furfural (MF) by hydrogenating it using PdCl<sub>2</sub>. The products so generated have high efficiency in ethanol production<sup>[23]</sup>.

Cellulose can be directly converted to 5-Hydroxy methyl furfural (HMF) using AlCl<sub>3</sub> as the catalyst in ionic liquids. 1-Butyl-3-methylimidazole chloride ([BMIM]Cl) in combination with Dimethyl sulfoxide (DMSO) is the most potent ionic liquid used for this purpose. [BMIM]Cl has a potential to act as a hydrogen bond acceptor by breaking the inter- and intra-molecular hydrogen bonds in cellulose, thereby making it convenient for the catalyst, AlCl<sub>3</sub>. AlCl<sub>3</sub> acts as a Lewis acid that catalyzes the isomerization of glucose to fructose. Sulfuric acid plays a key role in hydrolysis of cellulose into glucose. It is to be noted that 150°C is the optimum temperature for the conversion of cellulose to HMF. Lower temperature decreases the yield while elevating the temperature to 180°C makes the product, HMF unstable<sup>[24]</sup>.

Recent research has proved that Nano cellulose can be converted to ethanol for biofuel production in high yield (91%) by using *Saccharomyces cerevisiae* as the host organism. It has also got exceptional mechanical and optical properties<sup>[25]</sup>.

### ***Jatropha curcas***

The solid fatty acids from animal fats are difficult to produce biodiesel by trans-esterification process. They cause soap formation, consume catalysts and lower the efficiency of product formation. On the other hand, fatty acids from plants are much more efficient and are renewable. The oil extracted from *Jatropha curcas* is used for biodiesel production and is being highly favored because it is non-edible, thereby eliminating any compromise on edible oil<sup>[26]</sup>.

The process of biodiesel formation involves esterification of free fatty acids (FFA) with methanol in the presence of a catalyst. *Jatropha* oil contains about 14% free fatty acid which is far beyond the limit of 1% FFA level that can be converted into biodiesel by trans-esterification. The FFAs are pretreated with methanol using an acid catalyst ( $H_2SO_4$ ) and converted to esters. It is followed by trans esterification reaction to produce biodiesel. The reaction is carried out with methanol to oil ratio (5:1 molar ratio) using KOH as an alkaline catalyst. The two step process for producing biodiesel from *jatropha* oil gives a yield of over 99%<sup>[27]</sup>.

Oxidation stability is an important criterion in biodiesel production. Chemically biodiesel is an ester molecule and there is a high possibility that it will get hydrolyzed to acid or alcohol in the presence of oxygen. It is not possible to use biodiesel without anti-oxidants. Oxidation of FAME leads to the formation of a free radical. The anti-oxidant contains highly labile hydrogen that is easily attracted by the peroxide radical. The resulting anti-oxidant free radical is either stable or further reacts to form a stable molecule that does not contribute to the chain oxidation process<sup>[28]</sup>. Synthetic anti-oxidants like *tert*-butyl-hydroquinone (TBHQ), pyrogallol, *n*-propyl-gallate, 3-*tert*-butyl-4-hydroxyanisole (BHA) and 2,6-di-*tert*-butyl-4-methyl-phenol(BHT) could significantly improve the stability of biodiesel, even at low anti-oxidant concentrations<sup>[29]</sup>.

The by-products obtained by fermentation reaction can be further processed. The press cake obtained can be used as a fertilizer and the organic waste products can be digested to produce biogas ( $CH_4$ ). More importantly this plant has the potential to prevent and control soil erosion<sup>[30]</sup>.

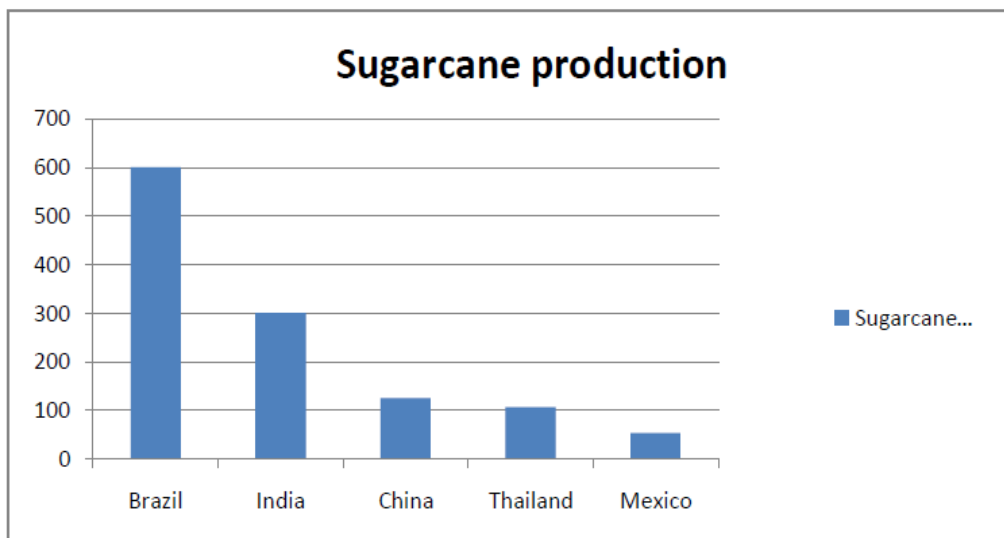
### **Sugar cane**

Brazil is the leading nation in ethanol production from sugar cane. Fermentation of sugar cane to produce ethanol as a fuel for motor vehicles reduces  $CO_2$  emission. This leads to a significant decrease in global warming<sup>[31]</sup>. The sugar cane bagasse contains lignocellulose which can be used for producing second generation biofuel. Simultaneous Saccharification and Fermentation (SSF) reduces the capital investment and is found to be highly efficient (51% conversion rate). Pre-treatment with  $SO_2$  can be carried out to improve the digestibility of cellulose and to hydrolyze xylan<sup>[32]</sup>.

Sugarcane is highly efficient in solar energy conversion and it can be harvested annually for a number of years without replanting. For these reasons sugarcane is an attractive component of global bioenergy future. An energy balance of 8U output per 1U input (8:1 ratio) have been reported in Brazil, which is in stark contrast with corn 1.25:1 (output to input ratio of corn). Mix-Stock is a trans genetic approach in which the degradative enzyme are incorporated into a separate transgenic plant variety (called the 'enzyme stock') that is then mixed with the bulk stock (sugarcane, corn, cassava, etc.) and fed into the refinery for ethanol production. This technique is adopted to prevent crop failure by genetic manipulation. A novel approach, flex-stock can alleviate problems of seasonal availability of a particular feedstock by having a refinery that can use multiple raw materials as input. This approach would be advantageous in economic terms as the refinery can be operated all year around, without having the constraint of seasonal crops as feedstock<sup>[33]</sup>.

The intrinsic recalcitrance of lignocellulosic biomass hinders the exploitation of stored chemical energy in sugarcane biomass. Cellulases can adsorb irreversibly to lignin during saccharification thereby reducing the enzymatic activity. Caffeic acid *O*-methyltransferase (COMT) is responsible for expression of lignin content in sugarcane. The RNAi –inducing transgene cassette should contain a highly conserved region of COMT that may suppress its activity. The promoter of *Oryza sativa C4H* gene induces the expression of RNAi-inducing trans gene in lignifying tissues. By successfully suppressing the activity of COMT gene by 67% to 97% lead to a reduction in lignin content from 3.9% to 13.7% respectively. Hence by using RNA interference, the yield of fermentable sugar increased considerably by 29% without pretreatment<sup>[34]</sup>.

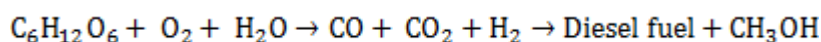
The below mentioned graphical representation is of great economic importance for the energy sector. On one hand it depicts huge chunk of sugarcane production by developing nations, it also creates a sort of alarm for other nations dependent on sugarcane for ethanol production, particularly Europe and North America. The primary reason is the cost incurred while importing the sugarcane materials like bagasse and cellulose that are used for fermentation process.



**Figure 2:** It represents the annual yield of sugarcane in million tons per year from various countries across the globe

### Syngas

There are many biomass sources like straw and wood that contain a large portion of materials that cannot be easily converted to ethanol by microorganisms or enzymatic treatment. An alternative might be to gasify organic biomass and to use the produced synthesis gas ( $H_2$  and  $CO$ ) as a feedstock to produce ethanol. Integrated technology works by coupling carbon monoxide with methanol to form dimethyl oxalate and subsequent hydrogenation to yield ethanol. It is highly efficient process (91%) and more industry friendly than the one step conventional process<sup>[35]</sup>. Syngas fermentation to ethanol is highly advantageous as: (1) it utilizes whole of the biomass including the lignin and cellulose irrespective of the quality, (2) it does not require complex pre-treatment process and costly enzymes, (3) it is independent of the  $H_2$ :  $CO$  ratio for bioconversion, (4) there is no issue of novel metal poisoning<sup>[36]</sup>.



**Figure 3:** Syngas is produced by gasification of a carbon containing source. Diesel fuel and methanol is produced from syngas by Fischer-Tropsch synthesis reaction. The optimum reaction temperature is around 250°C

The Fischer-Tropsch synthesis produces hydrocarbon of different length from a gas mixture of  $H_2$  and  $CO$  (syngas) from biomass gasification called as bio-syngas. Prior to synthesis the syngas can be conditioned using the water gas shift to achieve the required  $H_2$ :  $CO$  ratio. The large hydrocarbons so generated can be hydrocracked to form mainly diesel (sulfur free) of excellent quality<sup>[37]</sup>.

Microorganisms, particularly acetogens are capable of generating acetate as a product from syngas. The reaction is carried out in the absence of air (anaerobic). Ethanol can be produced either directly from acetyl-CoA in a 2 step reaction via acetaldehyde, or via acetate and subsequent reduction to acetaldehyde. The direct pathway requires an aldehyde dehydrogenase or a bifunctional aldehyde/alcohol dehydrogenase enzyme. The indirect route proceeds via a ferredoxin: aldehyde oxidoreductase coupled to  $CO$  oxidation. Acetaldehyde is finally reduced to ethanol by the enzyme, alcohol dehydrogenase<sup>[38]</sup>. The major drawbacks of using syngas to produce ethanol are: low productivity and poor solubility of gaseous substrates in the liquid phase.

## Bioreactors

Microalgae are an important biomass feedstock for biogas production. Cultivation of microalgae for the production of energy implies harvesting of solar energy over large areas, bringing light and CO<sub>2</sub> to the algae cells and care for moderate conditions inside the medium. A preferred bioreactor to increase the Photo Conversion Efficiency (PCE) is the flat panel air lift reactor. It suppresses fouling and reduces the hydrodynamic pressure in the reaction medium. It also increases light dilution over large reactor surface for better photosynthetic activity<sup>[39]</sup>.

Mass transfer limitation in large bioreactors creates heterogeneous growth conditions and micro-environmental fluctuations (such as suboptimal O<sub>2</sub> level and pH) that induce metabolic stress and genetic instability. In addition large bioreactors (e.g. A fed-batch bubble column reactor) feed the substrate from top and aeration from the bottom, creating opposite substrate and O<sub>2</sub> gradients. This can lead to excess accumulation of waste products. With the advancement in fermentation technology, *in situ* removal techniques such as gas stripping have alleviated product toxicity to a great extent. Moreover biosensor regulators can be used to promote or repress a biofuel pathway or substrate uptake according to its growth conditions (e.g. quorum sensing) or metabolite concentration<sup>[40]</sup>.

The production of biodiesel using a semi-batch two-phase carbon membrane reactor using either an acid- or base- catalyzed transesterification of canola oil can be increased significantly. Carbon membranes are of particular interest as they can be stable at high temperatures and resist chemical attack. The immiscibility of oil and methanol is the bottleneck for the mass transfer issue for biodiesel production. Due to immiscibility of canola oil and methanol, and due to various surface forces, the canola oil will exist in the form of an emulsion. On the other hand, fatty acid methyl ester is soluble in methanol and due to its small molecular size will pass through the membrane pores easily<sup>[41]</sup>. In the membrane bioreactor the reaction temperature should be kept as low as possible for control of two phases and avoiding the system at homogenous phase. Transesterification occurs at the surface of oil droplets suspended in methanol, hence heterogeneous phase is necessary for the operation of the membrane reactor. The membrane bioreactor is particularly useful in removing unreacted canola oil from the FAME product and shifting the reaction equilibrium to the product side<sup>[42]</sup>.

A few shortcomings encountered with membrane reactors are membrane clogging and gas cavitation due to high pressure and cross-flow velocities. These problems can be alleviated by using submerged membrane filters, which operate at low pressures, enable frequent back-pulsing for membrane clogging control and requires no recirculating pumps. Another fairly naïve technology which uses spin filter which operates on the basis of physical sieving of particles. The spinning action of the filter assists in the control of biomass accumulation on the surface of membrane<sup>[43]</sup>.

## Application of Nanotechnology in Bioenergy Sector

Nanotechnology, the control of materials and phenomenon at scales between 1 and 100 nanometers holds the key to many of the technological advancements in the energy sector. It involves the miniaturization as well as the manipulation of atoms and molecules to control their properties, which at this scale are so different from the bulk properties. Nanomaterials have very high surface area and are being exploited for various renewable energy applications<sup>[44]</sup>.

Photovoltaic (PV) cells are devices that convert light energy into electrical energy, the photons from light are converted into electrical energy. The PV cells are made up of silicon-wafers which are cost effective, however the conversion efficiency is below par. The inclusion of nanoscale components in PV cells is a way to reduce some of its limitation. Quantum dots are highly efficient light emitters because of their ability to emit multiple electrons per solar photon, with different absorption and emission spectra depending on the particle size. This inevitably increases the theoretical efficiency by adapting to the incoming light spectrum. Dye sensitized solar cells which are composed of colloidal titanium dioxide films is another application of nanotechnology in energy sector. These films are sandwiched between a transparent electrode acting as anode, which is based on a conducting glass, and a platinum electrode, which acts as a catalytic conductor. An electrolyte is placed between the film and the platinum electrode for transportation of electrons. The dye molecules absorb the light and excite the electrons into the conduction band of the semiconductor. Due to the high surface area of the nanoparticles there is a charge separation at the interface between titanium and dye molecules which ultimately leads to increase in light harvesting<sup>[45]</sup>.

Metal-TiO<sub>2</sub> nanostructures exhibit a remarkable photocatalytic activity for hydrogen evolution from water-alcohol mixtures. Experimentally it was found to be 50% more efficient than that of the metal containing nanocomposites. The metal cations used for the study were : Ni<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>, Ag<sup>+</sup>, Pb<sup>2+</sup>. The rate of the photocatalytic hydrogen formation in water-ethanol mixture strongly depends on the metal type, increasing from silver to nickel to copper. This can be deduced from the fact that there is a difference in the electronic interactions between metal nanoparticles and TiO<sub>2</sub> surface. One dimensional TiO<sub>2</sub> (nanowires, nanorods, nanotubes, nanofibres) are highly efficient in H<sub>2</sub> production as they have a high surface area and a high interfacial charge transfer rate<sup>[46]</sup>.

Enzyme based biofuel cells are highly promising in the field of bioenergy because of their inexpensive components. Enzyme is used to catalyze the reaction instead of expensive precious metals. However two critical issues, short lifetime and poor power density still needs to be addressed. Both these short comings are related to enzyme stability, electron transfer rate and enzyme loading<sup>[47]</sup>. Microencapsulation is an enzyme immobilization technique whereby the enzymes are incorporated into three dimensional constraint aggregates. The use of micelles can stabilize the enzyme to a great extent: pH and temperature stability. Micelles also increase the activity of enzymes as compared to water or buffer, a phenomenon known as 'super activity'<sup>[48]</sup>.

### **Biofuel Policies**

Governments can play a greater role in regulating the expansion of the biofuel industries to ensure the benefits are realized by local communities. They can provide incentive and funds for the establishment of R&D (Research and Development) center and industrial unit for biofuel production<sup>[49]</sup>. One such example is a FACT foundation, which provides knowledge and expertise on biofuel sector to the local communities. Bioenergy is still in the developing phase and it needs revised policies and encouragement for it to prosper. The main driving forces for the development of biofuel are: (1) to reduce dependence on oil imports and guarantee oil security, (2) to create more employment and develop agricultural sector, (3) to promote the development of low carbon and sustainable economy, and (4) to explore new industries and novel technologies so as to form a situation of diversified energy sources and production supply. Government needs to provide ample funds for research and development in the field of biofuel. The taxation policies should also be monitored and special subsidies should be provided to the producers and end users<sup>[50]</sup>.

The use of biofuels without much significant doubt has had a positive impact on the environment. It has led to a reduction in the greenhouse gases to large extent, thus preventing global warming. It has also led to a gradual improvement in the air quality, with the reduction in SO<sub>2</sub> and particulate emissions. The introduction of biofuel has also created employment opportunities, with many critiques arguing in its favor. However, taking into account the whole impact on the economy, biofuel production does not look all that positive. This is mainly because the raw materials for biofuel have to be imported as they are not equally distributed in all the countries. The cost of which is pretty high, leading to a net negative impact on the economy<sup>[51]</sup>. The impact of biofuel on the economy of under developed nations can also be realized by the fact that they can act as a source of raw material producers. It not only mobilizes farmers to grow feedstocks for biofuel production but also increase their per capita income. The Food and Agricultural Organization (FAO) report put forward an interesting finding: the growth of demand of biofuel would lead to an increase in the prices of relevant agricultural products and that would in turn increase farmer's income, particularly in developing countries. Additionally the use of agricultural by-products for ethanol fermentation would reduce waste accumulation and enhance resource usage (reuse, reduce, recycle)<sup>[52]</sup>.

There are different taxation policies adopted by various nations across the globe that directly or indirectly affect biofuel production or consumption. For example, Blender's tax credit is used in the United States, wherein credit is received by fuel blenders for each gallon of biofuel they blend with the conventional fuel. Since the tax credit is a subsidy to biofuel consumption, it benefits fuel consumers. Blenders are able to reduce the price of the final fuel by the amount of the tax credit, adjusted for the share of the biofuel in the fuel blend. A tax exemption in the European Union and Brazil represents a reduction in the biofuel in the fuel excise tax collected at the pump level. The European Union uses a blende mandate, requiring that the biofuel makes up a pre-specified minimum share of energy content of the fuel<sup>[52]</sup>.



## Conclusion

Biofuel seems to be a promising alternative for conventional fuel in the field of energy. It is not only a means of sustainable development but also reduces the constraints posed on the environment by conventional fuel. However it is not as cost effective as conventional fuel as lot of funds has to be generated for research and development on biofuel. With the advent of modern and sophisticated genetic engineering tools, biofuel production has become much more easier and cost effective. Microorganisms can be used as a host as their genetic machinery is better understood and their generation time is quite rapid. The introduction of nanotechnology has not only increased the rate of biofuel production (nanocatalysts) but has also improved the kinetics (less time) as well. With better design of bioreactors and optimized reaction conditions, the yield of biofuels has increased considerably. However some critical issues like food security and land usage for biofuel raw material production are still highly debatable. The efficiency of biofuel also needs to be upgraded to the level of conventional fuel so as to convince the energy market for its survival. Government policies like tax credits, subsidy for biofuel production and consumption, donation for research and development are deemed as encouraging move for the future of biofuel industry.

## References

1. Milano J., Ong H.W., Masjuki H.H., Chong W.T., Lam M.K., Loh P.K. and Vellayan V., Microalgae biofuels as an alternative to fossil fuel for power generation, *Renewable and Sustainable Energy Reviews*, 58, 180-197 (2016)
2. Liu T. and Khosla C., Genetic Engineering of *Escherichia coli* for Biofuel Production, *Annual Review of Genetics*, 44, 53-69 (2010)
3. Singh V., Chaudhary D.K., Mani I. and Dhar P.K., Recent advances and challenges of the use of cyanobacteria towards the production of biofuels, *Renewable and Sustainable Energy Reviews*, 60, 1-10 (2016)
4. Liao J.C., Mi L., Pontrelli S. and Luo S., Fuelling the future: microbial engineering for the production of sustainable biofuels, *Nature Reviews Microbiology*, 14, 288-304 (2016)
5. Enciso S.R.A., Fellmann T., Dominguez I.P. and Santini F., Abolishing Biofuel policies: Possible impacts on agricultural price levels, price variability and global food security, *Food Policy*, 61, 9-26 (2016)
6. Henstra A.M., Sipma J., Rinzema A. and Stams A.J.M., Microbiology of synthesis gas fermentation for biofuel production, *Current Opinion in Biotechnology*, 18, 200-206 (2007)
7. Kumar A. Ergas A. Yuan X. Sahu A. Zhang Q. Dewulf J. Macata F.X. and Langenhove H.V. , Enhanced CO<sub>2</sub> fixation and biofuel production via microalgae: recent developments and future directions, *Trends in Biotechnology*, 28, 371-380 (2010)
8. Kallio P., Pasztor A., Thiel K., Akhtar M.K. and Jones P.R., An engineered pathway for the biosynthesis of renewable propane, *Nature Communications*, 5, 1-8 (2014)
9. Wang Q., Huang X., Zhang J., Lu X., Li S. and Li J.J., Engineering self-sufficient aldehyde deformylating oxygenases fused to alternative electron transfer systems for efficient conversion of aldehydes into alkanes, *Chem communication*, 50, 4299-4301 (2014)
10. Lu X., Vora H. and Khosla C., Overproduction of free fatty acids in *E. coli*: Implication for biodiesel production, *Metabolic Engineering*, 10, 333-339 (2008)
11. Steen E.J., Kang Y., Bokinsky G., Hu Z., Schirmer A., McClure A., del Cardayre S.B. and Keasling J.D., Microbial production of fatty-acid-derived fuels and chemicals from plant biomass, *Nature Letters*, 463, 559-563 (2010)
12. Jones A., Davies H.M. and Voelker T.A., Palmitoyl-Acyl Carrier Protein (ACP) Thioesterase and the evolutionary origin of plant Acyl-ACP Thioesterases, *The Plant Cell*, 7, 359-371 (1995)

13. Dormann P., Voelker T.A. and Ohlogge J.B., Cloning and expression in *Escherichia coli* of a novel thioesterase from *Arabidopsis thaliana* Specific for Long-Chain Acyl-Acyl Carrier Proteins, Archives of Biochemistry and Biophysics, 316, 612-618 **(1995)**
14. Rodriguez J.O.V., Shi S., Siewers V. and Nielson J., Metabolic Engineering of *Saccharomyces cerevisiae* for production of fatty acid ethyl esters, an advanced biofuel, by eliminating non-essential fatty acid utilization pathways, Applied Energy, 115, 226-232 **(2014)**
15. Roermund C.W.T van, Hetteema E.W., Kal A.J., Berg M.V.D., Tabak H.F. and Wanders R.J.A., Peroxisomal  $\beta$ -oxidation of polyunsaturated fatty acids in *Saccharomyces cerevisiae*: isocitrate dehydrogenase provides NADPH for reduction of double bonds at even positions, The EMBO journal, 17, 677-687 **(1998)**
16. Yu C., Kennedy N.J., Chang C.C.Y. and Rothblatt J.A., Molecular Cloning and characterization of two isoforms of *Saccharomyces cerevisiae* Acyl-CoA: Sterol Acyltransferase, The journal of Biological Chemistry, 271, 24157-24163 **(1996)**
17. Aldiguier A.S., Alfenore S., Cameleyre X., Goma G., Uribe Larrea J.L., Guillouet S.E. and Jouve C.M., Synergistic temperature and ethanol effect on *Saccharomyces cerevisiae* dynamic behavior in ethanol biofuel production, Bioprocess Biosystems Engineering., 26, 217-222 **(2004)**
18. Runguphan W. and Keasling J.D., Metabolic engineering of *Saccharomyces cerevisiae* for production of fatty acid derived biofuels and chemicals, Metabolic Engineering, 21, 103-113 **(2014)**
19. Machado I.M.P. and Atsumi S., Cyanobacterial Biofuel production, Journal of Biotechnology, 162, 50-56 **(2012)**
20. Quintana N., Kooy F.V., Rhee M.D.V., Voshol G.P. and Verpoorte R., Renewable energy from Cyanobacteria: energy production optimization by metabolic pathway engineering, Applied Microbiology and Biotechnology, 91, 471-490 **(2011)**
21. Tamagnini P., Axelsson R., Lindberg P., Oxelfelt F., Wunschiers R. and Lindblad P., Hydrogenases and Hydrogen metabolism of Cyanobacteria, Microbiology and Molecular Biology Reviews, 66, 1-20 **(2002)**
22. Bioson G., Bothe H., Hansel A. and Lindblad P., Evidence against a common use of the diaphorase subunits by the bidirectional hydrogenase and by the respiratory complex I in cyanobacteria, FEMS Microbiology Letters, 174, 159-165 **(1999)**
23. Mascal M. and Nikitin E.B., Direct high yield conversion of Cellulose into Biofuel, Angew Chem, 120, 8042-8044 **(2008)**
24. Xiao S., Liu B., Wang Y., Fang Z. and Zhang Z., Efficient conversion of cellulose into biofuel precursor 5-hydroxymethylfurfural in dimethyl sulfoxide-ionic liquid mixtures, Bioresource Technology, 151, 361-366 **(2014)**
25. Zhu J.Y., Sabo R. and Luo X., Integrated production of nano-fibrillated cellulose and cellulose biofuel (ethanol) by enzymatic fractionation of wood fibers, Green Chemistry, 13, 1339-1344 **(2011)**
26. Koh M.Y. and Ghazi T.I.M., A review of biodiesel production from *Jatropha curcas* L. oil, Renewable and Sustainable Energy Reviews, 15, 2240-2251 **(2011)**
27. Tiwari A.K., Kumar A. and Raheman H., Biodiesel production from *Jatropha* oil with high free fatty acids: An optimized process, Biomass and Bioenergy, 31, 569-575 **(2007)**
28. Sarin R., Sharma M., Sinharay S. and Malhotra R.K., *Jatropha*-Palm biodiesel blends: An optimized mix for Asia, Fuel, 86, 1365-1371 **(2007)**

29. Schober S. and Mittelbach M., The impact of anti-oxidants on biodiesel oxidation stability, *European Journal of Lipid Science and Technology*, 106, 382-389 **(2004)**
30. Achten W.M.J., Verchot L., Franken Y.J., Mathijs E., Singh V.P., Aerts R. and Muys B., *Jatropha bio-diesel production and use*, *Biomass and Bioenergy*, 32, 1063-1084 **(2008)**
31. Claudio A., Growth of ethanol fuel stalls in Brazil, *Nature*, 491, 646-647 **(2012)**
32. Peterson A.M., Melamu R., Knoetze J.H. and Gorgens J.F., Comparison of second generation processes for the conversion of sugarcane bagasse to liquid biofuels in terms of energy efficiency, pinch point analysis and Life cycle analysis, *Energy Conversion and Management*, 91, 292-301 **(2015)**
33. Lam E., Shine J., Silva J., Lawton M., Bonos S., Clavino M., Carrer H., Silva-Filho M.C., Glynn N, Helsel Z., Ma J., Richard E., Souza G.M. and Ming R., Improving sugarcane for biofuel: engineering for an even better feedstock, *GCB Bioenergy*, 1, 251-255 **(2009)**
34. Jung J.H. Fouad W.M. Vermerris W. Gallo M. and Alpeter F., RNAi suppression of lignin biosynthesis in sugarcane reduces recalcitrance for biofuel production from lignocellulosic biomass, *Plant Biotechnology Journal*, 10, 1067-1076 **(2012)**
35. Yeu H. Ma X. and Gong J., An alternative synthetic approach for efficient catalytic conversion of syngas to ethanol, *Accounts of Chemical Research*, 47, 1483-1492 **(2014)**
36. Munasinghe P.C. and Khanal S.K., Biomass-derived syngas fermentation into biofuels: Opportunities and challenges, *Bioresource Technology*, 101, 5013-5022 **(2010)**
37. Daniell J. Kopke M. and Simpson S.D., Commercial Biogas Syngas Fermentation, *Energies*, 5, 5372-5417 **(2012)**
38. Demirbas A., Progress and recent trends in biofuels, *Progress in Energy and Combustion Science*, 33, 1-18 **(2007)**
39. Lehr F. and Posten C., Closed photo-bioreactors as tools for biofuel production, *Current opinion in Biotechnology*, 20, 280-285 **(2009)**
40. Hollinshead W., He L. and Tang Y.J., Biofuel production: an odyssey from metabolic engineering to fermentation scale up, *frontiers in Microbiology*, 5, 1-8 **(2014)**
41. Dube M.A. Tremblay A.Y. and Liu J., Biodiesel Production using membrane bioreactors, *Bioresource Technology*, 98, 639-647 **(2007)**
42. Cheng L.H. Yen S.Y. Su L.S. and Chen J., Study on membrane reactors for biodiesel production by phase behaviors of canola oil methanolysis in batch reactors, *Bioresource Technology*, 101, 6663-6668 **(2010)**
43. Levin D.B. Carere C.R. Cicek N. and Sparling R., Challenges for biohydrogen production via direct lignocellulose fermentation, *International Journal of Hydrogen Energy*, 34, 7390-7403 **(2009)**
44. Ramsurn S. and Gupta R.B., Nanotechnology in Solar and Biofuels, *ACS Sustainable Chemistry and Engineering*, 1, 779-797 **(2013)**
45. Serrano E. Rus G. and Martinez J.G., Nanotechnology for sustainable energy, *Renewable and sustainable Energy reviews*, 13, 2373-2384 **(2009)**
46. Zhu J. and Zach M., Nanostructured materials for photocatalytic hydrogen production, *Current opinion in Colloid and Interface Science*, 14, 260-269 **(2009)**

47. Kim J., Jia H. and Wang P., Challenges in Biocatalysis for enzyme based biofuel cells, *Biotechnology Advances*, 24, 296-308 **(2006)**
48. Cooney M.J. Svoboda V. Lau C. Martin G. and Minteer S.D., Enzyme catalyzed biofuel cells, *Energy and Environmental Science*, 1, 320-337 **(2008)**
49. Gilbert N., The seeds of an economy, *Nature*, 474, 18-19 **(2011)**
50. Su Y. Zhang P. and Su Yuq., An overview of biofuel policies and industrialization in the major biofuel producing countries, *Renewable and Sustainable Energy Reviews*, 50, 991-1003 **(2015)**
51. Santamaria M. and Azqueta D., Promoting biofuels use in Spain: A cost benefit analysis, *Renewable and Sustainable Energy Reviews*, 50, 1415-1424 **(2015)**
52. Ji X. and Long X., A review of the ecological and socioeconomic effects of biofuel and energy policy recommendations, *Renewable and Sustainable Energy Reviews*, 61, 41-52 **(2016)**
53. Rajcaniova M. Drabik D. and Ciaian P., How policy affect international biofuel price linkages, *Energy Policy*, 59, 857-865 **(2013)**