Review paper Integration of Biodiesel and Bioethanol Processes: Conversion of Low Cost Waste Glycerol to Bioethanol

Saifuddin Nomanbhay; Refal Hussain; Md. Mujibur Rahman; Kumaran Palanisamy

Department of Science and Mathematics; and
Department of Mechanical Engineering
College of Foundation Sciences and College of Engineering, Universiti Tenaga Nasional, Jalan IKRAM-UNITEN 43000, Kajang Selangor, Malaysia

Saifuddin Nomanbhay; Refal Hussain; Md. Mujibur Rahman; Kumaran Palanisamy: Review paper Integration of Biodiesel and Bioethanol Processes: Conversion of Low Cost Waste Glycerol to Bioethanol

ABSTRACT

The once abundant petroleum reserves now dwindling and oil prices continue to rise; the search for alternative fuels becomes more vigorous. The paper reviews current growing attention focusing on ethanol as a renewable fuel to substitute and/or complement gasoline fuel. This trend is due to, among other aspects, the growth of oil prices in the international market, and the pressure to reduce atmospheric emissions of CO₂ to mitigate the problem of global climate changes. Glycerol-rich streams generated in large amounts by during the production of biodiesel, present an excellent opportunity to establish biorefineries. This review covers the anaerobic fermentation of glycerol in microbes and the harnessing of this metabolic process to convert abundant and low-priced glycerol streams into higher value products, thus creating a path to viability for the biofuels industry. However, glycerol is rarely used as a carbon source in Escherichia coli fermentation because of its low yield of products. A native, nonpathogenic strain of E. coli, able to ferment glycerol to useful products under anaerobic condition is currently used by many researches. The key factor is not the type of strain, but rather on the appropriate environment including an acidic pH, avoiding accumulation of fermentation gas hydrogen and appropriate medium composition to achieve high yields. The process becomes increasingly attractive, using biocatalyst like (Alcohol Dehydrogenase) to metabolize these chemicals anaerobically to produce ethanol.

Key words: Bioethanol; biodiesel; crude glycerol; Escherichia coli.

Introduction

A growing population, increasing per capita income, infrastructural development and rapid socio-economic development has spurred an increase in energy consumption across all major sectors of the world economy. In 1895, a famous Swedish chemist, Svante Arrhenius, presented a paper to the Stockholm Physical Society titled On the Influence of Carbonic Acid in the Air upon the Temperature of the Ground, in which he argued that the use of fossil fuel for combustion would lead to global warming (Hoffert, et al., 2002; Somerville, 2006). Although he was right, the widespread availability of inexpensive petroleum during the 20th century seemed to disregard this theory. Today the earth must deal with the consequences of global climate change and somehow meet expanding energy needs while limiting greenhouse gas emissions. Global warming, depletion of fossil fuels and price rise of petroleum-based fuels are causing great concern, and the exigency of the situation has encouraged the search for alternative, sustainable, renewable, efficient and cost-effective energy sources with lesser greenhouse gas (GHG) emissions (Nigam and Singh, 2010). The transportation sector worldwide has become has most entirely reliant on petroleum-based fuels and is responsible for 60% of the world oil consumption (Balat & Balat, 2009). Furthermore, the transportation sector accounts for more than 70% of global carbon monoxide (CO) emissions and 19% of global carbon dioxide (CO₂) emissions (Goldemberg, 2008; Balat & Balat, 2009). Around the world, there were about 806 million cars and light trucks in 2007 and the number is expected to reach 1.3 billion by 2030 (Balat, 2011). This huge growth will influence the stability of ecosystems and global climate as well as global oil reserves.
Energy security and environmental concerns have led to a concerted search for alternatives to fossil fuels. Different types of renewable energy are currently being researched, namely solar, wind, geothermal, hydrothermal and biofuels. Biofuels are liquid or gaseous fuels produced from renewable resources that absorb carbon dioxide via photosynthesis as they grow. When biofuels are burned, only the carbon dioxide absorbed by the biomass is released, so the net production of greenhouse gases (GHG) in total can be lower than that of burning fossil fuels. Some of advantages of using biofuel are shown in figure 1 (IEA, 2011). This paper will review the major issues concerning the technology development and challenges in production of bioethanol. It will focus on the current status of glycerol fermentation using *Escherichia coli* to produce ethanol and other value added products.

**Fig. 1:** Environmental, social and economic aspects of biofuel and bioenergy production (IEA, 2011).

**Fuel Ethanol in the World:**

Worldwide interest has increased in identifying, developing and commercializing technologies for alternative renewable sources of energy. The most feasible biofuels for vehicles being considered globally are biodiesel and bioethanol. Its market grew from less than a billion liters in 1975 to more than 39 billion liters in 2006, and is expected to reach 100 billion liters in 2015 (Mussatto, *et al*., 2010). Brazil and the United States are the largest promoters of bio-fuels in the world. Between 2000 and 2009 fuel ethanol output experienced an increase from 16.9 to 72.0 billion liters while biodiesel grew from 0.8 to 14.7 billion liters (Brown, 2009). Figure 2 shows the annually ethanol and biodiesel production from 2000-2009 (Brown, 2009).

Pure ethanol is used rarely as a transportation fuel. Instead, it is mixed with gasoline, ranging from 10% to 85% in content, and is used mainly as a gasoline additive because it contains a high percentage of oxygen (35%) and provides a high octane rating (113) (Dias De Oliveira, *et al*., 2005). Bioethanol can be produced from a number of crops including sugarcane, corn, sorghum, grains, potatoes, etc. Biodiesel, however, is the fuel that can be produced from vegetable oils-edible and non-edible, recycled waste vegetable oils, and animal fat (Jianxin, *et al*., 2007; Agarwal, 2007). The transfer of crude oil-based refinery to biomass-based biorefinery has attracted strong scientific interest which focuses on the development of cellulosic ethanol as an alternative transportation fuel to petroleum fuels.

**Fig. 2:** World annual ethanol and biodiesel production, 1975–2009 (reproduced from Brown, 2009)
Global production of ethanol was about 17,335 million gallons (65,614 million liters) in 2008. The United States led the world in production of fuel ethanol and produced 9000 million gallon (34,065 million liters). Brazil was second with production of 6472 million gallon (24,497 million liters), followed by the European Union (733 million gallon, or 2777 million liters) and China (501 million gallon, 1900 million liters) (Renewable Fuels Associate, 2010). In the United States, more than 90% of fuel ethanol is derived from corn feedstock, while sugarcane and molasses are the primary sources for ethanol production in Brazil (Rushing, 2008).

In 1970, Brazil set up a National Alcohol Program (ProAlcool) focusing on the production of ethanol from sugar cane and today it is regarded as one of the more developed nation in ethanol production, being also one of the world’s largest producer of this bio-fuel (RFA, 2010). Since the introduction of ProAlcool policy in Brazil, a substantial increase in the ethanol production has occurred, from 555 million liters in 1975/76 to more than 16 billion liters in 2005/06 (Orellana and Bonalume Neto, 2006). Brazil has continued its ethanol expansion plans, by adding new sugar plantations and ethanol production capacity. Brazil’s ethanol is recognized as the most price-competitive biofuel in the world. According to de Almeida, et al. (2008) the average production cost of ethanol projects to be around US$0.37 per liter. These values would make ethanol competitive with oil prices at about US$42 per barrel. Another estimate by Kojima and Johnson (2006) has put fuel ethanol average production costs in Brazil at the range of between US$0.25 and US$0.29 per liter. Favorable climate conditions, abundant and productive land allow Brazil to produce over 30 million tons of sugar and 20 billion liters of ethanol annually (Cerqueira Leite, et al., 2009). Brazil’s low manufacturing expenses are the result of the production feedstock, sugarcane, which is relatively cheap. High levels of land productivity combined with almost no needs for irrigation gives cheap feedstock. In addition, the mills are able to satisfy almost all of their energy needs through co-generation power plants based on bagasse, a by-product of sugarcane (de Almeida, et al., 2008).

Similar to Brazil, the United States started to invest in the production of ethanol fuel as an alternative source of energy to rely less on oil. The ethanol industry in United States has shown a fast growth and USA capacity of ethanol production increased from 1.63 billion gallons in 2000, to 9 billion gallons in 2008, representing a 5.5-fold increase. In 2006, ethanol production in the United States overtook the Brazilian production, which was the world's largest producer for decades. The obligatory consumption of given biofuel volumes was first implemented with the inclusion of Renewable Fuel Standards (RFS1) in the Energy Policy Act of 2005. The objective was to employ 4 billion gallons of renewable in transport fuels in 2006 (RFA, 2010). The guiding principle of biofuel policies has been a reduction in the US’s dependency on oil. This speedy growth in ethanol production is predicted to continue at least until 2012, when the United States intend to attain an ethanol production of 28 billion liters/year (Solomon, et al., 2007). Currently, over 95% of ethanol production in the United States comes from corn, with the rest made from wheat, barley, cheese whey, and beverage residues (Solomon, et al., 2007).

The parliament of the European Union in April 2009 endorsed a minimum binding target of 10% for biofuels in transport by 2020 as part of the EU Directive 2009/28/EC on renewable energy. (Directive 2003/30/EC, 2009) The directive also specified a minimum 35% reduction in GHG emissions to be achieved by biofuels during their lifecycle, a target that is meant to increase to at least 50% starting from 2017. In several countries in Europe, tax reductions or exemptions have been implemented in order to support production or consumption of biofuels. In Europe, ethanol is mostly produced from wheat and sugar-beet. France, Germany and Spain are Europe’s most strongly committed nations to ethanol production (Prieur-Vernat & His, 2006).

China’s biofuel policy focuses on ethanol production. The Ethanol Promotion Program was launched in 2002 in order to make use of excessive maize stock-piles. In August 2007 the National and Development Reform Commission (NDRC) announced a Medium and Long Term Development Plan for Renewable Energy. Renewable energy as a share of total primary energy consumption should rise to 10% by 2010 and to 15% by 2020. Ethanol production in China is projected to reach 2 million tonnes by 2010 and 10 million tonnes by 2020 (Liu & Lin, 2009). Many projects recently have focused on sorghum bioethanol to replace the use of petroleum based oil (Liu & Lin, 2009). China has also increased subsidies, tax and VAT exemptions in both biodiesel and ethanol to promote this industry.

**Energy Balance, Basic Properties and Green House Gas Emission of Ethanol Fuel:**

Ethanol (Bioethanol; ethyl alcohol) is a clear, colourless, flammable oxygenated hydrocarbon, with the chemical formula CH\(_3\)-CH\(_2\)-OH. It is a liquid biofuel produced from various biomass feed stocks and conversion technologies. Bioethanol is an attractive alternative fuel because it is renewable resource oxygenated fuel, thereby providing the potential to reduce particulate emissions in compression-ignition engines (Balat & Balat, 2009). Much controversy over the energy balance of ethanol was created by Pimentel (1998), who claimed that ethanol production from corn had a negative energy balance of 56300 British Thermal Units (BTU) per gallon of ethanol produced. Table 1 summarizes the energy inputs and outputs of ethanol as mentioned in
recent large-scale studies of ethanol production in the U.S. and Canada. Why Pimentel’s ethanol energy balance is so different from other researches was well explained and rebutted by Graboski (2002). In light of the justified criticisms of Pimentel’s work, and the consensus among other ethanol researchers, it is clear that ethanol does have a positive energy balance.

Table 1: Comparison of Energy Balances from Recent Ethanol Studies (BTU/US Gallon) (Graboski, 2002)

<table>
<thead>
<tr>
<th>Study</th>
<th>Inputs-Corn Growing</th>
<th>Inputs-Ethanol Manufacture</th>
<th>Co-Products Credits</th>
<th>Total Inputs</th>
<th>Net Energy Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pimentel (1998)</td>
<td>55,300</td>
<td>74,300</td>
<td>Nil</td>
<td>129600</td>
<td>(53600)</td>
</tr>
<tr>
<td>Wang (2001)</td>
<td>21,896</td>
<td>41,400 (dry mill)</td>
<td>14 076</td>
<td>49220</td>
<td>26780</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40,300 (wet mill)</td>
<td>12 493</td>
<td>49703</td>
<td>26297</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49220</td>
<td></td>
<td>49703</td>
<td></td>
</tr>
<tr>
<td>Graboski (2002)</td>
<td>21,268</td>
<td>48,539 (dry mill)</td>
<td>14 829</td>
<td>54978</td>
<td>21022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60,658 (wet mill)</td>
<td></td>
<td>67097</td>
<td>8903</td>
</tr>
<tr>
<td>Levelton (2000)</td>
<td>17,775</td>
<td>50415</td>
<td>14055</td>
<td>54135</td>
<td>21865</td>
</tr>
</tbody>
</table>

Besides being a renewable fuel, with high octane, ethanol is a much cleaner fuel than petrol. Ethanol blends dramatically reduce emissions of hydrocarbons, (major sources of ground level ozone formation), cancer-causing benzene and butadiene, sulfur dioxide and particulate matter. Moreover, ethanol blends can be used in all petrol engines without modifications (Miller, 2003; Demirbas, 2008). On a life-cycle basis, not all biofuels are equal in terms of environmental benefits. Figure 3 demonstrates the lower GHG emissions resulting from the use of bioethanol from various sources compared to gasoline on a life-cycle basis. As Figure 3 shows, corn-based bioethanol offers rather limited benefits, as it reduces GHG emissions by only 18% compared to gasoline. In contrast, sugarcane and cellulosic bioethanol result in almost 90% lower emissions (Philippidis, 2008).

To make sure that ‘‘good’’ bioethanol is produced, with reference to GHG benefits, the following demands must be met (Börjesson, 2009): (1) bioethanol plants should use biomass and not fossil fuels, (2) cultivation of annual feedstock crops should be avoided on land rich in carbon (above and below ground), such as peat soils used as permanent grassland, (3) by-products should be utilized efficiently in order to maximize their energy and GHG benefits, and (4) nitrous oxide emissions should be kept to a minimum by means of efficient fertilization strategies, and the commercial nitrogen fertilizer utilized should be produced in plants which have nitrous oxide gas cleaning. Disadvantages of bioethanol include lower energy density than gasoline (petrol) which can reduce fuel economy as illustrated in Figure 4. However, ethanol has a higher octave rating than petrol, potentially improving engine performance, and engine manufactures are developing engines that, by taking advantage of ethanol’s octave advantage, will yield improved fuel economy when using E85. Other disadvantages are low flame luminosity, lower vapor pressure (making cold starts difficult), and miscibility with water, (Balat, et al., 2008). Some properties of alcohol fuels are shown in Table 2 (Demirbas, 2008).

Another factor which is of importance in the field of fuel ethanol is the difference between anhydrous and hydrous alcohol. Anhydrous alcohol is free of water and at least 99% pure. This ethanol may be used in fuel blends. Hydrous alcohol on the other hand contains some water and usually has a purity of 96%. In Brazil, this ethanol is being used as a 100% gasoline substitute in cars with dedicated engines. The distinction between anhydrous and hydrous alcohol is of relevance not only in the fuel sector but may be regarded as the basic quality distinction in the ethanol market. Anhydrous ethanol can be blended with gasoline (petrol) in various ratios for use in unmodified gasoline engines; the most common blend of bioethanol with petrol (gasoline) 10% of ethanol to 90% of gasoline (called E10) (Demirbas, 2008; Balat & Balat, 2009). Higher percentage of ethanol can be used in blended fuels such as E85. (a blend of fuel mixed with 85% bioethanol and 15% gasoline) (De Oliviera, et al.,...
2005). The biologically produced ethanol contains about 5% of water. This is an azotropic mixture. Therefore, simple distillation is not enough to clean it. Hydrated ethanol is not completely mixed with gasoline or diesel fuel. By using an appropriate emulsifier bioethanol could be mixed with diesel. A mixture of hydrated ethanol with diesel oil with the emulsifier is called diesohol. Diesohol is blended in concentrations of 84.5% of diesel, 15% of hydrated ethanol and 0.5% of emulsifier (Demirbas, 2008). Table 3 shows the properties of gasoline fuel blended with various percentages of ethanol (Tangka, et al., 2011).

![Fig. 4: Volumetric energy density of fuels adjusted for engine efficiency](image)

**Table 2: Fuel properties of gasoline, methanol, ethanol.**

<table>
<thead>
<tr>
<th>Property</th>
<th>Gasoline</th>
<th>Methanol</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C₄-C₁₂</td>
<td>CH₃OH</td>
<td>C₂H₅OH</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>95-120</td>
<td>32</td>
<td>46</td>
</tr>
<tr>
<td>Lower heating value (MJ/Kg)</td>
<td>44</td>
<td>20.26</td>
<td>27</td>
</tr>
<tr>
<td>Stoichiometric AFR (Kg/Kg)</td>
<td>14.8</td>
<td>6.52</td>
<td>9.05</td>
</tr>
<tr>
<td>Density (Kg/m³)</td>
<td>0.70-0.75</td>
<td>0.795</td>
<td>0.79</td>
</tr>
<tr>
<td>Boiling point (°C at 1 atm)</td>
<td>25-215</td>
<td>65</td>
<td>78</td>
</tr>
<tr>
<td>Self-ignition temperature (°C)</td>
<td>300-400</td>
<td>500</td>
<td>420</td>
</tr>
<tr>
<td>Latent heat of evaporation (KJ/Kg)</td>
<td>310-320</td>
<td>1100</td>
<td>862</td>
</tr>
<tr>
<td>Stoichiometric mixture heating value (KJ/m³ atm at 20°C)</td>
<td>3750</td>
<td>3557</td>
<td>3660</td>
</tr>
<tr>
<td>RON</td>
<td>90</td>
<td>110</td>
<td>106</td>
</tr>
<tr>
<td>MON</td>
<td>81-89</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td>Carbon (wt%)</td>
<td>85.5</td>
<td>37.5</td>
<td>52.2</td>
</tr>
<tr>
<td>Oxygen (wt%)</td>
<td>0</td>
<td>12.5</td>
<td>13</td>
</tr>
<tr>
<td>Hydrogen (wt%)</td>
<td>14.5</td>
<td>50</td>
<td>34.8</td>
</tr>
</tbody>
</table>

**Table 3: Properties of gasoline fuel blended with various percentages of ethanol (Average values) (Tangka, et al., 2011).**

<table>
<thead>
<tr>
<th>Sample code</th>
<th>% Ethanol</th>
<th>% Gasoline</th>
<th>Flash point (°C)</th>
<th>Auto ignition temperature (°C)</th>
<th>Vapor pressure (Kpa at 37.8°C)</th>
<th>Energy Density (MJ/L)</th>
<th>Octane number</th>
<th>Specific gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>E0</td>
<td>00</td>
<td>100</td>
<td>-65</td>
<td>246</td>
<td>36</td>
<td>34.2</td>
<td>91</td>
<td>0.7474</td>
</tr>
<tr>
<td>E10</td>
<td>10</td>
<td>90</td>
<td>-40</td>
<td>260</td>
<td>38.9</td>
<td>33.182</td>
<td>93</td>
<td>0.7508</td>
</tr>
<tr>
<td>E20</td>
<td>20</td>
<td>80</td>
<td>-20</td>
<td>279</td>
<td>39</td>
<td>32</td>
<td>94</td>
<td>0.7605</td>
</tr>
<tr>
<td>E30</td>
<td>30</td>
<td>70</td>
<td>-15</td>
<td>281</td>
<td>38</td>
<td>31.5</td>
<td>95</td>
<td>0.7782</td>
</tr>
<tr>
<td>E40</td>
<td>40</td>
<td>60</td>
<td>-13.5</td>
<td>294</td>
<td>35.6</td>
<td>30</td>
<td>97</td>
<td>0.7792</td>
</tr>
<tr>
<td>E50</td>
<td>50</td>
<td>50</td>
<td>-5</td>
<td>320</td>
<td>34</td>
<td>29</td>
<td>99</td>
<td>0.7805</td>
</tr>
<tr>
<td>E60</td>
<td>60</td>
<td>40</td>
<td>-1</td>
<td>345</td>
<td>31</td>
<td>28</td>
<td>100</td>
<td>0.7812</td>
</tr>
<tr>
<td>E70</td>
<td>70</td>
<td>30</td>
<td>0.00</td>
<td>350</td>
<td>28</td>
<td>27</td>
<td>103</td>
<td>0.7823</td>
</tr>
<tr>
<td>E80</td>
<td>80</td>
<td>20</td>
<td>5</td>
<td>362</td>
<td>24</td>
<td>26.5</td>
<td>104</td>
<td>0.7834</td>
</tr>
<tr>
<td>E90</td>
<td>90</td>
<td>10</td>
<td>8.5</td>
<td>360</td>
<td>18</td>
<td>23.6</td>
<td>106</td>
<td>0.7840</td>
</tr>
<tr>
<td>E100</td>
<td>100</td>
<td>00</td>
<td>12.5</td>
<td>365</td>
<td>9</td>
<td>23.5</td>
<td>129</td>
<td>0.7890</td>
</tr>
</tbody>
</table>
Major Issues Attributed to Fuel Ethanol Feedstock:

All plant and plant derived materials has great potential to provide renewable energy for the future. The complexity of the production process depends on the feedstock. The spectrum of designed and implemented technologies goes from the simple conversion of sugars by fermentation, to the multi-stage conversion of lignocellulosic biomass into ethanol. Feed stocks for the production of bioethanol can be classified into three categories namely; sucrose-containing feed stocks (e.g. sugar cane, sugar beet and fruits), starchy materials (e.g. corn, wheat, rice, potatoes, cassava, sorghum, sweet potatoes and barley), and lignocellulose biomass (e.g. wood, straw, and grasses, corn cobs and stalks) (Balat & Balat, 2009). Almost all fuel ethanol is currently produced is the first-generation ethanol (sugar and starch feed stock). About half the world’s bioethanol production uses sugar crops as feedstock, mostly sugarcanes but also beets. The majority of the remaining ethanol is produced from starch crops, mainly grains such as corn and wheat (Bai, et al., 2008). Estimates show that feedstock costs can sometimes represent three quarters of bioethanol production costs (Gnansounou & Dauriat, 2005). The use of herbicides, fertilizer, labor, firm machinery, electricity and water must be considered in the process of feedstock cultivation. Table 4 highlights some comparisons of the yield and cost of some common feedstock crops (Gnansounou & Dauriat, 2005).

There exist several reports on bioethanol production from lignocellulosic waste materials such as crop residues (Kim and Dale, 2004), municipal solid waste (Muti and Nakamura, 2005), forest products industry wastes (Kadar, et al., 2004; Fan, et al., 2003), leaf and yard waste (Lissens, et al., 2004), as well as a few studies involving dairy and cattle manures (Wen, et al., 2004; Chen, et al., 2003, 2004). Nonetheless, the feasibility of using these materials as a feedstock is often limited by the low yield and the high cost of the hydrolysis process based on current technologies. Lignocellulosic ethanol or also called as second-generation ethanol, is produced in almost the same way as first-generation ethanol (sugar and starch feedstock). The pre-treatment needed to access the fermentable sugars in the ligneo-cellulosic plant materials, however, is much more difficult and may, depending on the feedstock, require acid, pressurized steam, special enzymes or a combination of those. These methods can result in undesirable toxins that inhibit the following fermentation process (Mann, 2004). Once decomposed, the biomass requires a fermentation process in which both hemicellulose (C5) and cellulose (C6) sugars must be processed. The major advantage of cellulosic ethanol is the low cost of feedstock, which as mentioned can be agricultural or forestry residues or more dedicated energy crops such as willow and switchgrass. Another advantage is that second-generation production does not conflict, in the same way as first-generation ethanol, with production of human food. Unfortunately, the economics of cellulosic ethanol are currently at a stage where the low cost of feedstock does not outweigh the high cost of production (Gnansounou & Dauriat, 2005). Table 5 shows the costs of ethanol production that comes from corn and cellulosic materials.

Table 4: The production cost and bioethanol yield of various crops

<table>
<thead>
<tr>
<th>Type</th>
<th>Annual yield (ton/ha)</th>
<th>Conversion rate to sugar Or starch (%)</th>
<th>Conversion rate to ethanol (L/ton)</th>
<th>Annual Yield (kg/ha)</th>
<th>Cost (US$/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar cane</td>
<td>70</td>
<td>12.5</td>
<td>70</td>
<td>4900</td>
<td>-160</td>
</tr>
<tr>
<td>Cassava</td>
<td>40</td>
<td>25</td>
<td>150</td>
<td>6000</td>
<td>700</td>
</tr>
<tr>
<td>Sweet</td>
<td>35</td>
<td>14</td>
<td>80</td>
<td>2800</td>
<td>200-300</td>
</tr>
<tr>
<td>Corn</td>
<td>5</td>
<td>69</td>
<td>410</td>
<td>2050</td>
<td>250-420</td>
</tr>
<tr>
<td>Wheat</td>
<td>4</td>
<td>66</td>
<td>390</td>
<td>1560</td>
<td>380-480</td>
</tr>
</tbody>
</table>

Table 5: Costs of ethanol production from corn and cellulosic feed stocks (Collins, 2007).

<table>
<thead>
<tr>
<th>Type</th>
<th>Cost (US$/gallon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feedstock</td>
<td>1.17 (available at $3.22 per bushel)</td>
</tr>
<tr>
<td>Enzymes</td>
<td>0.04</td>
</tr>
<tr>
<td>Other costs</td>
<td>0.62</td>
</tr>
<tr>
<td>Capital costs</td>
<td>0.20</td>
</tr>
<tr>
<td>Total costs</td>
<td>2.03</td>
</tr>
<tr>
<td>By-product</td>
<td>-0.38</td>
</tr>
<tr>
<td>Net costs</td>
<td>1.65</td>
</tr>
</tbody>
</table>

The most common concern related to the current first generation biofuel systems is that as production capacities increase, the production of biofuels takes different dynamic in relation to fossil fuels, being based on agricultural products, where land is the main input (Rathmann, et al., 2010). This represents a shift in land use away from food production and poses a global dilemma, namely the need to feed humanity versus the greater monetary returns to farmers through the incorporation of lands for agro-energy (Schmidhuber, 2006). A number of studies have looked at the impact of bioethanol expansion on agricultural markets both at the national and global levels (ElObeid, et al., 2007; Hill, et al., 2006; Secchi and Babcock, 2007; Tokgoz, et al., 2007). These studies reveal that, in general, with increased ethanol expansion, the prices of both the agricultural feedstock commodities and their competing crops rise with implications for land allocations, food prices, and the
environment. While there is consensus on the impact of the growth in bioethanol on the prices of agricultural commodities, the debate as to whether the net effect on the economy and the environment is positive or negative is still continuing. Doornbosch and Steenblik (2007), for example, contend that the contributions of bioethanol to energy demands are very limited given their adverse effects on food prices and the environment.

**Biodiesel Production:**

Biodiesel is a suitable substitute for petroleum-derived diesel. It is biodegradable, almost sulfurless and a renewable fuel, though still not produced by environmentally friendly routes. This alternative fuel consists of methyl or ethyl esters, a result of either transesterification of triacylglycerides (TG) or esterification of free fatty acids (FFAs) (Maa & Hannab, 1999). Currently, biodiesel mostly comes from transesterification of edible resources such as animal fats, vegetable oils, and even waste cooking oils, under alkaline catalysis conditions (Chhetri, et al., 2008; Zheng, et al., 2006; Kima, et al., 2004). Figure 5 shows transesterification of vegetable oils or animal fats with the addition of alcohol.

![Fig. 5: Transesterification of triglycerides with alcohol (Demirbas, 2009).](image)

In general, the biodiesel has similar composition and characteristics when compared to petroleum-derived diesel such as cetane number, energy content, viscosity and phase changes. Therefore, when blended with petroleum-derived diesel, it can be used in any IC diesel engine without any modifications. Several of its distinct advantages such as lower greenhouse gases emissions, higher lubricity and cetane ignition rating compare to petroleum derived diesel have enabled biodiesel to become one of the most common biofuels in the world (Lim & Teong, 2010). The transesterification is an equilibrium reaction and the transformation occurs essentially by mixing the reactants. The presence of a catalyst (acid or base) could accelerate and control the equilibrium, to achieve a high yield of the ester, however the alcohol has to be used in excess is used to increase the yield of the alkyl esters and to allow its physical separation from the glycerol formed. Various other factors determine the extent of the reaction such as type of catalyst (acid or base), alcohol to vegetable oil molar ratio, temperature, purity of the reactants and free fatty acid content. The rise prices of vegetable oil and the occasional formation of soaps, and the low yields, make biodiesel currently more expensive than petroleum-derived fuel (Haas, 2005).

The development and commercial use of biodiesel has been rapidly expanding in Europe and US for over 10 years. The superiority of biodiesel over petroleum diesel towards health and environment (free sulfur content, low content of harmful emission, e.g. particulate matter, HC, CO, etc., better lifecycle of CO$_2$ for global warming alleviation) as well as engine performance (enhance lubricity, high cetane number for complete combustion) (Pramanik and Tripathi, 2005; Bournay et al., 2005) has prompted Asia to use biodiesel as alternative fuel. Figure 6 shows the world biodiesel production (Canakci, et al., 2009). In the EU, biodiesel is by far the biggest biofuel and represents 82% of the biofuel production. Germany produced 1.9 billion liters or more than half the world total. In February 2006, the European Union set the goal of fulfilling 5.75% of transportation-fuel needs with biofuels in all member states by 2010. Many countries have adopted various policy initiatives. Specific legislation to promote and regulate the use of biodiesel is in force in Germany, Italy, France, Austria and Sweden (Canakci, et al., 2009). By 2010, the United States is expected to become the world’s largest single biodiesel market, accounting for roughly 18% of world biodiesel consumption, followed by Germany. New and large single markets for biodiesel are expected to emerge in China, India and Brazil (Yusuf, et al., 2011).
Malaysia’s biodiesel production is mainly palm oil based and since 1980s, Malaysian Palm Oil Board (MPOB) in collaboration with the local oil company ‘‘Petronas’’ has begun Trans esterification of crude palm oil into palm biodiesel (also known as palm diesel) (Kalam & Masjuki, 2008). It is currently the world’s second largest producer, accounting for 42.3% of worldwide production and 48.3% of the world’s total exports of palm oil. The Malaysian Government has been researching the use of a B5 (5% processed palm oil and 95% diesel) blend for vehicles and industrial sectors (Zhou & Thomson, 2009). According to Malaysian Biodiesel Association (MBA), there are 10 active biodiesel plants in the country with a total annual biodiesel installed capacity of 1.2 million tons. An additional four biodiesel plants with combined annual capacity of 190,000 tons are on the way to be completed soon (Lim & Teong, 2010).

Alongside the rapid pace of biodiesel development and commercialization, there are several key challenges emerging and one of them is the inevitable low value production of glycerol as by-product of biodiesel from transesterification and esterification of vegetable oil. Stoichiometrically, glycerol is produced by 10 wt.% of total biodiesel production. The mass production of biodiesel would bring about surplus glycerol production which has 80–88% purity (Pramanik and Tripathi, 2005). The conventional application and current market of glycerol could not cope the excess production that need further costly purification step to meet the purity of crude glycerin of industrial grade (98% purity) (Kenkel and Holcomb, 2008). A report from Tyson (2003) indicated that by taking into account the value of glycerol, the biodiesel cost production could be reduced from US$ 0.63 per L to US$ 0.38 per L. Figure 7 represent the biodiesel production over the years and its impact on the price trend of glycerol (Yazdani and Gonzalez, 2007).
Glycerol, or its chemical name propa-1,2,3-triol, is the simplest trihydric alcohol. The glycerol molecule has two primary and a secondary hydroxyl groups. Glycerol is a water soluble, colorless, odorless, viscous, and hygroscopic liquid with a specific gravity of 1.261 g/mL, melting temperature of 18.2°C, and a boiling temperature of 290°C (accompanied by decomposition). Chemically, glycerol is available for reacting with a stable alcohol under most operation conditions, and it is basically non-toxic to human health and to the environment. The key feature of its usefulness is the particular combination among its physicochemical properties, compatibility with other substances, and easy handling. Due to these particular properties glycerol has found more than 1500 end-uses or large volume applications. Figure 8 shows traditional glycerol applications (Van Loo, 2006).

In general, about 10 kg crude glycerol is produced for every 100 kg of biodiesel. Crude glycerol generated by homogeneous base-catalyzed transesterification contains approximately 50 to 60% of glycerol, 12 to 16% of alkalis, especially in the form of alkali soaps and hydroxides, 15 to 18% of methyl esters, 8 to 12% of methanol, and 2 to 3% of water. In addition to that, crude glycerol also contains a variety of elements such as Ca, Mg, P, or S (Thompson & He, 2006). The wide range of the purity values can be attributed to different glycerol purification methods used by biodiesel producers and the different feedstocks used in biodiesel production. Despite the wide applications of pure glycerol in pharmaceutical, food, and cosmetic industries, the refining of crude glycerol to a high purity is very expensive, especially for small and medium biodiesel producers (Pachauri and He, 2006). To improve the economic feasibility of the biodiesel industry, alternate ways of using the crude glycerol phase have recently been studied. One of many promising applications of the glycerol surplus is its bioconversion to high value compounds through microbial fermentation. Bioconversion is a cheap way to obtain reduced chemicals (e.g., succinate, ethanol, xylitol, propionate, hydrogen, etc.), at higher yields than those obtained from sugars (Dharmadi, et al., 2006).

**Fig. 8:** Traditional glycerol applications (Van Loo, 2006).

**Glycerol as a new type of feedstock:**

The amount of raw glycerol increases constantly and this triol has become an attractive feedstock material. The substantial increase in crude glycerol has created a need for a quick conversion of large quantities of glycerol into useful products. Although combustion of glycerol for heat is one solution that can consume large quantities, it is not ideal from an economics perspective. To help make biodiesel plants more profitable, it is desirable to convert glycerol into a commodity chemical that brings a higher price and has (or could have) a large market (Anand & Saxena, 2011). Clearly, the development of processes to convert crude glycerol into higher-value products is a dire need. Table 6 shows contamination in raw glycerol obtained as a by-product of biodiesel production (Papanikolaou, et al., 2000).

**Table 6:** Residues in raw glycerol obtained as a by-product of biodiesel production (Papanikolaou, et al., 2000).

<table>
<thead>
<tr>
<th>Contamination</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na and K salts</td>
<td>4.5</td>
</tr>
<tr>
<td>Methanol</td>
<td>3</td>
</tr>
<tr>
<td>Heavy metals and lignin</td>
<td>1</td>
</tr>
<tr>
<td>Other organic materials</td>
<td>0.5</td>
</tr>
<tr>
<td>Water</td>
<td>26</td>
</tr>
</tbody>
</table>
Glycerol from biodiesel is considered as a cheap reagent in bio-chemistry and biotechnology for synthesis of value-added products. As shown in figure 9, glycerol can be fermented to syngas that can be used for the Fisher-Tropsch or methanol synthesis, or reformed to H₂ or CH₄ (Simonetti, et al., 2007). Dihydroxyacetone, a chemical which is used as a tanning agent, can be obtained both biochemically using bacteria and by oxidation over Pd, Pt, Au catalysts (Demirel, et al., 2007). Succinic acid obtained mainly via fermentation of glycerol is used as an intermediate in organic synthesis and as a monomer in polymer production (Song and Lee, 2006). 1,3-Propanediol, propylene glycol, acrolein and glycerol carbonate are used for synthesis of biodegradable polymers (Kurian, 2005). Many useful products like ethanol, butanol, lactic, propionic, acetic and butyric acids can be obtained via biological fermentation of glycerol (Silva, et al., 2009). One can say that the increased production of glycerol as a by-product has provoked a huge interest in its utilization for the synthesis of useful value-added products, gases and fuels. However, only processes with low cost are commercially viable.

6.1 Bacterial fermentation of glycerol:

Fermentation is one of the most convenient methods of conversion of glycerol. In the last few years glycerol conversion to 1,3-propanediol by anaerobic bacteria (Figure 10) was studied intensively due to the potential use of the product diol in the production of polymers (Kurian, 2005). Although many microorganisms are able to metabolize glycerol in the presence of external electron acceptors (respiratory metabolism), few are able to do so fermentatively (i.e., absence of electron acceptors). Fermentative metabolism of glycerol has been reported in species of the genera Klebsiella, Citrobacter, Enterobacter, Clostridium, Lactobacillus, Bacillus, Propionibacterium, and Anaerobiospirillum (Yazdani & Gonzalez, 2007). Enterobacteria of the genera Klebsiella, Enterobacter, Citrobacter and Clostridium are capable to ferment glycerol producing 1,3-propanediol as major product. The fermentative transformation of glycerol to 1,3-propanediol by C. butyricum bacteria attracts much attention compared to other strains (Klebsiella pneumoniae, Citrobacter freundii, Enterobacter agglomerans), because it allows to obtain high yield of the product. It is also more stable, since the key enzyme, glycerol dehydratase, is vitamin B12-independent, not deactivated by glycerol, and the toxic intermediate - 3-hydroxypropionaldehyde - does not accumulate in this process (Barbirato, et al., 1998). Fermentations of glycerol by Enterobacteria results in the accumulation of two main products, 1,3-propanediol and acetate, whereas byproducts, such as lactate, formate, succinate and ethanol, are differentially produced according to culture conditions (Fig. 11).
Fermentation of glycerol by Clostridium leads to 1,3-propanediol as a major component, two acids (butyrate and acetate) as byproducts and gaseous products, CO\textsubscript{2} and H\textsubscript{2}. The theoretical yield of 1,3-propanediol is 64 or 50 \%, if acetic or butyric acid is formed as the main by-product. Usually microorganisms use all the pathways simultaneously, therefore the total yield of 1,3-propanediol can vary (Biebl, et al., 1999; Saxena, et al., 2009). Industrial glycerol available directly from biodiesel production is frequently contaminated with salts and other residues. Despite the opinion that this glycerol is not suitable for biochemical processes, an example of using industrial glycerol for 1,3-propanediol production was demonstrated by Papanikolaou, et al., (2000). It is still unclear whether high concentration of 1,3-propanediol inhibits the consumption of glycerol or not, but undoubtedly pH plays a significant role, leading to the decrease in 1,3-propanediol production at low pH values due to the formation of acetic and butyric acids (Biebl, et al., 1999). High concentrations of glycerol up to 130 g/ L can be fermented in batch cultures with Clostridium bacteria, however, it was shown in batch cultures that the initial glycerol concentration higher than 150 g/L can cause prolonged lag phase and inhibits bacterial growth (Colin, et al., 2000). High concentrations of 1,3-propanediol up to 80 g/ L are likely to be non-inhibitory (Papanikolaou, et al., 2000).

To date, most research has focused on fermentation methods using pure microbial cultures (bacterial strains in the genera Citrobacter, Enterobacter, Ilyobacter, Klebsiella, Lactobacillus, Pelobacter, and Clostridium) (Pagliaro, et al., 2007). However, this involves expensive equipment and complicated protocols, and is costly. The use of microbes such as Escherichia coli, an organism very amenable to industrial applications, can easily help overcome the aforementioned problems (Murarka, et al., 2008). The non-native producers, Escherichia coli and Saccharomyces cerevisiae, have also been engineered for 1, 3-propenediol production. In S. cerevisiae, due to ineffective transport of vitamin B12 needed for 1, 3-propenediol synthesis, only low levels of the product has been obtained. On the other hand, E. coli has been metabolically engineered by DuPont and Genencor International, Inc., to produce 1, 3-propenediol at a concentration of 135 g/l, (Maervoet, et al., 2011) the highest reported so far in the industry. A major concern with the existing 1, 3-propenediol non-native producers is that a majority of them are opportunistic pathogens. The potentials for using these organisms at the industrial level could be limited due to issues that include pathogenicity, requirement of strict anaerobic conditions, need of
supplementation with rich nutrients, and unavailability of the genetic tools and physiological knowledge necessary for their effective manipulation. Besides the usual production of 1,3-propenediol from glycerol, Jarvis, et al. (1997) demonstrated that formate and ethanol are the major products of glycerol fermentation by *Klebsiella planticola* isolated from the rumen of red deer. Ito, et al. (2005) stated that *Enterobacter aerogenes* mutant can be used for the high-yield production of ethanol from biodiesel wastes glycerol. The task for the future is to find effective non-pathogenic microorganisms capable of producing 1,3-propenediol from glycerol (Saxena, et al., 2009).

By contrast, methods involving mixed cultures are simple to undertake, incur lower costs, and are not affected by contamination problems. The drawback is that productivity is lower than that with pure cultures. Because of the recent increases in glycerol production from biodiesel refining superior options for the biological conversion of glycerol into value added products are extremely important. There are various ways to improve the productivity of methods using mixed cultures. These include controlling substrate concentrations, light and temperature as well as using fermentation promoters. Studies have been done to develop a novel bioprocess system using mixed bacterial cultures for the anaerobic conversion of high-loading glycerol. A recent development in the microbial fermentation of glycerol is that *Escherichia coli* can ferment glycerol anaerobically (Dharmadi, et al., 2006; Tokumoto and Tanaka, 2012). A study by Tokumoto and Tanaka (2012), showed that the bacteria *Escherichia coli* and particularly *Schizosaccharomyces pombe* (found within sewage sludge) and glucose (found within bacterial components (i.e., cell walls)) acted as efficient promoters of fermentation. A native, nonpathogenic strain of *E. coli*, able to ferment glycerol to useful products under anaerobic condition without the need of genetic engineering is the main objective of current research by many researchers. The key factor is not the type of strain, but rather on the appropriate environment including an acidic pH, avoiding accumulation of fermentation gas hydrogen and appropriate medium composition.

6.2 Downstream processing of biotechnologically produced 1,3-Propenediol:

The recovery of 1,3-Propenediol from complex fermentation broth represents a true bottleneck in the development of a commercially viable bioprocess. This could be mainly attributed to its high boiling point and presence of two hydroxyl groups which make it strongly hydrophilic and therefore complicate its extraction. Therefore, its recovery from complex aqueous fermentation broth solutions containing macromolecules, salts, remaining substrates and by-products, is a difficult task. First, microbial cells are removed by filtration or centrifugation. Then, 1,3-propanediol and by-products are distilled. This step requires a substantial energy input which accounts for high cost of the final product. The application of relatively simple approaches of evaporation and vacuum distillation for the recovery of 1,3-PD has been attempted but appeared unattractive and uneconomical due to the requirement of large amounts of energy, desalination and low product yield (Hao, et al., 2006). Li, et al (2001) attempted to recover 1,3-propanediol by pervaporation through a X-type zeolite membrane from a model 1,3-propanediol/glycerol/water solution and broth solutions containing glucose. Extraction is the most promising and studied method with the ethanol/inorganic salts solution being the most effective (Hao, et al., 2006). The use of ion exclusion for extraction of 1,3-PD has been reported. The method consisted of desalination of the fermented broth by the aid of strongly cationic and weakly basic anionic resins with subsequent passage over a cationic exchange resin for purification of 1,3-PD (Hao, et al., 2006). Probably the easiest and the most effective method of 1,3-propanediol recovery is a two-phase aqueous extraction with ethanol (or methanol)/inorganic salts solution. This approach showed the highest recovery of 93.7 % of 1,3-propanediol from fermentation broth by a mixture of ethanol and saturated (NH4)2SO4 solution. Moreover, this method allowed the removal of cells and proteins from the fermentation broth. As one can see, the problem of the down-stream processing of 1,3-propanediol obtained via microbial fermentation has not been solved effectively so far. Extraction is the most promising and studied method with the ethanol/inorganic salts solution being the most effective.

7.0 Enzymatic process in cascade reactions:

There is an increasing demand for environmentally friendly technologies and processes in the chemical industry due to the growing concern about the irrevocable harmful anthropogenic impact on the environment. In some cases traditional organic/inorganic catalysts or stoichiometric reactions could be replaced by biocatalytic (or enzymatic) routes, which offer environmental benefits. Biocatalysts seem to satisfy the criteria of green chemistry in terms of biodegradability, high selectivity, mild reaction conditions and low amounts of waste and by-products. A concept of combining bio-catalysts within one system emerged as a possible way to develop a chain of feedstocks transformations to value-added products through multi-step reactions. Such systems also allow to reduce waste and to intensify chemical processes by avoiding extraction and purification of the intermediates. Enzymes can also be immobilized by: 1) ionic binding to ion-exchange supports (e.g. cellulose and carboxymethyl cellulose), 2) adsorption through van der Waals interactions to hydrophobic supports (e.g.
polypropylene and teflon), 3) covalent binding between the amino or carboxyl groups of amino acids and the support membrane (Giorno and Drioli, 2000). Despite some mass-transfer problems, immobilized systems offer considerable advantages in comparison with conventional free biocatalysts: a) prolonged stability of the biocatalyst, increased tolerance to high substrate concentrations and reduced end-product inhibition; b) high biocatalyst density per unit of a bioreactor volume, which leads to high volumetric productivity and short reaction times; c) easy product recovery without separation and filtration, thus reduction in the cost of equipment and energy; d) regeneration and reuse of the biocatalysts for extended periods of time.

The concept of developing a chain of feedstocks transformations to value-added products through multi-step reactions allows to reduce waste and to intensify chemical processes by avoiding extraction and purification of the intermediates. It also reduces time and labor required to effect a given transformation. A tandem or cascade reaction is a reaction in which several bonds are formed in sequence without isolating intermediates, changing reaction conditions, or adding reagents. A sequential transformation of 1,3-propanediol formed via a biocatalytic fermentation by a catalyst integrated within the same reactor system. Several approaches can be proposed to carry out multistep bio-chemical transformation within a single reaction system. This is usually based on the principles of spatial separation by using immiscible solvents or by supporting the catalyst or cell on a support (immobilization).

**Conclusion:**

Ethanol has experienced unseen levels of attention due to its value as fuel alternative to gasoline, the increase of oil prices, and the climatic changes, besides being a renewable and sustainable energy source, efficient and safe to the environment. Currently, worldwide ethanol production is in high levels, and corn is the main raw material used for this purpose, but this scenario may change due to technological improvements that are being developed for production of low cost cellulosic ethanol, as well as for ethanol production from microalgae. It is important to emphasize that, to be a viable alternative, bio-ethanol must present a high net energy gain, have ecological benefits, be economically competitive and able to be produced in large scales without affecting the food provision. The use of various wastes (such as crude glycerol from biodiesel production, wood, agricultural wastes) and unconventional raw materials (such as microalgae) can solve the problem without sacrificing food demands. Under anaerobic fermentation conditions, facultative anaerobes such as *Escherichia coli* are able to convert glycerol into soluble metabolite products and gaseous products, including 1,3-propanediol, 2,3-butanediol, ethanol, acetic acid, succinic acid, H\textsubscript{2}, and CO\textsubscript{2}. Glycerol the most effective carbon substrate for *Escherichia coli*, which can form 1,3-PDO and 2,3-BDO, gaseous (H\textsubscript{2}) and liquid (ethanol) biofuels. Notably, ethanol production from glycerol was greatly enhanced upon fermentation by using *Escherichia coli* and Enhance the enzyme by immobilization process generate a large amount of bioethanol and decrease the cost process. The usage of low-grade quality of glycerol obtained from biodiesel production is a big challenge as this glycerol cannot be used for direct food and cosmetic uses. An effective usage or conversion of crude glycerol to specific products will cut down the biodiesel production costs. Glycerol can cover possible conversion into useful products such as 1,3-propanediol, 1,2-propanediol, dihydroxyacetones, hydrogen, polyglycerols, succinic acid, polyesters, and ethanol.

**References**


