MICROBIAL BIO-FUELS: PAST, PRESENT AND FUTURE TRENDS : A REVIEW

Ahmed EL-Imam, A M.

Microbiology Department, Faculty of Science, University of Ilorin, Nigeria

+234(0)803 700 5448 amkmusty@yahoo.com

INTRODUCTION

The concept of global warming is one that was first brought to public consciousness by Dr. James Hansen of NASA who alerted the world to the issue of climate change at the U.S. Senate hearings in July 1988 (The Daily Independent, 2012). A large proportion of fuel production is aimed at producing fuels suitable for transportation and the electricity generation, these are the most important fuels to target in the efforts to cut down greenhouse emissions. This has been achieved by increasing interest in alternative energy sources such as microbially-produced biofuels such as the bio-alcohols, methane and hydrogen.

This review evaluates the history of biofuels, the current state of microbial fermentation to produce biofuels and some prospects which are still in the promising research prospects for future production of microbial biofuels. It also focuses on how the usage of alternative fuels produced by microorganisms in the past has shaped present biofuel use, and what the future portends for microbial biofuel production and use. Biodiesel use is also discussed in the review although it is not microbially.

What Are Biofuels?

A bio-fuel is described as a fuel whose energy is derived from biological carbon fixation (Demirbas, 2009). Biofuels are fuels derived from living plants, animals or their by-products which are not more than 20-30 years old. Biofuels contain stored solar energy and are a renewable source of energy, since the plants can be grown again, and are thus also referred to as Green Fuels. Unlike petroleum products, all biofuels are biodegradable and do not damage the environment when spilled (www.altenergy.in). Although fossil fuels also have their origin in ancient carbon fixation, they are not considered biofuels because they contain carbon that has been “taken out” of the carbon cycle for a very long time.

Bio-fuels could be biomass conversion products, solid biomass, liquid fuels or bio-gases. Also known as agro-fuels, these fuels are derived either from biomass or bio-waste. The most commonly utilized biofuels are the solid biomass such as wood, charcoal, sawdust, dried manure and dried grass. This is particularly true in the developing countries where they are primarily burnt to produce heat and in some of the least developed countries (LDCs) of Africa, it accounts for 70 to 90 percent of primary energy supply (U.N. DESA, 2007). Liquid biofuels include bioethanol, biobutanol, biomethanol, biodiesel and so on which are made from microbial fermentation of starch and sugars; and biodiesel which is chemically produced from the trans-esterification of vegetable oils. Alongside combustion, they were put to a variety of uses as solvents, greases, cleaners or as basic chemicals for the emerging chemical industry, until again, a cheaper source was found in fossil oil. They have the advantage of possessing certain chemical and physical properties such as stability, safety, energy density and predictable combustion required for safe and efficient engine applications.

Biodiesel is a liquid biofuel which can be used to replace petroleum diesel in diesel engines. The most common process for biodiesel production is extraction of oils from vegetable oil feedstocks, such as palm tree, soybean, and rapeseed, and converting the oil to biodiesel by trans-esterification of triacylglycerols (TAGs) with methanol and ethanol. Fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs) are synthesized by this reaction (Ma and Hanna, 1999).

Biogases include Hydrogen, methane and carbon dioxide produced by anaerobic digestion or fermentation of biomass.

Classification/Generations of Biofuels

Biofuels are generally classified into generations. First generation biofuels are also called conventional biofuels, and are biofuels made by the action of microorganisms or their enzymes on sugar, starch, and vegetable oil. The most commonly used organism is *Saccharomyces cerevisiae.* The production of bioethanol or butanol by fermentation of starch (from wheat, barley, corn, potato) or sugar (from sugarcane, sugar beet, etc.) have been blamed for pushing up food prices globally. The increased pressure on arable land currently used for food production can lead to severe food shortages, in particular for the developing world where already more than 800 million people suffer from hunger and malnutrition. In addition, the intensive use of land with high fertilizer and pesticide applications and water use can cause significant environmental problems (Schenk, 2008)

Second generation biofuels are biofuels produced from sustainable feedstock such as lignocellulosic biomass. Sustainability of a feedstock is defined among others by availability of the feedstock, impact on GHG emissions and impact on biodiversity and land use. Bioethanol and biodiesel produced from conventional technologies but based on novel starch, oil and sugar crops such as *Jatropha curcas*, cassava or *Miscanthus* or other non-food plants that do not compete with food production also fall in this category (Dragone, *et al.*, 2010). However, converting the woody biomass into fermentable sugars requires costly technologies involving pre-treatment with special enzymes such as lignases and cellulases. This is because while corn grain consists of starch and sugar cane of sucrose, biomass comprises mainly lignocellulose (typically 40-50 % cellulose, 25-35 % hemicellulose and 15-20 % lignin), which is the main component of the plant cell wall and therefore very resistant to degradation (Schubert, 2006; Gray et al., 2006). This implies that second generation biofuels cannot yet be produced economically on a large scale. Many second generation biofuels are currently under development such as cellulosic ethanol, algae fuel, biohydrogen, biomethanol, Fischer-Tropsch diesel, biohydrogen diesel, mixed alcohols, wood diesel, *Jatropha curcas* oil and biodiesel, etc (biofuels.org.uk).

Third-generation biofuels are algae-based. Examples are biodiesel from microalgae, bioethanol from microalgae and macroalgae (seaweeds), Hydrogen from green microalgae and microbes (Dragone, 2010).

Fourth-generation biofuels are either created using petroleum-like hydroprocessing, advanced bio-chemistry, or revolutionary processes like Joule's "solar-to-fuel" method which do not fit into any other category of biofuels (www.greentechmedia.com). These are all however mostly-theoretical or laboratory-scale processes.

PRODUCTION OF BIOFUELS

This involves any of the following processes:

Direct combustion

This is the most common and oldest form of conversion that involves burning organic matter in an oxygen-rich environment mainly for the production of heat. Examples of its applications include burning of biomass like wood, dung, and agricultural wastes in homes for cooking and heating, the burning of wood for heat in chemical industries, etc.

Thermo-chemical conversion: In contrast to direct combustion, this process utilizes heat and pressure in an oxygen-deficient environment to produce "synthesis gas". Syn-gas is composed mainly of carbon monoxide and hydrogen, and can either be combusted directly to produce heat or transformed to other fuels like ethanol and hydrogen.

Biochemical conversion: In contrast to the direct combustion and thermo-chemical conversion, biochemical conversion processes occur at lower temperatures and have lower reaction rates. Fermentation is a common biochemical conversion process employing bacteria, yeasts and other microorganisms in the conversion of sugar and starch, found in crops like sugarcane, corn, wheat, etc., to ethanol. The process is sometimes preceded by the acid or enzymatic hydrolysis of cellulosic feedstock to liberate more sugars from the lignocellulosic biomass. Anaerobic digestion is another biochemical process involving the bacterial breakdown of biodegradable organic material in the absence of oxygen to yield biogas, which is mainly methane and carbon dioxide with some impurities such as hydrogen sulfide.

The most common process for the production of biodiesel is the chemical transesterification of vegetable oils and animal fats. Waste cooking oil and fats set forth significant disposal problems in many parts of the world. This environmental problem could be solved by proper utilization and management of waste cooking oil as raw material for the production of biodiesel. There are four primary ways to make biodiesel, direct use and blending, microemulsions, thermal cracking (pyrolysis) and transesterification (Shalaby, 2011). There is an increasing interest in developing microbial fermentation processes for production of biodiesel as this will allow for the use of a wider range of raw-materials, including sugar cane, corn, and biomass. Production of biodiesel by microbial fermentation can be divided into two different approaches, (1) indirect biodiesel production from oleaginous microbes by in vitro transesterification, and (2) direct biodiesel production from redesigned cell factories (Shuobo, 2011).

4. **Transesterification**: This is the most common method of producing biodiesel today. Transesterification is a chemical process by which vegetable oils can be converted to methyl or ethyl esters of fatty acids also called biodiesel. Biodiesel is physically and chemically similar to petro-diesel and hence substitutable in diesel engines (Rajagopal and Zilberman, 2007).

The last two processes are more relevant to this write-up.

The Need for Microbial Biofuels

It is generally believed that the re-introduction of carbon entombed in fossil fuels into the carbon cycle today will have adverse effects on the climate through the production of greenhouse gases (GHGs) which trap heat in the atmosphere. Various processes such as the burning of plant and fossil fuels (IPCC, 2007) for electricity generation and transportation which produces anthropogenic carbon dioxide; animal husbandry which produces methane; and deforestation which reduces the natural carbon sinks available (www.planetsave.com; IPCC, 2001), all increase the GHGs. Since the transportation sector is growing at an annual average of 3% (EIA, 2010) and contributes the largest quota of GHGs to the atmosphere this could be counterbalanced by using alternative energy sources such as biofuels.

Microbial biofuels are simply fuels that are produced via microbial action on biomass. Microorganisms can ferment a vast array of carbohydrates to produce numerous fuel compounds (Table 1) including those suitable for transportation and electricity generation. Microbial biofuels provide a double environmental benefit in that, in addition to reducing GHG emissions, the production processes of microbial biofuels also reduce environmental waste by harnessing the ability of various microorganisms to utilize and break down agro-industrial wastes such as bagasses, molasses, stover, grain husks and seed cakes e.g. Jatropha seed cake. Microorganisms are ubiquitous and can be manipulated in an unlimited number of ways to improve certain characteristics.

Many countries are exploring the biofuel alternative to petroleum fuels to decrease their dependence on foreign oil and the attendant uncertainities in price and supply.

Table 1: Primary crops utilized as feedstock in biofuel production in select countries

COUNTRY BIOFUEL	FEEDSTOCK CROP(S)
USA Bioethanol, biodiesel	Sugar cane, maize, soybean
UK Bioethanol	Sugar beet
Germany Biodiesel	Rapeseed
Malaysia Biodiesel	Oil Palm

Indonesia	Oil Palm	Biodiesel
China	Maize	Biodiesel
Tanzania	Oil Palm, Jatropha	Biodiesel
Brazil	Cassava, soybean	Bioethanol, Biodiesel
India	Sugar cane	Bioethanol
Nigeria	Cassava	Bioethanol
France	Rapeseed	Biodiesel
South Africa	Sugarcane	Bioethanol
Global	Sorghum, wheat, maize, rice, cassava, sugarcane, grass	Bioethanol
Global	rapeseed, Jatropha, peanut, canola, animal fat, soybean	Biodiesel

Biological processes of producing bio-fuels have been used throughout history as mankind has been fermenting and distilling alcohol for millennia, even though it was meant for human consumption rather than for use in combustion engines. Most of these fuels were alcohols produced by the fermentation of substances like starch or sugars, or plant oils (Antoni *et al.*, 2007). Microorganisms convert almost any kind of biomass into chemicals that can be used as transport bio-fuels. Even though it may be argued that ethanol, methane, butanol and other biofuels can be produced almost as efficiently as refining products of crude oil, this process is not only blamed for accelerating global warming, but it is also deleterious to the environment.

THE HISTORY OF MICROBIAL BIO-FUELS

Until the beginning of the industrial revolution, most manufacturing processes were small-scale and the transportation of goods and humans was minimal. The Industrial Revolution of the 19th century however, saw manufacturing processes powered by the combustion of coal which resulted in the production and accumulation of gases collectively referred to as greenhouse gases (GHGs) because of their ability to absorb or trap some of the energy that is radiated back into space from the heat of the Earth's surface, thus raising the atmospheric temperature and causing the "greenhouse effect". Even though these gases existed in the atmosphere prior to industrialization, it has been reported that since then, concentrations of carbon dioxide have increased by nearly thirty percent, concentrations of methane have more than doubled, and nitrous oxide concentrations have risen by about fifteen percent due to the burning of fossil fuels such as oil, natural gas, and coal, which are used to operate cars and trucks, heat homes and businesses, and run factories (Martinez, 2005). Smoke and smog (a mixture of smoke and fog) became common in London and other industrial cities. This necessitated the need to search for alternative energy sources.

In 1834, the first US patent for alcohol as a lamp fuel was awarded to S. Casey. The first attempts to chemically create bio-fuels were ascribed to Duffy and Patrick, two chemists who in 1853 while experimenting with the trans-esterification of vegetable oils to make soap, produced a Fatty Acid Methyl Ester (FAME) which will later be known as biodiesel (Biodiesel Times, 2005). The application of microbial fermentation to biofuels production commenced with Louis Pasteur's discoveries of lactic acid fermentation in 1857, and discovered in 1862 that butanol can be formed by certain bacteria. The first use of bioethanol in transportation is attributed to Nikolaus August Otto in the 1860s (Antoni *et al.*, 2007). Between 1900 and 1930, the production of the fuels ethanol and butanol were the most important industrial fermentations in the world. Albert Fitz, Martinus Beijerinck and Sergei Winogradsky in their works on butanol fermentation further identified similar organisms which were given names like "*Granulobacter saccharobutyricum*", "*Amylobacter butylicus*", and "*Bacillus orthobutylicus*" but which all most likely belonged to the genus *Clostridium* (Dürre & Bahl, 1996).

Almost four decades after Otto's discovery, Dr. Rudolf Diesel patented a design for the compression ignition engine which used peanut oil as fuel. This vegetable oil fuel replaced the steam engine and gained popularity for a while until the 1920s when the manufacturers of diesel engines decided to alter their vehicles to utilise the far less viscous petro-diesel alternative which were also plentiful and cheap. In 1898, liquefaction of chemically produced hydrogen was achieved by James Dewar. In 1902 Deutz Gas Engine Works designed one third of their heavy locomotives to run on pure ethanol. Around 1925, ethanol was only added to gasoline as an anti-knocking additive because of increasing cost of production and by 1940 the price of petrol plummeted and ethanol became comparatively too expensive that its use was totally phased out. Increasingly more sophisticated fuel injection systems were then designed to run these fossil fuel-derived oils.

Over time there was increasing use of fossil fuels, leading to various air pollution disasters such as the Great Smog of 1952 which led to the death of thousands in London. This brought to the fore, the need for changes to be made in fuel consumption patterns. By the 1970's though, the global price of crude oil increased due to the oil crises of the 1970s caused by the oil embargo of Organization of Arab Petroleum Exporting Companies (OAPEC) and the Iranian Revolution. Consequently, the microbial production of biofuels was resuscitated in some parts of the world, such as the production of a large bioethanol industry in Brazil based on the fermentation of cane sugar by *Saccharomyces cerevisiae* (Basso, 2008), making Brazil the world's largest producer of bioethanol producing 13.7 billion liters by 1997 (IEA, 2004). Hydrogen research also received a boost and in 1988, the first hydrogen-powered aircraft was flown in the U.S. (Koskinen, 2008). In 1990, the American Clean Air Act of 1956 (and 1968) was amended and included more stringent restrictions on vehicle emissions thus increasing the existing interest in cleaner safer fuels; also there was an enormous rise of the crude oil prices since the late 1990s, and by 2006, the United States rapidly surpassed the Brazilian bioethanol production with an annual capacity of 18.4 billion litres. In the same year, Nigeria launched the pilot phase of its ethanol project which was to be based on the fermentation of cane sugar cultivated on 10,000 hectares in the North, the project has however been stalemated.

In 2005, David Ramey successfully toured the United States in a car fuelled by pure butanol showing that emissions of carbon monoxide (CO), hydrocarbons, and nitrogen oxides (NOx) were decreased enormously (Dürre, 2007). The next year, BP and DuPont commenced fermentative biobutanol production from sugar beet.

Anaerobic digestion of organic materials in industrial wastewater to methane has been utilized for years and is now used worldwide. This process convert all types of polymeric materials such as lipids, carbohydrates and proteins to methane and CO_2 in anaerobic conditions by a variety of microorganisms which include fermentative microbes (acidogens), hydrogen producing, acetate forming microbes (acetogens) and methane producing microbes (methanogens).

MICROBIAL BIOFUELS: WHERE WE ARE

Early in the third millennium, the rise in the prices of petroleum fuels became even steeper; and coupled with the greater global awareness on pollution and global warming, and the desire for self-sufficiency, bio-fuels continued to gain popularity. In 2011, the production level of bioethanol is at its highest level (Table 2) with the united states topping the list of producers.

Table 2: Bioethanol production output (in billions of litres) of selected countries in 2011

Country	bioethanol output
U.S.A.	13,900
China	555
Canada	462
Brazil	5,573
Nigeria	N.A.
India	N.A.
E.U.	1,199

Source: Adapted from www.en.wikipedia.org

Government subsidy of bio-fuel industries became common, particularly in developed countries such as the United States, the European Union. This has given the industry in these countries the economic security needed to invest in various bio fuels. In 2011 a policy was put in place in South Africa for the mandatory blending of 2% v/v bioethanol with petrol and 5% v/v biodiesel with diesel and this has provided the necessary incentive for investment and research in biofuels in that country (www.info.gov.za).

Today, the global bio-ethanol consumption in 2010 stood at 100 billion litres while the figure for biodiesel was 20 billion litres (OECD-FAO, 2011) and these figures are projected to continue to expand rapidly over the next ten years.

A less commonly utilized biofuel is bio-hydrogen gas (H_2) which is a clean fuel with a high energy content per unit weight (122 KJ g-1) and does not contribute particulate or GHG emissions into the atmosphere upon combustion. H_2 can be produced by nuclear or fossil fuel mediated electrolysis of water, coal gasification, and steam reformation of natural gas or through the action of biological systems. Production of H_2 using fermentative biological processes is potentially the most attractive of these strategies as it is not as energy intensive as other means and could potentially utilize refuse or agricultural waste-streams as the raw material. The biological production of H_2 is fundamentally dependent on hydrogen evolving enzymes known as hydrogenases. These enzymes catalyze the reversible oxidation of hydrogen gas. Collet *et al.* (2004) concluded that lactose fermentation by *C. thermolacticum* represents a cheap alternative to biohydrogen production that makes use of an otherwise polluting waste product. If harnessed properly, hydrogenases and/or hydrogenase-containing organisms could be used to supply affordable and renewable H_2 to be used as a biofuel to power vehicles or used in hydrogen fuel cells to generate electricity. Current production is limited by the cost of reactors required for photochemical synthesis and the low yields.

COMMONLY USED MICROORGANISMS IN BIOFUEL PRODUCTION

The most commonly utilized organism in the fermentative production of biofuels is *Saccharomyces cerevisiae* commonly referred to as baker's yeast. A wide variety of organisms are utilized in biofuel production (Table 4). Many of these organisms have been bio-engineered to produce strains with better biofuel production capacities than they are naturally capable of.

Table 3: Organisms used in the fermentation of various substrates for biofuel production

Organism type	Organism	Fermentable substrates	End product
Bacteria	*Zymomonas mobilis,*	Simple sugars	ethanol
	Clostridium acetobutylicum	sugars, starch	butanol, ethanol
	Escherichia coli,*	Glycerol	ethanol, (butanol)
	Bacillus subtilis		
	Klebsiella oxytoca,		
	C. butyricum	carbohydrates	hydrogen
	Thermoanaerobacterium aciditolerans	carbohydrates	hydrogen
	Clostridium ljungdahlii	Syngas	butanol
	C. cellulolyticum,		
	C. phytofermentans,		
	C.thermocellum,	Cellulose	ethanol
	C. thermolacticum	lactose	hydrogen
	C. uzonii	glucose	hydrogen
	C. beijerincki	sucrose	butanol
Yeasts	*Kluyveromyces fragilis, K. Lactus*	Lactose	ethanol
	*Saccharomyces cerevisiae**	Sugars	ethanol, (butanol)
	S. uvarum, S, diataticus	Dextrins	ethanol
	Pichia spp	Xylose	ethanol
Microalgae	*Scenedesmus obliquus*		
	Scenedesmus dimorphus		
	Euglena gracilis		
	Chlorella vulgaris		
	Spirulina maxima		
	Anabaena cylindrica	Salt water	biodiesel
Fungi	*Aspergillus niger,*	Palm oil	biodiesel

*engineered to produce the desired fuel; fuels in parentheses are products of engineered strains

LIMITATIONS TO THE PRODUCTION AND USE OF MICROBIAL BIOFUELS

- The primary hindrance to the use of microbial biofuels is an ethical one. There is a concern that the biofuel programmes have raised and will continue to raise food prices globally as food materials are diverted by companied and governments to provide power and transportation fuels. This is however being mitigated by the increased attention on secondary biofuels production. The transition from food to biomass-based feed-stocks for bioenergy production should greatly reduce the controversy over food vs. fuel, although a host of other factors limit the development of secondary biofuels.
- Biomass feedstocks (or the landscapes on which they are produced) are currently used for livestock feed. For example grasses and some crop residues (e.g., corn stover) are used for cattle feed. Agricultural landscapes that are less productive, subject to erosion, or in more marginal climates are often used to produce livestock forage, and these same landscapes are under consideration for biofuel feedstock production. Therefore, biofuel producers may compete with livestock producers for herbaceous feedstocks (grasses and crop residues).
- Expensive pre-treatment methods have also hindered the development of second-generation biofuels as this raises production costs. The average cost incurred to hydrolyse cellulose using *Trichoderma reesei* cellulases is U.S. cents 2.5-5/litre of ethanol produced (Michael *et al.*, 2010).
- The major setback though to the biodiesel production is that it has relatively low land-yield and is competing for agricultural land that can be used for food production, thus the cost of biodiesel, however, is a main hurdle to commercialization of the fuel. The oil is inedible because of the presence of anti-nutrient factors and thus its use in biodiesel production doesn't compete with food. That is the major advantage that *Jatropha curcas* oil offers. The University of Ilorin, has devoted hectares of land towards the research and development of *J. curcas* oil based biodiesel, the largest such plantation for dedicated to research in Nigeria. Adaption of continuous transesterification process and recovery of high quality glycerol from the glycerol by-product are primary options currently being considered to lower the cost of biodiesel and thus set the stage for competitive local production of biodiesel.
- In addition to the high cost and energy requirement of the trans-esterification process and the subsequent separation of the biodiesel from the glycerol are high, the availability of cheap vegetable oil feedstocks is also limited. To overcome these limitations, several studies have focused on enzymatic trans-esterification using lipase with whole cell biocatalysts technology and biodiesel production from microalgae (Connor, 2009).
- Also, not every car user is amenable to the use of ethanol in their vehicles; as a result the use in petrol/ethanol formulations has met with scepticism and rejection in some parts of the world. For instance, in Germany, drivers defeated a government mandated ethanol-blend program with a largely successful boycott which resulted in a setback for an aggressive biofuel program being pursued by the German government.
- Bioethanol as a transport fuel is not as efficient as petrol (Table 4). Too high alcohol-fuel blends are reputed to cause corrosion of aluminium fuel system components. These limitations are however not significant with the use of biobutanol which is less corrosive than ethanol and will thus not require the modification of existing structures such as pumps, tanks, pipelines etc (Sakuragi *et al.*,2011). Biobutanol is superior to ethanol as a fuel molecule and can replace petrol 100% or mixed at any concentration, unlike ethanol which can be mixed up to a maximum of 85%.
- Currently butanol production is not viable because of low butanol yield and will

only likely be economically viable at gasoline costs of $3.00 per gallon or more (Ramey 2004).

Table 4: Comparison of critical parameters of commonly used fuels

Fuel characteristic	Bioethanol	Biobutanol	Petrol	Diesel	Biodiesel
Cetane number	-	-	-	50-60	45-70
Octane number	85	10278	-	-	
Flash point	< -20	12	36	55-60	100-190
Heat of vaporisation	0.36	0.92	0.43		
Compression ratio	15	9	11	15	14
Mileage	100	65	87	100	90-100
Energy density	33	21	29	35-42	32-42
Vapour pressure	35-90	58	7	-	-
Viscosity (at 20^{o}C)	0.6	3.6	1-4		2-10
Environmental toxicity	high	low	moderate	high	low

Adapted from Balat *et al.*, (2008); Michael, *et al.*, (2011). Some figures have been rounded off.

• Currently the biodiesel production represents 82% of biofuel production in the European Union which is also the largest consumer of biodiesel. There have been attempts to cultivate oil-bearing crops in Africa for the production of biodiesel to serve the EU. While some have been disastrous such as the ambitious Jatropha biodiesel project of the London-based company, Sun Biofuels which was allocated over 8000 hectares of land in Kisarawe district of Tanzania. This project never kicked off, and this left the villagers impoverished, unemployed and without ancestral land to subsist on. The thirst for biofuels to meet the UK and EU's rising targets has led British companies to lead the charge into Africa. Half the 3.2m hectares of biofuel land identified is linked to 11 British companies, the biggest proportion of any country. ActionAid's estimate suggests that up to 6m hectares has been acquired continent-wide. But with landowners frequently illiterate and unaware of their rights, the potential for exploitation is high (http://www.guardian.co.uk). Similar problems abound in other countries like Nigeria where fraudulent people dupe unsuspecting farmers in the name of providing them with "special" varieties of *J. curcas* to grow and supply to the E.U.

FUTURE TRENDS IN MICROBIAL BIOFUEL PRODUCTION

The primary focus in biofuel research will be on the genetic modification of microorganisms to achieve greater product specificity and output. There will also be increased focus on higher level biofuels as the food vs fuel debate gains more momentum. The challenge of degrading lignocelluloses will be on the fore-front. The following are the developments which could better improve microbial biofuel production, affordability, and efficiency in the near future.

• *The Sustainable Use of Renewable Fuels (SURF)* is a consortium founded in 2010 by Cranfield University, U.K and comprises Airbus, British Airways among many others, with the aim of finding alternatives for aviation fuel. The project will be designed to use near-shore waters to rapidly grow microalgae at a faster rate than any other initiative and capture CO_2 from the atmosphere and seas at the same time. The greatest advantage of this project will be that it will not compete with agricultural land, does not require fresh water, does not result in deforestation and does not damage the environment (www.go-green.ae). Another similar consortium in the SkyNRG by KLM airlines.

- An EU-funded project, known as *DISCO* comprising research institutes, universities and industrial partners, is searching for microorganisms that can degrade lignocellulosic material. The overall aim of the biofuel project is to find a cocktail of microorganism derived enzymes that can simultaneously breakdown the complex lignocellulose into simple sugars, and enable yeast co-fermentation to produce bioethanol (www.renewableenergyfocus.com).

- The South African government plans to increase microbial biofuel production through the government-owned Industrial Development Corporation (IDC) and Central Energy Fund (CEF). The plan is to attain a two per cent penetration of biofuels in the national liquid fuel supply by 2012 (USDA,2009).

- Rainbow Nation Renewable Fuels Limited (RNRF) South Africa announced a R1.5 billion ($0.18bn) biofuels processing plant in the Eastern Cape part of South Africa. The facility will consume 1.36 million tons of soybeans annually producing 288 million litres of biodiesel making it the largest soybean processing facility in Africa (USDA, 2009).
- Many researchers now point to increasing evidence that commercial biofuel production can be reconciled with feeding humanity and preserving the environment, provided that we invest the time and effort needed to make the improvements necessary to achieve this goal (Lynd & de Brito Cruz, 2010).
- Engineered host fermentation by using *Escherichia coli* and *Saccharomyces cerevisiae* containing heterologous genes has also been shown to be involved in the production of biodiesel FAEEs. Researchers have also isolated from soil contaminated with petroleum waste products *Rhodococcus opacus*, a bacterium with a flexible appetite, capable of eating a number of sugars and toxic compounds and converting them to tryacylglycerols which can then be used to produce biodiesel (www.technologyreview.com)
- In January 2012, The *Science* Journal published that scientists engineered a new strain of *E. coli* capable of metabolizing the main sugar in seaweed (macroalgae), alginate by isolating genes used by the marine bacterium *Vibrio splendidus* to metabolise alginate and splicing it into *E. coli*. This new strain gives 80% of the theoretical maximum yield, converting 28% of the dry weight of the seaweed into ethanol. Natural seaweed species grow very fast - 10 times faster than normal plants - and are full of sugars thus making this a very significant development. This new development holds great potential in the future.
- Principle Energy in Mozambique is implementing a 20,000-hectare sugar cane plantation to provide 65 million gallons of ethanol and about 13 megawatts of exportable electrical power.
- Hydrogen gas is considered as a future energy because it is renewable, produces only water as a by product, does not evolve the "greenhouse gas (CO_2) in combustion, liberates large amounts of energy per unit weight in combustion and easily converted to electricity by fuel cells. The microbial hydrogen production by fermentative bacteria, photosynthetic bacteria and algae has been reported.
- Isobutanol is also currently under investigation as one of new biofuel targets. It is among branched C4 and C5 alcohols that are considered potential gasoline alternatives. Isobutanol has very similar characteristics to n-butanol, although it has a higher octane number than n-butanol (Lee *et al.*, 2002) which is significantly lower than petrol (Table 1).

- Improvements to conversion systems are needed to increase yield and minimize costs, while sizing systems for distributed processing. Efforts to improve the biochemical platform are focused on pretreatment strategies and engineering microbes and enzymes to deconstruct carbohydrate polymers and produce long chain hydrocarbons or alcohols. Thermo-chemical efforts are being directed towards integrated thermo-catalytic processes that can readily switch between a multitude of feedstocks. Conversion systems of the future must optimize value of products produced, minimize energy and water use, be scaleable to distributed processing networks (to minimize feedstock logistics challenges), and produce minimal wastes.
- Researchers and companies such as Solazyme are developing microbes (native or genetically modified) to ferment glucose into a range of infrastructure compatible, energy dense 4th generation biofuels such as longer chain alcohols, alkanes, and alkenes. The xylose fractions present a greater challenge, as fewer microbes have the needed metabolic machinery (Saha & Cotta, 2007).
- Bio-hydrogen and oil from algae will likely generate more research in future. Microalgae may be used as the primary energy source in future, as their yield per acre is the highest compared to other sources. Microalgae are currently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, CO2 fixation ability and high production capacity of lipids; they also do not compete with food or feed crops, and can be produced on non-arable land. Microalgae have broad bioenergy potential as they can be used to produce liquid transportation and heating fuels, such as biodiesel and bioethanol.
- A research team at the University of Cambridge, funded by the Biotechnology and Biological Sciences Research Council (BBSRC), has identified and studied the genes for two enzymes that toughen wood, straw and stalks making it difficult to extract sugars to make ethanol biofuel. The team studied *Arabidopsis* plants that lack two of the enzymes that build the xylan in lignocellulose. When attempting to extract biofuel from these plants, the researchers found that it takes less effort to convert all the xylan into sugar. Plans are underway towards developing new varieties of bioenergy crops such as willow and miscanthus grass with these properties (http://www.renewableenergyfocus.com).
- An alternative process to produce bio-ethanol from algae is being developed by the company Algenol. Rather than grow algae and then harvest and ferment it the algae grow in sunlight and produce ethanol directly which is removed without killing the algae. It is claimed the process can 56,000 litres per hectare per year compared with 400 (3,750 l/ha) for corn production (www.wikipedia.com).

CONCLUSION

The production of biofuels from microorganisms has come a long way since 1862 when Louis Pasteur discovered butanol fermentation by bacteria. The fluctuations in petroleum prices due to political unrest and foreign policy, coupled with the deleterious effects of the GHGs produced by the c ombustion of petroleum products has led to sustained interest in the search for a sustainable alternative. Microbially produced biofuels are constantly being produced or modified to decrease dependence on limited fossil fuel reserves and create jobs in addition to helping keep the planet healthier. Various organisms have been genetically modified with the aim of improving product specificity and output such as *Clostridium acetobutylicum*, *Rhodococcus opacus*, *Saccharomyces cerevisiae* and *Escherichia coli*. More research is underway to help reduce the production of undesired by-products, improve price and performance competitiveness of biofuels with fossil fuels, and reduce the impact of biofuel production on food prices. Hydrogen and other fuels will probably be expanded as aviation fuels and there is no limit to the developments that are possible in the production of microbial biofuels. This may in the long run, not only minimize, but may even reverse the deleterious effects of climate change

REFERENCES

Antoni, D., Zverlov, V., & Schwarz, W. (2007): Biofuels from microbes. Appl Microbiol Biotechnol **77**:23–35. DOI 10.1007/s00253-007-1163-x

Collet, C., Adler, N., Schwitzguebel, J. P., and Peringer, P. (2004): Hydrogen production by *Clostridium thermolacticum* during continuous fermentation of lactose. *International Journal of Hydrogen Energy*. 29 (14) p. 1479-1485.

Connor, M.R. and Liao, J.C. (2009): Microbial production of advanced transportation fuels in non-natural hosts. *Current Opinion in Biotechnology*. **20**: 3. pp. 307–315.

Cummins, C. Lyle (1993): *Diesel's Engine: Volume 1: From Conception To 1918*, Carnot Press. U.S.A.

Demirbas, A. . (2009). "Political, economic and environmental impacts of biofuels: A review". *Applied Energy* **86**: S108–S117.

Dragone, G. Fernandes, B., Vincente, A., Teixeira, J. (2010): Third generation biofuels from microalgae. *Curr. Res., Tech., and Educ. Topics in Appl. Microb and Biotech.*A. Mendez-Villaz Ed.

Dürre, P. & Bahl, H. (1996). Microbial production of

Balat, M., Balat, H., Oz, C. (2008): Progress in bioethanol processing. *Progress in Energy and Combustion Science*. **34:** (5). Pager 551-573.

Basso, L.C., de Amorim, H.V., de Oliveira, A.J., Lopes, M.L. (2008): Yeast selection for fuel ethanol production in Brazil. *FEMS Yeast Res*. **8: (**7). Page 1155-63.

Biofuels for transport: An international perspective, OECD Publishing, ISBN 92-64-01-51-24, Paris, France Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, UK, p 944. OECD-FAO Agricultural Outlook 2011-2020. acetone/butanol/isopropanol. In: Biotechnology, M. Roehr, (Ed.), VCH Verlagsgesellschaft: Weinheim, Vol.6, 2nd Ed., pp. 229-268.

Dürre, P (2007): Biobutanol: An attractive biofuel. *Biotechnology Journal.* Special Issue: Food *vs* Fuel**.**2:**12,** pages 1525–1534**.**

EIA (2006) International energy: outlook. Energy Information Administration, Office of Integrated Analysis and Forecasting. US Department of Energy, Washington, DOE/EIA-0484

Gray, K. A.; Zhao, L. & Emptage, M. (2006). Bioethanol. *Curr Opin Chem Biol*, Vol.10, pp. 141-146

Gibbons, W., Hughes, S. (2011):Distributed, Integrated Production of Second and Third Generation Biofuels, Economic Effects of Biofuel Production, Santos Bernardes (Ed.), retrieved from http://www.intechopen.com/books/economic-effects-of-biofuel-production/distributed-integrated-production-of-second-and-third-generation-biofuels.

IPCC; Houghton J.T., Ding Y, Griggs D.J., Noguer, M., van der Linden, P.J, Xiaosu, D. (eds) (2001): Climate Change: the scientific basis. Contribution of Working Group I to the Third Assessment
International Energy Agency.

IPCC, Synthesis Report, Section 1.1: Observations of climate change, in IPCC AR4 SYR 2007.

Koskinen, P. (2008): The development and microbiology of bioprocesses for the production of hydrogen and ethanol by dark fermentation. Thesis report. Tampere University of Technology, publication 751.

Lee, S.K., Chou, H., Ham, T. S., Lee, T.S. and Keasling, J.D. (2008): Metabolic engineering of microorganisms for biofuels production: from bugs to synthetic biology to fuels. *Current Opinion in Biotechnology*, **19**:6. pp. 556–563

Ma, F., and Hanna, M.A.(1999): Biodiesel production: a review. *Bioresource Technology*, **70**: 1, pp. 1–15.

Martinez, L.H. (2005): Post industrial revolution human activity and climate change: why the United States must implement mandatory limits on industrial greenhouse gas emissions. *Journal of Land Use* **20**:5.Page 407-426.

Michael K, Steffi, N., Peter, D (2011): The Past , Present , and Future of Biofuels – Biobutanol as Promising Alternative M. A. Dos Santos Bernades, ed. *History*, p.451-486.

Rajagopal, D., Zilberman, D. (2007): Review of Environmental, Economic and Policy Aspects of Biofuels. The World Bank Development Research Group Sustainable Rural and Urban Development Team. Retrieved from http://www.professorzilberman.com/wp-content/uploads/2008/09/wps4341.pdf

Sakuragi, H., Kuroda, K., Ueda, M. (2011):Molecular Breeding of Advanced Microorganisms for biofuel Production. *Journal of Biomedicine and Biotechnology. doi:10.1155/2011/416931*

Schenk P, Thomas-Hall S, Stephens E, Marx U, Mussgnug J, Posten C, Kruse O, Hankamer B(2008): Second generation biofuels: high efficiency microalgae for biodiesel production. *BioEnergy Research*. 1:20-43.

Schubert, C. (2006). Can biofuels finally take center stage? *Nature Biotechnol*, Vol.24, pp. 777-78.

Shalaby, E.A. (2011): Algal Biomass and Biodiesel Production, Biodiesel - Feedstocks and Processing Technologies, Dr. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-713-0, InTech, Available from: http://www.intechopen.com/books/biodiesel-feedstocks-and-processing-technologies/algal-biomass-and-biodiesel-production.

Shuobo, S., Valle-Rodríguez, J.O., Siewers, V., Nielsen, J (2011): Prospects for microbial biodiesel production. Biotechnology Journal. Special Issue: Systems biology for industrial applications. **6(** 3). pages 277–285. DOI: 10.1002/biot.201000117

Stern N (2006) The economics of climate change. The Stern Review. Cabinet Office – HM Treasury. Cambridge University
http://altenergy.in/biofuel.html

http://www.guardian.co.uk/environment/2011/oct/30/africa-poor-west-biofuel-betrayal retrieved on 1st April, 2012.

http://www.info.gov.za/view/DownloadFileAction?id=150835

http://www.go-green.ae/greenstory_view.php?storyid=1438

Press. Retrieved from:www.hmtreasury.gov.uk/independent_reviews/stern_review_economics_climate_change/stern_review_report.cfm on March 4th 2012

United Nations Department Of Economic And Social Affairs. Commission On Sustainable Development, Fifteenth Session 30 April-11 May 2007. New York

United states Department for Agriculture Foreign Agricultural Service (2009). *Biofuels Annuals*. Retrieved from: http://gain.fas.usda.gov/Recent%20GAIN%20Publications/General%20Report_Pretoria_South%20Africa%20-%20Republic%20of_5-28-2009.pdf.

Wargacki, A.J., *et al.* (2012): An Engineered Microbial Platform for Direct Biofuel Production from Brown Macroalgae. *Science 335(6066)* pp. 308-313. *DOI: 10.1126/science.1214547* . Retrieved from http://www.sciencemag.org/content/335/6066/308 on 12th April, 2012.

http://www.renewableenergyfocus.com/view/12976/hunting-microorganisms-for-lignocellulosic-biofuel/

http://www.technologyreview.com/biomedicine/23526/