*Available online*www.ijpras.com

International Journal of Pharmaceutical Research&Allied Sciences, 2017, 6(3):93-106

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Bioenergy: Microbial Biofuel Production Advancement

Naief H. Al Makishah*
Department of Environmental Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture
King Abdulaziz University, Jeddah, Saudi Arabia
****Corresponding Author Email**: nalmakishah @ kau.edu.sa*

ABSTRACT

It is vital to ensure the safe disposal of organic residues, in particular due to the accumulation of organic waste contributing to environmental pollution, while also spreading epidemics, diseases and unpleasant odours, and releasing ammonia and other harmful gases. This has led researchers to consider a number of direct applications for organic waste, including biotechnological applications. These are widely known to offer ecological and economic benefits, including: (1) limiting fossil fuel consumption; (2) reducing polluting emissions; (3) facilitating the production of cost-effective raw materials; and (4) developing a suitable substrate for a variety of microorganisms. Sustainable bioenergy (i.e. bio-gas, bio-diesel and bio-butanol) is an end product of biotechnology and an attractive solution for the disposal of organic feedstock. Carbohydrates form the primary ingredient of the organic fraction, while the majority of such polymers are readily microbial degradable. This review therefore focuses on isolated strains of different microorganisms with the capacity to utilise one or more forms of organic waste as a sole carbon source from different residue, in order to produce biofuel as its final products. The isolation involves: (1) the collection of random samples from soil exposed to organic waste; (2) purification of the microbial isolates; and (3) a comparison of the capabilities of the microbes, in order to identify the most valuable strain.

Keywords: *Bioenergy, Microbial biofuels, Fermentation, First generation biofuels, Environmental microbiology*

INTRODUCTION

Biofuel industries have previously been employed for the production of explosives during the First World War, due to the large demand for acetone as a solvent for the production of cordite [1]. Biofuel industries are once again attracting considerable interest, due to the global increase of the demand for energy (in particular by emerging economies) and the recent instability in global oil prices. The primary source of energy is currently crude oil, which is considered unsustainable, while world energy consumption is estimated to grow by 57% by 2030 [2]. In addition to concerns related to sustainability and economics, there has also been an increase in the environmental impact of petroleum being the sole source of energy. The burning of crude oil is currently increasing the emissions of harmful greenhouse gases in the atmosphere, thus contributing to air pollution [3]. Therefore, concerns related to environmental issues, along with the rising price of crude oil, have promoted research into alternative energy. One solution consists of bio-energy technologies, including the utilization of biomass by microorganisms. However, much work still needs to be done to achieve the target of converting the actual economic pattern into a bio-based economy. Microbial biotechnology approaches are currently being investigated for the use of microorganisms to produce various versions of biofuel (i.e. alcohols; hydrogen; biodiesel; and biogas) from various crude materials, i.e. carbohydrates; oil crops; agricultural and animal residue; and lignocellulosic biomass.

Bio-ethanol and bio-diesel are considered the main biofuels suitable for use in transportation, while biogas has also found a small number of large-scale applications in some European areas. Bio-butanol was recently considered a promising liquid biofuel, due to properties including its high energy levels, low volatility and hydroscopicity [4]. At the same time, it can be also used directly, or mixed with gasoline, without requiring any modification of existing engines [5].

These forms of fuels can be defined according to the conversion technique still under studying and improving, pilot or demonstration phases, and refers to 2nd or 3rd generation biofuels [6]. It is thus assumed that the future of the industry of bio-butanol, bioethanol and biodiesel (beside other developed biofuels) will depend on processes described as low CO2 emissions and by utilizing organic wastes differing from those traditionally related to the food-supply cycle.

MICROBIAL BIOFUEL

2.1. Biodiesel

Biodiesel production through the use of microorganisms is considered a promising alternative for the production of biodiesel. Well-known microbes (e.g. microalgae, bacteria, fungi and yeast) can amass intra-cellular lipids (primarily triacylglycerol) into a huge amount of their biomass. The oil of these oleaginous microbes has the potential to be employed as the crud biomaterial for biological diesel production during transesterification, at the same line with the plant-based processing [7]. The use of fast-growing microbes could prove particularly effective, as it is potential employ a large kind of feedstock (i.e. sugar-cane) with extraordinarily greater product per one-hectare in comparison with seeds of the rape plant and biological mass, and therefore able to produce biodiesel which uses a smaller proportion of arable land. Furthermore, interest has grown following molecular engineering and biosynthetic aiming to modify well investigated microbes (i.e. Escherichia coli and Saccharomyces cerevisiae) into bioenergy cell plants, by initiating an ester synthesizing route with the potential to result in the immediate product of fatty acid ethyl esters (FAEEs) by directly esterifying the bioethanol with the acyl-moieties of the CoA thioesters of fatty acids [8]. Oleaginous microorganisms (Fig.1) could also provide a new lipids raw material for biodiesel industry. Oils also can be extracted from fast stable growing microbes, then trans esterified through simple-chain alcohols, thus producing a value quality biodiesel ester that qualify with current standards [9]. The oils accumulate within specific organelles (i.e. lipid bodies) within the cell and provide sterols and fatty acids for microbial anabolism. The key parameters impacting on lipid accumulation in microorganisms are: (1) nitrogen; (2) carbon: (3) C: N Ratio; (4) pH; (5) agitation rate; (6) temperature; and (7) length of incubation. [10]

The yield and lipid types are dependent on number of factors, including culture conditions, types of organism, and selected substrate [11]. Microalgae is potentially the most favourable crude material to supply a high percentage of lipids, which can be directly synthesised into renewable biodiesel. Furthermore, high oil yields (i.e. 3.4 times) require only a small area of land area for cultivation in comparison to corn, thus becoming a promising source of biodiesel production. Microalgae oil generally consists of 20% to 60% of dry weight biomass, and can reach approximately 80% in some genera, i.e. Botryococcus, Nannochloropsis and Schizochytrium. Microalgae oils primarily consist of unsaturated fatty acids, i.e. linolenic acids, linoleic, oleic and palmitoleic. Saturated fatty acids are also produced in a low concentration, i.e. stearic and palmitic [12].

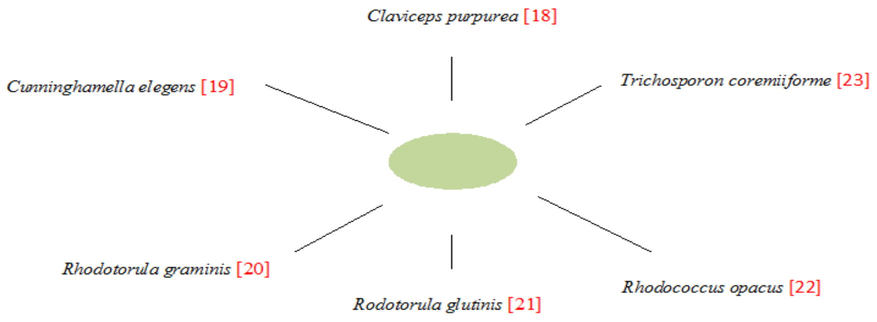


Fig 1: The familiar lipid profiles of oleaginous microorganisms.

On the other hand, a number of bacterial strains can also be used to produce lipids, in order to obtain the esters that can constitute biodiesel (Fig.2). The majority of bacterial strains primarily produce complex lipids, with a small number of species capable of producing lipids with the potential to be used as an indicator strain for biodiesel [13]. The sole resource of lipids in these particular strains are triacylglycerols (TAGs), while some specious of Actinomycetes can produce TAGs to great scales, as well other bacterial genera of Acinetobacter, Mycobacterium and Streptomyces [14]. The bacterial TAGs are specifically extracted inside the cell during standard cultivation conditions using simple uptake of available carbon under pressure. Furthermore, a number of studies have been employed as part of an extensive investigation into metabolic fatty acid in extensive bacterial strains to engineer Escherichia coli for biodiesel, which is directly produced from simple carbohydrates.

Similarly, fungus is one of the lipid-producing source for the biodiesel industry. Fungal genera capable of producing great amount of lipids (i.e. approximately 75%) include Humicolalanuginosa [15]. Biodiesel esters can also be extracted by Mucorcircinelloides species by estimated amount 19.9 % (by wt). Two procedures are followed to form the fungal esters: (1) conversion of accumulated lipids; and (2) lineal conversion of dry-weight fungal biomass. The product of the lineal method consists of fatty acid methyl-esters (FAME), with a high and pure product (over 99%) for all catalysts as opposed to those from the double convert stages (i.e. conversion of extracted lipids) process. Furthermore, the product of these forms of esters can be used as biodiesel. The new bifunctional wax is ester synthase/acyl-CoA:diacylglycerolacyltransferase (WS/DGAT), which is capable of synthesising wax esters from alcoholic solvents and fatty acid coenzyme A- thioesters (acyl-CoA) [16, 64]. The biodiesel yielded by mentioned pathway mostly ethanol-yielding fatty acid ethyl esters (FAEEs), which demonstrate an improved performance in comparison to methanol-yielded fatty acid methyl esters (FAMEs) [17].

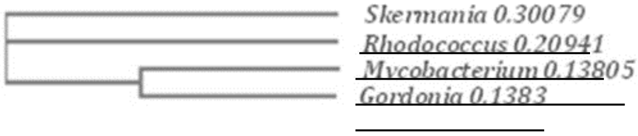


Fig 2: Phylogenetic tree demonstrating the selected bacterial strains capable of forming lipid profiles of oleaginous microbes in relation to the presentative protein sequence of bacteria. The neighbour-joining tree was constructed based on sequences of control taken from the NCBI database using clustal omega software.

2.2. Biogas

Biogas has emerged as an effective source of renewable energy, being a mixture of different gases produced from the action of anaerobic microorganisms on organic substrates, i.e. domestic and agricultural. It contains a low proportion of gases (i.e. CO_2, H_2, N_2, and O_2), whereas methane is present in a high proportion, i.e. 50% [24]. If the methane content is over 50%, then the mixture of gases in biogas becomes combustible [25]. The second larger proportion of biogas used as energy resource is hydrogen, whose biological production process is both a pollution decrease and local energy economy. Bioprocessing methods produce hydrogen that is low energy intensive than chemo-electrical methods, due to biological style being generally undertaken at ambient temperature and pressure [26]. Hydrogen product by means of anaerobic microbes is practically simpler than other bio processing, due to proceeding at a higher rate while lacking any need for a light source [27].

2.2.1. Stages of biogas production (H_2 and CH_4)

Biogas is produced in a series of different metabolic reactions involved in anaerobic decomposition, i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis [28]. Hydrolysis and methangensis are of greater importance, due to their association with the production of hydrogen and methane, respectively. Hydrolysis is a biologiclytic process whereby the polymers (i.e. lipids, polysaccharides and proteins) of which the substrate is made are degraded back to their original water-soluble building blocks (i.e. oligomers and monomers: long-chain fatty acids, monosaccharides and aminoacids). Hydrolysis of complex molecules is catalysed by extracellular enzymes, some of which contain cellulase, amylase, protease, and lipase. In general, organic residue stabilisation does not happen through hydrolysis, and then the organic materials are merely transformed into a dissolvable form capable of being utilised by the bacteria to produce H_2 and CO. The last phase is methane formation, which is the final product of fermentation processing. Formic acid, acetic acid, methanol, and hydrogen can be used as energy resources by several methanogens [29].

A number of different groups of microorganisms are involved in the degradation of the organic substrate into volatile fatty acids (i.e. actinomyces, thermonospore and shewanella), however the primary contribution in methane production is by Methanbrevibacter, Methanosarcina [30]. Approximately 70% of methane production is due to acetoclastic and CO_2 methanogens, which convert acetate into methane [31]. For some methanogenic bacteria, the optimal growth temperatures are: 37-45^{0}C for mesophilicMethanobacterium; 37-40^{0}C for Metanobrevibacter; 35-40^{0}C for Metanolobus, Methanococcus, Methanoculleus, Methanospirillum and Methanocorpusculum; and 50-55^{0}C for thermophilicMethanohalobium and Methanosarcina [32]. Anaerobic digestion is generally undertaken at mesophilic temperatures [33].

The production of methane is a complete process operated under anaerobic conditions, however, a two-stage anaerobic digester is used to collect both H_2 and CH_4 in a single process. In a two-stage reactor, the acidogenesis and methanogenesis processes operate separately, potentially increasing the stability of the complete process by controlling the acidification phase in the hydrogen-producing stage, and hence preventing the inhibition of the methanogenic population during the methane-producing stage [34]. Therefore, reactor respective gases can be collected at different stages and used as an energy source. In general, hydrogen production from organic substrate is accompanied by the production of acetate or butyrate, which can prove beneficial for methane production. A series of reactions for H_2 and CH_4 is given below: [35].

$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH$, $C_6H_{12}O_6 + 2H_2O \rightarrow 2C_4H_8O_2 + 2H_2 + 2CO_2$

2.2.2. Substrate for biogas production

A large number of factors are impacted by biogas yield, including the type and composition of: substrates; temperature; moisture; bioreactor design; and microbial composition. However, biogas contains the potential for considerable variation due to the type of substrate employed. As demonstrated in Table 1, a wide range of substrates have been used for the production of biogas, including plant and animal waste, along with industrial waste (i.e. brewery waste and carbonated soft drinks' sludge) [35]. Similarly, a number of researchers have reported the potential of obtaining biogas from various materials, including: rice straw [36]; municipal solid waste [37]; dairy manure [38]; poultry waste [39]; and food waste [40]. A comprehensive review of the potential of different substrates for biogas production is outlined in Table 1, which reveals that biogas can be efficiently produced from a large number of organic and waste resources. Overall, there is no doubt of the potential for the anaerobic digestion process to produce biogas to be used as alternate source of sustainable heat and power. Nevertheless, further studies are required to increase the potential for the application and commercial use of biogas. Further potential could be harvested by isolating novel bacterial strains that are capable of biogas production in harsh conditions. Similarly, field studies are required to optimise the factors impacting on anaerobic digestion, in order to ensure maximum conversion of substrate into biogas.

Table 1: A comprehensive review of the potential of different substrates for biogas production

Substrate	Temperature.	Digestion Time	References
Rice straw	37	40	41
Corn straw	37	35	42
Wheat straw	35	45	43
Municipal solid	35	200	44
waste Fruit and vegetable wastes	35	70	45
FW and dairy manure	36	160	46
Food Waste	37	225	47
Goat manure	35	55	48
Poultry manure	55	20	49
Waste activated sludge	37	10	50
Asparagus stem	35	60	51

2.3. Bioethanol

Due to fossil fuels being the major contributor to global warming, there is currently a need to establish the cleanest liquid fuel possible with the least carbon emissions. Increasing attention has therefore been paid to the conversion of biomass into fuel ethanol, with advanced technology being developed to produce ethanol from the fermentation of biomass. The conversion of biomass into ethanol requires a group of microorganisms that produce the enzyme cellulase for the hydrolysis of the lignocellulose of plant materials. In order to produce large amounts of energy, enzyme producing microorganisms are immobilised on a stable surface for saccharification and for the fermentation of sugar to convert into ethanol with high levels of efficiency and in a cost effective manner. A number of microorganisms are potentially involved in the decay of a dead body, and are able to produce ethanol. Approximately fifty-eight bacterial species, seventeen strains of yeasts and twenty-four species of mould capable to produce ethanol, in addition to other metavolatiles, during multiple intricate metabolic routes [52].Clostridia has the capability to produce 1-butanol during the butyrate and butanol-acetone anaerobic route that proceed at the same time of ethanol production. The higher-alcohols and 1-butanol might be generated together with ethanol from all suitable organic residue by various complicated bioprocessing and biochemical pathways [53]. Notable sides of their formation involved a dependence on glucose uptake system, with their appearance coinciding with the produce of ethanol and the predominance of 1-propanol mostly, spite of that the amount of their production is unstable. The appearance of 1-butanol during production processing has, in parallel, been showed as a clear reflection of ethanol production. A number of organisms have been investigated for ethanol production, i.e. Zymomonasmobilis; Corynebacteriumglutamicum; Pichia stipites; Clostridium thermocellum; Clostridium phytofermentans; Imported Saccharomyces cerevisiae and Escherichia coli [54].

2.4. Biobutanol

Butanol can, in a similar manner to ethanol, be made by the microbial fermentation of sugar, starch or cellulosic feedstocks. Biobutanol forms an alternative to conventional transportation fuels, with its benefits including:

- A higher energy content: the energy density of biobutanol is between 10% and 20% lower than that of gasoline. Moreover, the energy content of biobutanol is relatively high among gasoline alternatives.
- Lower reid vapour pressure: biobutanol has, in comparison to ethanol, a less (RVP), leading to decrease the volatility and evaporative emissions.
- Increased energy security: biobutanol has the capacity to be produced domestically from a variety of feedstocks.
- Fewer emissions: carbon dioxide is captured by growing feedstocks, thus leading to a reduction in overall greenhouse gas emissions.

Biobutanol production is based on a fermentation process by solventogenic Clostridium sp. bacteria (Weizmann organism) and other microbial species, as shown in Figure 3 (below).

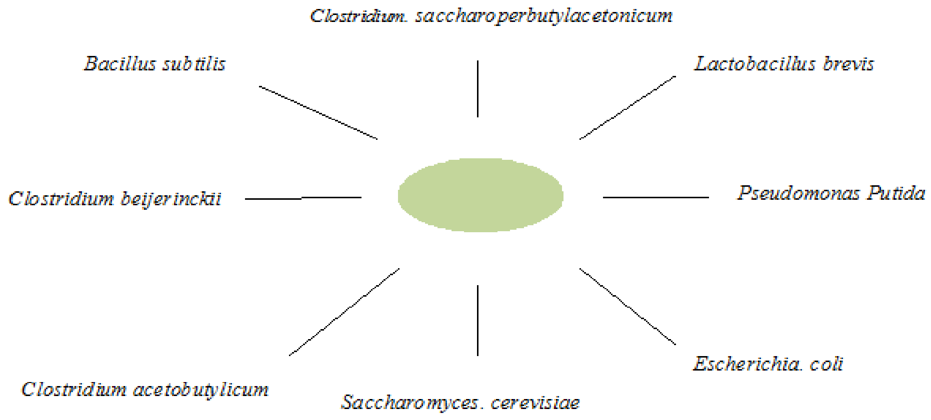


Figure 3. The familiar Biobutanol production of microorganisms [57, 58, 59, 60]

Moreover, in order to ensure that the production process is economical, sugars extracted from the pretreatment of lignocellulosic biomass are employed as substrates for fermentation. The solventogenic fermentation process is frequently limited by a number of specific factors, i.e. substrate inhibition, butanol toxicity in the medium, slow growth, and hence, lower cell density. Apart from these limitations, biobutanol yield is also reduced as a result of the production of other end products, e.g. acetone and ethanol. In order to address these issues, researchers have genetically developed a number of microbial strains in order to increase their capacity for improved biobutanol yield with several bacterial species, as demonstrated in the phylogenic tree (Figure 4) [55, 56].

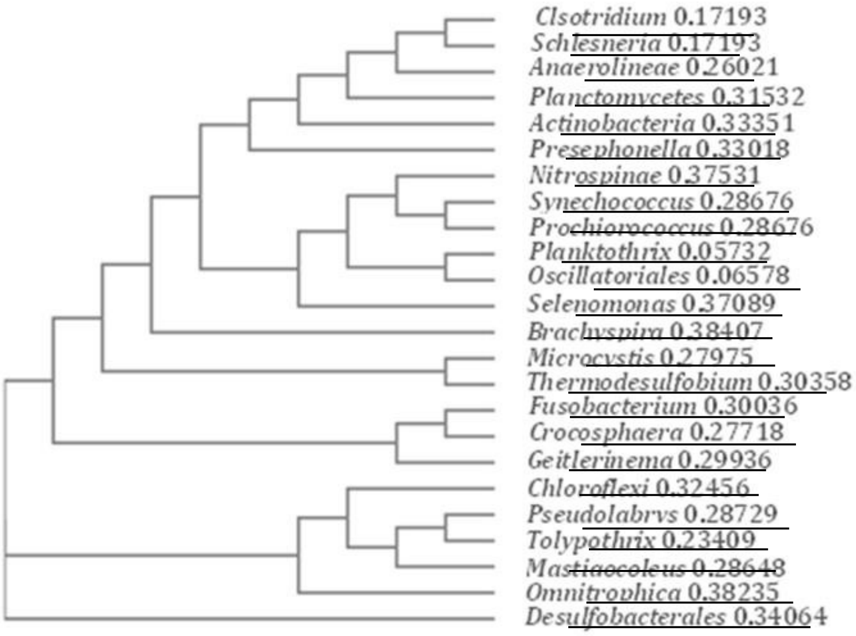


Fig 4: The Phylogenetic tree, demonstrating the selected bacterial strains capable of forming bio-butanol production in relation to the presentative protein sequence of bacteria. The neighbour-joining tree was constructed based on sequences of control taken from the NCBI database and by using clustal omega software.

2.5. Microbial Lipids

Bacteria, yeasts, fungi and the microalgae have been widely used over previous decades as sources of both lipids and enzymes. They were first used in the 1960s, for the production of laundry-grade lipases, used in conjunction with detergents and other cleaning agents. In the 1980s, a microbial oil appeared as a food supplement (i.e. as a source of gamma-linolenic acid), followed by a number of further major developments. Microorganisms are now regarded as the source of choice for long-chain polyunsaturated fatty acids used as high-grade nutraceuticals for human (including infant) and animal consumption.

Over the previous decade, microbial lipids have become available resource for the sustainable production ofbiofuels and value-added bio-products, to act as an alternative (and replacement) ofharmful petro-based chemicals. Several microorganisms (generally known as oleaginous microorganisms) have, under specific conditions, the ability to accumulate substantial amounts of lipids. Microorganisms containing over 25% of lipids in their cell biomass are generally known as an oleaginous [61]. There are a number of microorganisms containing sufficient oil content to be used for oil production (oil content-% dry wait), including: (1) Microalgae (Botryococcus braunii-25–75%; Cylindrotheca sp.-16–37%, Neochloris oleoabundans-35–54%, Schizochytrium sp.-50–77%); (2) Bacteria (Arthrobacter sp. > 40%, Acinetobactercalcoaceticus 27–38%, Rhodococcusopacus 24–25%); (3) Yeast (Cylindrotheca sp. 16–37% Chlorella sp. 28–32%, Rhodotorulaglutinis 72%); and (4) Fungi (Aspergillus oryzae-57%, Mortierella isabellina-86%, Humicola lanuginose-75%, Mortierella vinacea-66%) [62]. Lipid production in microorganisms commences when the carbon source is present in large amounts and other nutrients (primarily nitrogen) are running out in the culture media. A high C/N ratio of the culturing medium is required to ensure favourable lipid accumulation. The excessive carbon stored in the cell converts it into lipids in the form of triacylglycerol (TAG). Wu et al. [63] demonstrated lipid production using wastewater as a raw material, establishing that phosphate and sulphate limitation can also induce the accumulation of lipids in oleaginous yeast Rhodotorulaglutinis with the presence of excess nitrogen and other nutrients.

A number of low cost materials (i.e. cheese whey, wheat bran and sewage sludge) are currently being explored in an attempt to reduce the cost of lipid production. Table 2 summarizes the waste materials employed, along with microorganism and lipid production.

Table 2: Overview of microorganism and lipid production using raw substrates

Microorganism	Source	Lipid production	Reference
Morterellaisabellina	Hydrolysate of cohydrolysis of switch grass, miscanthus, giant reed and corn stove.	4.4 g/L	[64]
Cryptococcus curvatus	Corncob	61.3%	[65]
Trichosporonfermentas	Waste sweet potato wine	9.68 g/L	[66]
Mortirellaisabellianna	Wheat straw	39.4%	[67]
Chlorella protothecoides	Molasses	40.8 g/L, 57.6%	[68]
Lipomycesstarkeyi	Sewage sludge	68%, 1 g/L	[69]
Mordellistena. isabellina	Rice hull	64.3%	[70]
Chlorella protothecoides	Sorghum juice	5.1 g/L,52.5%	[71]
Rhodotorulaglutinis	Starch wastewater	35%	[72]
Polyculture of algae	Municipal wastewater, dairy wastewater	14–29%	[73]

There are a number of major challenges involved in lipid extraction from microbial biomass, i.e. limited lipid yielding, low mass transfer, and the formation of stable emulsions. A number of methods have been investigated, and co-solvent methods have proved efficient for total lipid accessibility at experimental level, but require additional research focusing on their practical application for industrial biofuel production. Future view on commercial biofuel industry needs to contain solvents with the characteristics capable of evaluating lipids recovery from microbial biomass.

FIRST GENERATION BIOFUELS

The recent fluctuation in the price of crude oil has prompted a number of investigations into alternative and supporting energy sources. Biofuels can compete with the rise in oil price to at least provide fuel for transport vehicles. This has led to a global increase in research into biofuels. There are three types of biofuels, including biodiesel (the first biofuel to be used commercially), along with ethanol, biogas and more recently bio butanol. These products have been produced in large quantities and become an essential aspect of the production process of many countries in the world. Biodiesel is produced by the transesterification processing of animal fats, plants and other remnants oils. This has resulted in simple modifications to engines that allow them to use biodiesel. Nevertheless, it is possible to use a complete substitute for ethanol and gasoline in several vehicles, and which can also be used as a crude material to produce three-butyl ethyl ether, which is easy to blend with gasoline. Bioethanol is currently used for producing three-ethyl butyl ether. Biogas and bio-methane are also used in gasoline as a biofuel for vehicles with some modifications. They are produced by biological processing, including the (AD) technique of liquid manures and other harvested raw material [74]. The capability has also recently been established to produce bioethanol, biodiesel and biogas using agricultural crops also used for human nutrition. However, the demands of agricultural crops grown for producing edible oils face several difficulties when it comes to biofuel production. There are a number of multipurpose oily crops (i.e. seeds) capable of being used in the industrial production of biofuels. In India, however, there has been a focus on the production of biodiesel from Jatropha, a perennial shrub or small tree that grows to a height of up to six meters, and capable of producing a type of vegetable oil mixed with biofuel. Jatropha oil is an environmentally safe, low cost substrate, and a promising source of energy from non-edible plant materials. Other solid waste can similarly be used for producing of biomaterials, including lignocellulosic, which is capable of being used in the second generation biofuel industry.

3.1. Manufacturing industry for producing first generation biofuels

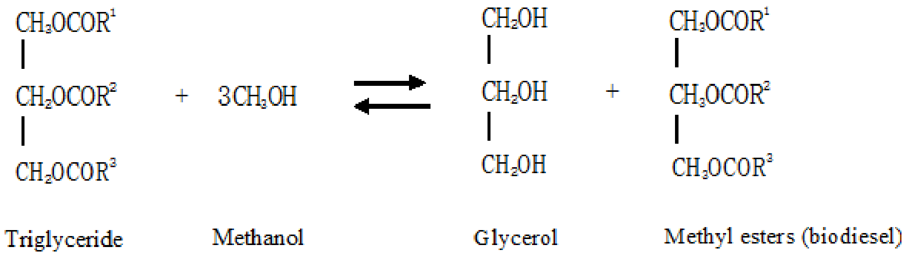
3.1.1. Transesterification

Esterification of fatty acids and vegetable oils forms the basis for obtaining commercially viable biodiesel, which is one of the most important biofuels, as well as being environmentally safe. Biodiesel is an alternative energy obtained from many natural materials through biochemical reactions via alcohol, homogeneous and heterogeneous catalyst. Thus, the result of reaction is a blend of methyl esters, including a high value of biodiesel and glycerine [75, 76].

3.1.2. Homogeneous catalysis

Homogenous catalysis is a preferred method of processing during the reaction of its Transesterification (base or acid) counterpart at a slower rate, as shown in Eq (1). This results from mixing the reactants and the replacement of alcohol from the ester by another alcohol using a same method as for hydrolysis, apart from the employment of alcohol rather than water [75].

Eq (1):



$$\begin{array}{l} CH_2OCOR^1 \\ | \\ CH_2OCOR^2 \\ | \\ CH_2OCOR^3 \end{array} + 3CH_3OH \rightleftharpoons \begin{array}{l} CH_2OH \\ | \\ CH_2OH \\ | \\ CH_2OH \end{array} + \begin{array}{l} CH_3OCOR^1 \\ | \\ CH_3OCOR^2 \\ | \\ CH_3OCOR^3 \end{array}$$

3.1.3. Heterogeneous catalysis

The interactions of saponification result in a number of difficulties in converting the high oil content of free fatty acids into methyl ester. It is therefore more effective to use acidic solid catalysts, which play a strong role in reactions for transesterification of triglycerides and simultaneously convert free fatty acids into methyl ester using Lewis acid. Esterification takes place among free fatty acids (RCOOH) and methanol (CH_3OH), while the esterification that takes place between triglyceride RCOOR and methanol is absorbed in the acidic sites (+L) from the surface of the catalysts. During the esterification of tetrahedral, it can be removed by a water molecule to form one mole of ester RCOOCH3. It can also be used as an esterification mechanism for bilateral and trilateral triglycerides. It is well known that the esterification is a gradual sequential reaction in which triglycerides switch to mono triglycerides and glycerol [76].

3.1.4. Ethanol production processes

There are a number of types of sugars capable of being used as a raw material for producing bioethanol by industrial fermentation. These materials can be classified into three basic types

- The first group consists of sugar yield, including: cane; wheat; beetroot; fruits; and palm juice.
- The second group consists of a starchy yield (e.g. grain) derived from root plants (e.g. potato and cassava) and crops, including: wheat; barley; rice; sweet sorghum and corn.
- The third group consists of cellulosic biomass, including: wood and wood waste; cedar; pine; and agricultural residues and fibers.

Both alcohols are produced by biochemical process from lignocellulosic biomass (i.e. rice straw and wheat straw residue), and are known as bioethanol or biomass ethanol.

The chemical composition of starch or amylum containing a large number of glucose polymer is unable to be fermented directly by classical processing. It can be initially hydrolyzed to the simplest chains of glucose by adding 15-20% of starch with water and treating the mixture by means of a high temperature, followed by treatment with hydrolyses enzymes. Amylase is a familiar enzyme of hydrolyzed starch molecules, which form simple chains of glucose through the process of liquefaction. Otherwise, the dextrin and oligosaccharide is hydrolyzed by the enzymes pullulanase and glucoamylase, respectively, by means of saccharification processing. The grind is then cooled by around 30°C and microorganisms added to ensure fermentation. Ethanol is generally produced by the enzymatic hydrolysis of several carbohydrates, i.e. maize and wheat. Maize ethanol industry methods can be divided to dry and wet mill processes. (i) Ethanol dry mills tend to have less capacity and are primarily built solely to manufacture ethanol, along with the production of ethanol and animal feeds. (ii) The new wet mill unit is capable of producing one ethanol gallon by consuming 35150 Btu of thermal energy and 2134 KWh of electricity, and can also produce a number of valuable products, including: pharmaceuticals; nutraceuticals; organic acids; and solvents. [77, 78, 79]

3.1.5. The fermentation industry

The fermentation process consists of the metabolic pathway of organic materials by microorganisms through chemical changes due to the activity of their enzymes. Fermentation processing consists of two main methods of bio-processing (i.e. aerobic and anaerobic), according to the presence of oxygen. Several microorganisms found in nature have the capacity for fermentative changes, a number of which are able to produce ethanol by utilizing many types of carbohydrate polymers, i.e. yeasts, bacteria and fungi. In general, during the fermentation reaction, microorganisms break down the different carbohydrates molecules under aerobic and anaerobic atmospheres, resulting in ethanol production. Almost 40-48% of fermented glucose is converted to ethanol by a fermentation efficiency of 46%, i.e. 1000kg of sugar in the fermentation industry is capable of producing 583L of pure ethanol [78].

3.1.6. Anaerobic digestion

The anaerobic digestion of biomass has attracted considerable attention as an alternative method for producing fuel and biofertilizer by means of organic cultivation. In this application, an identical processes is used as for biogas production, by breaking down the organic substrates and producing methane and CO_2 gases at a ratio of 60–70% methane and 30% CO_2 [80]. This ensures that the anaerobic digestion of biological waste is considered a promising processing method for the production of biofuel and biofertilizer. Anaerobic digestion can be used as an initial source of methane production from municipal solid waste. These compounds can be used alongside atmospheric nitrogen, carbon dioxide and traces of organic substrate known as (LFG) or land fill gas. The Environmental Protection Agency (EPA) has stated that every pound of biodegradable organic waste can produce between ten and twelve standard cubic feet of biogas. However, methane has the same low-quality experienced with natural gas, due to the need to remove volatile organic contaminants and CO_2 in order to achieve a high-quality commercial product. Landfill gases require highly efficient separation technology to produce natural gases. There is considerable potential to use gas production sites to generate electricity by means of internal combustion engines, turbines, micro turbines, direct use in boilers, dryers, furnace, and home ovens. Due to the high cost of production and purification, there has recently been an additional focus placed on researching the use of gas present in the ground as a fuel generating liquid, rather than gaseous fuel achieved through anaerobic fermentation. Liquid methanol production has a number of advantages, as outlined below:

- A low Sulphur content
- A low ash content
- It can be used on a commercial scale.

  The liquid fuel is easy to transport and store, in comparison with gaseous fuel.

Lignocellulosic biomass is, by means of the anaerobic fermentation process, used in liquid fuels and biofertiliser employed for agriculture [78].

3.2. Whole-crop biorefinery

The biorefinery processes of oil crops are undertaken to obtain beneficial products, with seeds being used as raw materials and intermediates in bio refineries. This has resulted in the use of the oilseed plant Jatropha (as previously discussed) as a nucleus containing the seeds of Jatropha oil at 35-40%, with potentially 1-1.5 tons of oil produced per hectare. In order to convert the biomass into energy, its useful parts can be separated into various components, which then can be treated separately. Following the biomass separation, the yield of oil is used as the main (or basic) material for biodiesel production, or can be treated by a number of chemical modifications to produce many oleochemical products. On the other hand, solid de-oil material is used in the production of primary crude materials for the composition of chemical structures or biological gases. Lignocellulosic feedstock obtained through refinery industries can serve as a beginning substrate for biorefineries in order to increase gas production [81].

3.2.1. Oleochemicals

The term oleochemicals refers the fatty acids and glycerol derived from oils and fats of both plant and animal origin, and also includes derivatives from the modification of the carboxylic acid group of the fatty acids by chemical or biological treatment. Oleochemicals are categorized into a number of basic oleochemicals, including: (I) fatty acids; (II) fatty methyl esters; (III) fatty alcohols; (IV) fatty amines; and (V) glycerol. Till the1980s, approximately 95% of natural oils were used for the production of food, with only a small percentage used for alternative applications, e.g. soap manufacturing and drying oils. Following the 1980s, oleochemicals were employed for a wide range of uses in both food and non-food products, and have recently been used in many applications, including the following: foods; particularly fats; washing soaps and cleaning materials; cosmetics and care products; lubricants and greases; various drying oils; coatings materials; and some polymers [82].

3.2.2. De-oil residual protein

The production of amino acids of de-cake oil has increased the value of these solid wastes, as a result of such oil crops being globally produced on a large scale. Jatropha oil seeds (i.e. neem and karanja) have been used to produce biopesticides, 1, 2-ethanediamine and 1, 4-butanediamine from amino acids, i.e. serine and arginine, respectively. There are additional limitations on the use of oil-cake for producing biodiesel, due to the process requiring several million tons of residual protein in comparison to the derivatives of fatty acids. The residual protein used also to produce the basic amino acids both for people consumption and for animal feeding [83].

CONCLUSION

This research discussed the concepts surrounding microbial biofuels, including biological refineries, biofuels, and their related challenges. Focus has been on to the first generation of biofuels, primarily due to their direct impact on agricultural products and thus food prices. In addition, there is currently a resistance to biofuels from poorer countries. Research is now attempting to identify a number of solutions to establish a sustainable and environmentally safe energy source, along with the most effective means of avoiding the negative effects of biofuel production from food supplies, through providing fuel for agricultural residues and lignocellulosic compounds in as short a period as possible (i.e. second generation biofuels). However, as the current production of first generation biofuels includes introducing logistical changes to their use, this will ensure that a transition to the second generation of biofuels becomes more economically feasible.

REFERENCES

1. Demain, A. L. (2000). Microbial Biotechnology. Trends on Biotechnology, 18(1), pp26-31.
2. U. S. Energy Information Administration [EIA] (2007) http://large.stanford.edu/courses/ 2012/ph240/ park1/docs/0383-2007.pdf).
3. Intergovernmental Panel on Climate Change (2007). Climate Change 2007 - The Physical Science Basis, Cambridge University Press, ISBN 978 0521 88009-1, New York, NY, USA.
4. Dürre, P. (2007) Biobutanol: an attractive biofuel. Biotechnol J 2: 1525-1534.
5. Pfromm PH., Amanor-Boadu V., Nelson R., Vadlani P., Madl R. (2010) Bio-butanol vs. bio-ethanol: a technical and economic assessment for corn and switchgrass fermented by yeast or Clostridium acetobutylicum. Biomass Bioenerg 34: 515-524.

6. Balan, V., Chiaramonti, D., Kumar, S. (2013) Review of US and EU initiatives toward development, demonstration, and commercialization of lignocellulosic biofuels. Biofuels Bioprod. Bioref. 2013, 7, 732–759.
7. Meng et al., Meng, X., Yang, J., Xu, X., Zhang, L. et al. (2009) Biodiesel production from oleaginous microorganisms. Renew. Energ. 2009, 34, 1-5.
8. Steen et al., Steen, E., Kang, Y., Bokinsky, G., Hu, Z., et al. (2010)Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 2010, 463, 559-562.
9. Vicente et al., Vicente, G., Bautista, L. F., Gutiérrez, F. J., Rodríguez, R. A. et al. (2010) Direct Transformation of Fungal Biomass from Submerged Cultures into Biodiesel. Energy Fuels 2010, 24, 3173-3178.
10. Yang et al., Yang, S., Wang, W., Wei, H., Van Wychen, S., Pienkos, P.T., Zhang, M., Himmel, M.E. (2016). Comparison of nitrogen depletion and repletion on lipid production in yeast and fungal species. Energies 9, 1–12.
11. Ledesma-Amaro et al., Ledesma-Amaro, R., Dulermo, R., Niehus, X., Nicaud, J.-M. (2016). Combining metabolic engineering and process optimization to improve production and secretion of fatty acids. Metab. Eng. 38, 38–46.
12. Meng et al., Meng, X., Yang, J., Xu, X., Zhang, L., et al. (2009) Biodiesel production from oleaginous microorganisms. Renew. Energ. 2009, 34, 1-5.
13. haojin et al., Shaojin, Y., Yiping, Z. (2006) Research and Application of Oleaginous Microorganism. China Foreign Energy 2006, 11, 90-94.
14. Olukoshi et al., Olukoshi, E. R., Packter, N. M. (1994) Importance of stored triacylglycerols in Streptomyces: possible carbon source for antibiotics. Microbiology 1994, 140, 931-943.
15. Meng et al., Meng, X., Yang, J., Xu, X., Zhang, L., et al. (2009) Biodiesel production from oleaginous microorganisms. Renew. Energ. 2009, 34, 1-5.
16. Clark JH.,Buldarni V., Deswarte, FIE. (2006) Green chemistry and the biorefinery: a partnership for a sustainable future. Green Chem 2006; 8:853–60.
17. Kalscheuer, R., Steinbuchel, A. (2003) A Novel Bifunctional Wax Ester Synthase/Acyl-CoA: DiacylglycerolAcyltransferase Mediates Wax Ester and Triacylglycerol Biosynthesis in Acinetobactercalcoaceticus ADP1. J. Biol. Chem. 2003, 278, 8075.
18. Thevenieau et al., Thevenieau, F. and Renaud J. (2013). Microorganisms as sources of oils. OCL, 20, 6, D603.
19. Zheng, Y., Xiaochen Yu, Zeng, J., Chen, S., (2015). Feasibility of filamentous fungi for biofuel production using hydrolysate from dilute sulfuric acid pretreatment of wheat straw. Biotechnol Biofuels 5, 50.
20. Galafassi, S., Cucchetti, D., Pizza, F., Franzosi, G., Bianchi, D., Compagno, C. (2012) Lipid production for second generation biodiesel by the oleaginous yeast Rhodotorulagraminis. Bioresour. Technol. 111, 398–403.
21. Qiang Li et al. (2008).
22. Thevenieau, F and Renaud J. (2013). Microorganisms as sources of oils. OCL, 20, 6, D603.
23. Zheng, Y., Xiaochen Yu, Zeng, J., Chen, S. (2015). Feasibility of filamentous fungi for biofuel production using hydrolysate from dilute sulfuric acid pretreatment of wheat straw. Biotechnol Biofuels 5, 50.
24. Kalia, V. C., Sonakya V. and Riazada, N. (2000). Anaerobic digestion of banana stems waste. Bioresour. Technol., 73:191-193.
25. Agunwamba, J. C. (2001). Waste Engineering and Management Tool. Immaculate Publication Limited, Enugu.
26. Jo, J.H., Jeon C.O., Lee D.S, Park J.M. (2007). Process stability and microbial community structure in anaerobic hydrogen-producing microflora from food waste containing kimchi. J Biotechnoldoi: 10.1016/j.jbiotec.2007.07.492.
27. Thompson, R. S. (2008). Hydrogen production by anaerobic fermentation using agricultural and food processing wastes utilizing a two-stage digestion system.
28. Nazari, L., Yuan, Z., Santoro, D., Sarathy, S., Ho, D., Batstone, D., Xu, C.C. and Ray, M.B. (2016). Low-temperature thermal pre-treatment of municipal wastewater sludge: Process optimization and effects on solubilization and anaerobic degradation. Water Research.

29. Thompson, R.S. (2008). Hydrogen production by anaerobic fermentation using agricultural and food processing wastes utilizing a two-stage digestion system.
30. Ike, M., Inoue, D., Miyano, T., Liu, T.T., Sei, K., Soda, S., Kadoshin, S. (2010). Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. Bioresour. Technol. 101, 3952–3957.
31. Zinder S. H. (1993). Physiological ecology of methanogens, in Methanogenesis: Ecology, Physiology, Biochemistry, and Genetics, ed Ferry J. G., editor. (New York, NY: Chapman and Hall; 128–206.
32. Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L. (2008). Optimization of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928–7940.
33. El-Mashad, H.M., Wilko, K.P., Loon, V., Zeeman, G, (2003). A model of solar energy utilisation in the anaerobic digestion of cattle manure. Biosyst. Eng. 84, 231– 238.
34. Fu, S.F., Xu, X.H., Dai, M., Yuan, X.Z. and Guo, R.B. (2017). Hydrogen and methane production from vinasse using two-stage anaerobic digestion. Process Safety and Environmental Protection, 107, pp.81-86.
35. Nualsri, C., Reungsang, A., Plangklang, P. (2016). Biochemical hydrogen and methane potential of sugarcane syrup using a two-stage anaerobic fermentation process. Ind. Crop. Prod. 82, 88–99.
36. Khalid, A., Arshad, M., Anjum, M., Mahmood, T. and Dawson, L. (2011). The anaerobic digestion of solid organic waste. Waste management, 31(8), pp.1737-1744.
37. Gu, Y., Chen, X., Liu Z., Zhou, X., Zhang, Y. (2014) Effect of inoculum sources on the anaerobic digestion of rice straw. BioresourTechnol 2014; 158:149–55.
38. Fernández-Rodríguez, J., Pérez M., Romero L. (2014) Dry thermophilic anaerobic digestion of the organic fraction of municipal solid wastes: solid retention time optimization. ChemEng J 2014; 251:435–40.
39. Marañón, E., Castrillón, L., Quiroga, G., Fernández-Nava, Y., Gómez, L., García M. (2012) Co-digestion of cattle manure with food waste and sludge to increase biogas production. Waste Manage 2012; 32:1821–5.
40. Zhang T., Liu L., Song Z., Ren G., Feng Y., Han X. et al. (2013) Biogas production by co-digestion of goat manure with three crop residues. PLoS One 2013;8: 66845.
41. Nagao N., Tajima N., Kawai M., Niwa C., Kurosawa, N., Matsuyama, T. et al.(2012) Maximum organic loading rate for the single-stage wet anaerobic digestion of food waste. BioresourTechnol 2012; 118:210–8.
42. Gu Y., Chen X., Liu Z., Zhou, X., Zhang, Y. (2014) Effect of inoculum sources on the anaerobic digestion of rice straw. BioresourTechnol 2014; 158: 149–55.
43. Song Z., Liu, X., Yan, Z., Yuan, Y., Liao, Y. (2014) Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion. PLoS One 2014;9: e93801.
44. Cheng, X., Zhong, C. (2014) Effects of feed to inoculum ratio, co-digestion and pretreatment on biogas production from anaerobic digestion of cotton stalk. Energy Fuels 2014.
45. ernández-Rodríguez, .J, Pérez, M., Romero, L. (2014) Dry thermophilic anaerobic digestion of the organic fraction of municipal solid wastes: solid retention time optimization. ChemEng J 2014; 251:435–40.
46. Scano E.A., Asquer, C., Pistis, A., Ortu, L., Demontis, V., Cocco, D. (2014) Biogas from anaerobic digestion of fruit and vegetable wastes: experimental results on pilot-scale and preliminary performance evaluation of a full-scale power plant. Energy Convers Manage 2014; 77:22–30.
47. Marañón, E., Castrillón, L., Quiroga, G., Fernández-Nava, Y., Gómez, L., García, M. (2012) Co-digestion of cattle manure with food waste and sludge to increase biogas production. Waste Manage 2012; 32:1821–5.
48. Nagao, N., Tajima, N., Kawai, M., Niwa, C., Kurosawa, N., Matsuyama, T. et al. (2012) Maximum organic loading rate for the single-stage wet anaerobic digestion of food waste. BioresourTechnol 2012; 118:210–8.
49. Zhang, T., Liu, L., Song, Z., Ren, G., Feng, Y., Han, X. et al. (2013) Biogas production by codigestion of goat manure with three crop residues. PLoS One 2013;8: e66845. Abouelenien F, Fujiwara W, Namba Y, Kosseva M, Nishio N, Nakashimada Y.
50. Improved methane fermentation of chicken manure via ammonia removal by biogas recycle. BioresourTechnol 2010; 101:6368–73.
51. Hasegawa, S., Shiota, N., Katsura, K., Akashi, A. (2000) Solubilization of organic sludge by thermophilic aerobic bacteria as a pretreatment for anaerobic digestion. Water SciTechnol 2000; 41:163–9.

52. Chen, X., Gu, Y., Zhou, X., Zhang, Y. (2014) Asparagus stem as a new lignocellulosic biomass feedstock for anaerobic digestion: increasing hydrolysis rate, methane production and biodegradability by alkaline pretreatment. BioresourTechnol 2014; 164:78–85.
53. Corry, J.E.L. (1978) Possible sources of ethanol ante- and postmortem: its relation to the biochemistry and microbiology of decomposition, J. Appl. Bacteriol. 44 (1978) 1–56. Boumba, V.A., Ziavrou, K., Vougiouklakis, T. (2008) Biochemical pathways generating post-mortem volatile compounds co-detected during forensic ethanol analysis, Forensic Sci. Int. 174 (2008) 133–151.
54. Gruszecki, A.C., Robinson, C.A., Kloda, S., Brissie, R.M (2005) High urine ethanol and negative blood and vitreous ethanol in a diabetic woman: a case report, retrospective case survey, and review of the literature, Am. J. Forensic Med. Pathol. 26 (2005) 96–98.
55. Alper, H., Stephanopoulos, G. (2009) Engineering for biofuels: exploiting innate microbial capacity or importing biosynthetic potential? Nature Reviews, 2009, 7, 715-723.
56. Bharathiraj, B., Jayamuthunagai, J., Sudharsanaa, T., Bharghavi, A., Praveenkumar, R., Chakravarthy, M., Yuvaraj, D. (2017) Biobutanol – An impending biofuel for future: A review on upstream and downstream processing techniques. Renewable and Sustainable Energy Reviews 68 (2017) 788–807.
57. Kumar Mahapatra, M. Kumar, A. (2017) A Short Review on Biobutanol, a Second Generation Biofuel Production from Lignocellulosic Biomass. Journal of Clean Energy Technologies, 5, 1, 2017-27-30.
58. Jang, Y-S., Malaviya, A., Cho, C., Lee, J., Lee, S. (2012) Butanol production from renewable biomass by clostridia, Bioresource Technology, vol. 123, pp. 653–663, 2012.
59. Kumar, M., Gayen, K. (2011). Developments in biobutanol production: New insights. Applied Energy 88: 1999–2012.
60. Berezina, O. V., Zakharova, N. V., Brandt, A., Yarotsky, S. V., Schwarz, W. H., Zverlov, V. V. (2010). Reconstructing the clostridial n-butanol metabolic pathway in Lactobacillus brevis. Applied Microbiology and Biotechnology 87: 635–646.
61. Ezeji, T., Qureshi, N., Blaschek, H. P. (2007a). Butanol Production From Agricultural Residues: Impact of Degradation Products on Clostridium beijerinckii Growth and Butanol Fermentation. Biotechnology and Bioengineering 97: 6.
62. Ratledge C. (2004) Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie. 2004; 86:807–815.
63. [62] Meng, X., Yang, J., Xu, X., Zhang, L., Nie, Q., Xian, M. (2009) Biodiesel production from oleaginous microorganisms. Renew Energy. 2009; 34:1–5.
64. Wu, S., Hu, C., Jin, G., Zhao, X., Zhao, Z.K. (2010) Phosphatelimitation mediated lipid production by Rhodosporidiumtoruloides. Bioresour Technol. 2010; 101:6124– 6129. Wu S, Zhao, X., Shen, H., Wang, Q., Zhao, Z.K. (2011) Microbial lipid production by Rhodosporidiumtoruloides under sulfatelimited conditions. Bioresour Technol. 2011; 102:1803– 1807.
65. Ruan, Z., Zanotti, M., Zhong, Y., Liao, W., Ducey, C., Liu, Y. (2013) Cohydrolysis of lignocellulosic biomass for microbial lipid accumulation. BiotechnolBioeng. 2013; 110:1039–1049.
66. Chang, Y.H., Chang, K.S., Hsu, C.L., Chuang, L.T., Chen, C.Y., Huang, F.Y., Jang, H.D. (2013) A comparative study on batch and fed-batch cultures of oleaginous yeast Cryptococcus sp. in glucose-based media and corncob hydrolysate for microbial oil production. Fuel. 2013; 105:711–717.
67. Zhan, J., Lin, H., Shen, Q., Zhou, Q., Zhao, Y. (2013) Potential utilization of waste sweetpotato vines hydrolysate as a new source for single cell oils production by Trichosporonfermentans. Bioresour Technol. 2013; 135:622–629.
68. Zeng, J., Zheng, Y., Yu, X., Yu, L., Gao, D., Chen, S. (2013) Lignocellulosic biomass as a carbohydrate source for lipid production by Mortierellaisabellina. Bioresour Technol. 2013; 128:385–391.
69. Yan, D., Lu, Y., Chen, Y.F., Wu, Q. (2011) Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production. Bioresour Technol. 2011; 102:6487–6493.
70. Angerbauer, C., Siebenhofer, M., Mittelbach, M., Guebitz, G.M. (2008) Conversion of sewage sludge into lipids by Lipomycesstarkeyi for biodiesel production. Bioresour Technol. 2008; 99:3051–3056.
71. Economou, Ch. N., Aggelis, G., Pavlou, S., Vayenas, D.V. (2011) Single cell oil production from rice hulls hydrolysate. Bioresour Technol. 2011; 102:9737–9742.

72. Gao, C., Zhai, Y., Ding, Y., Wu, Q. (2010) Application of sweet sorghum for biodiesel production by heterotrophic microalga Chlorella protothecoides. Appl Energy. 2010; 87:756–761.
73. Xue, F., Gao, B., Zhu, Y., Zhang, X., Feng, W., Tan, T. (2010) Pilotscale production of microbial lipid using starch wastewater as raw material. Bioresour Technol. 2010; 101:6092– 6095.
74. Woertz, I., Feffer, A., Lundquist, T., Nelson, Y. (2009) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng. 2009; 135:1115–1122.
75. www.task39.org.
76. Kulkarni, M., Gopinath, R., Meher, L.C. Dalai, A.K. (2006) Solid acid catalyzed biodiesel production by simultaneous esterification and transesterification. Green Chem 2006; 8:1056–62.
77. Meher, L.C., Vidyasagar, D., Naik, S.N. (2006) Technical aspects of biodiesel production by transesterification - a review. Renewable Sustain Energy Rev 2006; 10: 248–68.
78. helley M., (2006) Alcoholic Fuels. Boca Raton, FL 33487-2742, USA: CRC Taylor and Francis Group; 2006.
79. Lee, S., Speight, J.G., Loyalka, S.K. (2007) Handbook of Alternative Fuel Technologies. USA: CRC Taylor and Francis Group; 2007.
80. Shapouri, H., Duffield, J.A., Graboski, M.S. (1995) Estimating the net energy balance of corn ethanol, U. S. Department of Agriculture, Agricultural Economic Report Number 721, 1995.
81. Naik, S.N. a,b, *, Vaibhav, Goud, V. b,c , Prasant, K., Rout, B., Ajay, K., Dalai, B. (2010) Production of first and second generation biofuels: A comprehensive review. Renewable and Sustainable Energy Reviews 14 (2010) 578–597.
82. Pradhan, R.C., Naik, S.N., Bhatnagar, N., Vijay, V.K. (2009) Moisture -dependent physical properties of jatropha fruit. Ind Crop Prod 2009; 29:341–7.
83. Soon, T.K. (2000) An overview of the Asian Oleochemical market. The Second World Oleochemicals Conference, Amsterdam; 2000.
84. Sanders, J., Scott, E., Weusthuis, R., Mooiboek, H. (2007) Bio-refinery as the bio-inspired process to bulk chemicals. MacromolBiosci 2007; 7:105-17.