

Review

Production of liquid biofuels from renewable resources

Poonam Singh Nigam^{a,*}, Anoop Singh^b^a Faculty of Life and Health Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland, United Kingdom^b Biofuels Research Group, Environmental Research Institute, University College Cork, Ireland

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ABSTRACT

This article is an up-to-date review of the literature available on the subject of liquid biofuels. In search of a suitable fuel alternative to fast depleting fossil fuel and oil reserves and in serious consideration of the environmental issues associated with the extensive use of fuels based on petrochemicals, research work is in progress worldwide. Researchers have been re-directing their interests in biomass based fuels, which currently seem to be the only logical alternative for sustainable development in the context of economical and environmental considerations. Renewable bioresources are available globally in the form of residual agricultural biomass and wastes, which can be transformed into liquid biofuels. However, the process of conversion, or chemical transformation, could be very expensive and not worth-while to use for an economical large-scale commercial supply of biofuels. Hence, there is still need for much research to be done for an effective, economical and efficient conversion process. Therefore, this article is written as a broad overview of the subject, and includes information based on the research conducted globally by scientists according to their local socio-cultural and economic situations.

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* Corresponding author.

E-mail addresses: p.singh@ulster.ac.uk (P.S. Nigam), apsinghenv@gmail.com (A. Singh).

1. Introduction

The increasing industrialization and motorization of the world has led to a steep rise for the demand of petroleum-based fuels [1]. Today fossil fuels take up 80% of the primary energy consumed in the world, of which 58% alone is consumed by the transport sector [2]. The sources of these fossil fuels are becoming exhausted and found major contribution in greenhouse gas (GHG) emissions by consumption of fossil fuels to fulfill the energy demand [3–5], which leads to many negative effects including climate change, receding of glaciers, rise in sea level, loss of biodiversity, etc. [6]. Increasing energy demand leads to an increase in crude oil price, directly affected to global economic activity [7]. Progressive depletion of conventional fossil fuels with increasing energy consumption and GHG emissions have led to a move towards alternative, renewable, sustainable, efficient and cost-effective energy sources with lesser emissions [4,5,8,9].

Among many energy alternatives, biofuels, hydrogen, natural gas and syngas (synthesis gas) may likely emerge as the four strategically important sustainable fuel sources in the foreseeable future. Within these four, biofuels are the most environment-friendly energy source. As concern about global warming grows, there is increased interest in biofuels, which also stems from the fact that home-produced fuels relieve, to some extent, the reliance on imported oil and political vagaries in its supply and price [10]. Hence, biofuels are being explored to replace fossil fuels. Biofuels are favourable choice of fuel consumption due to their renewability, biodegradability and generating acceptable quality exhaust gases [11].

1.1. Biofuels an answer to a sustainable fuel

Biofuels are referred to liquid, gas and solid fuels predominantly produced from biomass. A variety of fuels can be produced from biomass such as ethanol, methanol, biodiesel, Fischer-Tropsch diesel, hydrogen and methane [12].

Biofuels have emerged as one of the most strategically important sustainable fuel sources and are considered an important way of progress for limiting greenhouse gas emissions, improving air quality and finding new energetic resources [13]. Renewable and carbon neutral biofuels are necessary for environmental and economic sustainability. People will always need fuel for living and heating, and since the demand for oil has increased, production from large oil fields is declining at the rate of 4–5% annually, subsequently the world production of oil is expected to peak in coming years. A worrying statistic is that global production of oil and gas is approaching its maximum and the world is now finding one new barrel of oil for every four it consumes [14]. Hence, as an alternative to fossil fuels, biofuels have been portrayed as a future leading supplier of energy sources that have the ability to increase the security of supply, reduce the vehicle emissions and provide a steady income for farmers.

1.2. Background

Biofuels are important because they replace petroleum fuels. An increasing number of developed and developing countries found biofuels as a key to reducing reliance on foreign oil, lowering GHG emissions and meeting rural development goals [15–17]. Between 1980 and 2005, worldwide production of biofuels increased by an order of magnitude from 4.4 to 50.1 billion litres [16,18], with further dramatic increases in future [19].

Biofuels are considered to be most promising in the short-term as their market maturity is above those of the other options [20]. The European Council in March 2007 endorsed a mandatory target

of a 20% share of energy from renewable sources in overall energy consumption by 2020 and a mandatory 10% minimum target to be achieved by all Member States for the share of biofuels in transport sector by 2020 [21]. In order to achieve these targets, Member States apply a wide variety of measures, comprising command and control instruments (e.g. standards, quotas) as well as economic and fiscal measures, such as tax exemptions or reductions, and communication and collaborative measures [22]. Furthermore, current EU legislation that limits the blending of biofuels into fossil fuels to 5% (v/v) is being revised to allow for higher shares [20].

Different countries have adopted different measures to introduce biofuels. The economics of each fuel vary with location, feedstock, and several other factors. Political agendas and environmental concerns also play a crucial role in the production and utilization of biofuels. Fitting biofuels into the enormous current fuel distribution and vehicle infrastructure challenged the stakeholders. In addition, important technical and commercial limitations on existing biofuels have resulted in significant resources being invested in researching and implementing new feedstocks and production technology [23].

Alcohol fuels can substitute for gasoline in spark-ignition engines, while biodiesel, green diesel and dimethyl ether (DME) are suitable for use in compression ignition engines. The Fischer-Tropsch process can produce a variety of different hydrocarbon fuels, the primary one of which is a diesel-like fuel for compression ignition engines [24]. There are a number of technologies existed and several under development, for production of biofuels such as fermentation of sugar substrates, catalytic technology to convert ethanol to mixed hydrocarbon, hydrolysis of cellulose, biobutanol by fermentation, transesterification of natural oils and fats to biodiesel, hydrocracking of natural oils and fats, pyrolysis and gasification of various biological materials, etc. Fig. 1 presents a mapping of various technologies and fuel products, positioned relative to their current and potential feedstocks.

2. Classification of biofuels

Biofuels are broadly classified as primary and secondary biofuels. The primary biofuels are used in an unprocessed form, primarily for heating, cooking or electricity production such as fuelwood, wood chips and pellets, etc. The secondary biofuels are produced by processing of biomass e.g. ethanol, biodiesel, DME, etc. that can be used in vehicles and various industrial processes. The secondary biofuels are further divided into first, second and third-generation biofuels on the basis of raw material and technology used for their production (Fig. 2).

Biofuels are also classified according to their source and type. They may be derived from forest, agricultural or fishery products or municipal wastes, also including by-products and wastes originated from agro-industry, food industry and food services. Biofuels can be solid, such as fuelwood, charcoal, and wood pellets; or liquid, such as ethanol, biodiesel and pyrolysis oils; or gaseous, such as biogas (methane).

2.1. Primary biofuels vs. secondary biofuels

Primary biofuels are natural and unprocessed biomass such as firewood, wood chips and pellets, and are mainly those where the organic material is utilised essentially in its natural and non-modified chemical form. Primary fuels are directly combusted, usually to supply cooking fuel, heating or electricity production needs in small and large-scale industrial applications.

Secondary fuels are modified primary fuels, which have been processed and produced in the form of solids (e.g. charcoal), or liquids (e.g. ethanol, biodiesel and bio-oil), or gases (e.g. biogas,

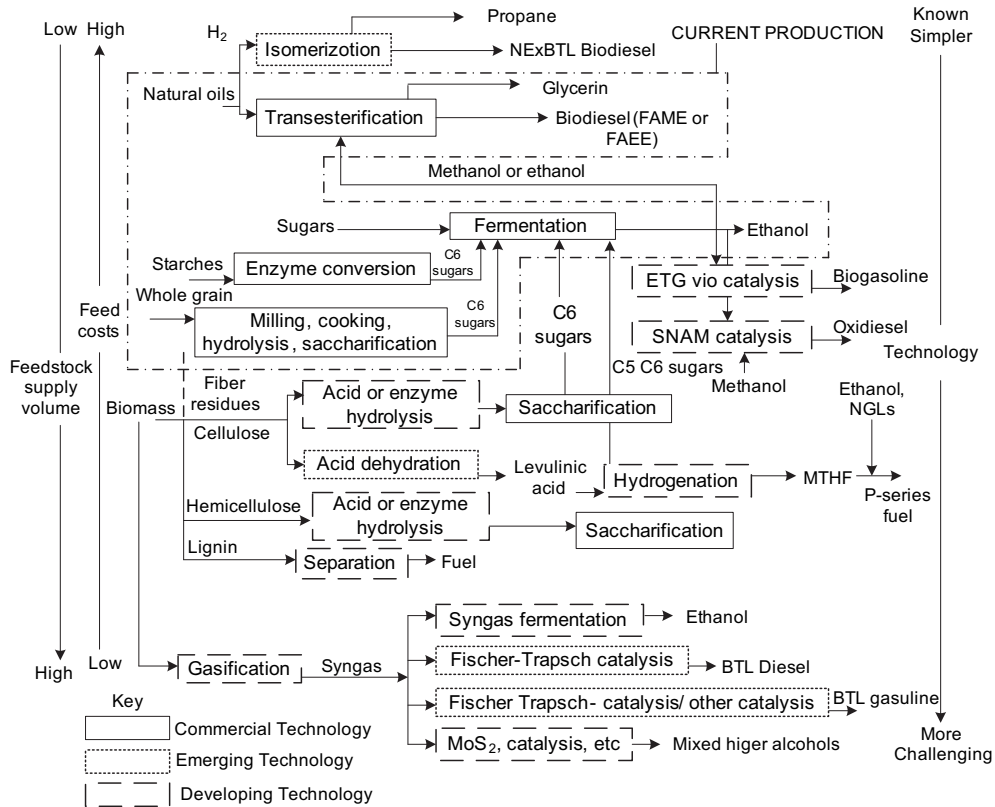


Fig. 1. Various technological routes for biofuels production (Adopted from NEXANT [23]).

synthesis gas and hydrogen). Secondary fuels can be used for multiple ranges of applications, including transport and high-temperature industrial processes. Now more advanced and efficient conversion technologies exist for the extraction of biofuels – in solid, liquid and gaseous forms – from materials such as wood crops and waste material as stated in the 2008 report of The state of food and agriculture [25].

2.2. Advantages and challenges of biofuels

Biofuels offer the promise of numerous benefits related to energy security, economics, and the environment. At the same time, several challenges must be overcome to realize these benefits

[26]. The main advantages and challenges in the production and consumption of biofuels are highlighted in the Table 1.

The key advantage of the utilisation of renewable sources for the production of biofuels is the utilization of natural bioresources (that are geographically more evenly distributed than fossil fuels) and produced bioenergy provides independence and security of energy supply. Utilising agricultural residual and waste substrates as raw materials will minimize the potential conflict between food and fuel and also produced the biofertilizer and biopesticides. Biofuels produced from lignocellulosic materials generate low net GHG emissions, hence reducing environmental impacts.

In a report by the United States Department of Agriculture (USDA) [27] the benefits of biodiesel use as fuel included are: it is renewable, suitable replacement for petroleum derived diesel,

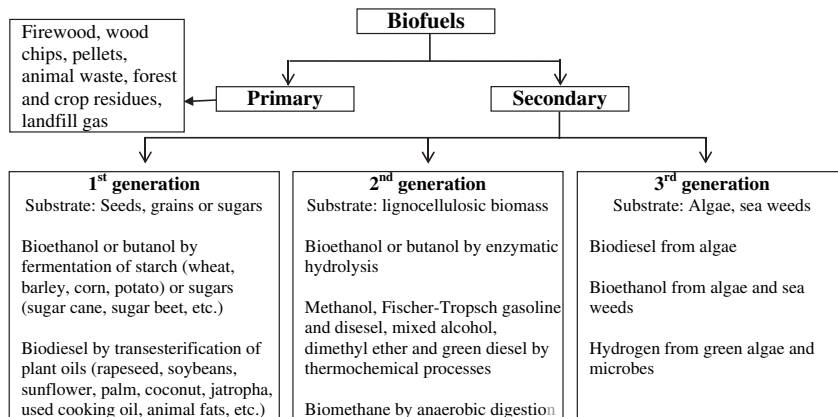


Fig. 2. Classification of biofuels.

Table 1
Potential benefits and challenges of biofuels.

Benefits	Challenge
Energy Security	Feed stock
Domestic energy source	Collection network
Locally distributed	Storage facilities
Well connected supply-demand chain	Food-fuel competition
Higher reliability	Technology
Economic stability	Pretreatment
Price stability	Enzyme production
Employment generation	Efficiency improvement
Rural development	Technology cost
Reduce inter-fuels competition	Production of value added co-products
Reduce demand-supply gap	Policy
Open new industrial dimensions	Land use change
Control on monopoly of fossil rich states	Fund for research and development
Environmental gains	Pilot scale demonstration
Better waste utilization	Commercial scale deployment
Reduce local pollution	Policy for biofuels
Reduce GHGs emission from energy consumption	Procurement of subsidies on biofuels production
Reduction in landfill sites	Tax credits on production and utilization of biofuels

suitable to use in most diesel engines with no or very little modification, has the potential to reduce GHG emissions, biodegradable with little or no toxicity and can be made from agricultural or other recycled sources. Through experiments involving biodiesel produced from different oil types it was found that biodiesel had lower carbon dioxide and polycyclic aromatic hydrocarbons (PAHs) emissions [28]. Biodiesel is considered a “carbon neutral” fuel, as any carbon dioxide released from its burning was previously captured from the atmosphere during the growth of the vegetative crop that was used for the production of biodiesel. Biodiesel is said to have a lower flash point than petroleum derived diesel so its transport is safer and easier [28,29].

Besides having several benefits, the production and utilization of biofuels also have several challenges. An improved biomass waste collection network and their storage is the main challenge for establishment of commercial biofuel plant. A strong policy is needed for organic waste collection and blending of biofuels at higher rate. The subsidy for establishment of biofuel plants will accelerate the production of biofuels and tax credits for utilization will create the market for the biofuel. The technological improvement could help to improve the system efficiency and provide value added co-products, which will reduce the production cost.

3. Liquid biofuels

Liquid biofuels are being researched mainly to replace conventional liquid fuels (diesel and petrol). A recently popularised classification for liquid biofuels includes “*First-Generation*” and “*Second-Generation*” biofuels [24]. The primary distinction between them is in the feedstock used. Research work is in progress for the production of “*third-generation of biofuels*” [30].

3.1. First-generation liquid biofuels

The first-generation liquid biofuels are the type of liquid fuels generally produced from sugars [31–38], grains or seeds [3,39–41] and requires a relatively simple process to produce the finished fuel product. The most well-known first-generation biofuel is ethanol made by fermenting sugar extracted from crop plants and starch

contained in maize kernels or other starchy crops [24]. Bioethanol is usually produced out of organic based matter with high contents of sugars fermentation by enzymes produced from yeast. The yeasts convert six-carbon sugars (mainly glucose) to ethanol, because starch is much easier than cellulose to convert to glucose. Initially the sugar of raw materials is separated after that fermentation processes use yeast to convert the glucose into ethanol. The distillation and the dehydration are used as the last steps for reaching the desired concentration (hydrated or anhydrous ethanol) that can be blended with fossil fuels or directly used as fuel. When the used raw materials are grains, usually hydrolysis is used for converting the starches into glucose [42]. The conventional processes used only the germs of the seeds or grains for ethanol production that represents a small percentage of the total mass of the plant, generating a significant amount of residue [2].

Biodiesel produced from straight vegetable oils of oleaginous plants by transesterification processes or cracking is the another well known first-generation biofuels. Transesterification can use alkaline, acid or enzymatic catalyzers and ethanol or methanol, and produces fatty acid (biodiesel) and glycerine as by-product [2]. In the biodiesel production process also a small fraction of plant biomass is used and left a large fraction as residue.

First-generation fuels are in existence and being produced in significant commercial quantity in a number of countries. The viability of the first-generation biofuels production is, however, questionable because of the conflict with food supply [30]. The utilization of only a small fraction of total plant biomass reduced the land use efficiency. The first-generation biofuels have high production cost due to competition with food. The rapid expansion of global biofuel production from grain, sugar, and oilseed crops has raised the cost of certain crops and food stuffs. These limitations favour the search of non-edible biomass for the production of biofuels.

3.2. Second-generation liquid biofuels

Second-generation liquid biofuels are generally produced by two fundamentally different approaches i.e. biological or thermochemical processing, from agricultural lignocellulosic biomass, which are either non-edible residues of food crop production or non-edible whole plant biomass (e.g. grasses or trees specifically grown for production of energy). The main advantage of the production of second-generation biofuels from non-edible feedstocks is that it limits the direct food versus fuel competition associated with first generation biofuels. Feedstock involved in the process can be bred specifically for energy purposes, enabling higher production per unit land area, and a greater amount of above-ground plant material can be converted and used to produce biofuels. As a result this will further increase land use efficiency compared to first generation biofuels. As stated by Larson [24], it is believed that the basic characteristics of feedstocks holds potential for lower costs, and significant energy and environmental benefits for the majority of second-generation biofuels.

It appears evident from literature [43] that production of second-generation biofuel requires most sophisticated processing production equipment, more investment per unit of production and larger-scale facilities to confine and curtail capital cost scale economies. To achieve the potential energy and economic outcome of second-generation biofuels, further research, development and application are required on feedstock production and conversion technologies. The future production of ethanol is expected to include both the use of traditional grain/sugar crops and lignocellulosic biomass feedstocks [43–46]. Second-generation biofuels share the feature of being produced from lignocellulosic biomass, facilitating in the use of low cost, non-edible

feedstocks, resulting in a limit between direct food and fuel competition [47].

Second-generation biofuels can be further classified in terms of the process or method used to convert biomass to fuel, i.e. biochemical or thermochemical. Few second-generation biofuels such as ethanol and butanol are produced through the biochemical process (these will be discussed in detail later in this article), whereas all other second-generation fuels are produced thermochemically. Many second-generation thermochemical fuels are currently being produced commercially from fossil fuels. These thermochemical fuels include methanol, refined Fischer-Tropsch liquids (FTL), and dimethyl ether (DME). Unrefined fuels (e.g. pyrolysis oils) are also produced thermochemically, but require additional and considerable refining before they can be utilised in engines [24].

Thermochemical biomass conversion involves processes that require much more extreme temperatures and pressures than those found in biochemical conversion systems. Certain essential characteristics differentiate thermochemical process from biochemical process, including the flexibility in feedstock that can be accommodated with thermochemical processing and the diversity of fuel outcome that is produced [48]. Thermochemical production of biofuels begins with gasification or pyrolysis. The former is generally more capital-intensive and requires large-scale production for economic benefit; however, the final product is a clean finished fuel that can be used directly in engines. Fischer-Tropsch liquid (FTL) is a mixture of mainly straight chained hydrocarbon compounds that resembles a semi-refined crude oil, that can be shipped to a conventional petroleum refinery for processing or refined on site into “clean diesel”, jet fuel or other fractions [48]. FTL is synthesised by catalytically reacting CO and H₂, thus any feedstock that can be converted to produce CO and H₂, can be used to produce FTL. In particular, coal, natural gas and biomass can be used as a feedstock for FTL production [24,48]. Starting in the 1990s, there has been renewed interest globally in FTL synthesis, producing liquids from large reserves of remote “stranded” natural gas, that have under valuing due to their distance from the market. Growing interest remains with the production of middle distillate fuels with high cetane number, little or no sulphur or aromatics that contribute to exhaust pollution. Such environmental factors, together with increasing crude oil prices, are encouraging the major expansion in the global capacity for FTL production [48].

The utilization of total above-ground biomass in the production of second-generation biofuels provide a better land use efficiency in comparison to first generation biofuels. Additionally, lower cost of feed material and use of non-edible biomass favours to promote the second-generation biofuels.

3.3. Third-generation liquid biofuels

Alternate energy resources akin to first generation biofuels derived from terrestrial crops such as sugarcane, sugar beet, maize and rapeseed place an enormous strain on world food markets, contribute to water shortages and precipitate the destruction of the world's forests. Second-generation biofuels derived from lignocellulosic agriculture and forest residues and from non-food crop feedstocks address some of the above problems; however, there is concern over competing land use or required land use changes [49]. The latest generation of biofuels researchers are now directing their attention past agricultural substrates and waste vegetable oils to microscopic organisms. Therefore, on the basis of current scientific knowledge and technology projections, third-generation biofuels specifically derived from microbes and microalgae are considered to be a viable alternative energy resource that is devoid of the major drawbacks associated with first and second-generation biofuels.

3.3.1. Biofuel from microbes

Recent advances have shown that some microbial species such as: yeast, fungi and microalgae can be used as potential sources for biodiesel as they can biosynthesise and store large amounts of fatty acids [50] in their biomass. Recently in 2009 Huang et al [51] have reported on microbial oil production from waste rice straw. Microbial oil can be produced from sulphuric acid-treated rice straw hydrolysate (SARSH) by cultivation of a microorganism *Trichosporon fermentans*. Fermentation of SARSH without detoxification gave a poor lipid yield of 0.17%, w/v (1.7 g l⁻¹). Huang et al. [51] worked on the improvement process to improve this yield. The detoxification pretreatment, including overliming, concentration, and adsorption by Amberlite XAD-4 improved the fermentability of SARSH significantly. The pretreatment process helped in increasing the lipid yield by removing the inhibitors in SARSH. A total microbial biomass of 28.6 g l⁻¹ could be obtained in 8 days fermentation, with a lipid content of 40.1% (corresponding to a lipid yield of 11.5 g l⁻¹) after cultivation of *T. fermentans* on the detoxified SARSH. Moreover, besides SARSH, *T. fermentans* was also found capable of metabolising other sugars such as mannose, galactose, or cellobiose, available in hydrolysates of other natural lignocellulosic materials used as the single carbon source. This organism was capable to grow and utilise rice straw hydrolysate to accumulate lipid within its cell biomass with a high yield, e.g. a minimum of 10.4 g l⁻¹. Therefore, this organism can be used as a promising strain for microbial oil production.

Zhu et al. [52] have worked on production of microbial biofuel from waste molasses and have reported that lipids produced in microbial biomass can be utilised for biodiesel production. In this project researchers have optimised the growth medium components for culture cultivation and studied the effects of culture conditions on microbial biomass and lipid production by a microbial strain of *T. fermentans*. The optimal nitrogen source, carbon source and C/N molar ratio for best lipid yields were found to be peptone, glucose and 163, respectively. The most favourable initial pH of the cultivation medium and temperature were 6.5 and 25 °C. Under these optimized conditions, a microbial-culture cultivated for 7 days produced a microbial biomass yield of 28.1 g l⁻¹, containing lipid content of 62.4%, which were much higher than the original values (19.4 g l⁻¹ and 50.8%) and the results reported by other groups [50,52]. *T. fermentans* could be cultivated in a medium consisting of waste molasses from sugar industry. A lipid yield of 12.8 g l⁻¹ was reported with the bioconversion of waste molasses consisting of 15% total sugar concentration (w/v) at pH 6.0. This report is claimed by authors representing the best result with oleaginous microorganisms on agro-industrial residues [52].

The accumulation of lipid within microbial cells could be efficiently enhanced [53,54] by addition of various sugars to the pretreated molasses and the lipid content was increased as high as above 50% of cell mass. The microbial lipid, similar to vegetable oils, mainly contained palmitic, stearic, oleic and linoleic acid with the unsaturated fatty acids amounting to about 64% of the total fatty acid content. In these experiments microbial oil with an acid value of 5.6 mg KOH g⁻¹ could be effectively transesterified to produce biodiesel following a process of base catalysis after removal of free fatty acids, and subsequently, a high methyl ester yield of 92% could be obtained [52].

The ability of yeast to grow well on pretreated lignocellulosic biomass could efficiently enhance the lipid accumulation, provides a promising option for the production of economically and environmentally sound microbial oil from agricultural residues.

3.3.2. Biofuel from algae

Algae are recognised as one of the oldest life-forms [55] and are present in all existing earth ecosystems, representing a big variety of

species living in a wide range of environmental conditions [56]. They are primitive plants (thallophytes), i.e. lacking roots, stems and leaves, have no sterile covering of cells around the reproductive cells and have chlorophyll *a* as their primary photosynthetic pigment [57]. Under natural growth conditions phototrophic algae absorb sunlight, and assimilate carbon dioxide from the air and nutrients from the aquatic habitats [49]. Microalgae can produce lipids, proteins and carbohydrates in large amounts over short periods of time. These products can be processed into both biofuels and valuable co-products [49]. However, the production of lipids, proteins and carbohydrates may be limited by available sunlight due to diurnal cycles and the seasonal variations; thereby limiting the viability of commercial production to areas with high solar radiation [58].

Microalgae can fix CO₂ from three different sources, viz. atmosphere, discharge gases and soluble carbonates [59]. Under natural growth conditions, microalgae assimilate CO₂ from the air and can tolerate and utilise substantially higher levels of CO₂ (up to 150,000 ppmv) [60]. Therefore, in common production units, CO₂ is fed into the algae growth media either from external sources such as power plants [61,62] or in the form of soluble carbonates such as Na₂CO₃ and NaHCO₃ [63,64]. Other inorganic nutrients required for algae production include nitrogen, phosphorus and silicon [65]. Algal cells are veritable miniature biochemical factories, and appear more photo-synthetically efficient than terrestrial plants as these are very efficient CO₂ fixers. The ability of algae to fix CO₂ has been proposed as a method of removing CO₂ from flue gases from power plants, and thus can be used to reduce emission of green house gas (GHG). Many algae are exceedingly rich in oil, since their algal cells have been found heavily enriched with oil globules, which can be converted to biodiesel [30].

Three distinct algae production mechanisms, photoautotrophic, heterotrophic and mixotrophic are in use, all of which follow the natural growth processes. Photoautotrophic production is autotrophic photosynthesis, heterotrophic production requires organic substances (e.g. glucose) to stimulate growth, while some algae strains can combine autotrophic photosynthesis and heterotrophic assimilation of organic compounds in a mixotrophic process [49]. Many microalgae strains have high lipid content (20–50% dry weight), it can be enhanced by optimising the growth determining factors [66].

Miao and Wu [67] demonstrated that algae, when starved of a suitable source of nitrogen, produced mainly oil, whereas in the presence of sunlight algae produce sugars and proteins from carbon dioxide. The microalgae *Chlorella protothecoides* when grown under autotrophic and heterotrophic conditions accumulate lipids, which can be used for biodiesel production. The most effective method of improving microalgae lipid accumulation is nitrogen limitation, which not only results in the accumulation of lipids, but also results in a gradual change of lipid composition from free fatty acids to triacylglycerol (TAG) [68]. TAGs are more useful for conversion to biodiesel [69].

The conversion technologies for utilising microalgae biomass can be separated into two basic categories of thermochemical and biochemical conversion (similar to terrestrial biomass). Thermochemical conversion covers the thermal decomposition of organic components to fuel products, such as direct combustion, gasification, thermochemical liquefaction and pyrolysis [70]. The biological process of energy conversion of biomass into other fuels includes anaerobic digestion, alcoholic fermentation and photobiological hydrogen production [71].

Grant has recently published an article in Scientist [72] and emphasized that biofuels made from algae are the next big thing on the alternative energy horizon and has questioned that if this group of biofuel can make us free from our addiction to petroleum. The basic concept for the production of biofuels using algal cells as mini

factories is very simple. The cells of microalgae are naturally able to bio-synthesize and store lipids similar to those types present in vegetable oils. But actual research would be required to achieve the commercially viable levels of fuels. This could be done by genetically modifying the oil-storing capabilities of algal cells making them more efficient than they are in nature [72].

Ideally for efficient oil production algae should be able to accumulate more than 30% of their cell weights in oils. The micro-algal cells are tiny fuel factories and more significantly they manufacture those compounds naturally, which are chemically similar to petroleum-based fuels. According to Walter Kozumbo, manager of the Air Force Office of Scientific Research's scientific program, the triglycerides accumulated by photosynthetic algae resemble to JP8 which is kerosene-based jet fuel of choice for military aircrafts. These algal cells not only synthesize and store these useful oils, they can also crank them out. According to USDOE microalgae have been identified to have the potential to synthesise 100 times more oil per acre of land than any other plant and they are even better than soybeans [72].

Currently, few private companies and some publicly-funded research groups are working on algae cultivation similar to those contained in Cargill ponds near the southern horn of San Francisco Bay and working on the aspect of bringing the cost of algal oil manufacture down to levels of affordable gasoline prices. *Solix Biofuels*, a Colorado-based company plans on firing up a closed-tank bioreactor system that utilises waste carbon dioxide generated from beer making. A New Zealand company, *Aquaflow Binomics* is working on the production of biofuels by harvesting wild algae from the polluted waterways [72].

A biofuel company *Solazyme* was started in 2003 by a microbial geneticist. After realising that the productivity of the algae was not enough to yield appreciable amounts of fuel, company switched to heterotrophic species of algae in order to utilise carbon-based compounds rather than passively fixing carbon dioxide from the atmosphere. The advantage of using this type of algae is that heterotrophic algae are all time bathed in their energy source as compared to photosynthetic strains of algae. The algal strains are able to consume anything from waste glycerols and sugar cane to sugar beet pulp and molasses [72].

The specific algal strains of *Chlamydomonas* can synthesize some hydrocarbons but they produce triacylglycerides on large extent. In natural environment algal cells can synthesise rarely above 30% oil, therefore for fuel production yields of 50–60% oil from dry weight algal cells should be considered as excellent. *Solazyme's* algal strains have been designed to synthesise and store 75% oil of dry weight of algal cells. The biodiesel is synthesised using the glyceride backbones from these triacylglycerides by adding methanol. The fatty acid methyl ester is used in the process of hydrotreatment, which involves the stripping off oxygen and saturation of the molecule with hydrogen. This saturation process generates a straight-chain alkane, which is similar to the diesel available in distribution pumps [72].

Mata et al. [56] suggested that further efforts on microalgae production should concentrate in reducing costs in small and large-scale systems. This can be achieved by using cheap sources of CO₂ (flue gas), nutrient-rich wastewaters, inexpensive fertilizers, cheaper design culture systems with automated process control, greenhouses and heated effluents to increase algal yields. Apart from saving costs of raw materials, these measures will also help to reduce GHG emissions, waste disposal problems and the feed cost. This will raise the availability of microalgae biomass for different applications and will contribute to the sustainability and market competitiveness of the microalgae industry. The main aspects in this direction to be studied by scientists would involve: the selection of most efficient algal strains; cultivation of selected strains at best growth rates; designing the metabolic pathways by

engineering those reactions that control lipid synthesis to produce algal cells saturated with desirable lipid contents; the optimization of lipid extraction process to standardise an efficient and economical method of oil recovery from the algal cells.

4. Biochemical liquid fuels

In the following paragraphs three important types of liquid biofuels, which are second-generation fuels and are produced biochemically (further to Section 3.2 have been discussed).

4.1. Bioethanol

Ethanol if it is produced using a renewable biomass, it is named as bioethanol. Use of bioethanol as biofuel is both renewable and environment-friendly [73]. Global production of first generation bioethanol in 2006 was about 51 billion litres, 35% of the total. China and India contributed 11% to global ethanol production in 2006, and production levels were much lower in other countries. Many countries are now getting involved in the expansion of first generation ethanol production, with the USA and Brazil displaying the largest of expansion plans [24]. Ethanol can be combined and blended with petrol or burned in its pure form within modified spark-ignition engines. In comparison, a litre of ethanol contains 66% of the energy provided by a litre of petrol but has a higher octane level, and when mixed with petrol for transportation it improves the performance of the latter. Ethanol also improves the fuel combustion in vehicles, thereby reducing the emission of carbon monoxide, unburned hydrocarbons and carcinogens. In comparison to petrol, ethanol contains only a trace amount of sulphur. Therefore, mixing ethanol with petrol helps to reduce the fuel's sulphur content and thereby lowers the emissions of sulphur oxide, a major component of acid rain, and a carcinogen [25,55]. Currently bioethanol is being used as fuel in Brazil where it is produced from sugar cane and it accounts for 40% fuel needs for cars, lorries and buses. Brazil is now the largest sugar cane producer in the world, and is also rapidly becoming the world's largest bio-fuel producer. In September 2008 lawmakers in India stated that they wanted adoption of second-generation biofuels from non-food crops. In America the law requires that 36 billion gallons of ethanol be produced by 2022; 15 billion gallons from corn kernels and 21 billion from cellulosic feedstock, such as corn stover, prairie grass, or poplar trees. The financial incentives to harvest cellulosic biomass provided by the 2008 farm bill may stimulate corn production due to demand for corn grain for feed and ethanol and corn residues for advanced biofuels [74].

Crops such as sugarcane, wheat and corn are most essential types of natural bioresources that are exploited for bioethanol production. Compared with corn based or sugar beet-based ethanol, Brazil's sugarcane-based ethanol yields considerably more favourable results in terms of energy balance and reductions in greenhouse gas emissions. Feedstock containing significant amounts of sugar, or materials that can be converted into sugars, such as starch or cellulose, can be used in the production of ethanol [44–47]. Ethanol available within the biofuel market is derived mainly from sugar or starch [25,55]. Common sugar crops used as feedstocks are sugar cane, sugar beet and, to a much lesser extent sweet sorghum [44]. Common starchy feedstocks include maize, wheat and cassava.

The utilisation of biomass containing sugars that can be directly fermented to ethanol is the least complex method used in producing ethanol [45,46]. Plant biomass consist of cellulose microfibrils embedded in lignin, hemicellulose and pectin with an altered amount of each compound among plant species and plant parts [4]. Cellulose is crystalline glucose polymer and hemicellulose is amorphous polymers of xylose, arabinose, and lignin a large poly

aromatic compounds [75]. The general steps for producing ethanol include pretreatment of substrates, saccharification process to release the fermentable sugars from polysaccharides, fermentation of released sugars and finally distillation step to separate ethanol (Fig. 3). Pretreatment is designed to facilitate in the separation of cellulose, hemicellulose and lignin, so that complex carbohydrate molecules constituting the cellulose and hemicellulose can be broken down by enzyme-catalysed hydrolysis into their constituent simple sugars. Cellulose is a crystalline polymer of long chains of glucose sugar molecules (6-carbon). Its complex structure makes it difficult to depolymerise into simple sugars, but once the polymer structure has been broken down, the sugar molecules are simply fermented to ethanol using fermentative microorganisms [47].

Hemicellulose consists of 5-carbon sugars, which although are easily broken down into its constituent sugars such as xylose and pentose, the fermentation process is much more difficult, and requires efficient microorganisms that are able to ferment 5-carbon sugars to ethanol. Lignin consists of phenols, and for practical purposes is not fermentable, although it can be recovered and utilised as a fuel, providing process heat and electricity for the alcohol (ethanol, butanol) production facility. Bioethanol is produced similarly to other alcohols such as spirits using natural products like wheat, maize and sugar beet. Hence, the suitable raw materials required for bioethanol production could be any of those materials which contain considerable amounts of carbohydrates to provide fermentable sugars for bioconversion into bioethanol. Then an optimised microbial fermentation process can be used for the bioconversion of sugars released from carbohydrates into ethanol [57,76–78].

The hydrolysis is usually, catalyzed by cellulase enzymes and the fermentation is carried out by yeast or bacteria. The factors that affect the hydrolysis of cellulose include porosity, i.e., accessible surface area of the waste materials, cellulose fiber crystallinity and lignin and hemicellulose content [79]. The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult. The lignin and hemicellulose removal, reduction of cellulose crystallinity and increase of porosity in pretreatment processes can significantly improve the hydrolysis.

The cellulose crystallinity can be reduced by a combination of chipping, grinding and milling [80]. Steam explosion is the most commonly used method for pretreatment of plant biomass [79]. Addition of $H_2SO_4/SO_2/CO_2$ in steam explosion can effectively improve enzymatic hydrolysis by decreasing the production of inhibitory compounds and lead to more complete removal of hemicellulose [81]. Ammonia fiber explosion (AFEX) improve the saccharification rates of various herbaceous crops and grasses significantly [9]. CO_2 explosion would form carbonic acid and increase the hydrolysis rate. Ozone can be used to degrade lignin and hemicellulose [82,83]. The acid hydrolysis used concentrated acids, that are toxic, corrosive and hazardous and requires special reactors (resistant to corrosion) and need to recover used acid [84]. Thereafter, dilute acid hydrolysis has been developed for pretreatment of lignocellulosic materials. The mechanism of alkaline

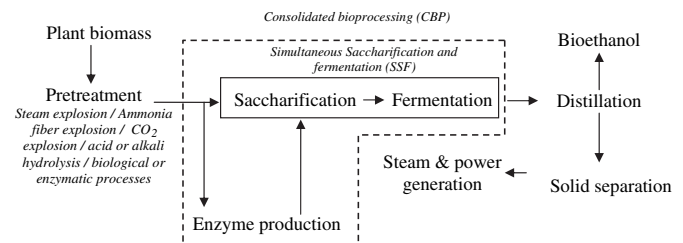


Fig. 3. Various processes for the production of bioethanol from biomass.

hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicellulose and other components [9] that increases the porosity of lignocellulosic materials [85]. Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H_2O_2 [86].

Microorganisms such as brown, white and soft rot fungi are used in biological pretreatment processes to degrade lignin and hemicellulose [87]. Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. The white rot fungus *Phanerochaete chrysosporium* produces lignin-degrading enzymes, lignin peroxidases and manganese-dependent peroxidases, during secondary metabolism in response to carbon or nitrogen limitation [88]. Other enzymes including polyphenol oxidases, laccases, H_2O_2 producing enzymes and quinone-reducing enzymes can also degrade lignin [89]. The advantages of biological pretreatment include low energy requirement and mild environmental conditions, but the hydrolysis rate is very low [80].

Furfural is an important inhibitor of ethanol production from hemicellulose hydrolysate [90], even at low concentrations [91,92]. Various bacteria and yeast have been reported to partially transform furfural to either furfuryl alcohol or furoic acid, or a combination of both [93–95]. Enzymatic hydrolysis of cellulose is carried out by cellulase enzymes [96], a mixture of several enzymes, viz. endoglucanases (attacks regions of low crystallinity in the cellulose fiber, creating free chain ends); exoglucanase or cellobiohydrolase (degrades the molecule further by removing cellobiose units from the free chain ends); β -glucosidase (hydrolyzes cellobiose to produce glucose) [97], glucuronidase, acetyltransferase, xylanase, β -xylosidase, galactomannanase and glucomannanase (attacks hemicellulose) [98]. Bacteria (*Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora* and *Streptomyces*) [99] and fungi (*Sclerotium rolfsii*, *P. chrysosporium* and species of *Trichoderma*, *Aspergillus*, *Schizophyllum* and *Penicillium*) [9,98] can produce cellulases. A few microbial species such as *Neurospora*, *Monilia*, *Paecilomyces* and *Fusarium* have been reported to hold the ability to ferment cellulose directly to ethanol by simultaneous saccharification and fermentation (SSF) [100]. Consolidated bioprocessing (CBP) featuring cellulase production, cellulose hydrolysis and fermentation in one step, is an alternative approach with outstanding potential [101].

The factors that affect the enzymatic hydrolysis of cellulose include substrates, cellulase activity and reaction conditions [80]. Cellulase activity is inhibited by the cellobiose and to a lesser extent by glucose. Several methods have been developed to reduce the inhibition, including the use of high concentrations of enzymes, the supplementation of β -glucosidases during hydrolysis and removal of sugars during hydrolysis by ultrafiltration or SSF [102,103]. Genetic techniques have been used to clone the cellulase coding sequences into bacteria, yeasts, fungi and plants to create new cellulase production systems with possible improvement of enzyme production and activity and also reduce the cost of production. The constructed operons encoding xylose assimilation and pentose phosphate pathway enzymes were transformed into the bacterium *Zymomonas mobilis* for the effective fermentation of xylose to produce ethanol [104]. The recombinant strain of *E. Coli* with the genes from *Z. mobilis* for the conversion of pyruvate into ethanol has been reported by Dien et al. [105]. A key challenge to commercializing production of fuels and chemicals from cellulosic biomass is higher processing costs [106,107]. Biological conversion opens such low costs production path as it has the potential to achieve a higher yield and the modern tools of biotechnology can improve key process steps.

The authors of this review have worked extensively on the utilisation of agricultural wastes and residues [4,9,108,109] and bioconversion of the sugar industry byproducts to produce

bioethanol. A range of residual substrates such as sugarcane bagasse, sugarcane molasses [57,76–78], and starch [44] has been found suitable for the bioconversion of available carbohydrates in these substrates to produce ethanol. A variety of mesophilic and thermophilic microorganisms were employed to optimise the fermentation process [108–114], which could be practically viable in different climatic conditions, particularly to reduce the cost of temperature maintenance in large fermenters operating in warmer countries in summer months [76,113,114].

The research group of Huang have been working on enhanced bioethanol production in a fermentation process utilising rice straw and have reported their findings just very recently in 2009 [115]. The group has successfully developed an enhanced inhibitor-tolerant strain of *Pichia stipitis* through its adaptation to a medium containing acid-treated rice straw hydrolysate. The ethanol yield obtained by fermentation of NaOH-neutralized straw hydrolysate without a detoxification step using the adapted *P. stipitis* was comparable to the yield obtained in fermentation of overliming-detoxified straw hydrolysate. The ethanol yield of $0.45 \text{ g}_p \text{ g}_s^{-1}$ could be achieved using the adapted *P. stipitis* with rice straw hydrolysates at pH 5.0, which is equivalent to 87% of the maximum possible ethanol conversion. This research team found that the newly adapted *P. stipitis* demonstrated significantly enhanced tolerance to sulphate and furfural and could effectively grow in the hydrolysate when both inhibitors had not been removed from the hydrolysate by NaOH neutralization. Huang et al [115] have claimed that the ethanol conversion could be maintained at 60% and above, when the neutralized hydrolysate contained the two inhibitors, sulphate (3.0%) and furfural (1.3 g l^{-1}).

Sukumaran et al. [116] have recently (2009) reported on bioethanol production from the saccharification of wheat bran, a lignocellulosic waste. The cost of cellulase enzymes is a major factor in the enzymatic saccharification of agricultural biomass, which contains lignin. Production cost of cellulases and hence ultimately the cost of ethanol production may be brought down by multifaceted approaches. One important approach is the use of cheaper lignocellulosic substrates for the biosynthesis of the enzyme, and second strategy is the use of cost efficient fermentation process such as solid state or solid substrate fermentation at much cheaper cost. In their study, wheat bran as a cheaper substrate was used and the cellulolytic enzymes for the hydrolysis of wheat bran were produced using solid state fermentation. Crude cellulolytic enzyme were produced using the fungi *Trichoderma reesei* and *Aspergillus niger*, which were subsequently used for the cost-effective saccharification of three different materials, i.e. sugar cane bagasse, rice straw and water hyacinth biomass. In such enzymatic hydrolysis process high yields of fermentable sugars were obtained, 26.3 g l^{-1} from rice straw and 17.79 g l^{-1} from sugar cane bagasse. The enzymatic hydrolysate of rice straw which consisted of 2.63% fermentable sugars was used as substrate for ethanol production by *Saccharomyces cerevisiae*. The team has reported yield of ethanol up to 0.093 g per gram of pre-treated rice straw [116].

Whilst bioethanol production has been greatly improved by development of new technologies but there are still challenges that need further improvements in the developed technology to bring forward to commercial scale. These challenges include maintaining a stable performance of the genetically engineered microorganisms and developing more efficient pretreatment technologies for the lignocellulosic biomass and integrating the optimal components into economic ethanol production systems.

4.2. Butanol

Butanol is a four carbon alcohol ($C_4H_{10}O$). It contains more hydrogen and carbon [117]. Consequently it is easier to blend with gasoline and other hydrocarbon products and also contains more

heat energy than ethanol, which equates to a 25% increase in harvestable energy (Btu's) [118]. Butanol contains 110,000 BTUs per gallon, closer to gasoline's 115,000 BTUs, and is safer to handle with a Reid Value of 0.33 psi, which is a measure of a fluid's rate of evaporation when compared to gasoline at 4.5 and ethanol at 2.0 psi [118,119]. Butanol is far less corrosive than ethanol and can be shipped and distributed through existing pipelines and filling stations. An 85% butanol/gasoline blends can be used in unmodified petrol engines [120] and butanol is much less evaporative than gasoline or ethanol, making it safer to use and generating fewer volatile organic compound (VOC) emissions [121]. Butanol contains 22% oxygen making it a beneficiary fuel extender that is cleaner burning than ethanol [122]. Ramey [118] also reported that when it consumed in an internal combustion engine yields only carbon dioxide, makes it more environmental friendly biofuel.

The four isomers of butanol exist, viz. *n*-butanol $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$ (normal-butanol), 2-butanol $\text{CH}_3\text{CH}_2\text{CHOHCH}_3$ (secondary-butanol), *i*-butanol ($\text{CH}_3)_2\text{CH}_2\text{CHOH}$ (iso-butanol) and *t*-butanol ($\text{CH}_3)_3\text{COH}$ (ter-butanol). All contain about the same energy. They are essentially identical in blending with gasoline and in combustion. However, their manufacturing methods are very different [117]. The *t*-butanol is a petrochemical product and there is no known biological process can produce *t*-butanol. *n*-butanol is a fermentation production. In fact, it is a very old industrial product. Before the rise of petroleum industry, *n*-butanol has been manufactured by fermentation of sugar or starch. The process for its production is very complicated and difficult. It produced by the anaerobic conversion of carbohydrates by strains of *Clostridium acetobutylicum* into acetone, butanol and ethanol as clostridia secrete numerous enzymes that facilitate the breakdown of polymeric carbohydrates into monomers (Fig. 4) [117,120,121,123]. This fermentation is known as the "Acetone–butanol–ethanol fermentation or ABE fermentation", with a product ratio of 3:6:1 [119]. Furthermore, *n*-butanol is toxic. When there are accumulated 12 grams of *n*-butanol per liter of fermenting broth, *n*-butanol will inhibit the bacterial cells from producing more *n*-butanol [117]. Upon completion of a fermentation run, there is relatively little product in the final broth that contains also ethanol and acetone. Recovery and purification of *n*-butanol from the fermented broth is difficult and costly. There has been some progress made with the final *n*-butanol concentration reaching about 20 grams per liter of fermentation broth. Currently, there are several organizations including, DuPont, BP, GEVO and so on are interested in developing iso-butanol. Most ordinary wine making yeast cultures do produce small amounts of iso-butanol. Careful distillation is needed to remove iso-butanol and methanol to achieve beverage products of high quality. iso-butanol is also toxic even though less than *n*-butanol [117]. 2-Butanol is not made directly by fermentation. It involves first a bacterial fermentation that can convert glucose (from starch or cellulose) and all members of the mixed sugars (from hemicellulose) to an intermediate product. Through additional chemical conversion done with directly in the fermented broth, this intermediate product is converted to 2-butanol. The fermentation process is of high conversion efficient of about 90–95%. This intermediate product is not toxic and can accumulate in the fermenting broth to as high as 110 g l^{-1} . Conversion of this intermediate product to 2-butanol is also of high conversion efficiency with about 95% yield. From about three tons of raw materials (sugar, starch, cellulose and/or hemicellulose), one ton of 2-butanol can be produced, much higher than what can be done with the other butanol isomers. 2-Butanol has a boiling point lower than that of water; while both *n*-butanol and iso-butanol boil at higher temperature than water. One needs to spend a lot of heat energy to remove water when recovering *n*-butanol or iso-butanol from aqueous fermentation broths [117].

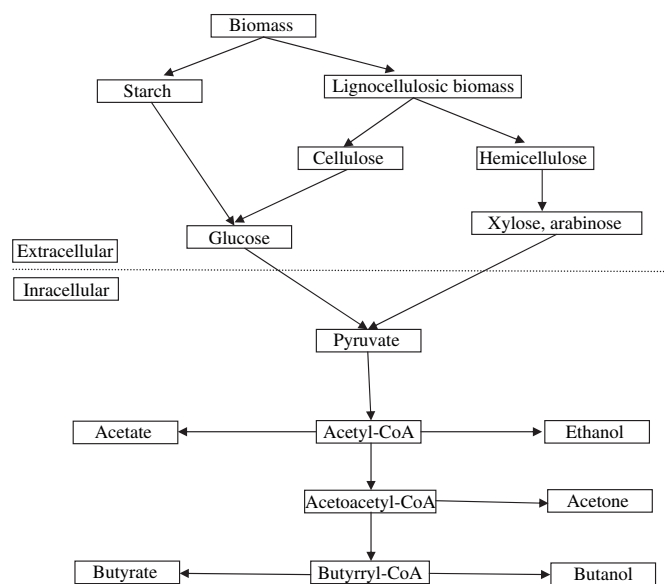


Fig. 4. ABE production pathway (Adopted from Ezeji et al. [123]).

In the past 20 years, research and development efforts have focused on various aspects of the ABE process. Molecular biology research has achieved major breakthroughs in strain/mutant development that dramatically improved microbial tolerance to butanol toxicity, which resulted in a significant increase in ABE solvent production yield [121]. Biobutanol production is a biphasic fermentation where acetic and butyric acids are produced during the acidogenic phase followed by their conversion into acetone and butanol (solventogenic phase). At the end of the fermentation, cell mass and other suspended solids are removed by centrifugation and can be sold as cattle feed [123]. In several recent approaches, agricultural waste such as packing peanuts, orchard waste, DDGS, corn fiber, wheat straw, barley straw, grass, etc. have been used as substrates [122,124–128]. Huang et al. [129] reported an experimental process that uses continuous immobilized cultures of *C. tyrobutyricum* and *C. acetobutylicum* to maximize the production of hydrogen and butyric acid and convert butyric acid to butanol separately in two steps. Extensive research has been performed on the use of alternative fermentation and product recovery techniques for biobutanol production. These techniques have involved the use of immobilized and cell recycle continuous bioreactors and alternative product recovery technique, for example adsorption, gas stripping, ionic liquids, liquid–liquid extraction, pervaporation, aqueous two-phase separation, supercritical extraction, and perstraction, etc. [123].

Ezeji et al. [127] found that long-term continuous cultivation of *Clostridium beijerinckii* BA101 in a degermed corn based medium was not possible due to the instability of the gelatinized degermed corn starch during storage often called "retrogradation". While agricultural residues such as straws (wheat and rice) and corn fiber are economically available, these materials must first be subjected to pretreatment and enzymatic hydrolysis to produce hydrolysates for fermentation. The processes used to produce these hydrolysates often result in the generation of chemical byproducts that inhibit cell growth and fermentation. Such inhibitors include salts, furfural, hydroxymethyl furfural (HMF), acetic, ferulic, glucuronic, and *r*-coumaric acids, and phenolic compounds [127]. There are a number of approaches available to reduce inhibitory effects of hydrolysates on cultures including dilution of the hydrolysate, removal of inhibitors using overliming and/or adsorbent resin/molecular sieve (XAD-4) Qureshi et al. [124], and development of inhibitor-tolerant/

metabolizing strains. Qureshi et al. [122] reported that fermentation of lime treated dilute sulfuric acid barley straw hydrolysate (BSH) by *C. beijerinckii* P260 resulted in the production of 26.6 g l⁻¹ ABE yield and productivity of 0.39 g l⁻¹ h⁻¹, superior to glucose and untreated BSH. Qureshi et al. [126] found that inhibitors present in corn stover hydrolysate were removed by overliming of hydrolysate and *C. beijerinckii* P260 was able to produce 26.27 g l⁻¹ ABE after inhibitor removal, and suggested that cultures resistant to inhibitors and able to produce butanol at high concentrations may be another approach to improve the current process. A comparison of use of different substrates (corn fiber, wheat straw) and different pretreatment techniques (dilute sulfuric acid, alkaline peroxide) suggests that generation of inhibitors is substrate and pretreatment specific [125].

In a biobutanol batch process, reactor productivity is limited to less than 0.50 g l⁻¹ h⁻¹ for a number of reasons, including low cell concentration, down time, and product inhibition [130]. In a batch reactor a cell concentration of <4 g l⁻¹ is normally achieved, that might be increased by immobilization or cell recycle technique. In a study Huang et al. [129] immobilized cells of *C. acetobutylicum* in a fibrous support and used these in a continuous reactor to produce ABE and obtained 4.6 g l⁻¹ h⁻¹ productivity. Cells are returned to the bioreactor using a filter and clear liquid is removed in cell recycle technique. Using this approach, reactor productivities up to 6.5 g l⁻¹ h⁻¹ (compared to <0.5 g l⁻¹ h⁻¹ in batch fermentation) were achieved in biobutanol fermentation [130]. Gas stripping is a technique that can be applied for in situ butanol recovery during the ABE fermentation. The ABE fermentation is associated with the generation of CO₂ and H₂ gases. The utilization of fermentation gases to recover butanol during simultaneous fermentation and in situ recovery by gas stripping can make the ABE recovery process simpler and more economical [130–132]. The ABE is a water-insoluble organic extractant is mixed with the fermentation broth in liquid–liquid ABE recovery process. Butanol is more soluble in the organic phase than in the aqueous phase; therefore, butanol selectively concentrates in the organic phase [130]. The fermentation broth and the extractant are separated by a membrane in perstractive separation. The membrane contactor provides surface area where the two immiscible phases can exchange butanol. As there is no direct contact between the two phases, extractant toxicity, phase dispersion, emulsion and rag layer formation are drastically reduced or eliminated [130].

Qureshi et al. [133] studied five different processes to produce ABE from wheat straw (WS) by *C. beijerinckii* P260 viz. fermentation of pretreated WS, separate hydrolysis and fermentation of WS without removing sediments, simultaneous hydrolysis and fermentation of WS without agitation, simultaneous hydrolysis and fermentation with additional sugar supplementation and simultaneous hydrolysis and fermentation with agitation by gas stripping. On the basis of results obtained they suggested that simultaneous hydrolysis of WS to sugars and fermentation to butanol/ABE is an attractive option as compared with more expensive glucose to ABE fermentation. Further development of enzymes for WS hydrolysis with optimum characteristics similar to fermentation would make conversion of WS to butanol/ABE even more attractive.

The cost issues, the relatively low-yield and sluggish fermentations, as well as problems caused by end product inhibition and phage infections reduces the process efficiency, but with continuous fermentation technology, butanol can be produced at higher yields, concentrations and production rates. James Liao at the University of California developed *E. coli* strains with genes coding for 2 enzymes that converted keto acids into aldehydes, and aldehydes into 1-butanol. When further manipulated, the microbes were able to produce butanol at much higher efficiencies, suitable for industrial production. Research is also being carried out into the

production of 2,3butanediol (a potential biofuel) from agricultural residues (e.g. hydrolysis of hemicellulose-rich fractions by *Trichoderma harzianum* followed by fermentations using *Klebsiella pneumoniae*) [120]. The limitations of microorganisms to tolerate at less than 1% butanol made process inefficient. The extraction is the only economical means of isolating butanol from the fermentation broth. DMIM TCB (1-decyl-3-methylimidazolium tetracyanoborate) has an excellent capacity for butanol and is polar enough to offer sufficiently high selectivity of butanol over water. Ionic liquids made with other anions just cannot compete with the capacity of TCB for butanol. And because DMIM TCB is a non-volatile Ionic Liquid, the recovery of butanol by distillation leads to a high purity product [134].

Butanol is mainly used as a solvent, but experts believe butanol could potentially be used in conventional automobiles in place of gasoline. Today, the recovery and purification of butanol account for about 40% of the total production cost, engineers at Ohio State University (OSU) have found a way to double production of the biofuel butanol – another prospect to replace gasoline in automobiles [135]. The OSU researchers say their process improves on the conventional method for brewing butanol in a bacterial fermentation tank. Normally, bacteria are capable of producing a certain amount of butanol – up to 15 g for every liter of water in the fermentation vessel – before the system would become too toxic for the bacteria to survive. This research group has developed a mutant strain of the bacterium *C. beijerinckii* in a bioreactor containing bundles of polyester fibres, where the mutant bacteria produced up to 30 g of butanol per litre. Because in this process butanol is produced at higher concentrations, the costs related to recovery and purification can be reduced to make biofuel production more economical. The research group is applying for a patent on the mutant bacterium and the butanol production methodology and will work with industry to develop this technology [135].

The synthesis of butanol or 2,3-butanediol (2,3-BD) can be achieved from the bioconversion of biomass in a bioprocess as reported recently in 2009 by Celinska and Grazek [136]. Biotechnological production of 2,3-BD from the agricultural wastes and excessive biomass is a promising and attractive alternative to its production in traditional chemical synthesis [136]. In the face of scarcity of fossil fuel supplies the bio-based process is significantly important, since 2,3-BD may have multiple practical applications (e.g. production of synthetic rubber, plasticizers, fumigants, as an antifreeze agent, fuel additive, octane booster, and many others). Although the 2,3-BD pathway is well known, microorganisms able to transform biomass to 2,3-BD have been characterised, and attempts of pilot scale production of this compound were made, but the processes lacked desired profitability. Both wild type and genetically improved strains have been employed in biotechnological production of 2,3-BD. These strains are able to produce 2,3-BD under different operating conditions applied for the transformation of different sources of biomass used [136]. Hydrolysis of cellulosic raw materials prior to butanol conversion potentially offers greatly increased yields. In a research published by the USDA in 2007, wheat straw was hydrolyzed to lignocellulosic component sugars (glucose, xylose, arabinose, galactose, and mannose) prior to their conversion to butanol, by *C. beijerinckii* P260. The rate of production of wheat straw hydrolysate to butanol was 214% over that from glucose [137]. In another research project the production of 2,3 butanediol has been achieved from agricultural residues through the hydrolysis of hemicellulose-rich fractions by a fungal strain *T. harzianum* followed by fermentations using *K. pneumoniae* [138].

Either directly as biobutanol or indirectly through biobutanol's synergy with ethanol can be facilitated into gasoline. Biobutanol will help expand the biofuels market, which will directly affect the

markets for related agricultural substrates, thus enhancing the finances for farmers. Renewed interest in butanol as a sustainable vehicle fuel has led to the development of improved biobutanol production process by two big companies DuPont and BP. They are making biobutanol the first product of their joint effort to develop, produce, and market next-generation biofuels. This research started due to the desired fuel characteristics of butanol, such as: it can be easily added to conventional gasoline, due to its low vapor pressure. The energy content of butanol is closer to that of gasoline than ethanol so consumers face less of a compromise on fuel economy – this would be of particularly importance when the amount of biofuel in the fuel blend will increase. Moreover, butanol can be blended at higher concentrations than bioethanol for use in standard vehicle engines. Currently biobutanol can be blended up to 10% (v/v) in European gasoline and 11.5% (v/v) in US gasoline. Butanol has been reported to be less susceptible to separation in the presence of water than ethanol/gasoline blends, and therefore the industry's existing distribution infrastructure can be used without requiring modifications in blending facilities, storage tanks or retail station pumps. In June 2006, DuPont and BP formed a partnership to develop new biobutanol production technology using lignocellulosic feedstocks. In July 2009 the partnership was cleared to take over the US company Biobutanol LLC. On 25 September 2009, BP and Mazda announced an Ethanol Biobutanol blend would be used in the Petit Le Man Race, US [138].

Since biobutanol is produced from the same agricultural feedstocks as bioethanol (i.e. corn, wheat, and sugar beet and sugar-cane), it is a good proposition for global farmers. This would provide another marketing opportunity for key agricultural residual products, thus enhancing value to farmers.

4.3. Biodiesel

Various researchers have discussed fairly extensively, the advantages of using vegetable oils as diesel [139]. Such properties include liquid nature for transport, heat content, and that they are renewable and readily available. Though there are some disadvantages such as higher viscosity, lower volatility and the reactivity of unsaturated hydrocarbon chains.

Shahid and Jamal [140] have reviewed a range of vegetable resources including sunflower oil, cottonseed oil, rapeseed oil, soybean oil, palm oil and peanut oil and their usefulness for biodiesel production. They concluded that using a mixture of petroleum diesel and biodiesel, at an 80:20 ratio (B20) was the most successful. Most of the vegetable oils showed promising potentials in the manufacture of biodiesel but there were reoccurring engine problems with all of them. It may be worth pointing out that most of the testing in engines was carried out in larger agricultural engines more than in smaller ones used in common road use. One of the outcomes highlighted in the research conducted by Bajpai and Tyagi [29] was that although rapeseed oil showed excellent potential as a biodiesel fuel, there was doubt cast over its long-term economic viability. The over extensive use of it tended to have a 'wear and tear' effect on the engines resulting in a lot of erosion of parts and carbon build up. This is an interesting point considering that most of Europe's biodiesel production uses rapeseed oil [29].

Vegetable oils can not often be used directly as an energy source in an engine due to the higher level of viscosity, the lower volatility and the reactivity of the unsaturated hydrocarbon chains within oils [28,141]. Direct use of vegetable oils has been deemed unsatisfactory, as due to the high viscosity, Free Fatty Acid (FFA) content and the matter of carbon deposits, their use has been limited to a considerable extent [139]. Many technologies and methods have been employed to try and reduce the viscosity of the oil; these

include microemulsion, pyrolysis (thermal cracking), catalytic cracking and transesterification [29,139,142]

Microemulsion is mixing of the vegetable oils with straight chain alcohols such as methanol or ethanol [28]. It reduces thickness and increases spray patterns of the biodiesel thereby making it more suitable for use in motor engines. Ma and Hanna's findings [139] are in accordance with this, the use of micro emulsion biodiesel was successful in lab tests but it may be appropriate to mention that durability in engines was not tested.

Other methods that manufacturers have tried to exploit are pyrolysis and catalytic cracking. Pyrolysis is the conversion from vegetable oils to biodiesel using heat or heat plus a catalyst. It is not very selective and a wide range of compounds is usually obtained. Depending on the triglyceride (TG) source and the pyrolytic method employed alkanes, alkenes, aromatic compounds, esters, CO₂, CO, water, and H₂ are produced. Oxygen removal from substrate molecules is another downside of pyrolytic production methods. Fuels obtained by pyrolysis are less environmental friendly than fossil fuels in terms of oxygen content, and also solid residues and carbon created during pyrolysis require additional separation steps [143]. In a study by Demirbas [28] it was found the liquid derived from pyrolysis of vegetable oils has similar properties to diesel. Several studies have been carried out using palm and copra oil using pyrolysis and catalytic conversion. The products consisted of gasoline, kerosene, diesel, and water [28]. Catalytic cracking has been used in an effort to control the types of products generated by TG cracking, using a vast variety of catalysts [144,145] and a gasoline-like fuel is more likely to be formed than a diesel-like fuel [146].

Amongst the four techniques, transesterification is the most promising solution. The transesterification of oil with alcohol in the presence of a catalyst produced biodiesel and glycerol. The reaction is normally a sequence of three consecutive reversible reactions [147]. In this process triglyceride is converted stepwise into diglyceride, monoglyceride, and, finally, glycerol in which 1 mol of alkyl esters is formed in each step [143].

Biodiesel is used to replace diesel and is produced by transesterification of vegetable oil and waste fats hence, biodiesel can be defined as "mono alkyl esters of fatty acids derived from vegetable oil or animal fats". It is the similarities in the constitution of the vegetable oils/animal fats and petroleum derived diesel that make the vegetable oils suitable for conversion to biodiesel [28,29,139]. The vegetable oils/animal fats are naturally insoluble in water and are hydrophobic substances. Their general make up consists of one glycerol to three fatty acids thereby they are frequently referred to as triglycerides [139]. The characteristics of the fat are influenced by the nature of the fatty acids attached to the glycerine; the nature of the fatty acids can have a knock on effect on the characteristics of the biodiesel. In excess of 350 oil bearing crops have been identified of which only a handful are considered viable for conversion to biodiesel, a table of these crops has been recently published by Demirbas [28].

Most of the research on biodiesel production [139] has concentrated on palm oil, soybean oil, sunflower oil, coconut oil, rapeseed oil and tung oil. A recent report has confirmed that most biodiesel production is now from soybean, rapeseed and palm oils [28]. The most commonly considered animal fats consist of those derived from poultry, pork and beef [142]. While one research group [29] reported on the conversion of animal fats for biodiesel, but then other group [139] have argued that although animal fats are mentioned regularly, their uses are limited as some of the methods for converting vegetable fats are not applicable to animal fats due to the natural differences between the two types of fats.

Used vegetable fats can be recycled for biodiesel production, but the quality of the oil will have a knock on effect on the quality of the biodiesel produced [148]. The one author of this review has studied

the use of waste cooking oils for biodiesel production in a project funded by Higher Education initiative fund of the University of Ulster, UK in 2007–2008 [149]. The aim of this project was to recycle the waste cooking vegetable oils generated in the University canteen, mainly from the frying process of potato-chips and other food-items. These left-over oils were not any more suitable for further cooking and were waste, which required an environment safer disposal. These non-edible oils were subjected to transesterification process for biodiesel formation. Certain factors affecting the various stages of this production process were optimised for an economical and practical process and the results of this project were presented in the International ECI-USA Biofuel conference [150]. This consisted of the chemical conversion of the oil to its corresponding fatty esters. Specifically the triglycerides are subjected to react with alcohol to produce alkyl esters of fatty acids (chemical name for biodiesel) and glycerol. Depending on the alcohol used, biodiesel with different types of chemical composition is formed i.e. if methanol is used, methyl esters are formed, if ethanol is used, ethyl esters are formed [29]. In the reactions alcohol replaces the glycerol, which can then be collected as a by-product and used for other applications such as cosmetics (mainly soaps).

The selection of catalyst depends on the amount of free fatty acid (FFA) present in the oil [143]. Generally, the catalysts are base, acid, or enzyme. For triglyceride stock having lower amount of FFAs, base-catalyzed reaction gives a better conversion in a relatively short time while for higher FFAs containing stock, acid-catalyzed esterification followed by transesterification is suitable [151]. The stoichiometric reaction requires 1 mol of triglyceride and 3 mol of alcohol. However, excess alcohol is used to drive the reversible reaction forward in order to increase the yields of the alkyl esters and to assist phase separation from the glycerol [151].

The transesterification process is catalyzed by alkaline metal alkoxides and hydroxides, as well as sodium or potassium carbonates [143]. Alkali-catalysed transesterification method is generally preferred, which includes the use of a homogenous catalyst such as sodium hydroxide or potassium hydroxide etc. The different levels of catalysts and alcohols have been used to obtain the highest yield from different types of oil [142]. Meher et al. [152] found 1% potassium hydroxide catalyst was the optimum concentration needed to obtain an 83% yield from crude Karanja (*Pongamia pinnata*) oil. In contrast, Karmee and Chadha [153] obtained a 99% yield from refined Karanja oil using 0.5% of sodium hydroxide. The alkaline catalysts normally show high performance when vegetable oils with high quality is used [143]. However, when the oils contain significant amounts of free fatty acids, they cannot be converted into biodiesels but to a lot of soap [154]. These free fatty acids react with the alkaline catalyst to produce soaps that inhibit the separation of biodiesel, glycerin and wash water [155]. Alkaline metal alkoxides are most active catalysts, since they give very high yields in short reaction times even if they are applied at low molar concentrations. Alkaline metal hydroxides (KOH and NaOH) are cheaper than metal alkoxides, but less active [143]. The industrial processes usually favor base catalysts because they are less corrosive than acidic compounds. One advantage of acid catalysts over base catalysts is their low susceptibility to the presence of FFA in the starting feedstock. However, acid-catalyzed transesterification is especially sensitive to water concentration. The transesterification of small esters under acid-catalyzed conditions can be retarded by the presence of spectator polar compounds [143].

When super critical alcohol was used in place of alcohol (and with no catalyst), nearly complete conversion was achieved by Sharma et al [142]. Although, nearly complete conversion can be achieved without a catalyst, but higher temperature and pressure conditions would be required for the reaction to reach completion.

Furthermore, Rathore and Madras [156] synthesised biodiesel from palm oil and groundnut oil using supercritical methanol and ethanol without any catalyst at temperatures of 200–400 °C and at 200 bar. On further analyses of their results they found that yield could be affected by temperature and molar ratios of alcohol and oil. Sharma et al. [142] discussed similar concepts but they elaborate and mention the effect of stirring during production. Meher et al. [152] carried out esterification experiments with mixing of reaction mixture at 180, 360 and 600 revolutions per minute. An incomplete reaction was recorded with 180 rpm, while yields obtained with 360 and 600 rpm were equal. Sharma et al. [142] found that using a mechanical stirrer (1100 rpm) increased biodiesel yield to 89.5%.

The operation and design of reactor is one of the important issues in catalytic biorenewable processes. Reactors are usually characterized as batch or continuous that can be either a continuous stirred tank reactors or plug flow reactors [157]. Batch processes allow high flexibility with respect to composition of the feedstock [158]. The transesterification is performed using an acid or base catalyst [159,160]. Nevertheless, the equipment productivity is low and the operating costs are high [161]. Moreover, the use of liquid catalyst has severe economical and environmental penalties [162]. Continuous processes combine the esterification and transesterification steps, allowing higher productivity [158]. However, most of these processes are still plagued by the disadvantages of using homogeneous catalysts [163] although solid catalysts emerged in the last decade [164–168]. Nevertheless, integrated processes based on reactive distillation have been also reported [166–170]. Moreover, an innovative process – known as ESTERFIP-HTM was developed for the transesterification with methanol by the French Institute of Petroleum. The process is based on heterogeneous catalyst based on zinc and aluminum oxides and it is currently being applied in commercial plants [171]. However, it requires relatively high temperature (210–250 °C) and pressure (30–50 bar).

Transesterification reaction is controlled by three mechanisms: mass transfer, kinetic and equilibrium. The mass transfer becomes slow if the immiscibility of the two reactants (i.e. methanol and triglycerides) is poor. On the completion of the mass transfer, the ensuing process is controlled by the kinetic. Both kinetic and mass transfer of the reaction can be improved by increasing the reaction temperatures and vigorous mixing [172,173]. The overall reaction kinetics is dependent on the individual rate constants for the conversion of triglyceride to diglyceride, monoglyceride and alcohol ester. Alternatively, vigorous mixing can be utilized to increase the rate of collision between the reactants and to homogenize the reaction mixture [143]. Vigorous mixing increases the mass transfer rate by dispersing the alcohol as fine droplets in the triglyceride phase, thereby increasing the contact surface area between the two immiscible reactants [174]. In a study Vicente et al. [172] found that the formation of methyl ester increased as the impeller speed was increased from 300 to 600 rpm. A co-solvent (e.g. tetrahydrofuran) can also be used as an alternative to create a homogeneous phase. The co-solvent improved the solubility of the alcohol in the triglyceride phase, allowing better mixing of the two phases and hence more reactions to take place [143]. Vacuum distillation step is used for methanol recycle prior to glycerin purification. The remaining base catalyst in the crude glycerin is commonly neutralized with low cost mineral acids, such as phosphoric acid. After neutralization, three distinct phases are formed: a low density (top) layer containing FFAs, a dense (bottom) liquid layer composed of glycerin, water and alcohol, and a third layer made of salt precipitates. These three phases are then separated with the non-glycerin layers being treated as waste. Glycerin is further purified by distillation to remove water and alcohol [143].

Hydrolysis and esterification processes are simpler processes as the glycerides are hydrolyzed first to fatty acids that are esterified in a second step to fatty esters [175,176]. Such processes have become very attractive and gain market share due to obvious advantages. High purity glycerol is obtained as by-product of hydrolysis step. Tailored properties of the biodiesel fuel are possible using esterification [177]. Moreover, the esterification step can be performed using solid acid catalysts [166–168] in an integrated reactive separation setup [159,167,178,179]. The usage of heterogeneous catalysts avoids the neutralization and washing steps, leading to a simpler and more efficient process. Compared to homogeneously catalyzed process, the transesterification with solid catalyst can tolerate more extreme reaction conditions, because the solid catalyzed process is an immiscible liquid/liquid/solid 3-phase system that is highly mass transfer limited [180]. Heterogeneous catalyst is reportedly used in the fatty acid methyl ester (FAME) plant of Diester Industrie (Paris) at Sete, France. The Esterfip-H process produces FAME by esterification of plant oils. The purity of methyl esters exceed 99%, and produced glycerol with purity of more than 98% compared to about 80% from homogeneous process. The overall production economic improves through the utilization of the by-product [143]. Choudary et al. [181] reported that thermally activated Mg–Al hydrotalcites with various Mg–Al ratios were effective catalysts for transesterification of tributyrin with methanol, with increasing catalytic activity. In the hydro-pyrolisis processes triglycerides are converted to fuel by hydrogenation followed by pyrolysis. The key difference is that the fuel product is a mixture of long-chain hydrocarbons instead of the conventional fatty esters. The process is known as NEXBTL (biomass to liquid) and invented by the Finnish company Neste Oy. While it has clear advantages, this process requires more complex equipment and implies the availability of a low-cost hydrogen source [158].

Enzymatic processes have low energy requirements, as the reaction is carried out at mild conditions, ambient pressure and a temperature of 50–55 °C. However, due to the lower yields and the long reaction times the enzymatic processes can still not compete with other processes at industrial scale [12,161,182]. The main purpose of enzymatic methanolysis using lipases for biodiesel production is to overcome the issue involving recovery and treatment of the by-product that requires complex processing equipment [183] and the main drawback is the high cost of the lipases as catalyst [59]. In order to reduce the cost, enzyme immobilization is introduced for ease of recovery and reused [59,184]. The low solubility of glycerol in biodiesel also poses a challenge in enzymatic transesterification because it reduces the enzymes activity [184]. This problem can be overcome by simply using 1,4-dioxane as co-solvent to solubilize methanol. An alternative technology for biodiesel production is non-catalytic transesterification with methanol under supercritical conditions [185]. Supercritical processes were developed to solve the problem of miscibility of oil and alcohol that hinders the kinetics of transesterification, as well as to take advantage of not using a catalyst. However, the operating conditions are severe ($T > 240$ °C, $p > 80$ bar) and therefore require

special equipment [186,187]. Compared to chemical reactions using catalysts, the supercritical method offers a few advantages, viz. catalyst is not needed in the reaction, making the after-production process much simpler since the separating process of the catalyst and saponified products from methyl esters becomes unnecessary. The waste water containing acid or alkali resulted from the after-production process can also be avoided. Supercritical reaction takes a shorter reaction time than the traditional catalytic transesterification reaction at higher conversion rate [185] and neither acidity nor water content influences the reaction in supercritical method [188]. This also allows a variety of resources to be used as feed materials. The disadvantages of the supercritical methods is the requirement of high pressure and temperature [189], high methanol-to-oil ratios made biodiesel production expensive. Table 2 summarizes various technologies used for the production of biodiesel.

Key reactor variables that dictate conversion and selectivity include temperature, pressure, reaction time and degree of mixing. The ideal biodiesel process would involve a continuous flow reaction that neither deactivate nor consume the catalyst and that minimizes or eliminates the need for multitudinous downstream separation and purification steps [143]. Hence, a choice of alcohol, FFA content, catalyst and reaction conditions affects the whole process of transesterification and the yield of biodiesel.

5. Debate on “food vs. fuel”

Biofuels will only be beneficial if they are cultivated in a sustainable way with both biodiversity and the “food vs. fuel”-debate in mind. One particular review by Groom et al. [190] concentrates solely on managing the production of oil crops in a resourceful and environmental friendly way. They recommend about growing biofuel crops with biodiversity in mind and recommend promotion of sustainable and low impact feedstocks such as Karanja, Jatropha and Switchgrass, maintenance of essential and native food crops and promotion of carbon neutral biofuel crops. Fargione et al. [191] and Demirbas [28] have also talked about the ‘food vs. fuel’ debate. Some food crops such as corn, sugarcane, soybeans and palms are being used in the synthesis of biodiesel, the dispute then arises as to if these crops should be designated to grow foods or be used in biodiesel production. Escobar et al. [2] suggested that the agricultural land in the world is limited therefore it is necessary to define the fraction of farmland that could be used for the production of biofuels. Cereals are the most important source of nourishment in the world [192], either for direct human consumption or indirectly, for feeding livestock. Therefore, variation in the availability and prices of cereals may be crucial for the world’s food supply. The use of farmland and grains that could be consumed by humans for biofuel production is already sending warning signals in some places of the world [2].

With an ever-rising world population and increasing number of motor vehicles on the roads, it is obvious an alternative solution will have to be found. In answer to this, reports by Sharma et al. [142], and Groom et al. [190] have special mention of Karanja,

Table 2
Summary of various biodiesel technologies (Adopted from Helwani et al. [143]).

Variable	Base catalyst	Acid catalyst	Lipase catalyst	Supercritical alcohol	Heterogeneous catalyst
Reaction temperature (°C)	60–70	55–80	30–40	239–385	180–220
Free fatty acid in raw material	Saponified products	Esters	Methyl esters	Esters	Not sensitive
Water in raw materials	Interfere with reaction	Interfere with reaction	No influence		Not sensitive
Yields of methyl ester	Normal	Normal	Higher	Good	Normal
Recovery of glycerol	Difficult	Difficult	Easy		Easy
Purification of methyl esters	Repeated washing	Repeated washing	None		Easy
Production cost of catalyst	Cheap	Cheap	Relatively expensive	Medium	Potentially cheaper

Jatropha, Switchgrass and prairie grasses, these oil-producing crops can be grown on waste agricultural land and require low inputs for growth such as low fertilizers and can use waste water for growth. Jatropha in particular is a perennial crop, which means it can be planted once but get yields oil for over 30 years or more. Groom et al. [190] have explained the potential of corn as a material for biodiesel but they feel this is only valuable as a short-term answer and it would be better if the crops were grown on waste land or unfertile agricultural land.

Recent technology advancements have made it possible to produce biofuels, called cellulosic biofuels, from inedible biomass, i.e. the stems, stalks and leaves of plants. By applying advanced plant breeding and biotechnology to dedicated energy crops, sustainable energy solutions can be delivered that: displace vast amounts of fossil fuels, provide greater energy security and create new economic opportunities for farmers and rural communities. Energy crops could meet 75% of the current gasoline demand.

High-yielding energy crops such as switchgrass, giant miscanthus, sorghum and other various energy crop species that can be produced as feedstock for cellulosic ethanol production [4,5,9] and additional biomass conversion processes. Switchgrass (*Panicum virgatum*) considered as an energy crop, is tolerant to a wide range of environmental conditions compared to many other perennial grasses and conventional crop plants, it produces large amounts of biomass under good and poor growing conditions. Genetic improvement of switchgrass, as a dedicated bioenergy feedstock crop, is necessary to reduce delivered feedstock cost.

The National Renewable Energy Laboratory (NREL) of the United States Department of Energy projects that by 2030, “technology developments will enable yields of ethanol to approach 400 l per dry metric ton of biomass feedstock converted, compared with about 270 l per ton that can be achieved with known technology today”. In pursuit of such a goal, the Department of Energy announced financial rewards in support of the establishment of three major bioenergy research centres and several major commercial-scale projects which are aimed at demonstrating the viability of cellulosic ethanol. While cellulosic ethanol is currently being produced, producing it competitively from lignocellulosic biomass still requires significant and successful research, and development [24].

One concept currently under review is the use of algae as an oil producer for the manufacture of biodiesel. Research has shown that oil content of algae per hectare can be a staggering 200 times more than the most productive land based crop (algae are the fastest growing photosynthetic organisms and have the potential to produce 46 tons of oil/hectare/year). This is a promising lead for new generation biofuels, without compromising with food supply as these can be cultivated on non-agricultural lands.

6. Concluding remarks

The consideration of any process for the biotransformation of substrates and realise what are the current research challenges to be faced in the production of biofuels needs critical evaluation. Despite so many obvious advantages, still no large-scale production facilities have been established using biotransformation of lignocellulosic materials. Bioethanol is currently being produced from sugar cane and starch-containing substrates. Although there are similarities in between the lignocellulosic and the starch process, the technical and economical challenges in bioconversion of lignocellulosic substrates are large. Though several options are available and reported by different researchers for a lignocellulose-to-ethanol process but, for any option which is chosen, the following factors are required to be carefully assessed in comparison with a well-established ethanol production using sugar or starch substrates. (i) A cost-effective strategy of transformation of cellulose and hemicellulose to

soluble sugars. (ii) A maximum fermentation-efficient process transforming a hydrolysate consisting of mixed sugars, containing hexoses and pentose sugars in presence of fermentation inhibitory compounds. (iii) A state-of-the-art process integration to minimize the demand of overall process energy. Following four major research challenges need to be considered for process optimization to produce a sustainable biofuel are:

- (1) The process of enzymatic hydrolysis of agricultural substrates needs to be improved, which can be approached with the use of cheaper and of higher specific activity crude enzymes, by synthesis of enzyme in a process of reduced production cost and by novel technology for the handling of large amounts of solids.
- (2) The development of such microbial strains which are not only robust fermenting organisms, but also are at the same time more tolerant to inhibitors present in substrate-hydrolysates. These specially developed strains should be able to ferment all sugars available from the raw material in concentrated hydrolysates, giving high productivity of alcohols and withstanding high alcohol concentration in the medium.
- (3) A well-thought strategy for the process integration to reduce the number of steps involved in overall production process.
- (4) Working on 3-R strategy: Recycling, Reduction and Reuse of any byproducts and wastes generated in the process to reduce the energy demand and protect the environment.

With an ever-increasing market for biofuels globally there is a definite need for a realistic solution to be reached. Many of the reports reviewed here have offered recommendations on how this can be employed. There are many proposals in place for using biodiesel and a lot of Governments worldwide are becoming more interested with promoting biofuel use. Obviously, each country will have to grow oil-producing crops suitable for its climate and in accordance with available land use but maybe a lot can be learned from countries like Brazil where biofuels have been in widespread use since 1970s and all fossil fuels must contain a minimum of 25% blend of biofuel. It has been well accepted globally that there is much potential for the biofuel market and it is only matter of time before they are more available than petroleum-based fuels. The development and use of biofuels as an alternate to fossil fuels, still require a more advanced technological development to increase their feasibility by enhancing the energy balance and reducing the emissions and production cost, are true alternatives that complete the biofuels future scheme.

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Dr. Ms Poonam Singh Nigam BSc MSc PhD CBiol PGCUT MILT FHEA Ph.D.(1985) Postdoctoral Fellow (1985–1988) Germany; Faculty of Life & Health Sciences, University of Ulster, Northern Ireland, UK (Jan 1990–continued); Chartered Biologist (1992) Institute of Biology London; Associate Advisor in Biotechnology British Council UK (2002–cont.); Member Institute Teaching & Learning in Higher Education UK (2001–); Fellow Higher Education Academy UK (2007–); Member Editorial Boards of six International Biosciences Journals (2000–), included in European database of women experts in Science www.shu.ac.uk/witec, Member various International conferences - Scientific & Organising Committees; Managed Research & Educational Grants and European Social Funds; External examiner 12 PhD; Supervisor of 9 Postdoctoral, & 12 PhD; Course-Director and Advisor of Postgraduate studies of two Master courses in Biotechnology & Food-Biotechnology, Senior Lecturer in Biotechnology, Author of >121 Scientific publications, 2 Biotechnology Books, 1 European Patent, 35 Reviews, 30 chapters, 57 conferences presentations, Recipient of research/academic grants from EU, European Social Fund, British Council, Northern Ireland Government, Pharmaceutical & Textile companies, UNESCO, Royal Society London, FEMS, and CSIR. Research, Teaching and publications are in the area of in Industrial, Environmental and Pharmaceutical Biotechnology.

Dr Anoop Singh has an MSc and a PhD in Environmental Sciences. He is a research fellow in bioenergy and biofuels in the ERI. He has previously worked at a number of research institutes in India (TERI, IARI, BHU and VBSPU). He has published 40 peer review journal papers and two books. He has visited several countries and participated in many international conferences. He is a member of several scientific communities. He is serving as Editor for the Journal of Agricultural, Food and Environmental Sciences besides being a reviewer for a number of International Journals. His research interests are focused on the utilization of industrial, agricultural and household waste for eco-friendly energy production and their life cycle analysis.