Proc Indian Natn Sci Acad **81** No. 4 September 2015 pp. 765-773
© *Printed in India*.
DOI: 10.16943/ptinsa/2015/v81i4/48295

Review Article

Biofuels: Engineering and Biological Challenges

PURNENDU GHOSH*
Birla Institute of Scientific Research, Statue Circle, Jaipur 302 001, India

(Received on 30 March 2014; Accepted on 02 August 2015)

The second generation biofuel technologies are evolving rapidly to provide solutions for the partial replacement of fossil fuels. Both bioethanol and biodiesel have great potential in India. Both the technologies, however, have to overcome various bottlenecks before they become commercial technologies. In this regard, several critical questions, besides science and technology, need to be resolved. This will require new ways of thinking about agriculture, energy infrastructure and rural economic development.

Keywords: Biofuels Technology; Bioethanol; Biomass; Algal Biofuel; Bioenergy

Introduction

In recent times, a great concern about fossil fuels supplies, their non-renewable nature and environmental consequences of their use has driven interest in biofuel programmes all over the world. There is no doubt that the "best substitute for petroleum is petroleum" and, as one analyst puts it, replacement of fossil fuel by biofuel is not possible,but augmentation of fuel supply probably is. As Church and Regis (2012) write in their book *Regenesis*, "We're now in a transitional period, caught between the age of fossil fuels and the age of biofuels."

It is believed that a partial transition from oil to biofuels can stabilize the energy market significantly. To be a viable alternative, a biofuel should provide a net energy gain, have environmental benefits, be economically competitive and be producible in large quantities without affecting food security of the country.

The National Policy on Biofuels of India (2009) proposes a target of 20% blending of bioethanol by 2017. A target of 10% petrol blending seem more realistic for 2017. Even this seems a difficult proposition keeping in view the present supply and demand situation. The intermediate target of 5% and 10% blending by 2007-2008 has not been achieved. The government is unable to implement compulsory blending of 5% ethanol in petrol.

First and Second Generation Biofuels

The basic routes for converting biomass to biofuel are biochemical and thermochemical. The two classic thermochemical options, namely, gasification and pyrolysis produce different intermediates. Gasification involves rapid heating and partial oxidation to produce syngas, which is largely carbon monoxide and hydrogen. The high oxygen content of biomass results in the production of significant quantities of carbon dioxide, which reduces carbon efficiency. Also the sulphur, nitrogen, phosphorous, potassium, and mineral content of biomass complicates matters further. In pyrolysis, lower temperatures are used to break down biomass into smaller molecules such as oxygenated aromatics, ketones, organic acids, and other oxygenates, as well as light hydrocarbon gases. In addition to the lower energy input to achieve biomass deconstruction, pyrolysis has a high theoretical yield for liquid products.

**Author for Correspondence: E-mail: ghoshiitbisr@gmail.com; Phone: 0141-2385283*

In biochemical processing, biomass is typically processed to yield monosacchrides, which are then converted by microbes to produce fuels. Though bioethanol is an established biofuel, there is an alternate view that it would be a good idea to look at the conversion of lignocellulose into organic acids rather than sugars.

Another biochemical route uses anaerobic digestion to produce biogas, a mix of methane and carbon dioxide. Here, natural consortia of bacteria decompose organic matter into methane in the absence of oxygen. Although much of the biomass resource might be dedicated to biofuel production (thus diminishing its role in electricity generation), biogas technologies could provide a small but nontrivial part of a renewable electricity portfolio, particularly given their flexibility and potential for distributed generation.

The feedstock for first generation biofuels produced through biochemical routes are primarily food crops, such as sugar cane, grain (corn), oil seeds and vegetable oils. Their limited contribution to meet the energy demands of the future has raised questions about their role in the transport fuel mix of the future. This makes the need for second generation biofuel technologies inevitable and desirable.

The feedstock for second generation biofuels is non-food biomass, such as lignocellulosic materials (bagasse, cereal straw, forest residues, and short-rotation energy crops). The second-generation biofuel production has the potential to provide benefits such as consuming waste residues and making use of abandoned land. Job creation and regional growth are probably the most important drivers for the implementation of second-generation biofuel projects in major economies and developing countries.

According to the estimates of International Energy Agency (2010) biofuels are expected to provide 9% (11.7 EJ) of the total transport fuel demand (126 EJ) in 2030, 26% (29 EJ) of total transportation fuel (112 EJ) in 2050, with second-generation biofuels accounting for roughly 90% of all biofuels .

Biofuels derived from lignocellulosic biomass and algae are promising additional sources to meet energy demands of the country. Both can play a significant part to solve energy supply picture in the future provided key obstacles are overcome. Both, however, are future technologies as there are no commercial plants, but a considerable number of pilot and demonstration plants have been planned or set up in recent years, mainly in North America, Europe, Brazil, China, India and Thailand.

In India, the commercial viability of both the options is highly dependent on the future price of oil and the government policy. There is thus a promise as well as an uncertainty. The promise is to significantly reduce our dependence on imported oil, create new jobs, improve rural economies, reduce greenhouse gas emissions, and enhance national fuel security. The major uncertainties are feedstock availability and cost, conversion technologies and cost, and the impact of technologies on the environment. The milestones (USDOE, 2006) that are suggested for the development of biofuels are provided in Fig. 1.

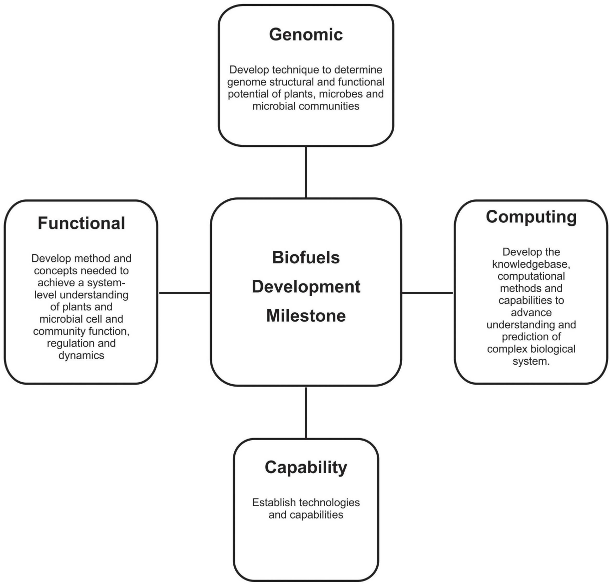

Fig. 1: Biofuels development milestones (USNAS, 2012)

A brief overview on the future of cellulose and algae-based biofuels is given here.

Cellulose-Based Biofuel

Production of ethanol from biomass follows various conversion routes (Fig. 2). Ethanol is produced in India

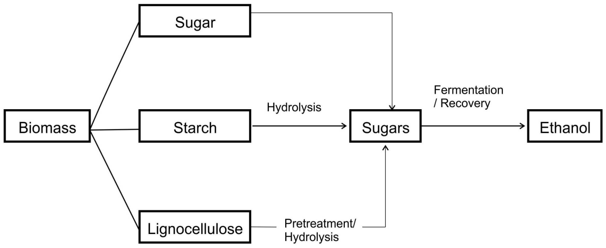

Fig. 2: Biomass-based bioethanol conversion routes

from cane molasses. Efforts to produce ethanol from other sugar-based feedstock such as sweet sorghum, sugar beets, and sweet potatoes are at present in the experimental stage. Lower molasses availability and consequent higher prices impact ethanol's cost of production, thereby causing a disruption in the supply of ethanol at pre-negotiated fixed ethanol prices.

All the countries in the world are looking for solutions for their growing energy needs using sustainable and renewable resources. The first-generation technologies for bioethanol production based on sugars and starches cannot provide long-term solution. They compete for land with food crops, resulting in misleading cost-benefit analysis. What we need is a cheap, abundant and renewable raw material that does not interfere with food production. Lignocellulosic biomass (LCB) is such a feedstock for the production of second-generation bioethanol.

Supply of Biomass

The global supply of cellulosic biomass is estimated to contain energy that is equivalent to much more than the world's current annual consumption of transportation fuel. The sources of cellulosic biomass include crop wastes, forest residues, and dedicated energy crops. Lignocellulosic biomass (LCB) is less expensive than sugar or starch-based feedstock, but its conversion to ethanol at present is more costly. The commercialization of this technology thus has to overcome various bottlenecks. These include feedstock availability, scale of operation, cheaper and effective pretreatment technologies, efficient hydrolytic agents, availability of recombinant organisms capable of co-fermenting the whole range of sugars at a temperature compatible to optimum hydrolysis, and better co-product value (Ghosh and Ghose, 2003).

Supply of biomass is one of the most critical factors for the development of a viable bioethanol technology. Three distinct goals need to be met for the development of biomass-based biofuels, namely, maximizing the total amount of biomass produced per hectare per year, maintaining sustainability while minimizing inputs, and maximizing the amount of fuel that can be produced per unit of biomass. Exact values for each of these parameters would vary, depending upon the type of energy crop and the growing zone. Logistics of raw material supply (availability, collection, storage and handling) to meet large demands of biofuels is a major issue of concern. In addition, the availability of the feedstock on a sustainable basis would need either large storage facilities or availability of plants to operate on multiple feedstock for their continued operation throughout the year.

Ideal Pretreatment Technology

Pretreatment of LCB continues to be a major barrier for the development of a viable technology. In the LCB-based bioethanol technology, cellulose and hemicellulose present in the lignocellulose are hydrolysed to sugars (hexoses and pentoses) using acids or enzymes. Lignin is the major interference in the hydrolysis of native lignocellulose. In the enzymatic process, the LCB is pretreated in order to increase the accessibility of cellulolytic enzymes (cellulases) to the substrate. Typically, hydrolysis yields in the absence of pretreatment are less than 20% of theoretical yields, whereas yields after pretreatment often exceed 90%. The rationale for pretreatment has thus been to separate individual components of LCB with minimum component losses, concomitant with an increase in surface area and a decrease in crystallinity.

An ideal technology is expected to produce a reactive fibre that will require little or no size reduction, and can be operated at a high solid/liquid ratio. One needs to ascertain what is more important for enzymatic hydrolysis – the extent of delignification that requires harsher conditions for complete lignin separation or loosening of cellulose – hemicellulose-lignin bonds under milder conditions. The benefits of lignin solubilization need to be weighed against the potential for fermentation inhibition by soluble lignin derivatives.

Various mechanical, physical, chemical, and biological approaches, either singly or in combination have been attempted to meet these objectives, but none has shown the promise expected from an ideal pretreatment technology. Development of LCB-based energy plants with traits such as increased cellulose and hemicellulose and less lignin not only has the potential to improve ethanol yields, but also the possibility of application of much simpler pretreatment technologies. Metabolic engineering of the lignin biosynthetic pathway has been suggested as a method for modifying lignin content in the feedstock.

Enzyme and Enzymatic Hydrolysis

The important parameters of enzymatic hydrolysis are sugar yield, duration of hydrolysis, enzyme loading, characteristics of substrate cellulose, and enzyme cellulases.

The most desirable attributes of the enzyme cellulases include the ability to produce a complete cellulase system with high catalytic activity against crystalline cellulose, thermal stability, decreased susceptibility to enzyme inhibition by the products of hydrolysis (glucose, and cellobiose), selective adsorption of the enzyme on cellulose, and the ability to withstand shear forces. Strategies to improve cellulases include discovering new enzymes through bioprospecting, creating new/better mixtures of enzymes, and developing improved expression systems through protein engineering. *De-novo* and *in-silico* designing of improved cellulases are also being attempted. Creating a more effective cellulose binding domain in the enzyme molecule is another approach to increase enzyme efficiency.

A critical element for the success of bioethanol technology is the availability of cheap cellulases. Industrial enzyme producers are trying to achieve reduction in enzyme cost in order to support an economical and robust cellulose biorefinery. Cellulase enzymes are too expensive for bioethanol. For example, costs of amylase enzymes for converting grain starch to ethanol are about ten times cheaper than the most optimistic cost estimates for cellulase preparations. There is, however, a good possibility of producing effective cellulases at a much reduced cost. For the hydrolysis of pretreated biomass, extremely complex cellulases may not be required; simpler cellulase systems may serve the purpose. The major market for cellulase enzymes is the textile industry, and the enzymes produced are tailored to meet the requirement of this industry. It is important to recognize that biofuels application needs are significantly different from textile applications.

An Ideal Ethanol Producing Organism

The bioethanol process needs an efficient organism with capability to convert sugars (both hexoses and pentoses) to ethanol. An ideal ethanol producing organism should have characteristics such as high ethanol tolerance, capacity to withstand high osmotic pressure, high temperature, and low pH, high cell viability, appropriate flocculation and sedimentation characteristics, capability to ferment broad range of sugars mainly to ethanol and possibly negligible levels of by-products (such as acids and glycerol), and resistance to inhibitory compounds present in the pretreatment/hydrolysis stream. A strategy for increasing ethanol tolerance or other traits uses evolutionary engineering concepts and methods. This strategy allows the microbial process to evolve under proper selective pressure (in this case, higher ethanol concentrations) to increasingly higher ethanol tolerances.

Conversion of cellulose and hemicellulose to ethanol comprises hydrolysis followed by fermentation of hexoses and pentoses by ethanol producing organisms. Simultaneous saccharification and fermentation (SSF) integrates the processes of hydrolysis with fermentation. The development of thermophilic ethanol-producing organisms for use in SSF could allow the consolidated process to run at higher temperatures, thus realizing significant savings by reducing cellulase enzyme requirements. Combining cellulase production, cellulose hydrolysis, and co-fermentation of hexose and pentose sugars in a single step, called “consolidated bioprocessing”, is considered the ultimate low-cost configuration for cellulose hydrolysis and fermentation.

Companies are engaged in creating synthetic microbes to accelerate the conversion of agricultural waste to ethanol. Diverse microbial strains collected from the seawater is being used to create new synthetic microbes. The hunt is on for a better microbe that will cheaply and efficiently break down cellulose to sugars and then ferment those sugars into ethanol. Such designer organisms are yet not available.

Commercialization of Technology

The projected cost of ethanol from LCB has declined significantly in the last ten years. Further cost reduction is needed. This is possible by employing a cellulose and hemicellulose rich, but lignin lean feedstock.

Relatively large investments are required to install LCB-based bioethanol plants. In India ethanol plants are comparatively small in capacity. This brings to the fore another related issue, namely, scale of operation vis-à-vis feedstock availability. Keeping in view the logistics of feedstock procurement, it is needed to decide if it is advisable to build very large plants as increased feedstock cost (due to collection and transport of large amounts of feedstock) may offset savings due to economies of scale.

Commercialization of ethanol needs the attention of researchers, entrepreneurs, and more importantly, the policy makers. India has the capacity to produce 4000 million litres of ethanol from molasses, but it produces around 2800 million litres. A sizable utilizable capacity needs to be utilized. It will depend upon how pricing issues are addressed.

A Global Market Survey (Ethanol 2020), reports that it is possible to replace 20% of gasoline consumption in the US, China, and India by 2020, if the promises of competitive, large-scale cellulosic ethanol production are realized, and if national import/export policies for biofuels are further liberalized.

These are big ‘ifs’. There are many questions that need to be resolved, both at the researchers and planners stage. Large-scale lignocellulose-based bioethanol technology will require major changes in supply chain infrastructure. It will require new ways of thinking about agriculture, energy infrastructure, and rural economic development.

Algal Biofuel

The National Bio-diesel Mission (NBM) has identified *Jatropha curcas* as the most suitable tree-borne oilseed for bio-diesel production on wastelands. Biodiesel production in India is very small due to inadequate supplies of Jatropha. NBM had set an ambitious target of covering 11.2 to 13.4 million hectares of land under Jatropha cultivation by the end of 2011-12.

The Government of India’s ambitious plan of producing sufficient bio-diesel to meet the mandate of 20% blending with diesel by 2011-12 has proceeded slowly. According to trade and industry estimates, Jatropha has been planted across 500,000 hectares of wasteland, of which 65-70% is estimated to be new plantation, and would take three to four years to mature. As a result, there are insufficient Jatropha seeds available for biodiesel production.

Lack of high-yielding, drought-tolerant Jatropha seeds, smaller land holdings, ownership issues with government or community owned wastelands, little progress made by state governments to meet large scale Jatropha plantations, and negligible commercial production of biodiesel have hindered the efforts and investments made by both private and public sector companies in this sector.

According to the report on Biofuels for Transportation Programme for 12th Five Year Plan prepared by the sub-group constituted by the Ministry of New and Renewable Energy, the absence of guaranteed national market due to the absence of minimum support price is bound to deter the investment especially in long duration crops with little history of cultivation such as *Jatropha curcas* or *Pongamia pinnata*.

There are about 20 large capacity biodiesel plants (10,000 to 100,000 tons per year) in India that produce biodiesel from edible oil waste (unusable oil fractions), animal fat and non-edible oils. Commercial production of biodiesel from Jatropha and non-edible oilseeds is small, with estimates varying from 140 to 300 million litres per year. The biodiesel produced is sold to the unorganized sector (irrigation pumps, agricultural usage, diesel generators, etc.), and to experimental projects carried out by automobile and transport companies. Biodiesel production cost is higher than the government notified purchase price. This is mainly due to the lack of supply of Jatropha seeds.

An Ideal Algae

Algae are an attractive way to harvest solar energy, and turn carbon dioxide into biofuel. Much money is thus being poured into the idea of turning algae into mini oil wells.

The algae-derived biofuel is projected to reduce fossil fuel consumption equivalent to 6% of road transport diesel by 2030. An efficient algae-based biofuel process promises around 40000 litres of oil per hectare of land. The worldwide microalgal manufacturing infrastructure is devoted to extraction of high value products such as carotenoids and omega 3 fatty acids used for food and feed ingredients. Although microalgae are not yet produced at large scale for bulk applications, recent advances, particularly in the methods of systems biology, genetic engineering, and biorefining present opportunities to develop this process in a sustainable and economical way, within the next twenty years. But the challenges are many.

Algae use carbon dioxide to produce oil molecules via photosynthesis. In non-stressed growing algae, lipids are mostly present in the form of phospholipids in the cell membranes. Some microalgae, when exposed to stress conditions (e.g., nutrient deprivation or high light intensities), accumulate lipids in the form of triacylglycerols in so-called oil bodies. This accumulation occurs at the expense of energy used for growth, leading to a decrease in growth rate and a consequent decrease in productivity.The carbon dioxide discharged from power plants and oil refineries can be captured by algae and used to produce biofuel, and thereby reducing carbon dioxide build up in the atmosphere. A schematic of algae-based biodiesel processing is provided in Fig. 3.

An ideal algae that can produce biofuel should have high yield on high light intensity, large cells with thin membranes, stable and resistant to infections, and insensitive to high oxygen concentration. Algae should be able to grow and produce lipids at the same time, should form flocs, and preferably should excrete oils outside the cells. Such a magic bug has not been discovered yet. Efforts, nevertheless, are being made to design such magic bugs.

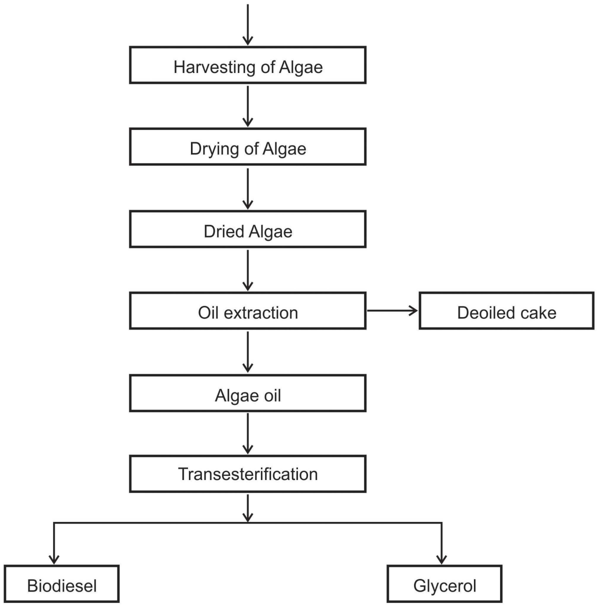

Fig. 3: Algae-based biodiesel processing

Knowledge of the biosynthesis mechanism of triacylglycerols and their accumulation in oil bodies is limited and often based on analogies with higher plants. If the mechanisms are known, it could open the possibility of inducing lipid accumulation in oil bodies without having to apply a stress factor. A detailed insight into metabolic pathways may lead to strategies to induce lipid accumulation based on process conditions, defined nutrient regimens, and/or the use of metabolic engineering techniques.

Mass Cultivation of Algae

Both open and closed mass cultivation systems can be used for growing algae. Table 1 summarizes the pros and cons of open and closed system. The obvious problems with open systems are low biomass growth

Table 1: Algal Biofuel. Open and closed systems (USDOE, 2006)

Parameter	Open system	Closed system
Cost	Lower	Higher
Pumping energy	Lower	Higher
Ease and scale up	Greater Lower	
Evaporative water loss	Higher	Negligible
Land area required	Higher	Lower
Contamination risks	Higher	Lower
Productivity	Lower	Higher
Productivity stability	More variable	Less variable
Sparged CO_2 loss	Higher	Lower

and biomass loss due to lesser control on growth parameters, including disrupted availability of sunlight. Prevention of algae from the predators and contamination by natural strains are other obvious difficulties (USNAS, 2012).

Closed and controlled photobioreactors are more efficient. The design and fabrication of efficient photobioreactors equipped with optimal lighting techniques and configurations with emphasis on light efficiency, less shear damage and low cell adherence at the surface of the bioreactor is quite expensive.Advantages and disadvantages of typical closed bioreactors for algal biofuel production are mentioned in Table 2 (Kunjapur and Eldridge, 2010).

In recent years, much effort is put into increasing photosynthetic efficiency of microalgae under oversaturating light (the normal condition on a sunny day). Certain strains of microalgae can harness 3% of the incoming sunlight to make plant matter, as opposed to roughly 1% for corn or sugar.

The photosynthetic efficiency can be increased by developing new microalgae strains with smaller antenna sizes, and by decreasing the light path of photobioreactors, while increasing mixing in high cell density cultures. Researchers obtained high photosynthetic efficiencies under bright sunlight in systems with lower energy requirements by reducing the light intensity at the reactor surface. To reduce the cost of manufacturing these systems, vertical panels made from thin plastic films such as polyethylene have been be used.

Table 2: Closed Bioreactors: Advantages and disadvantages (Kunjapur and Eldridge, 2010)

Reactor type	Advantages	Disadvantages
Flat plate	Shortest oxygen path Low power consumption	Low photosynthetic efficiency
Tubular	High volumetric biomass density	Oxygen accumulation Photoinhibition Most land use
Vertical	Greatest gas exchange Best exposer to light/ dark cycles Least land use High photosynthetic efficiency	Support costs Scalability

Arranging for carbon dioxide that could be utilized for commercial algae production is challenging; a total of 1.8 kg of carbon dioxide is needed to produce 1 kg of algal biomass. While carbon dioxide could be sourced from power plants for sequestration, arranging large quantities of fresh or saline water is extremely difficult. In addition, algae like any plant would require nutrients such as NPK and other micronutrients for optimal growth.

For sustainable production of biofuel from microalgae, it will be important to make use of residual nutrient sources, and to recycle nutrients as much as possible. Utilization of wastewater will also achieve twin objectives of algal biomass production and wastewater treatment.Waste-water may offer a useful point source, which can be either municipal, organic industrial (e.g., food processing), organic agricultural (e.g., confined animal facilities), or eutrophic waters with low organic content but high nutrient content (e.g., agricultural drainage, lakes and rivers). Microalgae can also grow in seawater. Even deserts would be suitable if there is access to salt aquifers.

Algae Harvesting and Processing

After the growth, the algal biomass needs to be harvested, the lipids extracted, and the remaining cell components recovered. Harvesting of microalgae is expensive because of the high energy requirements and capital costs involved.

Since most microalgae are small individual cells, centrifugation is often used as a preferred harvesting method. However, as the biomass concentration is generally low (<3 g/L), centrifugation of diluted streams requires a large capacity of the centrifuge, which makes the process energy-demanding and expensive. Flocculation, followed by sedimentation and flotation, before centrifugation or filtration will substantially reduce harvesting costs and energy requirements. Ideally, algae should flocculate spontaneously at a certain stage of the process.

The process involves extraction of stored oil from the algae (by breaking oil-rich algae). This adds to downstream processing cost. The algal oil is extracted from the algal cells and then converted into biodiesel by transesterification with short-chain alcohols or by hydrogenation of fatty acids into linear hydrocarbons.

After harvesting, the cells are disrupted and the oil extracted with solvents. Most microalgae strains are, in general, relatively small and have a thick cell wall. For this reason, very harsh conditions (e.g., mechanical, chemical, and physical stress) are needed to break the cells for extraction of the products. Excretion of the oils, in a manner similar to what naturally occurs in the microalgae *Botryococcus braunii*, will lead to a simplified biorefinery and improve downstream economics. However, it will not provide a complete solution because the remaining cell components still need to be recovered from the cells.

Thin cell membranes, such as those present in *Dunaliella*, strong enough to prevent shear damage during production, would facilitate cell disruption. Research is needed to explore mild cell disruption, extraction, and separation technologies that retain the functionality of the different cell components.

A genetically engineered bacteria (*E. coli*) has shown promise to convert sunlight, carbon dioxide and water into different hydrocarbons, including biodiesel. The bacterium grows happily (three times faster than the yeast) at tropical temperature. The designed organism secretes oil, instead of storing it inside the organism, so as to reduce downstream costs.

Concluding Remarks

It is now a well-established fact that fossil fuels are in short supply and have limited reserves. They need to be replaced. It must, however, be recognized that their full replacement is neither desirable, nor possible. Its partial replacement seems to be a reality. Among the alternatives available, biofuels have great potential in India, because of the availability of feedstock, environmental benefits, and the possibility of improved rural economies.

The potential of a technology is one thing and its availability at the desirable 'çost' is another. India has to overcome various bottlenecks before it becomes a commercial technology. The country has to answer several critical questions. The answer to these questions will decide the future of biofuels technology in India. The questions, related to technology and its dissemination, include: What kind of support, other than science, will be needed for its viability? Who will be the major promoter of policy – agricultural sector, sugar industry or petroleum industry? What kind of government support is needed to make this technology viable? Who will be the major promoter of large scale biofuel technology? Other than science, what kind of support is needed to make this a viable technology? Under what circumstances or conditions can refiners consider participation in the ethanol industry? These questions continue to bother researchers and planners. Large-scale lignocellulose based bioethanol technology will require major changes in supply chain infrastructure. It will require new ways of thinking about agriculture, energy infrastructure, and rural economic development.

References

Church G and Regis E (2012) Regenesis, Basic Books

Ethanol 2020, A global market survey, Emerging markets, online publication

Ghosh P and Ghose T K (2003) Bioethanol in India: Recent past and emerging future, In: Advances in Biochemical Engineering/Biotechnology (Eds: Ghose T K and Ghosh P), **85**, pp 1-27, Springer-Verlag

International Energy Agency (2010) Sustainable production of second-generation biofuel – potential and perspectives in major economies and developing countries

Kunjapur A M and Eldridge R B (2010) Photobioreactor design for commercial biofuel production from microalgae *Ind Eng Chem Res* **49** 3516-3526

National Policy on Biofuels (2009) Ministry of New & Renewable Energy, Government of India

US Department of Energy (USDOE) (2006) Biofuels Joint Roadmap

US National Academy of Sciences (USNAS) (2012) Sustainable development of algal biofuels.